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Obesity-Related Metabolic Syndrome: Investigations into Novel Clinical Markers and Possible Causes

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A thesis submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Sciences/Medicine and Health Science, The University of Auckland, 2011
ABSTRACT

Obesity-related metabolic syndrome (MetS) increases risks for degenerative cardiovascular disease (CVD), and has become widespread. Efforts to stem the increase of obesity are inadequate. New approaches to the problem may be required.

Novel CVD Risk and Prediction Markers were investigated for use in enhancing the utility of MetS. Subsequently, a unifying hypothesis on causes of MetS was developed.

MetS comprises central obesity, hypertension, dyslipidaemia and hyperglycaemia, but insufficiently reflects underlying oxidative, metabolic, inflammatory, and nutritional stresses. Novel CVD laboratory (1) Risk Markers; consisting of clinical routine screening haematology and biochemistry (CRShaem&Biochem): Leukocytes: neutrophils; monocytes; lymphocytes; eosinophils; erythrocyte sedimentation rate (ESR); haemoglobin A1c (HbA1c); urate; ferritin; liver function tests (LFT): alkaline phosphatase (AlkPhos); alanine transferase (ALT); aspartate transferase (AST); gamma glutamyl transferase (γGT); bilirubin (2) Protective Markers; (a) serum fat cell-derived, adiponectin (Adpn) with oligomers (high, medium, low molecular weight (HMW, MMW, LMW) (b) serum fat soluble vitamins (sFSVitamins) beta carotene (sβCaro), vitamins (sVit)D, sVitA, sVitE, and VitK(INR) were investigated.

Baseline, and mean over 6months (m), oxidants HbA1c and urate were strongly related to MetS marker count, a MetS index. Over 6m, on wide-ranging multivariable mixed modelling, γGT and ESR changed with MetS marker count.

Adpn showed anti- and pro-inflammatory correlations. sVitA and sVitE correlated with MetS marker count, and dyslipidaemia. sβCaro and sVitD correlated with protective markers. Thus 1) HbA1c, urate, γGT, ESR, and 2) sβCaro, sVitD may be acceptable Novel CVD Risk and Protective Markers, respectively, for use with MetS.

The unifying hypothesis on obesity and MetS was predicated on humans evolving proportionately large, energy demanding brains requiring co-adaptive mechanisms to: (1) increase dietary energy by developing strong neural self-reward/motivation systems for
the acquisition of energy dense food and (2) economise on body energy metabolism by
the co-option of many of antioxidant phytonutrients to confer long-lived cell protection.

The study indicated that strong risk markers in MetS are oxidant, and associate with
degenerative change, and antioxidant cytoprotection is augmented by plant food
micronutrients, such as food-derived or dietary βCaro (fβCaro). The unifying hypothesis
was compatible with the study results. Whole-food diets need studying for prevention and
reversal of MetS.
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PUBLICATIONS ARISING FROM WORK DESCRIBED IN THE THESIS

Papers


Abstracts


# TABLE OF CONTENTS

**ABSTRACT** ........................................................................................................................... II
**ACKNOWLEDGEMENTS** ........................................................................................................ IV
**PUBLICATIONS ARISING FROM WORK DESCRIBED IN THE THESIS** ................................ V
**LIST OF TABLES** ...................................................................................................................... XII
**LIST OF FIGURES** .................................................................................................................. XIII
**LIST OF APPENDICES** .......................................................................................................... XIV
**LIST OF ABBREVIATIONS** ..................................................................................................... XIV

## CHAPTER 1. INTRODUCTION AND BACKGROUND ............................................................... 1

1.1 Introduction ..................................................................................................................... 1
   1.1.1 Scope ..................................................................................................................... 1
     1.1.1.1 Thesis Structure ......................................................................................... 2
   1.1.2 General Introduction ............................................................................................. 2

1.2 Obesity ............................................................................................................................. 6
   1.2.1 Obesity - Definitions ............................................................................................. 6
   1.2.2 Obesity - Epidemiology and Natural History ....................................................... 8
     1.2.2.1 Obesity - Ethnicity and Socio-Economic Status ........................................ 9
   1.2.3 Causes of Obesity ................................................................................................. 10

1.3 Central Obesity Related Metabolic Syndrome ............................................................. 11
   1.3.1 Definitions of the Metabolic Syndrome .............................................................. 13
   1.3.2 Obesity and Type II Diabetes .............................................................................. 14
   1.3.3 Obesity and Cardiovascular Disease .................................................................. 16

1.4 Physiology of Metabolic Syndrome – Adipose Tissue, Macro- and Micro-nutrients ...... 19
   1.4.1 Adipose Tissue - Body Fat Distribution and Metabolism ..................................... 19
   1.4.2 Nutrients in Metabolic Syndrome ......................................................................... 20
     1.4.2.1 Lipids ........................................................................................................... 20
     1.4.2.2 Protein ........................................................................................................ 23
     1.4.2.3 Carbohydrate ............................................................................................... 24
     1.4.2.4 Micronutrients ............................................................................................ 27
   1.4.3 Pathophysiology of Metabolic Syndrome ............................................................ 31

1.5 Novel Cardiovascular Disease Risk and Protective Markers ........................................ 32
   1.5.1 Novel Metabolic Cardiovascular Disease Risk Markers ......................................... 33
     1.5.1.1 Clinical Routine Screening Haematology and Biochemistry ......................... 33
     1.5.1.2 Haematology ............................................................................................... 33
     1.5.1.3 Biochemistry Long Term Plasma Glucose Indicator/Oxidant ....................... 34
   1.5.2 Novel Metabolic Cardiovascular Disease Protective Markers ............................... 37
     1.5.2.1 Adiponectin ............................................................................................... 38
     1.5.2.2 Serum Fat Soluble Vitamins ......................................................................... 39
     1.5.2.3 Vitamin D ................................................................................................. 40
     1.5.2.4 Carotenoretinoids – Beta-Carotene and Vitamin A ...................................... 41
     1.5.2.5 Tocopherols and Vitamin E ......................................................................... 43
1.6 Theories on the Causes of Obesity and Metabolic Syndrome ....................... 43
1.6.1 Rationale for studies .................................................................................. 44
  1.6.1.1 What is Known .................................................................................. 44
  1.6.1.2 What is Needed ................................................................................ 45
1.7 Hypotheses ..................................................................................................... 46
  1.7.1 Overarching Hypotheses ....................................................................... 46
  1.7.2 The Clinically Tested Hypotheses .......................................................... 47
    1.7.2.1 The Clinically Tested Primary Hypotheses ...................................... 47
    1.7.2.2 The Clinically Tested Secondary Hypotheses ................................. 47
  1.7.3 The Unifying Hypotheses ...................................................................... 48
  1.7.4 Aims and Objectives ............................................................................. 48

CHAPTER 2. METHODS .......................................................................................... 51
  2.1 Introduction ................................................................................................. 51
  2.2 Study Designs: Rationale and Development ............................................. 51
    2.2.1 Study Concept Development ............................................................... 51
      2.2.1.1 Novel CVD Risk Markers – Concepts and Statistical Analyses Rationale .. 52
      2.2.1.2 Novel CVD Protective Markers – Concepts and Statistical Analyses Rationale .. 52
      2.2.1.3 Diet&Health-Weight Loss Trial .................................................... 53
  2.3 Contributors to the Dietary Fibre and Lifestyle for Health Studies .......... 54
    2.3.1 Author’s Contribution to the Studies .................................................... 54
      2.3.1.1 Collaborations ............................................................................. 57
      2.3.1.2 Study Site .................................................................................. 58
  2.4 Dietary Fibre and Lifestyle for Health – Weight Loss Trial ...................... 59
    2.4.1 Study participants ............................................................................... 59
      2.4.1.1 Eligibility criteria ...................................................................... 59
      2.4.1.2 Ethical Approval and Study Registration .................................... 60
    2.4.2 Study Procedures ............................................................................... 60
      2.4.2.1 Study Visits and Procedures ....................................................... 60
    2.4.3 Anthropometry .................................................................................... 64
      2.4.3.1 Weight, Waist, Height, Bioelectrical Impedance and Blood Pressure .. 64
      2.4.3.2 Randomisation, Blinding, Medication Dosing and Dispensing .......... 66
      2.4.3.3 Trial Medication Details ............................................................... 66
    2.4.4 Laboratory ............................................................................................ 67
      2.4.4.1 Laboratory Sample Analyses ....................................................... 68
      2.4.4.2 Biochemical Analytes ................................................................. 68
    2.4.5 Fibre and Faecal Fat Loss Mechanism Substudy ................................. 68
      2.4.5.1 Method ....................................................................................... 69
      2.4.5.2 Procedures .................................................................................. 69
      2.4.5.3 Eligibility Criteria ...................................................................... 69
    2.4.6 Demographic, Health Status and Lifestyle Questionnaire Analyses ...... 70
2.4.6.1 Demographic, Lifestyle, Quality of Life and Eating Attitude Questionnaires - Analytical Methods ................................................................. 70
2.4.6.2 Adverse Events Protocols ........................................................................ 72

2.5 Dietary Fibre and Lifestyle for Health – Novel Cardiovascular Disease Markers 72
2.5.1 Design, Population, Eligibility, Procedures, Anthropometry ...................... 73
2.5.2 Novel Cardiovascular Disease Risk Markers - Clinical Routine Screening Biochemistry and Haematology .............................................................. 74
2.5.2.1 Laboratory Analyses .............................................................................. 74

2.6 Novel Cardiovascular Disease Protective Markers ............................................ 76
2.6.1 Adiponectin, Adiponectin and Oligomers: Novel Cardiovascular Disease Protective Markers ................................................................................ 76
2.6.1.1 Laboratory Analyses .............................................................................. 78
2.6.2 Serum Vitamin D, Beta Carotene, Vitamin A and Vitamin E..................... 78
2.6.2.1 Laboratory Analyses .............................................................................. 79

CHAPTER 3. THE DIETARY FIBRE AND LIFESTYLE FOR HEALTH – WEIGHT LOSS TRIAL: A RANDOMISED PLACEBO-CONTROLLED WEIGHT LOSS MODEL ........................................ 81

3.1 Introduction ........................................................................................................ 81
3.1.1 Hypotheses ..................................................................................................... 82
3.1.2 Aims............................................................................................................... 82
3.2 Methods .............................................................................................................. 83
3.2.1 Method Rationale & Summary .................................................................... 83
3.2.2 Statistical Analyses ...................................................................................... 84
3.3 Results ................................................................................................................ 86
3.3.1 Participant characteristics .............................................................................. 86
3.3.2 Primary Outcome .......................................................................................... 87
3.3.3 Secondary Outcomes ..................................................................................... 87
3.3.4 Other measures .............................................................................................. 93
3.4 Fibre and Faecal Fat Loss Mechanism Substudy ............................................... 95
3.4.1 Method.......................................................................................................... 95
3.4.2 Laboratory Work ........................................................................................... 95
3.4.2.1 Results ...................................................................................................... 95
3.5 Discussion ........................................................................................................... 97
3.5.1 General Comments ....................................................................................... 97
3.6 Conclusion ......................................................................................................... 103

CHAPTER 4. NOVEL CARDIOVASCULAR DISEASE RISK MARKERS IN THE METABOLIC SYNDROME ...................................................................................................................... 104

4.1 Introduction ........................................................................................................ 104
4.1.1 Hypothesis ................................................................................................... 106
4.1.2 Aims.............................................................................................................. 106
4.2 Methods ............................................................................................................ 107
4.2.1 Method Rationale & Summary .................................................................... 107
4.2.2 Laboratory Procedures and Analyses .......................................................... 108
4.2.3 Statistical Analyses ...................................................................................... 108
  4.2.3.1 General Comments ................................................................................. 108
  4.2.3.2 Demographic, Health Status and Lifestyle Questionnaires ............ 109
  4.2.3.3 Anthropometry ....................................................................................... 109
  4.2.3.4 Metabolic Syndrome Comparisons ........................................................ 109
  4.2.3.5 Clinical Routine Screening Haematology and Biochemistry, and
          Metabolic Syndrome ............................................................................. 111
4.3 Results .............................................................................................................. 113
  4.3.1 Demographic, Health Status, Lifestyle and Quality of Life ................. 113
    4.3.1.1 Baseline Characteristics – Anthropometry and Questionnaire Data .. 113
  4.3.2 Anthropometry Variability .......................................................................... 116
  4.3.3 Metabolic Syndrome Comparisons .............................................................. 116
    4.3.3.1 Metabolic Syndrome Comparisons ........................................................ 120
    4.3.3.2 Clinical Routine Screening Haematology and Biochemistry and
            Metabolic Syndrome ............................................................................. 127
4.4 Discussion ........................................................................................................ 135

CHAPTER 5. NOVEL CARDIOVASCULAR DISEASE PROTECTIVE MARKERS ............... 144
  5.1 Introduction ...................................................................................................... 144
    5.1.1 Hypothesis ................................................................................................... 146
    5.1.2 Aims ............................................................................................................. 146
  5.2 Methods ............................................................................................................ 147
    5.2.1 Method Rationale & Summary .................................................................... 147
    5.2.2 Clinical Study Procedures ............................................................................ 148
    5.2.3 Laboratory Procedures and Analyses .......................................................... 148
    5.2.4 Statistical Analyses ...................................................................................... 149
      5.2.4.1 Adiponectin and Oligomers ................................................................... 149
      5.2.4.2 The Fat Soluble Vitamins ...................................................................... 150
  5.3 Results .............................................................................................................. 152
    5.3.1 Adiponectin and Oligomers ......................................................................... 152
    5.3.2 The Fat Soluble Vitamins ............................................................................ 158
  5.4 Discussion ........................................................................................................ 170

CHAPTER 6. CAUSES OF OBESITY AND METABOLIC SYNDROME ......................... 181
  6.1 Introduction ...................................................................................................... 181
    6.1.1 Scope ............................................................................................................ 181
    6.1.2 The Need for a New Theory on Causes of Obesity ..................................... 181
    6.1.3 Background to Theory Development ......................................................... 183
  6.2 Aims ................................................................................................................. 185
  6.3 Methods ............................................................................................................ 185
  6.4 Influences from Thesis Study on the Unifying Hypothesis ............................. 187
    6.4.1 The Unifying Hypothesis on Causes of Obesity and Metabolic Syndrome 187
      6.4.1.1 Unifying Hypothesis Corollary: Evolutionary Adaptations Outwitted? 189
## LIST OF TABLES

Table 1-1. NCEP-Derived Metabolic Syndrome Definitions .......................................................... 4
Table 1-2. Classifications of Obesity ............................................................................................... 8
Table 1-3. Obesity in New Zealand Adults by Ethnic Group (Unadjusted) ...................................... 10
Table 2-1. NHANES BMI Classification .......................................................................................... 60
Table 2-2. Laboratory Test Collection and Analytes ........................................................................ 68
Table 2-3. Faecal Collection Time Line .......................................................................................... 69
Table 2-4. Laboratory Test Collection and Analytes ....................................................................... 75
Table 2-5. Criteria for Selecting the Adpn30 Subset for Oligomer Analysis ..................................... 77
Table 2-6. Laboratory Test Collection and Analytes ..................................................................... 78
Table 2-7. Laboratory Test Collection and Analytes: Fat Soluble Vitamins .................................. 79
Table 3-1. Baseline Characteristics: All, Chitosan vs. Placebo ..................................................... 88
Table 3-2. Change in Weight Sensitivity Analyses: Chitosan vs. Placebo ........................................ 89
Table 3-3. Change, Baseline to 6m BP & Lipids Sensitivity Analyses: Chitosan vs. Placebo .......... 91
Table 3-4. Secondary & other Outcomes - Anthropometry, BP & Laboratory Analytes over 6m: Chitosan vs. Placebo ................................................................. 92
Table 3-5. Adverse Events by Treatment Group ........................................................................... 94
Table 3-6. Baseline Characteristics of the Fibre&Fat Loss Substudy Completers: n=29 ............... 96
Table 3-7. Clinical Trials of Chitosan for Weight Loss or Cholesterol Lowering ............................... 99
Table 3-8. Summary of % Weight Loss and Cholesterol Lowering Trials ...................................... 101
Table 4-1. Baseline Characteristics .................................................................................................. 114
Table 4-2. Baseline MetS Marker & CRSHaem&Biochem............................................................... 120
Table 4-3. Baseline, NCEP, NCEP/ADA & IDF/JIS MetS: Gender & Age Groups ......................... 121
Table 4-4. Baseline, MetS Marker Frequency: NCEP, NCEP/ADA & IDF/JIS MetS ...................... 122
Table 4-5. 6m Final Visit NCEP, NCEP/ADA & IDF/JIS MetS: Gender & Age Groups .............. 123
Table 4-6. Change, Baseline to 6m. MetS, Marker & MetS Marker Count: Women n=117, Men n=30 .......................................................................................................................................... 123
Table 4-7. Baseline. MetS Marker Count, Excluding MetS Marker Waist by Gender .................... 124
Table 4-8. Baseline. MetS Marker Count, Excluding MetS Marker Waist by Ethnicity .................. 124
Table 4-9. Baseline. Clinical Routine Screening Haematology/Biochemistry vs. MetS Marker Count, Simple and Partial Correlations .................................................................................. 128
Table 4-10. Relationships of MetS Markers and MetS Marker Count with Demographic, Health Status and Lifestyle Parameters .......................................................................................................................... 132
Table 4-11. Relationships of MetS Markers and MetS Marker Count vs. Clinical Routine Screening Haematology/Biochemistry – Inflammatory ................................................................................. 133
Table 4-12. Relationships of MetS Markers and MetS Marker Count vs. Clinical Routine Screening Haematology/Biochemistry – Oxidant ................................................................................................... 134
Table 5-1. Baseline, 6m & Change - Adpn250 & Adpn30: All, Women & Men .............................. 152
Table 5-2. General Linear Model. Baseline Adpn250: Demography, Lifestyle & Quality of Life 154
Table 5-3. Baseline Adpn250 vs. Study Parameters - Correlations: All, Women & Men .............. 155
Table 5-4. Change in HMW30 vs. Study Parameters, Correlations: All, Women & Men ............. 157
Table 5-5. Baseline Adpn30 vs. Adpn30 - Correlations: All, Women & Men ................................. 158
Table 5-6. Serum Lipids vs Serum Fat Soluble Vitamins – Correlations ........................................ 158
Table 5-7. Baseline, 6m & Change – Serum, Dietary, & Numbers Taking Supplemental FSVitamins: All, Women & Men ................................................................. 161
Table 5-8. Demographic, Health Status, Lifestyle, Quality of Life and Eating Attitudes with Serum Fat Soluble Vitamins .................................................................162
Table 5-9. Baseline Serum Fat Soluble Vitamins vs. Study Parameters .....................................164
Table 5-10. Change in sβCaro in All & Women, & Change in sVitE in All & Men vs. Study Parameters .................................................................166
Table 5-11. Baseline Serum Fat Soluble Vitamin vs. Metabolic Syndrome Marker Count – Ordinal Logistic Regression .................................................169
Table 6-1. Estimate of General Oxidant and Inflammatory Properties of Laboratory Markers in the Diet&Health-Novel CVD Marker Study ...........................................188
Table 6-2. Patterns of Association with CVD Risk or Protective Markers by Gender ..................189
Table 6-3. Diet Comparisons: Forager and Westernised .............................................................225
Table 6-4. Energy Content Body Compartments of Female Hominoids ....................................236

LIST OF FIGURES

Figure 1-1. A Selection of Factors Involved in Hypertension ....................................................17
Figure 1-2. Structure of Chitin, Chitosan and Cellulose ...............................................................26
Figure 2-1. Clinical Studies – Plan of Components .....................................................................55
Figure 2-2. Diet&Health–Weight Loss Trial and Fibre&Fat Loss Study Timeline ......................56
Figure 2-3. Diet&Health-Novel CVD Risk and Protective Marker Studies Timeline ..................56
Figure 3-1. Study Recruitment ....................................................................................................86
Figure 3-2. Weight & Waist over 6m (A) Weight Observed (B) Weight LOCF, ITT & (C) Waist LOCF, ITT: Chitosan vs. Placebo .................................................................89
Figure 3-3. BMI over 6m (A) Observed and (B) LOCF, ITT: Chitosan vs. Placebo ..................90
Figure 3-4. BP over 6m ITT (A) SBP (B) DBP: Chitosan vs. Placebo ..........................................90
Figure 3-5. Serum Lipids over 6m (A) TC (B) LDL-C: Chitosan vs. Placebo (LOCF ITT) ...............92
Figure 3-6. Baseline, 6m & Change in Faecal Fat Excretion: Chitosan & Placebo, n=29 ..........96
Figure 3-7. Change in sFSVitamins: Fibre&Fat Loss Substudy, n=29 .........................................96
Figure 3-8. Change in Serum Fat Soluble Vitamins vs. Faecal Fat: Fibre&Fat Loss Substudy, n=29 ........................................................................................................97
Figure 4-1. Weight & Waist of Individuals at Baseline, 3m & 6m: Women, n=117 & Men, n=30 .................117
Figure 4-2. BMI & %Fat of Individuals at Baseline, 3m & 6m: Women, n=117 & Men, n=30 ......118
Figure 4-3. % Change Correlations Weight vs. Waist & %Fat: All, Women & Men .................119
Figure 4-4. Baseline (A) NCEP, NCEP/ADA & IDF/JIS MetSMCt & MetS .....................................121
Figure 4-5. Baseline to 6m Change, Weight & Waist in MetS: Women & Men .........................125
Figure 4-6. Change Baseline to 6m Correlations: Weight vs. MetSM, MetSMCt & MetS ..........126
Figure 5-1. Change in Adpn30 vs. Change in MetS Marker Count: Women & Men .................153
Figure 5-2. Baseline sFSVitamin vs. Waist .................................................................................165
Figure 6-1. A Version of Human Phylogeny ............................................................................183
Figure 6-2. Relative Brain Size & High Dietary Quality, or High Energy Diets, in Primates ..........184
Figure 6-3. Reward Pathways - Hypothalamic Nuclei to Frontal Cortex ....................................200
Figure 6-4. Excess Lipid in Adipocytes: Endoplasmic Reticulum & Mitochondrial Stress ..........212
Figure 6-5. Antioxidant Vitamins in Central Obesity ...............................................................214
Figure 6-6. Phytochemicals and Reactive Oxygen and Nitrogen Species: Antioxidant ..........215
LIST OF APPENDICES

Appendix 1. Questionnaire Forms ........................................................................................................ 255
Appendix 2. Adverse Events Form ......................................................................................................... 273
Appendix 3. Evaluable Numbers and Normal Range for Laboratory Tests .............................................. 275
Appendix 4. Expanded Tables ................................................................................................................. 276

LIST OF ABBREVIATIONS

ACE  Angiotensin converting enzyme
ADA  American Diabetes Association
adipocyte  adipose cell
Adpn  adiponectin
Adpn30  the subgroup of participants in whom Adpn oligomers were analysed at baseline (n=30) and 6 months (n=20)
Adpn250  the total study group of 250 participants
ADHB  Auckland District Health Board
AE/SAE  adverse event/serious adverse event
AGE  advanced glycation end-products
AHA  American Heart Association
Akt  serine/threonine kinase
Akt/PKB  serine/threonine protein kinase
ALE  advanced lipoxidation end-products
AlkPhos  alkaline phosphatase
ALT  alanine transaminase or transferase
AMP  adenosine monophosphate
AMPK  adenosine monophosphate activated protein kinase
ANCOVA  analysis of covariance
ANZFA  Australia and New Zealand Food Standards Code – see FSANZ
ANZOS  Australia and New Zealand Obesity Society
ARB  angiotensin receptor blocker
ASM  Annual Scientific Meeting
ASSO  Australasian Society for the Study of Obesity
AST  aspartate transaminase or transferase
ATP  adenosine triphosphate
AUC  area under the curve
B6  pyridoxine
B-cell  bone-cell
BIA  bioimpedance analysis
BMI  body mass index
BMR  basal metabolic rate
BP  blood pressure
C-C  carbon to carbon atom link
CABG  coronary artery bypass graft
CAM complementary and alternative medicine
CAT catalase
CHD coronary heart disease
CHO carbohydrate
CI confidence interval
CKD chronic kidney disease
CMP-1 chemoattractant protein-1
COX-2 cyclooxygenase-2 medications
CRP C-reactive protein
CRShem&Biochem clinical routine screening haematology and biochemistry
CVA cerebrovascular accident
CVD cardiovascular disease
d day
DAT dopamine transporter
DSMB data safety monitoring board
DBP diastolic blood pressure
DEXA dual-energy X-ray absorptiometry
DHA docosahexaenoic acid
DNA deoxyribonucleic nucleic acid
DsBA-L disulfide bond-A oxidoreductase-like protein
EAT-12 Eating Attitudes Test – 12 question
ECG epicatechin gallate
EDTA ethylenediamine tetra-acetic acid
EGCG (-)-epigallocatechin-3-gallate
EGIR European Group for the Study of Insulin Resistance
ELISA enzyme linked immunosorbent assay
EPA eicosapentaenoic acid
ESR erythrocyte sedimentation rate
ER endoplasmic reticulum (RER rough endoplasmic reticulum)
f food derived/dietary
FA/FFA fatty acid/free fatty acid
FBC full blood count
%Fat body fat percentage
FDA Food and Drug Administration
FMHS Faculty of Medical and Health Science
FPG fasting plasma glucose
FSANZ Food Standards Australia New Zealand
FSVitamin fat soluble vitamin
FRAP ferric acid reducing potential
GCP Good Clinical Practice
GLmM general linear mixed model
GLUT4 Glucose transporter type 4
GR glutathione reductase
GRAS generally regarded as safe
GSH reduced glutathione
GSK-3 glycogen synthase kinase
GSX glutathione peroxidase
H2O2 hydrogen peroxide
HbA1c haemoglobin A1c
HDL-C high density lipoprotein-cholesterol
HMW high molecular weight adiponectin
HNU Human Nutrition Unit
HO-1 haem oxygenase-1
HOMA-IR homeostasis model analysis-insulin resistance
HPA hypothalamic-pituitary-adrenal
HPLC high performance liquid chromatography
hr hour
HRC Health Research Council
IASSO International Association for the Study of Obesity
IB investigational brochure
OSQoL  obesity specific quality of life
OTC  over the counter (medication)
PA  physical activity
PGC-1α  peroxisome proliferator-activated receptor gamma coactivator alpha
PDK1/Akt  phosphoinositide 3-kinase dependant / serine/threonine kinase
phytochemical  (food) plant chemical
phytonutrient  (food) plant micronutrient
phytalexin  plant defence chemical (often antiyeast, antifungal or dehydration preventers)
PIS  participant information sheet
POMC  pro-opiomelanocortin
PPAR-α, δ, or γ  peroxisome proliferator-activated receptor alpha, delta or gamma
PT  prothombin time
PTH  parathyroid hormone
PUFA  polyunsaturated fatty acid
PVD  peripheral vascular disease
QALY  quality adjusted life year
QoL  quality of life
r  correlation coefficient
RAAS  renin-angiotensin-aldosterone system
RBC  red blood cell
RCT  randomised controlled trial
REE  resting energy expenditure
RER  rough endoplasmic reticulum
RIA  radioimmunoassay
RNA  ribonucleic acid
ROS  reactive oxygen species
RONS  reactive oxygen and nitrogen species
RXR  retinoid X receptor
S/DBP  systolic/diastolic blood pressure
SBP  systolic blood pressure
SAS  Statistical Analysis Software™
SCOTT  standing committee on therapeutic trials
s  serum
sd  standard deviation
sem  standard error of the mean
SF-36  short form – 36 -36 questions health related quality of life survey
SFB  Single frequency bioimpedance-3
Sir2  S. cerevisiae silent information regulator 2, gave name to family of Sirtuins
SIRT2  silent mating type information regulation 2 homolog 1
SOD  superoxide dismutase
SOP  standard operating procedure
sp  supplemental
TAS  total oxidant status
T-cell  thymus-cell
TG  triglyceride
TIA  transient ischaemic attack
TIDM  type I diabetes mellitus
TIIDM  type II diabetes mellitus
TNF-α  tissue necrosis factor-alpha
U  unit
UCP  uncoupling proteins
UGI  upper gastrointestinal
UK  United Kingdom
USA  United States of America
UV  ultraviolet (light radiation)
VitA  vitamin A, retinol
VitC  vitamin C, ascorbic acid
VitD  vitamin D₃, 25 hydroxyvitamin D₃ or calcifediol or calcidiol
VitDBP  VitD binding protein
VitE  vitamin E, α-tocopherol
VLDL-C: very low density lipoprotein-cholesterol
waist: waist circumference
weight: body weight
WBC: white blood cell
WHF: World Heart Federation
WHO: World Health Organisation
wk: week
XOR: xanthine oxido-reductase
γ: year
α-MSH: alpha melanocortin stimulating hormone
βCaro: beta carotene
γGT: gamma glutamyl transferase
δ or Δ: change
ω-3: omega-3 or n-3, as in ω-3 fatty acid
Chapter 1. Introduction and Background

1.1 Introduction

1.1.1 Scope

This thesis addresses obesity, in particular central obesity, and central obesity-related metabolic syndrome (MetS). MetS definitions have been devised to include a cluster of cardiovascular disease (CVD) risk factors or risk markers that tended to occur together. MetS was thought to have greater predictive power for disease than each of the factors used alone. New research shows that MetS is associated with a wider range of chronic, degenerative, non-communicable disease than CVD alone, and MetS has limitations in predicting them all.

The Dietary Fibre and Lifestyle for Health (Diet&Health) study was designed and conducted. It comprised two research themes. The randomised controlled trial (RCT), of weight loss over 6 months (6m) in overweight and obese women and men using a natural dietary fibre, provided the study model for the primary enquiry; the Novel Cardiovascular Risk and Protective Marker (Novel CVD Markers) investigation. Thus the clinical weight loss trial, comprising two research themes, was performed primarily to explore MetS, MetS definitions and Novel CVD Markers and their change with weight change over time. These markers could then be combined with MetS to increase prediction of obesity-related degenerative disease in clinical practice.

The Novel CVD Risk Markers were drawn from clinical routine screening biochemistry and haematology (CRSHaem/Biochem). The first Novel CVD Protective Marker study concentrated on a key adipocytokine (adipo- adipose; cytokine signalling molecule), adiponectin (Adpn), and the second group of Novel CVD Protective Markers were a nutritional and antioxidant group, the serum fat soluble vitamins (sFSVitamins).

Consequently, an investigation of human obesity and MetS was undertaken by exploring the multidisciplinary literature in order to perform a critical analysis of the causes of obesity-related MetS. The enquiry proceeded by examining the evolution and effect of human encephalisation and adaptive changes in energy metabolism pathways, energy balance, nutrition, immune system, locomotion, behaviour, and technology. A unifying
hypothesis of the causes of obesity and MetS was formed. Research ideas on models to further investigate the hypothesis and possible useful clinical dietary and energy metabolism modelling studies are outlined.

1.1.1.1 Thesis Structure

The first Chapter presents the thesis Introduction and Background. The Methods are covered in Chapter 2, although brief method summaries and statistical methods are found in the relevant Chapters. Chapters 3, 4 and 5 describe the one clinical study; the Diet&Health study. The Diet&Health study is further divided into the Diet&Health-Weight Loss trial detailed in Chapter 3, the Novel CVD Risk Markers study in Chapter 4 and the Novel CVD Protective markers studies in Chapter 5. Chapter 6 is the theoretical work that follows the study sections, and Chapter 7 contains the thesis discussion and conclusions.

1.1.2 General Introduction

Central obesity, a proxy marker for which is waist circumference (waist), has been shown to increase risk for CVD\(^1\). Hypertension or raised systolic and diastolic blood pressure (S/DBP), serum dyslipidaemia, namely decreased high density lipoprotein cholesterol (HDL-C) and raised triglyceride (TG), and impaired fasting plasma glucose (FPG) or FPG in the type II diabetes mellitus (TIIDM) range, are conditions which commonly occur together.

The latter 4 markers are the traditional or conventional CVD risk factors. Waist has been added as a 5\(^{th}\) member to this list since the obesity epidemic became universally recognised, in large westernised populations, in the mid 1990s\(^2\). All 5 of these conditions cluster together and therefore form a syndrome.

A syndrome is a cluster of signs and symptoms or markers which occur together more often than by chance alone. Diagnoses of medical syndromes are usually made on a selection of markers that are outside a cut-point, or are outside the normal or a specified range. The above-mentioned group of markers is referred to as MetS\(^3\,\,^7\).

There have been a number of definitions, as will be discussed, but MetS defined by the National Cholesterol Education Program (NCEP), Adult Treatment Panel III, using the above 5 markers, is the basis for MetS definitions used in this thesis (**Table 1-1**).
In order to be categorised as having MetS, individuals must have at least 3 of these 5 measures must be outside of the cut-points, the levels set where increased risk of CVD has been shown by meta-analyses used to define MetS. Note that central obesity need not be present in the NCEP MetS, but must be included in the NCEP-derived International Diabetes Federation (IDF) MetS, for which waist and 2 other of the MetS markers are mandatory for diagnosis (Table 1-1). The IDF MetS also has ethnic specific waist cut-points. More recently still the Joint Interim Statement (JIS) authors have modified the IDF MetS by revoking the mandatory inclusion of waist. The inclusion criteria are that any 3 markers (or more) outside the same cut points as for IDF defines the IDF/JIS MetS. The IDF/JIS MetS is probably the most appropriate definition for populations which are on average overweight, and is employed for the analyses in this thesis.

T2DM is predicted by MetS, and both the risk of completed CVD events, such as myocardial infarction (MI) or heart attack, and death, are greatly increased in individuals who have MetS, using the NCEP-based and/or other definitions, compared with those without MetS. The importance of MetS is that 4 of its components; (1) raised blood pressure (BP) (2) decreased HDL-C (3) raised TG, and (4) raised FPG, historically, comprise the classical or traditional CVD risk factors. It is not clear, yet, whether the 5 MetS components detailed above are causative, contributing to, or are only indicators of, CVD. All 5 will be referred to as MetS markers in this work (Table 1-1).

It is important to note that CVD is not the only non-communicable disease to occur at higher rates in centrally overweight and obese individuals. Most of the non-communicable diseases are degenerative, where degenerative is defined as ‘a disease characterized by progressive deterioration and loss of function in organs or tissues’ and are increased with central obesity.
### Table 1-1. NCEP-Derived Metabolic Syndrome Definitions

<table>
<thead>
<tr>
<th>Metabolic Syndrome</th>
<th>International Diabetes Federation /Joint Interim Statement 2009 (IDF/ JIS)</th>
<th>National Cholesterol Education Panel (NCEP)¹</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Diagnosis</strong></td>
<td>IDF requires waist plus any two of the disorders; JIS requires any 3 of the disorders, below.</td>
<td>Requires any 3 of the disorders below</td>
</tr>
<tr>
<td>Central Obesity</td>
<td>Population- &amp; country-specific definitions. ‘It is recommended that the IDF cut points be used for non-Europeans [for example Maori, Pacific peoples] &amp; either the IDF or AHA/NHLBI cut points used for people of European origin until more data are available’. IDF cut points: ‘Central obesity (defined as waist ≥ 94cm for Europid men &amp; ≥ 80cm for Europid women, with ethnicity specific values for other groups) i.e. Asian (based on a Chinese, Malay &amp; Indian Asian population) Male ≥90cm &amp; Female ≥ 80cm’.</td>
<td>Waist &gt;102 cm (40 in) men and &gt;88 cm (35 in) women</td>
</tr>
<tr>
<td>Hypertension</td>
<td>SBP ≥ 130 or DBP ≥ 85 mmHg. Medication for elevated BP is an alternate indicator.</td>
<td>S/DBP ≥ 130/85 mmHg</td>
</tr>
<tr>
<td>Dyslipidaemia</td>
<td>Raised TG ≥ 1.7mmol/L or specific treatment for this lipid abnormality or reduced HDL-C &lt;1.03mmol/L in men &amp; &lt;1.29mmol/L in women. Medications for decreased HDL-C or raised TG are alternate indicators. ‘The most commonly used medication for elevated TG and decreased HDL-C are fibrates and nicotinic acid. A patient taking 1 of these can be presumed to have high TG &amp; low HDL-C. High-dose ω-3 fatty acids presumes high TG’. (Note: raised TG &amp; decreased HDL-C are considered 2 separate factors counting toward the 2 of 4 necessary secondary factors for diagnosis)</td>
<td>Plasma TG &gt;1.7mmol/L (150 mg/dL), HDL-C&lt;1.03mmol/L(40 mg/dL) in men and&lt;1.29mmol/L (50 mg/dL) in women</td>
</tr>
<tr>
<td>Hyperglycaemia</td>
<td>FPG ≥5.6mmol/L (100mg/dL) or previously diagnosed TIIDM .Medication for elevated plasma glucose is an alternate indicator.</td>
<td>FPG &gt;6.1 mmol /L (110mg/dL )</td>
</tr>
</tbody>
</table>

¹Joint Interim Statement (JIS) Harmonizing the Metabolic Syndrome. A Joint Interim Statement of the International Diabetes Federation (IDF) Task Force on Epidemiology & Prevention; NCEP (ATPIII) National Cholesterol Education Program (Adult Treatment Panel III); American Heart Association (AHA); National Heart, Lung, & Blood Institute (NHLBI); World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity 

BP blood pressure; SBP systolic blood pressure; DBP diastolic blood pressure; TG triglyceride; HDL-C high-density lipoprotein cholesterol, IGT impaired glucose tolerance; BMI body mass index; FPG fasting plasma glucose; TIIDM type II diabetes mellitus.

The NCEP/ADA MetS is the same as the JIS MetS except the waist cut point remains as for the NCEP MetS. The IDF advises the following are studied with MetS: - 

“Abnormal body fat distribution; General body fat distribution; Dual Emission X-ray Absorptiometry (DEXA); Central fat distribution (CT/MRI); Adipose tissue biomarkers: leptin; adiponectin; Liver fat content (MRI); Atherogenic dyslipidaemia (beyond elevated TG and low HDL-C); ApoB (or non-HDL-C); Small LDL particles; Dysglycaemia; OGTT(oral glucose tolerance test); insulin resistance (other than elevated fasting glucose); Fasting insulin/proinsulin levels; HOMA-IR; Homeostasis insulin resistance by Bergman Minimal Model; Elevated free fatty acids (fasting and during OGTT); M value from clamp vascular dysregulation (beyond elevated blood pressure); Measurement of endothelial dysfunction; Microalbuminuria; Proinflammatory state; Elevated high sensitivity C-reactive protein (hsCRP); Elevated inflammatory cytokines (e.g. TNF-α, IL-6); Decrease in adiponectin plasma levels; Prothrombotic state; Fibrinolytic factors (PAI-1 etc); Clotting factors (fibrinogen etc); Hormonal factors; Pituitary-adrenal axis”. Adapted from NCEP³, IDF¹¹, Peters¹² and JIS⁵
The classical CVD risk and waist markers of the MetS are very useful concepts which have made clinicians and researchers aware of the importance of central obesity. However, it is clear that there is significant residual risk\textsuperscript{13,14} of CVD after medication prescription and other standards of care are achieved. MetS does not fully predict metabolic decompensation and risk for progression to irreversible degenerative disease. Many CVD events occur in individuals who have had no risk factors suspected on screening, or any previously diagnosed\textsuperscript{15}.

In this thesis, it is contended that groups of novel risk and protective markers could be employed in conjunction with MetS to strengthen disease prediction early enough in the time course to effect illness prevention. Interestingly, the long definition of the IDF MetS states that disease prediction marker development should focus on some of the newly discovered biomarkers (Table 1-1). These include markers of a pro-inflammatory state, such as CRP, and the anti-inflammatory marker, Adpn, both of which are investigated in this thesis.

The first group, Novel CVD Risk Markers, is derived from well-known CRSHaem&Biochem and includes markers often manifesting oxidant properties: haemoglobin A\textsubscript{1c} (HbA\textsubscript{1c}), urate, ferritin, liver function tests (LFT); and inflammatory markers: white blood cells (WBC) or leukocytes and serum and acute phase proteins, albumin, globulin, C-reactive protein (CRP) and the erythrocyte sedimentation rate (ESR).

Adpn and its different variants, or oligomers, are chosen as the first group of Novel CVD Protective Markers (Table 1-1). Decreased plasma levels of Adpn oligomers, and altered pathways appear to be involved in the profound metabolic disruption of obesity and MetS (Table 1-1).

The second group of Novel CVD Protective Markers, the nutritional, ‘antioxidant’ and obesity related serum fat soluble vitamins (sFSVitamins) are assessed. Causative factors involved in cellular dysfunction in MetS are then more likely to be identified, with the hope that endogenous, genetic and epigenetic, and exogenous, environmental, influences can be modified.

Finally, as the fundamental metabolic defects and initial causes of obesity-related MetS are not known, a new composite unifying hypothesis, predicated on new human evolutionary and genetic/epigenetic findings, is proposed.
1.2 Obesity

Obesity is the accumulation of excess body fat to the extent that it is likely to adversely affect health. Instances of obesity have been recorded for thousands of years, as documented by Hippocrates\textsuperscript{16}. In Great Britain (UK) pre 1900, obesity was present, but not common. It was captured in paintings of the upper class, as well the merchant class, and food and service industry individuals\textsuperscript{17}. Obesity and associated ill health was well recorded by physicians and others attending the affluent\textsuperscript{16}. In other cultures obesity was an induced condition for specific cultural or fertility practices\textsuperscript{18}.

Obesity rates have increased over the last few decades probably in response to the rapid increase in technologies for large-scale, cost-effective economy-of-size, food production systems\textsuperscript{19}. Food refining and energy concentration and preservation, has allowed large quantities of energy-dense food to be transported to mass markets\textsuperscript{20}. Television watching, and screen-based computer and phone work and entertainment technologies all serve to reduce physical activity (PA), compared to the past\textsuperscript{21}. Concurrently, advances in public and general medical health services have contributed to a reduction in the communicable disease load, allowing individuals longer life spans, but also increasing time in which to accumulate degenerative change and increase disease risk factors\textsuperscript{22}. More accurate clinical indicators to help improve prediction of chronic degenerative diseases associated with central obesity are required and are the subject of this thesis.

1.2.1 Obesity - Definitions

**Body Mass Index**

An approximate measure of fatness or adiposity can be classified by a body mass index (BMI, weight/height\(^2\), kilogram/metre\(^2\), kg/m\(^2\)) where BMI \(\geq 25\) kg/m\(^2\) denotes overweight, and BMI \(\geq 30\) kg/m\(^2\), denotes obesity (grade I), as shown in Table 1-2. Morbid obesity is defined as BMI \(\geq 40\) kg/m\(^2\) (grade II obesity), and a BMI of \(\geq 45\) kg/m\(^2\) (grade III) is classified as super obesity. This group has commensurate rates of disability and death\textsuperscript{23}. Most of the CVD risk studies had been in Europeans. BMI is very commonly recorded but people of different ethnicities have different body sizes and composition so adjustments are required for classification\textsuperscript{24}. The World Health Organisation (WHO) has suggested that researchers use narrower subcategories\textsuperscript{25} (Table 1-2). The shorter/slighter less muscular groups tend to be the East Asian, South Asian/Indian, and also include
various current or recent hunter-gatherer groups, as for example Australian aborigine\textsuperscript{26-28}. Their overweight status may start at approximately 20-23kg/m\textsuperscript{2} and obesity at 25kg/m\textsuperscript{2}. Of the taller, larger, more muscular groups the African American, Hispanic, and Maori and Pacific have been studied, and at this time are grouped with European\textsuperscript{29,30}. BMI proves to be a crude measure of different CVD risks\textsuperscript{31,32}. Measures of central adiposity may relate more closely to degenerative morbidity and mortality than BMI\textsuperscript{33,34}. Waist is a surrogate for, and gives an indication of unhealthy, visceral and, to a lesser extent, subcutaneous, central adipose tissue depots\textsuperscript{35,36} and liver fat.

\textbf{Waist and Waist Indices}

These measures relate to degenerative disease, particularly T2DM and CVD\textsuperscript{33,37}. Waist, and especially the related indices, waist/hip, waist/thigh and waist/height, may be more appropriate than BMI as health indictors for certain ethnic groups\textsuperscript{34,38-42}. Waist/hip and waist/thigh give a ratio of the central adipose tissue to the healthy peripheral hip and thigh fat\textsuperscript{43,44}. This is an index that may be particularly appropriate for Asian ethnic groups where modest appearing central abdominal adipose tissue is proportionately large compared with minimal peripheral fat, in both women and men. However, in mostly European populations, BMI and waist based indexes add no more to the prediction of CVD if lipid measurements, history of diabetes and BP are available\textsuperscript{45}.

\textbf{Body Fat Percentage}

Total body fat percentage (%Fat) measurements have been used to estimate morbidity and mortality risk, although studies give contradictory results\textsuperscript{34,46}. %Fat as measured indirectly by bioimpedance analysis (BIA), the relative resistance of fat to lean tissue to a small electric current, or dual-emission X-ray absorptiometry (DEXA) is frequently performed.

Body composition varies widely across ethnic groups and the genders. Individuals of some ethnic groups can accumulate massive peripheral fat stores, whereas some Asian groups (East and South/Indian), and various hunter-gatherer group individuals gain weight mainly by increasing central fat depots\textsuperscript{47}. There are notable exceptions as seen with sumo wrestlers and others. Thus, %Fat may be an adequate indicator of CVD in the groups whose individuals have minimal peripheral adipose tissue. As noted above, these same groups have a high central adipose tissue/height ratio and high rates of CVD at lower BMI\textsuperscript{24,48}. 
Table 1-2. Classifications of Obesity

<table>
<thead>
<tr>
<th>Classification</th>
<th>BMI (kg/m²)</th>
<th>Principal Cut-Points</th>
<th>Additional Cut-Points for Ethnic Differences</th>
</tr>
</thead>
<tbody>
<tr>
<td>Underweight</td>
<td>&lt;18.50</td>
<td>&lt;18.50</td>
<td></td>
</tr>
<tr>
<td>Severe thinness</td>
<td>&lt;16.00</td>
<td>&lt;16.00</td>
<td></td>
</tr>
<tr>
<td>Moderate thinness</td>
<td>16.00 - 16.99</td>
<td>16.00 - 16.99</td>
<td></td>
</tr>
<tr>
<td>Mild thinness</td>
<td>17.00 - 18.49</td>
<td>17.00 - 18.49</td>
<td></td>
</tr>
<tr>
<td>Overweight</td>
<td>≥25.00</td>
<td>≥25.00</td>
<td></td>
</tr>
<tr>
<td>Pre-obese</td>
<td>25.00 - 29.99</td>
<td>25.00 - 27.49</td>
<td>27.50 - 29.99</td>
</tr>
<tr>
<td>Obese</td>
<td>≥30.00</td>
<td>≥30.00</td>
<td></td>
</tr>
<tr>
<td>Obese class I</td>
<td>30.00 - 34.99</td>
<td>30.00 - 32.49</td>
<td>32.50 - 34.99</td>
</tr>
<tr>
<td>Obese class II</td>
<td>35.00 - 39.99</td>
<td>35.00 - 37.49</td>
<td>37.50 - 39.99</td>
</tr>
<tr>
<td>Obese class III</td>
<td>≥40.00</td>
<td>≥40.00</td>
<td></td>
</tr>
<tr>
<td>Ethnic Group</td>
<td>Taller/Larger (European, African-American/black, Maori/Pacific)</td>
<td>Smaller/Slighter (Asian/East, Asian/Indian, Australian Aboriginal, Hunter-gatherer (forager))</td>
<td></td>
</tr>
<tr>
<td>Waist cut-points¹</td>
<td>&gt; 80-88cm</td>
<td>&gt; 80cm</td>
<td>&gt; 80-88cm</td>
</tr>
<tr>
<td>Women</td>
<td>&gt; 94-102cm</td>
<td>&gt; 90-94cm</td>
<td></td>
</tr>
</tbody>
</table>


1.2.2 Obesity - Epidemiology and Natural History

In many westernised countries, including relatively recently colonised countries such as New Zealand (NZ) and the United States of America (USA), obesity rates have been estimated to be approximately 3% at the turn of 20th Century, but no measured data was retained. Overweight began to be recognised as a health problem, perceptibly reducing life-expectancy. In 1943 Metropolitan Life (MetLife), a life insurance company, using actuarial data collected from 1943 European clients who could afford life insurance since the 1930’s, published the first tables of Desirable Weights for Height and Framesize. These tables were updated in 1983. The tables indicated that above certain weights for women and men, significant increases in morbidity and mortality could be expected. A weight 20% greater than average for a medium frame person was another definition for overweight. Introducing height into the equation was possible from data collected, and weight-to-height tables gave an improved estimation of risk of death for men and women. Obesity was then defined as BMI, or Quetelet’s index from MetLife data. Although in the early 1900’s survival in those with tuberculosis was
higher in those who were fatter\textsuperscript{56}, by the 1930’s links between obesity and premature mortality were also becoming recognised by some clinicians and surgeons.

In 1923, studies from diabetes clinics were reported where obesity was linked with degenerative CVD risk factors\textsuperscript{57} and atherosclerosis. The obesity rates increased rapidly towards the latter third of the 20\textsuperscript{th} Century and the WHO declared a ‘world epidemic of obesity in 1997’\textsuperscript{53}. Further, in 2008 the WHO noted that ‘globally, more than 1.5 billion adults were overweight - and at least 500 million of them are clinically obese’\textsuperscript{58}. USA obesity rates measured in a 2007-2008 National Health and Nutrition Examination Survey (NHANES) sample indicated that over 35\% of women and 32\% of men were obese\textsuperscript{59}. Combined overweight and obesity rates were 68\%\textsuperscript{59}. Morbid obesity, which had been very rare, showed a prevalence of 4.8\% in the USA in 2004\textsuperscript{60}.

In NZ, the reported obesity rates of women in 2006-2007 were 26\% for women and 25\% for men, and for morbid obesity a prevalence of 5\% in women and 2\% in men\textsuperscript{61}. The number of obesity-related quality adjusted life years (QALYs) lost doubled over 16y, and rose from an average of 0.0204 per person to 0.0464 during that time, an increase of approximately 127\%\textsuperscript{62}.

1.2.2.1 Obesity - Ethnicity and Socio-Economic Status

In many countries either the colonized aboriginals/first nation peoples, or immigrant worker groups, tend to be non-European groups, and often overlap with those who are relegated to lower socio economic positions. Individuals and families from lower socioeconomic groups have higher obesity rates than those in wealthier groups\textsuperscript{63}. There is often an ethnic gradient in BMI but disease may still be high in those with slight body frames, for example Chinese and Japanese\textsuperscript{25}, so BMI should be adjusted for ethnic grouping\textsuperscript{64} or the subcategories in \textbf{Table 1-2}. 
Table 1-3. Obesity in New Zealand Adults by Ethnic Group (Unadjusted)

<table>
<thead>
<tr>
<th>Ethnic group</th>
<th>Prevalence (95% CI)</th>
<th>Number of adults</th>
</tr>
</thead>
<tbody>
<tr>
<td>European/ Other</td>
<td>24.3 (23.1–25.5)</td>
<td>619,200</td>
</tr>
<tr>
<td>Maori</td>
<td>41.7 (39.8–43.7)</td>
<td>148,300</td>
</tr>
<tr>
<td>Pacific</td>
<td>63.7 (60.0–67.5)</td>
<td>104,900</td>
</tr>
<tr>
<td>Asian</td>
<td>11.0 (9.0–13.0)</td>
<td>30,800</td>
</tr>
</tbody>
</table>

From New Zealand Ministry of Health\(^{61}\)

Maori and Pacific in NZ had morbid obesity rates of 6.6% for women and 7.8% for men in a 2008 report\(^{61}\). Overall morbidity and mortality increases proportionately. The prevalence of obesity in the highest socioeconomic group in NZ was 20.4% for women and 21.5% for men\(^{61}\) and in the lowest group, 39.7% for women and 35.4% for men\(^{61}\).

Obesity prevalence rates have risen over the last century, initially in the wealthier, westernised, developed countries, but they are now epidemic in the poorer sections of developing countries. A significant cause is likely to be the move from traditional diets to the westernised processed, high saturated fat, high refined carbohydrate (CHO), and high salt diet. This is denoted the nutrition transition\(^{65}\).

### 1.2.3 Causes of Obesity

The causes of obesity are likely to be the result of gene-environment interactions and changes in this inter-action. Our environment has transformed over the last few hundred years but most particularly in the last 100y. The first law of thermodynamics states that energy cannot be created or destroyed so energy taken in by auxotrophs, organisms which do not make energy from light energy, must be expended or stored.

In obesity, a relative excess of energy is consumed from the macronutrients compared with energy expenditure. The macronutrients are composed of (1) CHO subgroups, starches and sugars (2) lipids (fats), and (3) protein; of which the latter is not usually ingested primarily for energy. Alcohol is metabolised immediately and unlike the other macronutrients is not directly, or indirectly stored as fat. Diets throughout the world have become westernised, which generally indicates they have a high proportion and levels of processed, refined, energy dense and over-palatable CHO and fat, with many non-nutritive sweeteners and additives, and are low in fibre and micronutrients\(^{66}\).

Basal metabolic rate (BMR) is the energy used by all the processes that keep homeotherms (warm-blooded animals), alive in the resting fasted state, and consists of
basal cell metabolism of maintaining electrical gradients. In the waking state, digestion and PA result in further energy expenditure. The potential energy of food macronutrients has been calculated from animal and human experiments since the mid 1900’s and confirmed many times since.

A decline in energy expended in PA may have occurred although not all researchers agree. Modest PA alone may not be very efficacious for weight loss or weight loss maintenance, but appears to prevent weight gain. Estimates for the average energy required for women and men to physically work efficiently was also estimated many years ago, but the early researchers and authors knew that there were many conditions, such as gender, age, temperature and other unknowns.

Techniques for the measurement of PA energy expenditure in individuals have become more accurate, and mechanisms and genetic influences for mobilising energy stores, switching energy substrates and energy loss through uncoupling proteins (UCP) in the obese and lean have been studied. Only recently, however, has the change in energy expenditure with change in fat mass over a weight loss period been integrated in time, and non-exercise activity thermogenesis (NEAT) been taken into account. There are still many unknown factors in the links between PA, energy expenditure and food intake. However PA will not be addressed in detail in this thesis.

Although there are many studies reviewing energy acquisition and expenditure, there are other hard-to-determine factors in both. There are other probable evolutionary, developmental, inadequate sleep related, psycho-behavioural, socioeconomic and physical factors, in addition to chemical additives/pollutants and other environmental determinants which may affect obesity development and prevalence.

1.3 Central Obesity Related Metabolic Syndrome

MetS predicts pre-diabetes, specifically impaired fasting glucose (IFG) and glucose intolerance, CVD risk markers, and other degenerative disease, including cancer. The prevalence of MetS is approximately proportional to obesity rates (34% in the USA, 2003-2006) but varies by definition and ethnicity from 25-50% in other countries. In a systematic review and meta-analysis, individuals with NCEP/American Diabetes Association (ADA) MetS had a 2-fold increased risk of CVD, and all-cause mortality of 1.5 fold compared to those without.
A few early TIIDM clinicians realised that the hyperglycaemia of adult onset diabetes and the CVD-risk factor of hypertension were related to obesity. Harrick made ‘...the observation that the association of high BP and increased concentration of glucose in the blood... (in) the majority of the cases belong in a quite definite group characterized by the four cardinal symptoms: hypertension, hyperglycaemia, obesity and arteriosclerosis (demonstrable in the retina)’\textsuperscript{95}. Furthermore he experimented and found that a CHO- but expressly not a protein- restricted diet (1600 kilocalorie (Kcal)) resulted in weight loss, and that this weight loss translated into decreases in hypertension and hyperglycaemia.

This was in the same year that Kylin, generally regarded as producing one of the earliest descriptions of the clustering of CVD risk factors, published his work on the ‘Hypertension, Hyperglycaemia and Hyperuricaemia Syndrome’\textsuperscript{96}. Hyperuricaemia has frequently been associated with MetS and is often on verge of being selected to be part of MetS\textsuperscript{97-107}. Hyperuricaemia was present in 2/6 patients\textsuperscript{95}. Gout was also recognised early as being related to CVD\textsuperscript{108,109}. Furthermore, Vague recognised that the central fat distribution seen more often in men was associated with CVD\textsuperscript{110}.

Over this time, obesity rates were gradually increasing and data continued to be collected on the association of obesity with TIIDM, the other CVD risk markers, and CVD events. Weight loss strategies, including prescribed medication\textsuperscript{111,112} to reduce weight in order to lessen CVD risk were studied \textsuperscript{113-115} before obesity rates began to accelerate, in the 1980’s. The realisation that central and/or upper body obesity was the adipose tissue depot of risk was rather slow\textsuperscript{116-120}, and there is still controversy about adipocyte size and location\textsuperscript{121}. As the rates of central obesity increased significantly, waist measurements were factored into analyses of risk, and waist was shown to be a significant and valid marker of CVD risk. Central obesity became factored into some indexes of cardiometabolic and CVD risk, although the Framingham equations omitted central obesity until as late as the 1990’s\textsuperscript{122}.

Various CVD risk marker combinations were formulated in MetS. Kaplan coined the idiom ‘deadly quartet’, an early MetS definition which included the upper body obesity marker, waist, dyslipidaemia, hyperglycaemia and hypertension\textsuperscript{123}, although he was keen that hyperinsulinaemia was considered \textsuperscript{124}. Other descriptive terms for this heterogeneous cluster of risk factors included, ‘the syndrome X’, ‘insulin resistance syndrome’,
‘plurimetabolic syndrome’, ‘dysmetabolic syndrome’, ‘cardiovascular metabolic syndrome’, ‘cardiometabolic syndrome’, and ‘hypertriglyceridaemic waist’125,126. Various definitions were promulgated after the WHO MetS was published. A number of definitions were proposed and MetS was accepted by most researchers as a useful clinical index for a common clustering of risks factors. However, MetS greatest contribution may have been to show in high relief that central adiposity or its causes are a serious risk to health.

### 1.3.1 Definitions of the Metabolic Syndrome

It is instructive to review MetS definitions, as they reveal why there is controversy in defining MetS, but also possibly a preference for insulin resistance as the core problem rather than obesity as being a risk factor. Although the WHO Diabetes Group (1999) made the first attempt to define the MetS in a formal manner, others were using ad-hoc clusters of risk factors in trial reports127. The cut-points, except waist, have been similar for all definitions.

The WHO MetS was primarily centred on the presence of insulin resistance. Diagnosis comprised impaired glucose tolerance (IGT), as measured by a hyperinsulinaemic euglycaemic clamp, where the insulin and glucose are infused intravenously at a level of insulin that keeps the plasma glucose constant. Insulinaemia is measured, or TIIDM is present, and at least 2 of the following risk factors: (1) obesity; waist-to-hip ratio and/or BMI >30kg/m² (2) dyslipidaemia; elevated plasma TG or decreased HDL-C levels (different levels for men and women); or (3) hypertension and (4) microalbuminuria7. Cut-points were derived from large pooled data sets or meta-analyses.

In 1999 the European Group for the Study of Insulin Resistance (EGIR)128,129 suggested that the terminology insulin resistance syndrome (IRS) should be used and defined as the upper quartile of a non-diabetic reference population. Their definition comprised waist, fasting hyperglycaemia, hypertension, TG, HDL-C. Also included was medication for hyperlipidaemia or hypertension.

The EGIR excluded TIIDM, and therefore the 3rd group of cardiometabolic medications; those for hypoglycaemia or diabetes. Ironically, this IRS-based MetS already included the 5 markers, in addition to central obesity for the first time, with cut-points, that would later
become the IDF/JIS MetS, although to most clinicians the IDF/JIS MetS appears to be NCEP MetS-derived\textsuperscript{4,7,130}. Microalbuminuria was discarded. In 2000, the ‘hypertriglycerideamic waist’\textsuperscript{131} phenotype was proposed. It included 3 non-traditional risk factors, which are not often clinically measured; small dense low density lipoprotein (LDL) particles, elevated plasma apolipoprotein (apo) B levels, and elevated plasma insulin levels. This definition would increase the 5y CVD risk 18-fold\textsuperscript{131}.

The NCEP MetS related definitions have been discussed above, and outlined in Table 1-1. In addition they have been widely publicised, debated and used\textsuperscript{132-141}. In 2010, the WHO, with the support of long-time proponents of MetS, surprisingly joined the North American endocrinologists by announcing that it did not support the concept, nor use of the term, MetS, and that newer biological markers derived from recent basic science should be used\textsuperscript{133}.

Articles still appear indicating that MetS is a useful concept and is likely to be hard to erase\textsuperscript{7,132}. Clinicians and researchers outside of the USA and Europe are much more inclined to use the NCEP derived MetS definitions, as they are cheap and easy to use. In some countries and ethnicities the ‘nutrition transition’, and consequent huge morbidity from TIIDM and CVD, is occurring rapidly. Young individuals transition quickly through MetS and develop TIIDM and CVD, as seen in South African and Fijian Indians\textsuperscript{142}. The IDF waist cut-point levels appear appropriate. With no provision to use new biological markers, clinicians in many situations have no reason for abandoning the concept or clinical use of MetS\textsuperscript{133}. Any index which gives prior warning of disease raises the likelihood of TIIDM and CVD prevention.

1.3.2 Obesity and Type II Diabetes

Type II Diabetes Mellitus - Diagnosis and Natural History

TIIDM is part of MetS spectrum of diseases and is diagnosed by an abnormally high plasma glucose measure. Hyperglycaemia, as determined by FPG >7mmol/L, random plasma glucose, or an oral glucose tolerance test (OGTT) of >11mmol/L, and/or glycated HbA\textsubscript{1c} >6.5% is used by the ADA\textsuperscript{143} to define this adult onset, degenerative condition. TIIDM is a later stage of MetS where hyperglycaemic problems are to the fore. Those with TIIDM are prone to coronary heart disease (CHD), and other CVD at double the rate
of those without TIIDM\textsuperscript{144}. If hyperglycaemia is not corrected, very high blood glucose can result in lethargy, dehydrating glycosuria and hyperosmolar states and coma.

Whilst early testing and oral hypoglycaemic and insulin sensitising medication now makes this presentation rare, chronic modest hyperglycaemia damages arterioles and capillaries, particularly those supplying the kidneys, retinas and nerves. Excess consumption of macronutrients (CHO, lipid and protein), and their subsequent metabolism is linked to abnormal micronutrient metabolism as seen, for example, with the transition metals\textsuperscript{145} in TIIDM.

**Type II Diabetes Mellitus - Epidemiology**

TIIDM accounts for approximately 90\% of all diabetes. It has increased drastically in the last 100y to a world prevalence calculated by the WHO at 220 million people in the early 21\textsuperscript{st} Century and numbers are predicted to rise\textsuperscript{146}. All cause- and vascular- mortality is doubled in people with TIIDM and 50\% of these die from CVD\textsuperscript{146,147}. Of people with TIIDM, 80\% are overweight or obese, and most of this obesity is central. In 2005, 1.1 million people were estimated to have died from diabetes complications, of which 80\% are in developing countries and 55\% are women. Projections show that by 2030 the death rate from TIIDM will be double these figures\textsuperscript{146}.

From NZ estimates the ‘most likely’ scenario reflects anticipated growth in the obesity epidemic along with improvements in both diabetes and general health care. The model estimates over 11,000 incident diagnoses, 180,000 prevalent diagnoses of NZ adults and 1900 deaths attributable to diagnosed TIIDM disease in 2011\textsuperscript{148}. Note that estimates of the prevalence of undiagnosed diabetes can be high, especially in obese groups and/or non-European ethnicities.

In a NZ Maori group who were on average obese, age adjusted undiagnosed TIIDM rates of approximately 5\% (4.2\% women and 6.5\% men) were recorded, 2003-2006\textsuperscript{149}. Furthermore, as CHD, chronic kidney disease (CKD) and end-stage renal failure and infection, gangrene, from peripheral vascular disease are common sequelae of TIIDM, many patients are recorded as dying from these diseases, not diabetes itself.
1.3.3 Obesity and Cardiovascular Disease

Cardiovascular Disease – Diagnosis and Natural History

CVD is the major degenerative disease pattern to have overtaken infectious diseases in the cause of morbidity and mortality, and is the main reason for the development for MetS definition. CVD predominantly presents as ischaemic heart disease (IHD), the symptoms and signs of which are 3 related IHD manifestations; (1) angina (2) MI (which is often fatal), and (3) chronic heart failure. Cerebrovascular accidents (CVA), transient ischaemic attacks (TIA) and multi-infarct dementia are other atherosclerotic cerebrovascular manifestations. Lastly PVD, especially arterial atheroma, cause claudication of lower limb muscle and severely reduced exercise tolerance. PVD contributes to lower limb (diabetic) ulcers and gangrene, sometimes necessitating amputation.

Historic physicians knew of atheromatous plaques and hardening of the aorta and coronary arteries (arteriosclerosis) as described by Krell in 1740 (Stamler J, p21). Prior to the 20th Century there is evidence that atherosclerotic IHD was rare compared with infectious disease.

Blood Pressure

Since late in the 19th Century BP has been able to be measured in patients, both as a marker of CVD, and for other circulatory problems, such as shock. Its importance to CVD was also appreciated since before the rapid increase in CHD. Raised BP is associated with MI, but post infarct, and in heart failure, cardiac output and BP may fall to low, inefficient levels. The rise in obesity has been associated with increases in, and difficulties in control of, BP. Outpatient S/DBP measurement is primarily used as a marker of atherosclerotic CVD. Ultimately factors affecting the narrowness and reactivity of the arteries in relation to the pump action of the heart control BP.

Various axes or systems are involved in BP control. The main function of mineralocorticoids, particularly aldosterone, is to regulate kidney sodium resorption and conservation, control fluid retention and hence maintain BP. Excess aldosterone and cortisol also increase BP. Plasma cortisol is associated with central obesity, and adrenal hypersecretion causes central obesity, as for example in Cushings Disease. However, cortisone is converted into cortisol locally in adipose tissue by 11β-hydroxysteroid
dehydrogenase enzyme 1 (11β-HSD1), thus there may be much activity in the large depots of an obese person, but no raised serum cortisol levels detectable. Some of the complex relationships in BP are shown in (Figure 1-1).

Activation of the adrenals occurs through many mechanisms: the hypothalamic-pituitary-adrenal (HPA) axis, the renin-angiotensin-aldosterone system (RAAS), and the sympathetic system. All of these processes seem to have systemic and tissue level subsystems, and often the tissue involved is visceral adipose tissue\(^{155}\). It explains phenomena such as the observation that angiotensin II blocking (ARB) agents reduce CVD risk irrespective of BP lowering\(^{156}\).

**Figure 1-1. A Selection of Factors Involved in Hypertension**

Other biochemical factors are also involved. Hypoadiponectinaemia in obesity is associated with reduced endothelium-dependent vasodilation and hypertension\(^{157}\). In addition, perivascular adipose tissue and its cytokines affect BP with Adpn having a hypotensive effect and leptin, hypertensive\(^{158}\). Endothelin modulates increased endothelium-dependent vasoconstriction in obesity\(^{159}\). Other obesity induced cardiomyopathies\(^{160}\) and non-obesity factors probably contribute in addition. In central obesity, there appears to be a simultaneous loss of nitric oxide (NO)-mediated relaxation in the large conduit arteries, loss of NO-independent, potassium-mediated relaxation in the small arteries, and lastly a perpetuation of insulin resistance by negative action on metabolism and haemodynamic coupling\(^{161}\).

Although those with high blood levels of antioxidants are known to be more protected from degenerative disease, and it seemed sensible to give targeted antioxidant supplements, most intervention studies have failed to decrease CVD\(^{162}\), BP and vascular
risk disorders such as pre-eclampsia\textsuperscript{163}. Hypertension treatment guidelines may need to change to accommodate MetS specific treatment of hypertension, in order to prevent CVD and TIIDM\textsuperscript{158,164}.

\textbf{Cardiovascular disease - Epidemiology}

CVD is the most common disease and cause of death in western post-industrial societies and is fast becoming so in non-western countries\textsuperscript{165}. “In 2005 CVD was the underlying cause of death in 864,480 of the approximately 2.5 million total deaths in the United States, and adults aged 65y or older accounted for 82\% of all deaths attributable to CVD\textsuperscript{166}. In the early 1920's rates of CVD started to accelerate. CVD presumably resulted from influences that had started some years before, perhaps in the 1880’s. From this period, all cause death rates were starting to fall more rapidly in westernised countries\textsuperscript{167}. The rate of heart disease increased steadily from the 1920’s and subsequently, so sharply between 1940-1967, that the WHO called this incline the world's most serious epidemic\textsuperscript{51,167}.

Environmental factors post the industrial revolution of the 19\textsuperscript{th} Century may be relevant\textsuperscript{153}. During the early to mid 20\textsuperscript{th} Century, various new environmental changes and industries engendered lifestyles that were seen as increasing CVD risk. Highly processed, hydrogenated, and heated fats and oils resulted in high saturated fat/\textit{trans} fat diets\textsuperscript{168}, although there is still doubt about saturated fat\textsuperscript{169-172}. Labour-saving devices and machines were associated with decreased PA\textsuperscript{173}. Environmental toxicants included tobacco smoke and other new man-made chemicals and particles\textsuperscript{174-181}.

The Framingham Heart Study was initiated in attempt to identify common factors that contributed to CVD by following its development over a long period of time in a modestly sized group of participants\textsuperscript{182}. The study found that together with the classic CVD risk factors, the postulated ‘genetic, ethnic, geographic differences, industrial exposures/ food additives/ chemical factors and coronary-prone (Type A) behaviour might be studied as risk factors for CHD\textsuperscript{183}. After 1965 rates of CVD started to drop, particularly in reasonably affluent European westernised groups, and continued into the present, but rather slower in the low socioeconomic groups. After the Surgeon General’s Report on Smoking\textsuperscript{184}, and public health campaigns, smoking slowly decreased in prevalence amongst the well-educated, and is thought to have contributed to the reduction in CVD prevalence.
Intensive commercial development of medication for the classical CVD risk markers resulted in drugs that tend to normalise BP, LDL-C, and hyperglycaemia, and modestly reduce post-event mortality. Further CVD rescue interventions such as coronary artery bypass grafts (CABG) and stents were introduced. Public health campaigns were developed to encourage low cholesterol- and low saturated fat-diets. Interestingly, the above interventions may have only been responsible for approximately half of the decline in CVD incidence\(^{185}\), and other reasons for change in CVD need investigating. However, increasing central obesity may be threatening the decline in CVD\(^{186}\).

1.4 Physiology of Metabolic Syndrome – Adipose Tissue, Macro- and Micro- nutrients

1.4.1 Adipose Tissue - Body Fat Distribution and Metabolism

It is important to discuss adipose tissue at this juncture in this thesis, as the type and distribution of fat has very different effects on MetS, CVD and other degenerative disease. Adipose tissue is necessary for many functions in mammals and is present in almost all tissues of the body. Fat cell number appears to be inherited and set by the late teenage years, and thereafter stays stable with about 8-10% turnover per year\(^{187}\).

Fat tissue is very important for structural and physical functions, including impact buffering as seen under the skin, subcutaneous adipose tissue, around the kidneys, and in the knee joint. It is therefore understandable that different depots perform different metabolic functions. Storage of excess dietary energy as lipid is an important, specialised function of most adipocytes.

The best adapted appear to be peripheral, subcutaneous adipocytes which can become quite large without causing metabolic upset. Fat serves as an efficient continuous and intermittent energy supply and store, as exemplified by bone marrow, as well as hip and thigh, fat. Hip and thigh subcutaneous adipose tissue depots in women are probably reserved for breast feeding or more severe energy shortages\(^{188}\). It has become clear that some adipose tissue is able to store large amounts of lipid with neutral or even positive metabolic effects\(^{189-193}\). Peripheral fat is less responsive to mobilisation than visceral fat on sympathetic stimulation\(^{194}\).
In contradistinction to peripheral subcutaneous adipose tissue, excess central and/or upper body adipose tissue depots are sites of metabolic risk\textsuperscript{195}. Lipid deposited in visceral tissue around the abdominal organs is thought to be temporary storage for rapid mobilisation. Large visceral adipose tissue depots contain adipocytes that tend to be metabolically active, but unlike peripheral adipocytes, appear unable to expand and function normally when lipid reaches high levels. Overfull visceral adipocytes appear to suffer a decline in the production of Adpn, an increase in secretion of the pro-inflammatory cytokines tumour necrosis factor alpha (TNF-\(\alpha\)) and interleukin 6 (IL-6), and are associated with MetS. They appear to be adipocytes that suffer lipotoxicity\textsuperscript{196}.

It was relatively recently that these upper body depots of fat were recognised as a problem\textsuperscript{116-120} and there is still controversy about fat cell size and fat cell location\textsuperscript{121}. It also appears that there are other ectopic sites of adipose tissue which associate with various organs and confer metabolic risk. Intra- and inter-muscular, and epi- or peri-cardial adipose tissue depots have been linked with adverse metabolic findings\textsuperscript{197-201}.

Most men and some women appear to have limited peripheral subcutaneous adipocytes with which to take up excess lipid metabolically safely. Consequently, in these individuals the body is said to suffer from lipid overspill\textsuperscript{202,203}. Excess lipid becomes ectopic. Lipid is forced into the upper body skin layers, liver and muscle\textsuperscript{204}, including heart muscle or myocardium. Lipid overspill causes lipotoxicity in cells not designed to function with large deposits of lipid in equilibrium between TG and non-esterified FA (NEFA)\textsuperscript{205}. Lipoxidation in lipoproteins, especially LDL-C, and other advanced lipoxidation end-products (ALE) become common in MetS\textsuperscript{206}.

\textbf{1.4.2 Nutrients in Metabolic Syndrome}

\textbf{1.4.2.1 Lipids}

\textit{Fat Absorption and Metabolism}

Lipids have low miscibility in water which means that their absorption is specialised. Chylomicrons are TG rich particles of dietary origin with minimal lipoprotein, synthesised in the intestinal brush border and secreted through the cell junctions into the lymph-stream, and via the thoracic duct into the blood circulation. Chylomicrons are transported to the liver for processing into the non-HDL-C, Apo-B-associated lipoproteins.
Apo A1 and Apo B are water soluble vehicles which transport TG and cholesterol. The HDL-C particles contain Apo A1 and the non-HDL-C lipoproteins (intermediate density lipoprotein-C (IDL-C), LDL-C and very low density lipoprotein (VLDL-C) and associated chylomicrons) associate with Apo B. Following a fatty meal, TG within chylomicrons rise, but are often also raised in obese persons with MetS and TIIDM when fasting. LDL-C in the obese is not uniformly raised, whereas dyslipidaemia associated with CVD in non-obese individuals has a high frequency of raised LDL-C.

The low HDL-C seen in MetS and TIIDM, and high TC/HDL-C ratio in CVD are often all part of the same problem. The one lipoprotein that tends to be low in MetS is the HDL-C. HDL-C and Apo A1 collect oxidised lipids from the artery lining endothelium and ‘foam cell’ monocytes, by reverse cholesterol transport using adenosine triphosphate (ATP) binding cassette transporters, which carry lipids back to the liver for ‘antioxidant treatment’ and recycling. HDL-C influences adipocyte metabolism. Raised serum TG and low HDL-C are pivotal components of MetS.

**Dietary Fats and Oils**

Dietary lipids derive from various groups. Many are involved in energy metabolism and relate to CVD risk. Glycerolipids, fatty acids plus glycerol are the main energy molecules ingested from the diet. Lipids are the most energy dense of the 3 main macronutrients, containing 38kJ/g of energy, as opposed to CHO and protein which only contain 17kJ/g. Other fatty acids obligatorily required from the diet in the glycerolipid group are arachadonic acid derivatives. The eicosanoids, obligatorily required from the diet, form eicosapentanoeic acid (EPA) and are required for prostaglandins and thromboxane which are involved in clotting and other vascular signalling. Docosahexanoeic acid (DHA), also a dietary requirement, is long chain ω-3fatty acid employed in the brain.

Whilst humans synthesise many dietary sterol lipids, cholesterol derived hormones, including VitD with sunlight exposure and signalling molecules, plant sterols (phytosterols) such as β-sitosterol have serum cholesterol lowering properties. Prenol lipids (carotenoids – βCaro, VitA, and VitE and VitK) are all required in the diet. Other classes of lipids such as phospholipids, sphingomyelins can be assembled in the body.

Dietary fats and oils are mixes of lipids that occur in both animal and plant foods.
Homeotherms (warm-blooded animals) such as mammals and birds tend to have fats that more or less solidify at room temperature, whereas most plant, usually seed, and marine animals have fluid fat or oil that only becomes solid at very low temperatures.

Both fatty and oily foods can be processed to produce much more pure fats such as lard and cooking oils. The dietary fats contain high proportions of saturated carbon-carbon (C-C) bonds. Oils usually contain mono- and/or poly-unsaturated C=C bonds. Saturated fat has been thought to be a major contributor to the dyslipidaemia of MetS and TIIDM, and dietary cholesterol to the LDL-C hypercholesterolaemia of CVD\cite{168}. The monounsaturated fatty acids (MUFA), present in olive oil appear to have an antiatherogenic profile and the omega-3 (\(\omega-3\)) long chain polyunsaturated marine FA, eEPA and DHA are antiatherogenic, anti-inflammatory, antithrombotic and possibly antiarrhythmogenic\cite{212,213} although may be diabetogenic\cite{214}.

A number of semi-saturated fats and oils, such as palm oil, are further industrially hydrogenated, and some polyunsaturated FA (PUFA) also, to form solid fats which are less likely to become rancid, to leak from packaging on travel and can be added to oils to form margarine-like spreads. This process tends to form \textit{trans}-fats and research shows that these industrial fats may increase atherosclerosis\cite{215-217}. Dietary advisories such as Food Standards Australia and New Zealand recommend that individuals now limit \textit{trans}-fats levels in foods, but decline to regulate industrial use\cite{218}. Interestingly, natural animal \textit{trans}-fats associated with saturated fats may be less atherogenic\cite{219}.

As energy density is greater for lipids than for CHO, high fat diets have been thought to be responsible for much of the obesity epidemic. As lipid metabolism is intimately connected with insulin action and pyruvate derived from CHO in the Krebs cycle can be used for the glycerol back-bone of TG, highly refined CHO diets can also lead to raised TG. Low fat diets have therefore been advocated for weight loss\cite{220}, although CVD risk factors do not always improve\cite{221}.

Up until approximately 10y ago, saturated fats diets were seen as atherogenic and prothrombotic. In addition, there have always been anomalous findings about atherogenic and thrombotic fats and diets\cite{214,222}, and there is far from complete resolution of obesity and improvement in CVD risk with low fat diets\cite{170}. Subsequently, many studies have
shown that it is energy reduction, irrespective of diet composition which results in negative energy balance and weight loss over 6-24m\textsuperscript{223-226}.

### 1.4.2.2 Protein

**Protein Metabolism and Functions**

Protein is the emergency macronutrient or energy source in humans. Proteins, formed by polymerisation of more than 20 amino acids into polypeptides, or amino acid polymers, are the products of genetic transcription. They are used for structural purposes, as enzymes and in many other functional roles as protein complexes, such as glycoproteins and lipoproteins.

A part of protein function consists in organising energy production and being up- or down- regulated depending on food type, balance and availability. However, the adverse milieu found in MetS is not ideal for either structural or functional proteins. In the hyperglycaemic context, protein is both glycated where glucose attaches to some proteins, such as HbA\textsubscript{1c}, and glycosylated where by covalent or other permanent binding occurs thus altering structure and function. These glycated proteins are oxidants in that they easily react with oxygen, particularly in the circulation. Similarly, in hyperlipidaemia, lipoproteins become oxidised, particularly in LDL-C where ALE form which exhibit increased and abnormal activity\textsuperscript{227-229}.

These altered proteins are involved in widespread oxidative stress and metaflammatory activation, especially in the liver mitochondrion and endoplasmic reticulum. Cytokines, which are messaging polypeptides, are involved in many energy metabolism processes, especially inflammation, including that at the endothelium. Much inflammation is mediated by acute phase proteins, such as CRP. The thrombosis cascade is another protein based system related to MetS.

**Dietary Protein**

Dietary protein intake levels have been controversial. They often carry or contain many nutrients, as for example iron in haemoglobin (Hb). Vegetable protein in human diets is important, and is usually concentrated with other digestible energy sources, lipid and CHO in seeds. Current protein intakes of 10-35% of total energy requirements are advised\textsuperscript{230}. There is evidence that pre-historic humans derived more energy from protein
and animal fat than hitherto thought. Forager societies probably consumed 19-38% protein from animal sources alone\textsuperscript{231}. Dietary protein peptides and amino acids interact with reducing CHO, leading to a train of reactions denoted as glycation, the Maillard reaction or ‘browning’. Fatty meat, which may also contain glycogen, degrades or breaks down to amino acids, sugars and oxidised lipids, which combine when left together forming advanced glycation end-products (AGE) and ALE. Formation is accelerated with high temperature frying or baking. Wheat protein and starch in baked breads and crackers form such reactions. For those with MetS, or mild to major hyperglycaemia, ingesting further preformed AGE/ALE’s may be ill-advised\textsuperscript{232-236}.

1.4.2.3 Carbohydrate

Carbohydrate metabolism

Once CHO is digested and sugars are absorbed, insulin and non-insulin dependent pathways allow glucose into the cell. Thereafter immediate oxidation, or glycogen, the animal storage polysaccharide, synthesis commences in the liver and muscle. Plasma glucose is the circulating form of CHO and control of its levels is complex, but insulin is the key regulator. It is at the insulin receptor, in the organs which have episodic large glucose uses (i.e. the liver and muscle), where insulin resistance is found. Initially the pancreas is forced to produce compensatory insulin, or hyperinsulinaemia, but after time, this fails and plasma glucose rises.

Abnormal insulin signalling, which involves many processes and controls, includes Adpn and receptor dysfunction. The glucose-insulin interaction is one of the overt problems in MetS but where the problem starts is not clear. Serum lipids are also affected by insulin and plasma glucose levels.

Dietary Carbohydrate

CHO comprises the easily digested starch polysaccharides and sugar disaccharides and monosaccharides. The starches are hydrolysed into absorbable monosaccharides, of which glucose is the main plasma sugar. The disaccharide, sucrose, or ‘table sugar’ is hydrolysed into glucose and fructose. The monosaccharide fructose is also a fruit sugar. Unlike protein and dietary fats, CHO as starch or sugars can be refined to be an extremely pure molecular type, such as amylose which is hydrolysed into glucose. In the past, CHO mixtures, typical of unrefined grains, were usually ingested along with fibre (non-
Dietary Fibre

The CHO group that is most helpful in prevention of obesity, MetS and TIIDM are the non-digestible soluble and insoluble polysaccharide dietary fibres. A definition that was arrived at for dietary fibre by the Australia NZ Food Authority (ANZFA) (now Food Standards Australia New Zealand, FSANZ), proposed in 2000, is one of the more recent listed by the Institute of Medicine of the National Academies of Science: ‘Dietary fibre is that fraction of the edible part of plants or their extracts, or analogous CHO, that are resistant to digestion and absorption in the human small intestine, usually with complete or partial fermentation in the large intestine’.237

The term includes cell wall polysaccharides or polyglycans; cellulose (insoluble), hemicellulose (non-glucose sugar links), hydrocolloids and sulphated polyglycans (many from seaweed), gums and mucilages, gel-forming β-glucans, pectins, polydextrose, resistant maltodextrins, the oligosaccharides (degrees of polymerization >2, usually fructo-oligosaccharides derived from inulin238 or galacto-oligosaccharides), lignins (polyphenol, phytoestrogens linked with cell wall polysaccharides), chitin β-pleated sheets mostly derived from the arthropod, crustacean exoskeleton, and chitosan, a partially deacetylated chitin, and non-CHO fibre.

A typical summary of dietary fibre functions is that fibre promotes one or more of these ‘beneficial physiological effects: laxation, reduction in blood cholesterol, and/or modulation of blood glucose’237,239-241. Currently, prebiotic fibre, which promotes the growth of useful gut bacteria, is under study. However, as all fibre has been a main part of whole food diets, it is not surprising that the healthier microbe patterns are seen in those who eat larger quantities of all fibre242. Other uses include bulking out of the diet and adding satiety properties to meals243,244. Increased satiety may mean weight loss is associated with increased fibre intake245, but more studies show improved MetS rather than weight loss246-250.

Increased fibre specifically purified from food sources has been associated with some CVD improvement (cholesterol-lowering) in some studies241, but not all251. In one recent study psyllium at very high dose of >50g/d was successful for weight loss and lipid
lowering\textsuperscript{252}. However, whilst some studies show positive effects with guar gum and β-glucans, others have not\textsuperscript{253}. The reduction in efficacy may have been due to changes associated with processing during extraction, preparation and formulation to refine the product for ease of administration, at least with β-glucans\textsuperscript{254}.

Chitosan, derived from chitin, is the fibre to be used in the weight loss model of MetS change employed in this thesis. It was chosen due to its unique properties in the intestine. Chitosan occurs in nature, but it is chitin which is predominantly found. Chitin is synthesised by fungi and invertebrates, including arthropods insects and crustaceans, and molluscs, thus has always contributed fibre to human diets. Commercial chitin is harvested from marine sources and in NZ from the squid fishery. The squid mollusc forms a chitin pen, which is industrially deaminated and acetylated in order to control its level of acetylation. The greater deacetylation gives chitosan its positive charge and activity (Figure 1-2). Although relatively non-toxic and biodegradable, it does have antimicrobial and immune enhancing effects and is used in agriculture and for industrial films. Chitosan is soluble in gastric acid.

Figure 1-2. Structure of Chitin, Chitosan and Cellulose

The polysaccharide beta pleated sheet structure of chitin and derived chitosan, and compared with cellulose. The acetyl group RC(O)H\textsubscript{3} is removed from amide, RC(O)NHR', leaving the charged amine (R'NH\textsubscript{2}) on the polysaccharide. C carbon, H hydrogen, N nitrogen. Graphic from Dalwoo 2009\textsuperscript{255}

If the level of deacetylation is at least 75%, putatively chitosan becomes sufficiently positively charged to bind negatively charged FA in the stomach and non-esterified cholesterol and bile acids in the duodenum\textsuperscript{256}. It is reported to absorb >200% of its weight
in water to form a gel around non-micelle associated lipids\textsuperscript{257}. The excreted gel is thought to remove free fatty acid (FFA)/cholesterol from absorption/enterohepatic circulation, thus decreasing serum cholesterol, %Fat and weight. Animal studies indicate that chitosan can reduce the amount of fat absorbed from the diet\textsuperscript{258} and can increase fat eliminated in the stool\textsuperscript{258,259}. In addition, plasma glucose was decreased in experimentally induced diabetes in rats\textsuperscript{260}. Clinical trials have reported decreased weight\textsuperscript{257,261,262} or lipid levels\textsuperscript{263,264}, while others have reported no effect on either outcome\textsuperscript{265,266}. In clinical mechanistic studies minimal\textsuperscript{267}, or no\textsuperscript{268}, faecal fat loss has been shown, although faecal cholesterol loss has only been recorded in rats\textsuperscript{269}.

**Fruit and Vegetable Fibre**

High fruit and vegetable diets are composed of large amounts of most of the fibre types referred to above. There have been numerous studies over more than 20y showing that the traditional Mediterranean diet, with its high fruit and vegetable and fibre levels is associated with decreased central fat and weight loss, maintaining a lower weight, lower CVD and cancer risk, and longevity\textsuperscript{248,270-276}. The fibre in these diets may be acting alone as volume enhancing fibres, and/or with prebiotic effects, and/or as a matrix associated with a large variety and volume of micronutrients and vitamins\textsuperscript{252}. All mechanisms are probable\textsuperscript{252,277}.

**1.4.2.4 Micronutrients**

Most micronutrients function as cofactors. Cofactors are non-protein activators of proteins. Many of these proteins are enzymes which catalyse biochemical reactions in living organisms.

Cofactors include groups which are tightly or loosely bound to the protein. Members of a group denoted as coenzymes are more loosely bound chemicals which bind with enzymes. Vitamins, especially antioxidant vitamins, are an important part of the coenzyme group, as are the metal ions and by definition all must be acquired from the environment\textsuperscript{278-280}.

Many of these vitamins and minerals are required in relatively small amounts, as they are restored to initial conditions, being directly or indirectly recycled\textsuperscript{281}. A number of the vitamins fall into the food plant chemical (phytochemical) or more aptly, phytochemical micronutrient (phytonutrient) groups. The are many other phytonutrients, which are not
essential for human life, but appear to be associated with significantly better health, less central obesity, less degenerative disease and reduced rates of neoplastic malignant disease\(^{281}\). Energy processing requires a complex array of micronutrients, most particularly for efficient macronutrient oxidation\(^{282}\). Antioxidant micronutrients are those that are thought to reduce free radical production in living systems. As discussed further, many vitamins, including those examined in this thesis, are ‘antioxidant’ and are employed in many biological systems mainly dealing with energy oxidation. This thesis investigates this function. However, ‘antioxidant’ has become a marketing term to indicate health. Recently, the European Food Safety Authority that deals with claims has termed antioxidants as “food constituent(s) with antioxidant properties”\(^{283}\).

1.4.2.5 Diets: Palaeolithic to Westernised, and Metabolic Health

It is important to note at this juncture that initially in CVD risk studies dietary leafy green vegetables and fruit were not particularly considered for dyslipidaemia or diabetes as sugar and fat macronutrients were seen as the problem\(^{284}\). Somewhat later, ironically, high fruit and vegetable intakes were promoted in hypertension studies in the form of the Dietary Approaches to Stop Hypertension (DASH) diet\(^{285}\). The AHA Step 1 & 2 diets, for dyslipidaemia and the many TIIDM dietary guidelines have changed over the years. The AHA Dietary Guidelines now put more stress on high fruit and vegetable\(^{286}\).

**Palaeolithic Diets**

Palaeolithic diets\(^{287,288}\), typical of forager and pre-agricultural societies, include hunted/scavenged/gathered animal and plant foods, some of which may have been preserved by drying, such as nuts and fruit, and which may be cooked, but not farmed/cultivated. Dairy, and often pulses, are excluded. Importantly, highly bred starch-packed grain and tuber foods that have developed in the last 10,000yr are not considered Palaeolithic.

**Mediterranean Diets**

The Mediterranean diet studies for CVD amelioration appeared promising in the late 1980s\(^{289}\), but this dietary pattern was possibly difficult for nations where agribusiness and food processing industries had firmly taken hold. Mediterranean diet studies have been studied more recently for central obesity-related MetS, even though they do not necessarily provide less energy\(^{240,248,273,290-301}\). Traditional Mediterranean diets were
typified by high volumes and varieties of fruits and vegetables, including frequently used phytonutrient (high polyphenolic)-containing olive oil. Low processed foods, such as modest amounts of fermented dairy (cheese, yoghurt) and fruit (wine, beer and other alcoholic drinks), seafood and meats were common. Most traditional fare comprised whole-food items.

**Whole-Food Diets**

There does not appear to be a single any agreed upon, whole-food definition, however most definitions indicate that it means ‘high in unprocessed fruit, vegetables and meats’ some of which is found in the traditional Mediterranean diet\(^{302-309}\). The inclusion of farmed foods, including diary, pulses and modestly developed fruit and vegetables for general nutrition, and low processed food preservation methods, differentiates a whole-food diet from a Paleolithic diet.

Thus whole-food diets include low processed foods, preferably local, sometimes organic, which are still raw, lightly cooked and/or preserved in salt, vinegar or unrefined oils. Fats and oils that are naturally present in nuts, seeds and animal products, or cold pressed/skimmed, especially if combined with high proportions of protein and phytonutrients are common whole-foods. Free range or wild animal products which tend to be lean, with high proportions of \(\omega-3\) fatty acids are eaten in modest amounts. Fruit (fresh, dried or preserved), which contain no added sugars and very modest amounts of starch, including fermented products, are a good source of energy and nutrients.

**Starch and Sugar Based Staples**

Whole grain breads were one of the early processed foods but included high levels of fibre and modest protein. However, these starch-based seeds, grains, which dry and store well, and tuber starch storage organs, such as potato, have been bred to produce greatly increased volumes and proportions of starch\(^{310}\).

Pre-agriculture starchy food was scarce. These food items have become very energy dense and phytonutrient-depleted; even the germ and skin largely diminished. Baked breads and crackers, even of the ‘whole grain’ type, contain much refined flour. White potato based foods, even with skins on, contain very high levels of starch energy. Whilst many studies include whole grain based foods as part of a ‘prudent diet’, and show health
benefits, when studied alone CVD risk markers did not improve on a whole grain augmented diet\cite{311,312}.

Whilst fruit may have been cultivated to be fleshier and contain more sugar they can still be eaten whole, often with skins and seeds; even preserved they retain fibre. Non fruit sources of mono- and di- saccharides (fructose, sucrose) such as from sugar cane and beet, have also been highly bred for their rich energy yield, and should be excluded from a whole-food diet.

Potato, and ‘whole grain’, should be classified with refined starch and sugar food ingredients, or foodstuffs, as producing too low a micronutrient/macronutrient ratio to be fairly called whole-foods that will reliably aid in metabolic health and prevent excess adipose tissue gain.

*Industrialised Fat, Cholesterol and Protein*

Oils, hydrogenated to prevent rancidity, some already saturated such as palm kernel, and animal fats are refined, losing associated micronutrients. Raised, and oxidised serum LDL-C is now known to largely be a function of an overall metabolic problem, although some researchers still advise low cholesterol diets, even to the extent of reducing egg yolk\cite{313,314}.

Meat producing animals are often grown in small confines on grain and/or soy based high-energy, processed diets, with varying degrees of antimicrobial and hormonal support, producing protein with abnormally high levels and ratios of $\omega$-6/$\omega$3 fatty acids\cite{309}, which may have inflammatory effects\cite{315}.

*Westernised Food*

In current westernised diets CHO, fats and oils, and proteins become foodstuffs, refined and industrialised. Highly bred grains for CHO, and pulses and waste seafood for protein, along with other additives are processed into feed for farmed meat/poultry/fish. Many food extracts and non food items are added to these ingredients and formed into unusual mixes for visual appeal, texture, taste, preservation and transport.

The above foodstuffs are manufactured into processed meat and textured vegetable protein dishes, sauces, pies, breads, pasta, crackers, crisps, biscuits, cake and
confectionary. Highly processed foods which are energy rich, highly refined/ and micronutrient depleted, with preservatives, colourants, artificial tastes for extra palatability or ‘nutrient(s) fortification’ or other production additives. They have been called ‘empty calorie’ or ‘junk’ food and have been shown to associate with MetS\textsuperscript{316}.

\subsection*{1.4.3 Pathophysiology of Metabolic Syndrome}

In central obesity excess macronutrients are ingested and energy is always in over-supply. Not only are lipids unable to be accommodated and oxidised in a timely manner in the body’s cells, but glucose uptake is also compromised. The pancreatic β-cells increase the tonic insulin production to increase the transport of glucose into cells as glycogen storage molecules in the liver. In MetS the normal, rapid first phase after meal or post-prandial rise of insulin seen in healthy slim people, is lost.

The liver develops insulin resistance, becomes lipid and glycogen laden, and subsequently malfunctions. Gluconeogenesis is no longer suppressed by insulin\textsuperscript{317,322}. Liver insulin resistance occurs initially, and post-prandial plasma glucose rises, but over time persistent hyperglycaemia supervenes. Glycolipotoxicity becomes widespread in the large organs that usually act as energy molecule processors namely liver and muscle tissue\textsuperscript{204,205,323,324}. As the liver is the powerhouse of energy metabolism and detoxification, pathologies in this organ have wide ramifications. Non-alcoholic fatty liver disease (NAFLD) of central obesity may or may not be pathologic, however non-alcoholic steatohepatitis (NASH) involves probable glycolipotoxicity and inflammation which can lead to fibrosis, and cirrhosis, which are unpredictable forms of progression of NAFLD in obesity. Such oxidative stressors are the likely cause of the liver enzymes leaching out of the liver. As the primary problem is with energy-yielding molecules, abnormal oxidation processes occur, are poorly controlled and oxidative stress prevails. As many immune responses are so closely related to oxidative signals, ‘inflammatory’ pathways are activated. It is important to note that this form of inflammation does not follow the classic 4 steps of inflammation (heat, redness, swelling and pain) arising from an insult such as injury or infection. It has appropriately been denoted as ‘metaflammation’ (metabolically triggered inflammation)\textsuperscript{325}. Another part of the immune disruption to which obesity appears to contribute involves the immune cells. Undifferentiated adipocytes and several of the leukocytes share many features\textsuperscript{326}. Large numbers of monocytes migrate to the central adipose tissue. Possibly due to poor angiogenesis and relative hypoxia, monocytes
become macrophages. Leukocytes and inflammatory processes are not fully effectual in obesity. Infection, auto-immune diseases, neoplastic and malignant change, or cancers, are increased in MetS.

1.5 Novel Cardiovascular Disease Risk and Protective Markers

With the above background in mind, some specific comments will be made on the Novel CVD Risk and Protective Markers that will be examined in this thesis.

There are many metabolic processes which appear to alter as individuals become obese and acquire the typical MetS markers. Firstly, there are the Novel CVD Risk Markers, which are novel in that they are a selection of commonly used CRSHaem&Biochem which have not as yet specifically been employed to yield information which may help with refining MetS prediction.

They are a diverse group of laboratory tests found to be useful for screening and tracking inflammatory, nutritional and metabolic dysfunction, which frequently occur together and almost always involve oxidative stress. They include markers of glucose metabolism, FPG, HbA1c, serum lipids, urate, liver function tests and acute phase proteins, including ferritin, leukocytes, ESR and are addressed below. The conditions of interest in this thesis are central obesity, MetS, TIIDM, CVD and cancer; examples of multiple dysfunction, hence the use of a battery of laboratory tests. How each CRSHaem&Biochem test fits into this battery will be described.

Secondly, in this thesis, Novel CVD Protective Markers will be explored. They comprise two groups.

Serum Adpn and its oligomers are peptides that are intimately related to obesity as they are synthesised in the adipose tissue, and secreted into the blood. They are denoted as experimental Novel CVD Protective Markers, as they are not used clinically at this stage.

The nutrition related serum fat soluble vitamins (sFSVitamins) are the second group of Novel CVD Protective Markers to be examined. As vitamins they are indispensable, but are altered in an environment of excess lipid.
1.5.1 Novel Metabolic Cardiovascular Disease Risk Markers

1.5.1.1 Clinical Routine Screening Haematology and Biochemistry

1.5.1.2 Haematology

Leukocytes

It is known that overall WBC is increased in obesity and in other CVD risk settings but immune function, such as macrophage cell chemotaxis, can be altered or impaired in these conditions.

Neutrophils

Neutrophils can be stimulated by activated adipose tissue pro-inflammatory cytokines to exocytose oxidant myeloperoxidase. Neutrophils from individuals with MetS have poorly controlled cell release of vesicles or granules (exocytosis) containing reactive chemicals. There is increased likelihood of exocytosis with their pro-oxidant and pro-inflammatory effects, especially at the endothelium.

Eosinophils

For many years the increase of eosinophils has been thought to be a signal of allergic activation. Eosinophilia is a typical response in atopy, and is well documented in asthma. Eosinophils can be raised in obesity, but high fat feeding may decrease the pulmonary eosinophilic response but increase it in spleen cells, possibly as these cells are not activating correctly.

Basophils

Basophils probably contribute to providing mediators in order to initiate immune responses. They are related to mast cells which classically, on noxious stimulation, degranulate large quantities of histamine. Basophils can also act as antigen presenting cells for certain T-helper cells on invasion by multi-cellular parasites and autoimmune diseases of the skin.

Monocytes

Overfull, large abdominal adipocytes appear to secrete monocyte chemoattractant protein-1 (MCP-1) and large central adipose tissue depots can amass large numbers of immigrant
monocytes, transforming them to macrophages and increasing many-fold the leukocyte numbers in this tissue.

**Lymphocytes**

Lymphocytes contribute to the innate part of the immune system as thymus (T)-cells and the adaptive immune system as T-cells which are in the cellular immunity part of the immune system and bone marrow (B)-cells which secrete immunoglobulins (Ig). The balance of T- to B- cells and their activity may also play a role in the hygiene theory, for example altering intestinal microbiota in obesity\textsuperscript{346}. Adpn probably regulates lymphocytes along with many other aspects of the immune system\textsuperscript{347}.

**Aggregation Factor**

**Erythrocyte Sedimentation Rate**

The ESR is the time it takes for the red blood cells (RBC) or erythrocytes to settle through the plasma to form stable stacks or rouloux. The rate is slowed by large molecules, such as complex inflammatory and glyco-oxidised plasma proteins\textsuperscript{348}. ESR is both an inflammatory and a rheological measure\textsuperscript{348-351}.

1.5.1.3 **Biochemistry Long Term Plasma Glucose Indicator/Oxidant**

**Haemoglobin A\textsubscript{1c}**

Hb is the protein responsible for blood oxygen carriage and delivery. A glycated form of Hb is present in the plasma, HbA\textsubscript{1c}. HbA\textsubscript{1c} is related to raised post-prandial\textsuperscript{352} and FPG levels. It indicates longer term plasma glucose levels over approximately 3-12wk preceding testing.

**Oxidant Markers in Metabolic Syndrome**

**Urate**

Urate is the final product of purine metabolism in higher order primates\textsuperscript{353}. Hyperuricaemia has been shown to be associated with hypertension and hyperglycaemia for eight decades,\textsuperscript{96} and has been proposed as a close fifth member\textsuperscript{354} to the deadly quartet of CVD risk factors which are usually listed as abdominal obesity, glucose intolerance/ hyperinsulinaemia/ TIIDM, dyslipidaemia and hypertension\textsuperscript{355}. 
Chapter 1. Introduction and Background

**Ferritin**

Ferritin is an iron storage protein released from the liver probably related to the effect of modest ‘oxidant iron’ overload. Ferritin is a pro-oxidant and inflammatory measure possibly due to insulin resistance set against a background of a large, activated hepatic fat depot\(^{356,357}\).

**Liver Function tests; Oxidant and Antioxidant**

**Bilirubin**

Bilirubin is the final excreted product of haem metabolism, is lipophilic and unlike the other LFT is an effective antioxidant. The biliverdin reductase cycle, which serves to amplify bilirubin levels 10,000-fold, provides a constant supply of intracellular bilirubin. The liver conjugates bilirubin and secretes it in levels at which it is an effective antioxidant. Higher bilirubin levels may help to delay atherosclerosis and aging, thus acting as a cardioprotective biochemical\(^ {358-360}\).

**Alkaline Phosphatase**

Alkaline Phosphatase (AlkPhos) is a universal animal enzyme known to de-phosphorylate proteins and deoxyribonucleic acid (DNA) in alkaline media. Recently, tissue AlkPhos has been isolated from pre-adipocytes and may form a significant proportion of total serum AlkPhos\(^ {361}\). Intestinal AlkPhos iso-enzymes are involved in chylomicron formation, FA metabolism and influence CHO levels.

**Transferases 1 – Alanine and Aspartate Transaminases**

The liver serum transferases are alanine transaminase (ALT), aspartate transaminase (AST), and gamma glutamyl transferase (\(\gamma\)GT). The transferases have been extensively reviewed in the literature with respect fatty liver in obesity, TIIDM and CVD, and have been used to predict disease risk\(^ {362-366}\). ALT and AST are involved in processing energy; ALT in gluconeogenesis and AST in lipid and glucose oxidation\(^ {367}\). A relationship exists between the transaminases and the severity of MetS-driven atherosclerotic CVD with ALT predicting TIIDM\(^ {362}\). Importantly, raised AST may be an indicator of the more serious oxidative stress- and liver fibrosis-related condition of NASH\(^ {363}\). An index of hepatic disease is a raised AST/ALT ratio, which is used in NASH, and may have CHD predictive properties over and above MetS\(^ {368}\).
Transferases 2 - Gamma Glutamyl Transferase

γGT, the nearly ubiquitous cell membrane enzyme which maintains cellular concentrations of glutathione, is an antioxidant, may be a sensitive measure of oxidative stress and yet may itself produce oxidative stress in central obesity\textsuperscript{369,370}. γGT independently predicts changes in BP, TIIDM\textsuperscript{370-373} and atherosclerotic CVD\textsuperscript{374}, irrespective of alcohol intake, and even before NAFLD or central obesity develops\textsuperscript{369,371,375}.

Classic inflammatory protein (Acute phase protein)

CRP

Acute phase proteins have been used as predictors for TIIDM and atherosclerotic CVD in previous studies\textsuperscript{376-378}. CRP is synthesised in the liver but has additionally been found to be secreted by adipocytes\textsuperscript{379-382}, and is known to be released by stimulation of inflammatory cytokines including TNF-\alpha. CRP is known to be related to waist circumference, MetS\textsuperscript{383,384}, TIIDM\textsuperscript{381,382,385} and atherosclerotic CVD\textsuperscript{386-388} but the relationship may be indirect, via the abdominal fat mass.

Variably inflammation sensitive serum proteins

Albumin

Albumin is the most abundant serum protein, existing in reduced, oxidised and glycated forms in normal serum, and has multiple functions. These include being an oxidant scavenger\textsuperscript{389} and a long chain FA transporter\textsuperscript{390}. Serum albumin levels are usually decreased in oxidative stress conditions, although they can initially be raised in young, obese diabetics with near-normal kidney function, possibly due to hyperinsulinaemia causing increased protein synthesis and viscosity\textsuperscript{391}.

Globulin

Serum globulins encompass a vast array of proteins with many forms and functions. The globulin class includes liver-derived sex and cortisol steroid binding globulins, which may be suppressed by obesity and hyperinsulinaemia\textsuperscript{392,393}. Plasma cell-secreted inflammatory Ig’s appear to be activated in MetS\textsuperscript{378,394}, but they can also be consumed in inflammation\textsuperscript{395}. Thus Ig levels vary greatly with inflammatory stimuli.
Insulin Resistance Marker

Insulin

Insulin is the classic and main hormone controlling entry of glucose into many cell types. It is formed in the pancreatic β-cells, and secreted along with other molecules, including amylin. The control of insulin action and signalling is very complex and depends on the insulin receptor complex, and many downstream substances and pathways, including Adpn. Reaven asserted that insulin resistance and compensatory hyperinsulinaemia was the primary and unifying pathology driving syndrome X or MetS\(^{396}\). This theory depended on tissue becoming less able to transport glucose into cells. Insulin resistant tissues included tended to be muscle and liver which undergo large changes in glucose and FFA flux.

Muscle has to contend with sudden large requirements with sudden bursts or long periods of contractile activity during exercise. The liver drains the intestine and experiences large portal vein monoglyceride and FFA loads after food is digested.

Homeostasis Model Assessment - Insulin Resistance

Insulin resistance can be assessed using a number of indices, but one frequently cited and used in this thesis is the homeostasis model assessment-insulin resistance (HOMA-IR). HOMA-IR is an index of FPG and fasting insulin originally described by Matthews et al\(^{397}\). HOMA-IR closely correlates with the insulin sensitivity index by the standard euglycemic hyperinsulinemic clamp. HOMA-IR is calculated using the following formula: HOMA-IR (mmol/L. μU/ml) = fasting glucose (mmol/L) x fasting insulin (μU/ml)/22.5. Most researchers now believe that metaflammation\(^{398}\) and acquired faulty insulin signalling can be caused by diet-induced obesity\(^{399}\), and that this precedes IRS rather than IRS being the initiating cause, and thus they no longer follow Reaven\(^{396,400}\)

1.5.2 Novel Metabolic Cardiovascular Disease Protective Markers

There are many known CVD protective markers, but they may not be available or economic for clinical use. In the present work Adpn, along with its oligomers, is a marker which is synthesised in adipose tissue, and has deep seated insulin sensitizing properties that modulate energy, lipid and glucose, metabolism. It also has anti-inflammatory effects\(^{401}\). Adpn is still an experimental polypeptide, although clinical use has been mooted. The sFSVitamins have been studied in CVD, as epidemiology shows that higher
levels of these are associated with lesser risk\textsuperscript{402-406}. However, they have not been routinely measured for nutritional diagnosis or tracking, and especially not with respect to MetS status. The Novel CV Protective Markers are presumed to be protective factors but, as with the risk markers, not enough is known to show clear causal effects.

1.5.2.1 Adiponectin

Adpn is an adipocytokine, or an adipose tissue derived polypeptide signalling molecule, secreted almost exclusively from adipose tissue\textsuperscript{407}. There is growing evidence to indicate that this bioenergetic homeostatic peptide may provide a causal link between obesity, TIIDM and CVD\textsuperscript{408}. The first description of the DNA encoding for Adpn was reported by Scherer and co-workers in 1995\textsuperscript{409}. The transcript encoded a hydrophilic protein with predicted size of 29kDa and was called adipocyte complement-related protein of 30kDa (Acrp30). Other groups subsequently reported both murine and humans forms\textsuperscript{407,410-412}. The Adpn gene is located in chromosome 3q27, a susceptibility locus for TIIDM and related MetS\textsuperscript{413-415}.

Low levels of total Adpn are correlated with increased adiposity\textsuperscript{416 407}, excess muscle cell lipid\textsuperscript{417}, NASH\textsuperscript{418}, dyslipidaemia (including low HDL-C\textsuperscript{419}), impaired endothelium-dependent vasodilation\textsuperscript{420} and vascular inflammation\textsuperscript{421}. Hence it is believed that this adipocytokine has an important role in cardio-protection. Certainly higher levels of serum Adpn have been associated with a reduced risk of MI, explained only partly by differences in serum lipids\textsuperscript{422}. The muddled physiology of MetS tends to be associated with low levels of Adpn. Animal models have shown that exogenous administration of Adpn can decrease atherogenesis and inflammation and hence improve CVD risk\textsuperscript{423}, as well as increasing fat oxidation and enhancing weight loss\textsuperscript{424}, decreasing hyperglycaemia and reversing insulin resistance\textsuperscript{425}. Modest to severe central obesity, CVD risk, and CVD, are characterised by large, poorly functioning central adipocytes, dyslipidaemia, insulin resistance and metaflammation\textsuperscript{426,427}. This adipose tissue may also be hypoxic, hence the widespread dysfunction of adipocytes\textsuperscript{327}, and it is likely that cellular dysfunction impedes Adpn synthesis.

On the other hand, Adpn may be pro-inflammatory in lean populations. Some chronic, highly inflammatory, catabolic, autoimmune and tissue-destructive conditions such as Crohns disease, rheumatoid arthritis, Type I Diabetes Mellitus (TIDM), liver disease (including cirrhosis), and in fact a number of conditions in old age appear to elicit or
associate with high levels of Adpn\textsuperscript{428}. It is possible that Adpn is involved in managing energy release from adipose tissue in lean and catabolic states.

**Adiponectin Oligomers**

Data are accumulating on the relationships of total Adpn and more specifically the variant forms of this peptide, the oligomers, with body weight and adiposity, or the established metabolic risk factors indicative of CVD.

High-molecular weight (HMW) Adpn is composed of 18+ multimers, medium molecular weight (MMW) hexamers and low molecular weight (LMW) trimers\textsuperscript{429}. HMW Adpn is more closely related to metabolic control than either trimer/hexamer forms, or total Adpn, hence it is important to investigate whether the isoform structure may change during a dynamic phase such as weight loss\textsuperscript{430,431}.

Sexual dimorphism of this hormone indicates that that females are more likely to have high levels of the HMW heterodimers or multimers which appear cardio-protective, whereas males have more hexamers which may be less cardio-protective\textsuperscript{432}. LMW Adpn may have protective anti-inflammatory properties, in addition\textsuperscript{433}.

**Adiponectin Receptors**

Adpn has two main receptors AdipoR1 and AdipoR2, with both receptors being prominent in muscle, but AdipoR2 being predominantly found in liver\textsuperscript{434}. Both receptors are found in the brain, but Adpn levels are 1/4000th that in serum, and HMW Adpn is not present in cerebral spinal fluid (CSF)\textsuperscript{434}. T-cadherin, which is expressed in endothelium and smooth muscle, appears to be an Adpn-binding protein with preference for HMW isomers\textsuperscript{434}.

**Adiponectin and Medication Effects**

Thiozolidinediones, insulin sensitizers, increase circulating Adpn\textsuperscript{435} as does niacin\textsuperscript{436,437}.

**1.5.2.2 Serum Fat Soluble Vitamins**

The second group of Novel CVD Protective Markers are the sFSVitamins. Those examined in relation to the MetS for this thesis were serum vitamin D (sVitD), sβCaro, vitamin A (sVitA), vitamin E (sVitE) and a surrogate for Vitamin K (VitK), the international normalised ratio (INR).
VitK is a key molecule acting on the blood clotting cascade factors, thus one of the factors prothrombin time (PT), or blood clotting time, and ratio is reported as the INR, a surrogate for VitK. By definition the FSVitamins relate to lipid, and are essential in humans. Part of the absorption, lymph and blood carriage of these vitamins is by the same lipoproteins as those which transport cholesterol, namely β-Apo A1 & Apo B\textsuperscript{438,439}, although sVitD has its own plasma VitD binding protein (VitDBP)\textsuperscript{440}. Often sVitE (and other sFSVitamins) are corrected for lipids due to their solubility, but this may disguise metabolic relationships as all the sFSVitamins have differing lipid affiliations and solubilities\textsuperscript{441}.

Healthy individuals, who have low risks of immune and oxidative-stress associated disease, TIIDM, CVD and neoplastic diseases, tend to have higher sFSVitamin levels. In people who are impoverished, or if general nutrition or environmental conditions are sub-standard, single or multiple sFSVitamin deficiencies manifest in a number of clinical diseases.

1.5.2.3 Vitamin D

VitD is synthesised from sterol precursors in the skin upon ultraviolet (UV) B exposure and is further metabolised to the circulating form, 25(OH) VitD\textsubscript{3}, mainly in the kidney, but in many other tissues as well. Insufficiency is seen in those not exposed to sufficient sunlight, as seen in many in office-based occupations and with the elderly, and is especially common in high and low latitudes in combination with winter and dark skin pigment\textsuperscript{442,443 443-446}.

There is minimal VitD in food, with oily fish being a main supply in some regions. In Europe, table spreads and milk are fortified. Definitions of sVitD insufficiency vary, but at levels of less than 50nmol/L, parathyroid hormone (PTH) levels tend to rise, increases calcium resorption from bone and bone turnover occur, and demineralisation increases\textsuperscript{447}. Recently, levels of 70-100nmol/L\textsuperscript{448} have been suggested as sufficient for humans to have improved all round health requiring oral intakes of 50μg-100μg/day, if there is minimal skin sun exposure.

Most studies\textsuperscript{449}, although not all\textsuperscript{450} have shown sVitD insufficiency to be linked with obesity and it has been hypothesised to be one of MetS abnormalities\textsuperscript{451}. More recently
SVitD has been found to be important for growth, immunologic modulating functions, cytokine inhibitory properties, reducing adenocarcinoma, hypertension and CVD risk; conditions which in turn are linked to MetS. For many years TIIDM has been related to s VitD insufficiency. SVitD as 1,25(OH)VitD₃ (calcitriol), influences insulin sensitivity and secretion. In addition, obesity has been associated with low calcium intake, and low calcium absorption is aggravated by low sVitD. In summary, there is now extensive evidence of roles for sVitD in aiding the prevention of degenerative disease with immune and macronutrient disturbances via gene regulation.

SVitD modulates proliferation, differentiation, and apoptosis of many cell types and has effects on immune function, and insulin release. In European women and men aged 55-70y, after 8y of observation the hazard ratio of all cause death was 2.0, and for CVD deaths 2.2 for the lowest quartile group with mean sVitD approximately 20nmol/L compared with those in the top quartile whose sVitD was approximately 70nmol/L.

1.5.2.4 Carotenoretinoids – Beta-Carotene and Vitamin A

Beta-Carotene

βCaro is the most well-known carotenoid. βCaro is a coloured prenol tetraterpenoid, and can be measured in the clinical setting. Other carotenoids are the alpha and gamma carotenes, lutein, xanthines, and lycopene. Although other food sources have been found to contain βCaro in some form, they have usually been from a fruit or vegetable source. Good sources of βCaro are raw vegetables and fruits which usually provide many other carotenoids that may all confer health benefits.

βCaro is found in the cellular membranes of tissues and is an antioxidant. It is an excellent free radical trapper, which has singlet oxygen-quenching effects in vitro. It is thought to protect against both lipid peroxidation and DNA oxidation. sβCaro is associated with less CVD and less cancer. For this reason it was thought that people at high risk of carcinogenic change would benefit from high dose βCaro supplements. However, a number of studies and meta-analyses have shown that lung cancer rates in smokers were increased. High dose (20-50mg) βCaro supplementation increased all-cause mortality odds ratios (=1.07) and CVD mortality (= 1.1; p=0.003), and was higher in smokers. However, dietary carotenoids may help reduce smoking related lung cancer.
The calculated antioxidant content of a 3d food recall intake predicted levels of sβCaro, rather than the converse, thus it may be the food type, including a mix of other carotenoids, that confer metabolic advantages rather than βCaro\textsuperscript{466}. The idea of nutrient synergy, where the context and other co-ingested nutrients affect absorption and action may apply to βCaro\textsuperscript{467}. βCaro may split asymmetrically producing carotenals which have profound effects on nuclear signalling, both inhibitory and stimulatory\textsuperscript{468}.

βCaro is pro-VitA and often retinol equivalents are given when assessing VitA sufficiency\textsuperscript{469}. Whilst dietary fVitA and spVitA can cause toxicity, sβCaro only renders the hairless skin yellow. sβCaro deficiency is not well characterised. sβCaro is transported in the serum by HDL-C\textsuperscript{470}.

\textit{Vitamin A}

In affluent populations preformed VitA is usually plentiful in fatty animal foods. VitA can be synthesised by the body from two symmetrical βCaro units, or ingested as one of three biologically active molecules: retinol, retinal (retinaldehyde) and retinoic acid. Although βCaro is required for formation of VitA, sβCaro and SVitA are virtually never correlated even in populations marginal for intake of both\textsuperscript{471}. Other carotenoids such as lutein may be more highly correlated\textsuperscript{471}.

VitA deficiency has received much attention as causing severe consequences, initially to the eye and vision, as can be seen in populations with marginal food quantity and quality\textsuperscript{472}. Higher levels of sVitA and sVitE may be acquired from fortified, preserved and processed food. The adverse associations of hypervitaminosis A have been well reported, and include liver damage, failure of immune functions, growth deformities and foetal malformation\textsuperscript{473}. Retinol is thought to inhibit lipid peroxidation by scavenging peroxyl radicals by electron transfer and chain breaking\textsuperscript{474}. Retinaldehyde may repress adipogenesis and diet-induced obesity via retinol-binding proteins and suppress the retinoid X receptor (RXR)\textsuperscript{475}. SVitA is transported by serum lipids, especially LDL-C.
1.5.2.5 Tocopherols and Vitamin E

**Vitamin E**

VitE is found in many foods such as meats and nuts and other fatty foods. The vitamer, one form of the vitamin, α-tocopherol, is one of seven other fat soluble tocopherols; and water soluble derivatives which appear in the blood, are less well studied. The tocopherols have been found to have many functions, only some of which are antioxidant. α-tocopherol prevents AGE formation in native LDL-C and inhibits its oxidation, but does may not reconstitute oxidised LDL-C nor glycated LDL-C efficiently. VitC can regenerate α-tocopherol from its oxy free radical.

γ-tocopherol is highly added to the American diet as a fat antioxidant, but in fried food it has negative health effects. Some therapeutic trials have used the synthetic all-rac α-tocopherol, not the d-form (and some in high dose) and have observed a possible increase in haemorrhagic stroke. Prevention and treatment trials (for heart failure and cancer prevention) which have used α-tocopherol supplements show no benefits, or borderline negative results.

1.6 Theories on the Causes of Obesity and Metabolic Syndrome

At least two authors of recent papers have called for a unifying theory on the causes of obesity, and also for researchers in the area to cross disciplines to work on the root causes of the widespread occurrence of obesity. Part of the problem may have been that the metabolic decompensation associated central obesity is pivotal, and the processes behind MetS and central obesity and are inseparable, although differing in degree. Pure peripheral subcutaneous obesity must be differentiated, and the locomotor and psychological issues associated with this adipose tissue distribution, treated in a different arena.

Obesity and MetS have always been related to a relative excess of macronutrient intake. A question that has not been answered adequately is why do humans have such an acute sense of taste, and motivation for very highly energy dense food? It is known that humans have been going to extraordinary lengths for thousands of years to use technologies to set up agricultural processes of horticulture and animal husbandry, and breeding
programmes, in order to yield increasingly concentrated starches, sugars and fatty or oily foodstuffs.

Do humans eat enough protein if there is excess energy dense food, or does this refined food effectively exclude adequate uptake of protein and other useful nutrients? What other micronutrients are required? Some excess energy is acquired from animal fat or processed saturated and trans fats, has been associated with oxidative stress and LDL-C lipoxidation.

Antioxidants have been of interest for many years and in epidemiological studies the higher the levels of antioxidant vitamins the lower the rate of the degenerative diseases, CVD and cancer. Intervention studies of the vitamins, as mentioned, have not been successful. However, many medications are phytochemicals, and some have anticancer effects as shown by paclitaxel, and can control neoplasia recurrence in breast cancer. Cancer is known to be associated with failure of the immune system to check and control aberrant replication. Cancer is thought to arise in an environment of oxidative stress as seen in those exposed to pollutants, tobacco products and other exogenous stressors. Endogenous oxidative stress conditions include T1IDM, chronic autoimmune inflammation as found in ulcerative colitis and chronic viral hepatic infection, and inflammation. It now appears that a similar constellation of influences is present in central obesity, MetS and CVD to those of cancer. Multiple food micronutrients exert beneficial preventative effects in all these conditions.

There seem to be two processes at play. Humans seem to have a very high drive, and possibly requirement for energy dense food, and yet they seem to have better health, and less degenerative decline, with a high level of food micronutrients. Reasons for why humans appear to have contrary drives and needs may be rooted in evolution, and may be contained in those aspects of evolution which are unique to humans.

1.6.1 Rationale for studies

1.6.1.1 What is Known

Chronic, degenerative disease, particularly CVD, the most common manifestation of which is atherosclerotic CHD, has been a major cause of death in westernised populations since the beginning of the 20th Century. Central obesity rates have been increasing over
this period, in addition, but they have only accelerated in since the 1980’s. Central obesity had been mainly associated with the non-insulin requiring type of diabetes, TIIDM prior to this time. TIIDM, often precedes or is concurrent with CVD and they are both are linked by common risk markers of hypertension, dyslipidaemia, hyperglycaemia, and latterly central obesity. Having multiple moderately abnormal risk markers was found to increase CVD risk, and it had been apparent for some years that they were clustering together, and forming a syndrome, subsequently labelled MetS. Medical treatment for significantly abnormal risk markers had been shown to normalise them, but not necessarily reduce CVD events and death.

Categorising easily measured and well known clinical CVD risk markers into MetS was certainly of interest for public health planners, but managing or preventing TIIDM and CVD risk in individuals proved difficult in the clinical setting. Environmental and behavioural contributions to MetS included energy dense, refined diets, lack of physical exercise and pollutants, including smoking. The decrease in CVD after the 1960’s has been attributed to dietary improvement, smoking cessation and medical and surgical therapies, although combined they may have only contributed modestly. Central obesity rates are still accelerating in many parts of the world, TIIDM rates are rising together with the associated CVD risk markers, and CVD is expected to increase again. New research is finding novel metabolic pathways that involve profound modulation of macronutrient oxidation and immune system disturbance that may explain CVD.

1.6.1.2 What is Needed

Serum oxidant and metaflammatory markers can be used to detect metabolic stress. Current clinical routine screening tests could be re-interpreted for useful information. Key novel, yet well researched, metabolic, hormone and cytokine, or small molecule signalling, peptides and markers could also be assessed for early screening of CVD risk.

Nutritional markers could be employed to assess metabolic health. All of the above could be used together with MetS, and the most useful risk markers should be able to track increases, in addition to decreases, in degenerative disease risk. Their timely introduction into clinical care, in conjunction with MetS, for early detection of increased or decreased decline in CVD risk may be especially important for the increasing number of young centrally obese individuals. The importance to this group is a long lead time in which there is the opportunity to effect change and prevention of permanent end organ damage.
and completed CVD events and death. Furthermore, if protective CVD markers are found to be associated with prudent diets, then rather than just preventing manifestations of CVD risk, health could be realistically optimised by making much more of the whole-foods type diet.

There is a need for novel uses of clinical screening tests, new key metabolic pathway markers and greater use of dietary indicators to be used with central obesity-related MetS definitions. Other disciplines are revealing interesting findings in evolutionary, genetic/epigenetic, nutritional and behavioural sciences, which, if coordinated and analysed, may shed light on the root causes of obesity and related MetS.

An overview of the traditional CVD risk factors has been given in the introduction. The importance of the energy macronutrients, CHOs, lipids and protein and their contribution to oxidative stress and metaflammation to MetS has been outlined and limitations of MetS definition reviewed. Properties of possible CVD risk markers taken from CRSHaem&Biochem are discussed. An experimental adipokine, Adpn and oligomers, the sFSVitamin micronutrients, and the dietary fibre, chitosan, as CVD protective markers were reviewed with respect to prevention and management of MetS. Various properties of well-known dietary patterns have been discussed.

1.7 Hypotheses

1.7.1 Overarching Hypotheses

The overarching hypotheses of this thesis are that;
As human central obesity-related MetS, and its predicted diseases of TIIDM, CVD, and probably cancer, are degenerative conditions, which have increased in the westernised environment and for which we do not know the cause or have adequate treatments, re-evaluation of previous assumptions on human evolution, energy balance and nutrition together with re-interpreting current tests and introducing additional tests suggested by new science, will illuminate some causal aspects of obesity and aid in clinical MetS monitoring.

Results of the above clinical studies, when incorporated into a wide-ranging inquiry and research of contemporary science from many different disciplines (human evolution, human brain development, appetite and the mesolimbic system, nutrition, basic
biochemistry, epi/genetics, systems mathematical modelling), will indicate how humans have diverged from other mammals with respect to metabolism and energy management.

Various co-adaptations that allowed development of the unique energy demanding human brain, which functioned well in hunter-gather environments of the past, together with the human intellect and use of dexterous prehensile forelimbs (hands). These new traits drove technological development for producing, refining and adding appetisers, storing, transporting and marketing energy dense food, in addition to myriads of other labour-saving devices. These all contribute to MetS.

The results of the clinical studies together with the literature search evidence from human brain, energy and nutrition evolution and new basic bioscience, and their ramifications, will result in strategies for obesity related MetS prevention and management strategies in future.

### 1.7.2 The Clinically Tested Hypotheses

#### 1.7.2.1 The Clinically Tested Primary Hypotheses

The clinically tested primary hypotheses are that:

In the Novel CVD Risk and Protective Marker study which assessed MetS change over 6m in overweight and obese participants who attempt to lose weight

1) Novel CVD Risk Markers, composed of a battery of CRSHAem&Biochem, which exhibit inflammatory and oxidant properties, are independently and together, strongly related to obesity-related MetS and degenerative disease risk and should be used to enhance MetS disease prediction, and track CVD risk.

2) Novel CVD Protective Markers, comprising (a) Adpn and its 3 oligomers and (b) the sFSVitamins, known for their anti-inflammatory and antioxidant attributes, are strongly inversely related to obesity-related MetS and degenerative disease risk, and should be used to enhance CVD prediction, and track protection from CVD.

#### 1.7.2.2 The Clinically Tested Secondary Hypotheses

The clinically tested secondary hypotheses are that:

In the Diet&Health Weight Loss RCT used both as a model for Novel CVD Risk and Protection studies and to test a natural non systemic weight loss agent
1) participants randomised to the fibre supplement chitosan and given best practice dietary and moderate PA guidelines for 6m lose more weight and have a greater decrease in TC and LDL-C than those randomised to placebo
2) chitosan’s putative mechanism of action consists in binding intestinal lipid, forming a gel complex which is excreted and results in greater faecal lipid loss, and thus decreases in circulating blood lipids and weight are observed in participants randomised to chitosan rather than to placebo.

1.7.3 The Unifying Hypotheses

3) Causes of central obesity-related MetS stem from the evolution of the large human brain, in proportion to the body, requiring very high levels of energy. Hypothesised adaptations that developed to support this organ acted (a) to increase energy content of the diet by developing a very strong neural self-reward/motivation system to drive the search for, and acquisition of energy dense food and (b) to further economise on general body energy metabolism by the co-option of a wide variety and volume of antioxidant phytochemicals occurring in a whole-food diet, conferring energy-efficient, long-lived cell protection.

Obesity and MetS develop with over-palatable, and for some individuals addictive, refined, energy dense foodstuffs, without the modulating antioxidant/antitoxicant phytochemicals required for efficient energy oxidation and cytoprotection.

1.7.4 Aims and Objectives

Primary Aims

To establish a model of weight loss in overweight and obese individuals in order to investigate changes in MetS, and therefore CVD and degenerative disease risk

- To investigate which markers from a battery of commonly used CRShaem&Biochem, especially markers with inflammatory or oxidant properties, associate the most highly with MetS, and whether they as Novel CVD Risk markers could be used together with changes in MetS to increase disease prediction and track disease changes
- To investigate whether any of the adipocytokine, Adpn and oligomers, and the sFSVitamins, strongly and consistently associate with various CVD protective
markers, and negatively with MetS, and can be used as Novel CVD Protective markers together with changes in MetS to predict CVD protection and track improvements in health

- To interrogate the medical and scientific literature on composite theories of obesity and MetS, then explore the multidisciplinary literature on human evolution and nutritional and metabolic co-adaptations to the large human brain, and relate these to humans in the current environment in order to formulate a unifying hypothesis, and propose further research to test the hypothesis and report the above using narrative method.

Other Aims

- To assess the difference of serum lipids and weight, and mechanism of action, of chitosan compared with placebo in an RCT
- To compare the utility of MetS definitions as defined by NCEP, IDF and JIS.
- To assess the variability of anthropometric change as individuals attempt to lose weight in a community study with advice on diet and PA
- To assess the quality of life and eating attitudes in a group of overweight and obese individuals at the same time as investigating MetS markers

In order to test the first two hypotheses on Novel CVD Risk and Protective markers and their relationship to MetS over time, random samples from a large community would be ideal if equal numbers of adult individuals are weight stable, gaining or losing weight, and there was no secular weight gain trend. As this pattern is not the case a weight loss study model was required where there was minimal metabolic interference, and weight loss counselling could be established as could occur in primary care. A Weight Loss RCT as a parallel study investigating the difference of weight loss between the treatment and placebo groups, using a mild natural non systemic agent over time would be appropriate.

This model would comprise best practice, dietary fat restriction/increased intake of fruit and vegetables, and moderate PA, with the natural dietary fibre chitosan, hypothesised to bind fat within the gastrointestinal tract and enhance weight loss. Increased dietary fibre has been shown to improve MetS markers\textsuperscript{252,487}. Chitosan, which may be either naturally or industrially derived from chitin, is thought to achieve weight loss and cholesterol lowering based on information from animal and preliminary short term human studies, as detailed above\textsuperscript{488-493}.
Demographics, medical history and lifestyle have significant impacts on MetS. However, once established MetS has a significant effect on lifestyle and quality of life (QoL), thus validated questionnaires assessing these parameters were required. A prudent lifestyle study with dietary, PA and motivational counselling on a regular basis was established, to emulate what may occur in community primary care settings. Since most weight is lost in the first 3-6m, a 6m time frame was chosen for the intervention. It was predicted that modest weight change and improved diet seen in community weight loss studies would produce a model by which anthropometry, MetS and novel CVD markers could be assessed.

The detailed method rational and specific methods for the types of clinical studies needed to answer the hypotheses and fulfil the aims, is developed in Chapter 2. Chapters 3-5 include the statistical analyses methods required for each data topic for the Chapter. The methods used to develop the composite unifying theory of obesity are outlined in Chapter 6.
Chapter 2. Methods

2.1 Introduction

This chapter outlines the thinking behind the study designs and then presents the common general and specific methods employed in performing the Diet&Health-Weight Loss trial and Diet&Health-Novel CVD Markers studies.

The study nomenclature and relationships of the studies and topics in this thesis are shown in Figure 2-1, Figure 2-2 & Figure 2-3. The study design rationales and development comprise the first part of the current chapter. The common methods are presented as the Diet&Health-Weight Loss trial, which is the model for the main investigation; Novel CVD Risk and Protective Markers studies. The specific Diet&Health-Novel CVD Marker study methods are presented separately and follow the Diet&Health-Weight Loss trial (Figure 2-1).

The statistical methods details are discussed and included in the relevant chapters, unless they are required for more than one chapter, as for example the Demographics, Health Status, Lifestyle and QoL analyses section.

2.2 Study Designs: Rationale and Development

The studies were designed from the outset to fulfil the requirements, concurrently, for 1) the Novel CVD Risk and Protective Markers studies, whereby hypotheses were generated or developed and 2) the Diet&Health-Weight Loss study, whereby a typical treatment comparison RCT was performed.

2.2.1 Study Concept Development

As a primary care clinician, the author was routinely attempting to manage MetS or CVD risk markers in patients, with or without obesity. The author’s supervisor had used an *ad hoc* MetS definition in one of her previous studies, prior to the NCEP MetS definition publication, so an interest in MetS in the author’s team was already present. MetS was the major outcome variable.
2.2.1.1 Novel CVD Risk Markers – Concepts and Statistical Analyses

Rationale

The Novel CVD Risk Markers, or CRShaeem/Biochem in this case, were novel in the sense that they are not typically used to track improvements or decline in metabolic status or MetS. They were not novel in the fact that, although not included in current MetS definitions, their associations with MetS are well enough known to possibly be included in a MetS definition or used with MetS. Such use had been suggested with CRP, urate and liver enzymes\textsuperscript{96,495}.

Analyses were planned to ascertain whether singly or group relationships existed with respect to MetS marker count change. However, some Novel CVD Risk Markers could be more strongly related to individual MetS markers over and above others. It was unknown which Novel CVD Risk Markers would perform better in relating to cross sectional data, or, more importantly, which would be more effective as possible tracking markers of MetS change.

**Novel CVD Risk Markers - Statistical Analyses Rationale**

Statistically, analysis of the Novel CVD Risk Markers were expected to correlate with each MetS marker and with a MetS index. Partial correlations formed should rank the most highly related Novel CVD Risk Markers to MetS over and above other Markers. In addition, mixed modelling over time should allow both the assessment of the independent relationship of within person changes in markers with changes in MetS, as well as to the overall mean whilst also including other common and important explanatory variables, such as age, gender and treatment group. The effect size is important to record as well as the p value. Factor analysis was not appropriate as 1) neither exploratory nor confirmatory analysis was required\textsuperscript{496,497} and 2) factor analysis cannot be used longitudinally

2.2.1.2 Novel CVD Protective Markers – Concepts and Statistical Analyses

Rationale

With respect to the Novel CVD Protective Marker, Adpn and its oligomers the author works in a team which has been studying Adpn in vitro and laboratory animals for many years, and various clinical studies were planned as part of a programme.
Novel CVD Protective Markers comprising the sFSVitamins, were familiar to the author who had already worked as a clinician on a study of a non-systemic weight loss agent where sFSVitamins analyses were performed to indicate whether faecal fat loss decreased FSVitamin status. In using the natural food fibre, chitosan, for the current study, and its possible faecal fat loss effect, correlational analyses of sFSVitamins was decided upon. However, the more likely value of the precautionary analysis of the sFSVitamins was soon perceived by the author as their being used as Novel CVD Protective Markers, and factored into the study design.

**Novel CVD Protective Markers - Statistical Analyses Rationale**

The statistical analysis for the Novel CVD Protective Markers would differ slightly in quality from the Novel CVD Risk Markers. This was to be a more hypothesis-generating analysis than that performed for the CRSHAem/Biochem.

Thus, the analyses chosen were broad and wide-ranging correlational arrays of Adpn and the sFSVitamins with known CVD protective and risk markers, including the MetS markers and MetS marker count. Although this array was to be broad it was not random, as all the correlation parameters related to CVD risk or protection. The effect size was as even more important in the Adpn30 oligomer group owing to 1) the limitation of sample number that was able to be analysed, and small sample number was also why participants with extreme outcomes would be chosen, and 2) the very different gender groups’ size or number of participants.

As the sFSVitamins are inter-correlated, mixed modelling would be fitted with the sFSVitamins and common explanatory variables, to attempt to ascertain the relative strength of each sFSVitamin, on an overall mean and intra-person basis, with the MetS markers and MetS index. Extra sVitD analyses would be required due to the previously established seasonal and skin pigmentation effects on dermal photosynthesis of this vitamin.

**2.2.1.3 Diet&Health-Weight Loss Trial**

The RCT was to test a treatment, chitosan against placebo, for outcomes of weight loss and traditional CVD marker improvement. Typical RCT statistical methods of comparisons between outcomes, and various sensitivity tests on all randomised participants as well as per protocol analyses would be run. As this was to be a treatment
decision trial, probabilities for statistical relevance need to be set beforehand and are usually p<0.05 for the main outcomes. However, effect size would influence decisions on clinical use of the chitosan.

2.3 Contributors to the Dietary Fibre and Lifestyle for Health Studies

There were a number of contributors to the Diet&Health study.

2.3.1 Author’s Contribution to the Studies

The author formulated the hypothesis, designed and wrote the draft protocol for the Diet&Health- Weight Loss and Novel CVD Marker studies.

She researched the information on inflammatory and experimental markers such as CRP, Adpn and, from pre-clinical and clinical research, and determined that the metabolic and nutritional markers in clinical use needed specific study in obesity-related MetS. The Diet&Health-Novel CVD Risk and Protective Markers studies were entirely designed, written and undertaken by the author, with the supervisor particularly advising on the Adpn section. The author designed and organised Adpn the clinical and procedural practical standard operating procedures (SOP).

The chitosan Investigational Brochure (IB) and Standing Committee on Therapeutic Trials (SCOTT) applications were written by the author. She wrote the first draft of the Ethics Committee approval application to the Auckland Ethics committee. The laboratory and health status questionnaire coding was overseen by the author, and she set the safety ranges of laboratory tests.

The author designed the participant interview protocol, and the examinations and procedures standard operating procedures, performing many of them herself. She taught these procedures to research assistants, and guided and monitored their performance.
Chapter 2. Methods

Figure 2-1. Clinical Studies – Plan of Components

The Clinical Study

Component Studies

Chapter 3
Double Blind, Parallel, Randomised, Placebo-Controlled Study: Dietary Fibre Intervention
Fibre & Fecal Fat Loss Mechanism (Fibre&Fat Loss) Substudy

Chapter 4
MetS & Novel CVD Risk Markers: Clinical Routine Screening Haematology/Biochemistry (CRSBiochem/Haem)

Chapter 5
MetS & Novel CVD Protective Markers:
1) Adiponectin (Adpn250) & Adpn Oligomers (Adpn30)
2) Fat Soluble Vitamins (FSVitamin)-Vitamin (Vit)D (VitD), beta-carotene (βCaro), VitA, VitE & VitK

Chapter 6
Theories: Causes of Obesity & MetS Energy for Human Brain: Evolution
1) Energy Uptake: Energy Food Reward & Motivation ...Addiction
2) Energy Economy: Phytonutrient Antioxidants & Energy Modulation ...Loss of Cytoprotection

Chapter 7
Discussion and Conclusion
Figure 2-2. Diet&Health–Weight Loss Trial and Fibre&Fat Loss Study Timeline

Recruitment Phone or Email Reply
Screening/Registration -2 wk Visit
Randomisation (n=250)
Visit Baseline
Blood test
Anthropometry
Bioimpedance
Faecal Fat Collect (n=51)
1m Visit Anthropometry
2m Visit Anthropometry
3m Visit Blood test
Anthropometry
Bioimpedance
4m Visit Anthropometry
5m Visit Anthropometry
6m Visit (n=164)
Blood test
Anthropometry
Bioimpedance
Faecal Fat Collect (n=29)
Collect Questionnaires

Figure 2-3. Diet&Health-Novel CVD Risk and Protective Marker Studies Timeline

Recruitment Phone or Email Reply
Registration -2wk Visit
Recruitment (n=250)
Visit Baseline
Blood test
Anthropometry
Bioimpedance
Collect Questionnaires
1m Visit Anthropometry
2m Visit Anthropometry
3m Visit Anthropometry
Bioimpedance
4m Visit Anthropometry
5m Visit Anthropometry
6m Visit (n=164)
Blood test
Anthropometry
Bioimpedance
Collect Questionnaires
Simple statistical calculations and correlations were performed by the author for the Fibre&Fat Loss substudy, and the Diet&Health-Novel CVD Risk and Protective Markers studies. She discussed, with a Faculty of Medical & Health Sciences (FMHS) biostatistician, Ms Joanna Stewart, the type of analyses appropriate for the questions posed by the Diet&Health-Novel CVD Marker study, comprising CRSHAem&Biochem, Adpn30 and the sFSVitamins sections. Ms Stewart ran the more complex analyses, as detailed below in Sections 2.3.1.1, 4.2.3.5 and 5.2.5.

The theoretical Chapter was the author’s idea with the co-supervisors guiding on scientific interpretation of the literature, ensuring energetics was addressed, and the human side of addiction was acknowledged.

2.3.1.1 Collaborations

**Collaboration with the Clinical Trials Research Unit**

The Clinical Trials Research Unit (CTRU) at the University of Auckland became collaborators. Their contribution encompassed editing the protocol and data management, including setting up the databases and double data entry. General, anthropometric and laboratory data entry and statistics were undertaken by the CTRU for the Diet&Health-Weight Loss trial only. CTRU staff chose the Obesity Specific Quality of Life (OSQoL) QoL and Eating Attitudes 12 question (EAT 12) the questionnaires. The CTRU staff contributed to the conception and design of the Fibre & Fat Loss substudy. The manufacture, quality control and randomisation of the chitosan and placebo were the responsibility of CTRU, and they had no contact with participants. Data monitoring and audit was overseen by the CTRU.

**Bioimpedance Collaboration**

Dr Leigh Ward (University of Queensland) was responsible for the establishment of the methods for the multifrequency bioelectrical impedance analysis (MFBIA, Impedimed, Australia). He analysed the raw data in order to generate the calculated %Fat values.

**Laboratory Analyses Collaborations**

Two groups provided laboratory analyses.
Chapter 2. Methods

1) Laboratory staff and clinicians at the Auckland District Health Board (ADHB) LabPlus were contracted to perform the CRSHAem&Biochem and specially adapted sFSVitamin analyses. Faecal fat samples were analysed by this laboratory.

2) A School of Biological Sciences post-doctoral scientist, Dr Yu Wang, performed the baseline Adpn analysis of the 250 samples from all participants, Adpn250, in Auckland. She analysed the Adpn oligomers from the 30 subgroup participant samples, Adpn30 (30 baseline, 20 6m) at her current workplace, the University of Hong Kong.

Statistical Analyses Collaborations

CTRU

CTRU statisticians performed the Diet&Health-Weight Loss study repeated measures last value carried forward (LOCF) and sensitivity tests. The statistical package used was SAS v8.0 (SAS Institute Inc, Cary, NC, USA, 2001)

University of Auckland, Faculty of Medicine and Health Science Biostatistician

Ms Joanna Stewart collaborated with, advised and was directed by, the author to 1) run the combined Pearson and partial correlations and the least square means analyses and 2) fit the general linear models, ordinal logistic regressions, and general and generalised mixed models. She used the statistical package SAS v9.1 (SAS Institute Inc, Cary, NC, USA, 2006)

2.3.1.2 Study Site

The University of Auckland Human Nutrition Unit

The study was undertaken at the Human Nutrition Unit (HNU), a converted two-storey wooden villa. The HNU is a community-based facility designed to carry out controlled nutrition, supplement and medication interventional, publicly funded and commercial trials. The HNU is located in a quiet inner Auckland suburb, with easy access to public transport and private car parking. It provides a comfortable, non-clinical-appearing environment for the participants of research trials. There are communal lounges/sitting/waiting rooms for reading of ‘Participant Information Sheets’ (PIS). There are separate private interview rooms. The HNU is Good Clinical Practice (GCP) compliant. Private clinical rooms for anthropometry are available. These are provisioned with electronic BP machines, body weight (weight) scales, stadiometers, non-stretch tape...
measures and other basic equipment. Portable electrical bioimpedance or other equipment is procured and utilised as necessary. A basic laboratory is set up for study staff to process human tissue, usually blood, and other specimens. Laboratory equipment includes -20°C freezers and centrifuges. The specimens are prepared for same day analysis and transport or storage at other laboratories for specialist analyses. Staff include study managers and research assistants who are contracted as needed, and research students in masters and PhD programmes.

2.4 Dietary Fibre and Lifestyle for Health – Weight Loss Trial

The Diet&Health-Weight Loss trial was a community based, 6m weight loss, parallel, double-blind, randomised, placebo-controlled, intervention trial of a modified dietary fibre, chitosan, as an example of a weight loss agent (Figure 2-1). This Diet&Health-Weight Loss was also the model used for the main investigation into MetS and Diet&Health-Novel CVD Markers.

Fibre and Faecal Fat Loss Mechanism Substudy

Refer to Section 2.4.5 and Figure 2-2.

2.4.1 Study participants

Two hundred and fifty overweight and obese women and men who desired to lose weight, and were living in the Auckland city area were invited to join the study.

2.4.1.1 Eligibility criteria

Inclusion

Participants were required to be over 18y, have a BMI between 28 and 50kg/m² (Table 2-1), and not be actively trying to lose weight. The classification used to select the range of BMI levels utilised one of the lower definitions of obesity at 28kg/m² or above the NHANES II definition of overweight⁴⁹⁸ (Table 2-1). An upper level of 50kg/m² was thought to encompass most ambulant participants.
Exclusion

Participants were excluded if they: were on treatment with crustacean- or mollusc- marine fibre chitosan-containing supplements; were on current or recent treatment with weight loss medications; were on a current, or had recently attended a commercial weight loss programme/clinic; had allergies to seafood; were pregnant or breastfeeding; had active gastrointestinal disease or previous obesity surgery; had involvement in another clinical trial and were individuals judged to be unlikely to comply with study treatment and follow-up procedures.

Table 2-1. NHANES BMI Classification

<table>
<thead>
<tr>
<th>Adult BMI kg/m²</th>
<th>Women</th>
<th>Men</th>
</tr>
</thead>
<tbody>
<tr>
<td>Underweight</td>
<td>&lt; 19.1</td>
<td>&lt; 20.7</td>
</tr>
<tr>
<td>In normal range</td>
<td>19.1 - 25.8</td>
<td>20.7 - 26.4</td>
</tr>
<tr>
<td>Marginally overweight</td>
<td>25.8 - 27.3</td>
<td>26.4 - 27.8</td>
</tr>
<tr>
<td>Overweight</td>
<td>27.3 - 32.3</td>
<td>27.8 - 31.1</td>
</tr>
<tr>
<td>Very overweight or obese</td>
<td>&gt; 32.3</td>
<td>&gt; 31.1</td>
</tr>
</tbody>
</table>

From NHANES II (1976-1980)\textsuperscript{498}

2.4.1.2 Ethical Approval and Study Registration

Ethical approval for the study was obtained from the Auckland Ethics Committee (currently Northern X Ethics Committee), Auckland, NZ\textsuperscript{499}. All participants were given detailed information on the studies. They provided written informed consent. The study was registered as a clinical trial with the International Standard RCT Register # 96702062\textsuperscript{500}

2.4.2 Study Procedures

2.4.2.1 Study Visits and Procedures

Participants were seen at 8 scheduled clinic visits during the study. Time periods were measured in months as visits could vary by a week (wk) or two. These were held at registration (-2wk), at baseline (0m and randomisation), and at 1m, 2m, 3m, 4m, 5m and 6m following randomisation. Eligible registrants were enrolled (-2wk, registration) and randomised at baseline. Participants visited the HNU monthly for anthropometry and advice on prudent diets and increasing PA.
Written lifestyle advice on diet comprised standardised items: recipes, energy content guides, fruit and vegetable information, PA (including a frequency x intensity x duration counter) and stress management and motivation sheets were given at the standardised visits. Fasting blood tests were drawn and body composition was performed at baseline, 3m and 6m. Food recall, PA and QoL questionnaires were administered at baseline and 6m$^{501}$.

Questionnaires provided included (1) a validated 24Hour (Hr) Food Recall record (Appendix 1) and instruction booklet (2) The Life in New Zealand (LINZ) validated PA questionnaire$^{502,503}$ (Appendix 1) (3) the Short Form – 36 question QoL Health Survey (SF-36) questionnaire (Appendix 1) (4) the OSQoL questionnaire (Appendix 1) and (5) the EAT-12$^{504}$ questionnaire (Appendix 1).

The questionnaires were given to participants at the screening/registration visit for completion and return at the baseline visit. Questionnaires were reviewed with participants to check for understanding prior to uplifting, and on return to check completion. Participants were not permitted to have used any formal weight control methods 2m prior to, or during the study. At each visit until 5m participants were given systematic dietary and PA advice, and enough capsules in a large bottle for 12 treatment or placebo capsules daily.

A personalised diary was distributed to all of the participants at the screening/recruitment (-2wk) visit so they could book all their visits in advance, and be reminded forthcoming visit times and procedures and record any AE. Additionally, visit specific instructions, recorded in the participant diary, were annotated, with prompts to attend fasted for blood tests. Study staff gave phone call reminders the day before visits, and rescheduled visits within the pre-set limits. A travel allowance and gratuity was given at each visit.

**Recruitment**

Overweight and obese women and men were recruited through local city and suburban newspaper advertisements. The advertisements were also posted around University campuses, supermarkets and other public places. Email advertisements were sent around tertiary institutions. Participants were invited to join a weight loss trial investigating the use of chitosan, a dietary fibre, as an adjunct to weight loss. Refer to **Section 2.4.2.1**.
**Screening/Recruitment -2wk Visit**

At the screening visit the prospective participants read the PIS and completed the Informed Consent Form (ICF). The study protocol, questionnaires, and capsule regimen were all explained in detail. Once the ICF was signed, body weight was measured, a health status questionnaire administered and registration completed. Those registered were given placebo capsules and instructed to take 4 capsules three times a day for the following 2wk, and return the capsule bottles at the next visit to allow assessment of compliance, whereby staff counted the capsules. The participants were also requested to have the questionnaires completed, and with them, when they returned 2wk later. An introduction was given on how to make changes towards prudent low fat diets and increased PA. This 2wk period was the formal Diet&Health-Weight Loss trial single-blind placebo pre-randomisation run in.

**Two Week Run-In**

Only those participants who took greater than 85% of their study medication (4 capsules, 3 times a day, based upon capsule count), in the run-in phase were eligible to take part in the 6m randomised intervention. A number of the registered participants either discontinued, or were excluded as they did not complete the placebo capsule course during the run in period.

**Baseline Visit (0m)**

Randomisation occurred at the baseline visit. Participants were asked whether they thought they could continue the regimen of 12 x 250mg capsules/d, and visit monthly. Adverse events (AE) were recorded. The run in capsules were emptied onto a pill counter tray and counted. Those who had taken >85% of their capsules or who had returned <15% and wished to continue were randomised to chitosan treatment or placebo. Computer generated randomisation codes were supplied by the CTRU staff. A month’s supply of capsules was dispensed.

All questionnaires (Refer to Section 2.4.2.1) were collected and checked for missed questions by study staff. Any outstanding questions were completed with the participant, where possible. One-on-one counselling was given on lifestyle changes for weight loss. ‘Best practice’ advice centred on low fat dietary change, increasing fruit and vegetable intake, and increasing moderate intensity PA to at least 1/2hr a day. Motivational
Chapter 2. Methods

techniques were employed to assist modest lifestyle change. All information was
standardised so each participant received the same advice in written material, including
recipes, at the same stage of the trial. Pamphlets given at Visit 2 included: ‘National Heart
Foundation - I’m walking’, ‘Tips on exercise’ generated by study staff, ‘Step up to the
Challenge (healthy meals)’, ‘Healthy Lifestyles for a healthy weight’, and ‘Fat content of
foods (Weet-Bix™ comparison)’.

The baseline anthropometry was performed. Weight, height, waist and BP were measured
and %Fat was assessed indirectly by multi-frequency BIA (MFBIA, Impedimed,
Australia).

Blood was collected following a 12hr overnight fast using (1) an ethylene diamine tetra
acetic acid (EDTA) tube for a full blood count (FBC) and stored for later HbA1c batch
analysis (2) an ESR tube for ESR measurement (3) a serum tube for lipid: TC, HDL-C
and TG measurement and same day analysis (LDL-C and TC/HDL were calculated), and
(4) a serum tube wrapped in silver paper to prevent light exposure for batched
sFSVitamin analyses. All 1)-4) were sent to LabPlus. A fluoride oxalate tube for FPG and
two other serum tubes for CRSHAem&Biochem and cytokines were taken, spun and
pipetted into microtubes and frozen at -80ºC for later batch analyses.

1m Visit

Anthropometry (weight, waist and BP) was performed, a capsule count made, and any AE
recorded. Refer to Section 2.4.6.2. All participants were given standardised, visit-specific
low-fat dietary and prudent activity advice in the form of one-to-one sessions with
investigators throughout the trial. Written information provided at this visit to each
participant was ‘NZ National Heart Foundation Food Guide: ways to reduce your risk’,
‘Be Active Every Day’ (Exercise Pyramid)’, and ‘SPARK NZ, Push Play Information’.
The study staff did not give personalised lifestyle advice at any visit

2m Visit

The protocol for this visit was as for the 1m visit. The written lifestyle advice given was
‘Reading Food Labels’ (information taken from various sources) and ‘National Heart
Foundation Number Crunching’ pamphlets.
3m Visit

The protocol for this visit was as for the baseline visit. Anthropometry and body composition were taken as for the baseline visit. Blood was drawn for analyses. The written lifestyle advice given was ‘Reasons for eating’ sheet and ‘Causes of Weight Gain’ sheets.

4m Visit

The protocol for this visit was as for the 1m visit. The written lifestyle advice given was ‘Motivation Wheel’ and ‘Motivation Explanation Sheet’ derived from Prochaska and DiClemente⁵⁰⁵ and a Buttercup pumpkin recipe.

5m Visit

The protocol for this visit was as for the 1m visit. The written lifestyle advice given was ‘Satiety Index’ and an ‘Insulin Resistance model graphic’ which was derived from various sources. Questionnaires were given to participants with instructions on completion and return at the 6m visit.

6m Final Visit

The 6m visit was as for the baseline visit. No further lifestyle advice was given.

2.4.3 Anthropometry

2.4.3.1 Weight, Waist, Height, Bioelectrical Impedance and Blood Pressure

Participants were measured when lightly clad, without jackets/coats, head coverings, hair clips or shoes and with empty pockets. The following measurements were taken and recorded on the case report forms. All measurements were performed in duplicate.

Body Weight

Weight was measured on calibrated digital scales (Seca, Model 708, Germany) to the nearest 0.1kg.

Height

Height was recorded at baseline in centimetres (cm) to the nearest millimetre (mm) using a wall-mounted stadiometer (Seca, Model 222, Germany).
Body Mass Index

BMI was calculated by using the standard equation BMI = weight/height² (kg/m²)

Waist Circumference

Waist was recorded to the nearest mm. It was measured midway between the last rib and the crest of the ileum at the natural point of waist narrowing using a non-stretch tape measure. In the very obese if there was no waist narrowing, the point predicted as the lower point of the ribs, laterally, was chosen and the tape passed horizontally around the body by having participants anchor the tape against their bodies at the correct level and slowly turn on the spot, until the tape was brought around to the start.

Bioelectrical Impedance

BIA was measured using a Seac bioimpedance multi-frequency meter MFBIA (Impedimed, Australia). Participants were measured at the baseline, 3m and 6m visits. They were fasted and had refrained from drinking for at least 2hr prior and from strenuous exercise on the day of measurements. Women were asked if they were pregnant, and if so, together with those with pacemakers, were excluded. The presence of any metal implants such as pins or plates was noted. The arm and leg lengths, and sitting height, were measured. Participants lay supine with limbs not touching the body. Electrodes were placed on the right wrist, dorsum on the right hand, the right ankle and the right foot. The electrodes of the MFBIA meter was positioned to perform separate whole body, leg, arm, and trunk measurements and recorded on the CRF. The drive electrodes were not moved in position during the measurement but the position of the sensor electrodes was moved for each measurement.

The measurement was made directly after the participant’s characteristics were completely entered. Characteristics measured were resistance R (ohms)=U (voltage drop Watts)/I (electrical current Amp) and impedance Z=height (of body)²/resistance), Z=(constant)k.h²/R. %Fat was calculated at the end of the study.

Blood Pressure

S/DBP was measured at every visit. Measurements were made after the participant had been sitting for 5min with an appropriately sized cuff (pneumatic bag 20% wider than the diameter of the upper arm – usually the large size) using a functional, calibrated blood
pressure monitor Dinamap XL machine (model 9300 series, Palo Alto, USA). Each measurement was made on the non-dominant arm. Readings were taken before blood was drawn. The blood pressure monitor was set so that time between deflation steps depends upon the frequency of these matched pulses or pulse rate of participant. If either of the diastolic or systolic values differed by more than 10mmHg for each reading, additional readings were obtained until values from two consecutive measurements were within 10mmHg.

2.4.3.2 Randomisation, Blinding, Medication Dosing and Dispensing

Study participants were randomised in a 1:1 ratio to receive chitosan or placebo capsules. Staff at the HNU dispensed the study medication under blinded conditions using a randomisation sequence generated by a computerised random-number generator with mixed block sizes to prevent unblinding. There was no stratification by gender or other demographic variables. Treatment assignment codes were not available to the investigators, research staff or data entry staff at any point during the study and were held centrally by the Diet&Health-Weight Loss trial statistician, at the CTRU, University of Auckland.

2.4.3.3 Trial Medication Details

The chitosan used in the study was a β-chitosan (pleated sheet, poly-[1→4]beta-(D-glucosamine) derived from NZ squid pen chitin, and supplied by Healtheries, a food and supplements company. Independent analysis verified that the level of deacetylation was 75.5%, which conformed to prior specifications.

The study medication was dispensed in identical capsules, each capsule containing either 250mg chitosan or 250mg placebo (maize corn flour). Participants were instructed to take 4 capsules with a glass of water 3 times daily before main meals such that a total of 12 capsules (3g/d) of either chitosan or placebo were consumed. Treatment allocation was confirmed by independent assessment of capsule content in a sub-set of 25 participants during the first 4wk of the trial. The CTRU statistician reviewed this data and confirmed the treatment allocation.
2.4.4 Laboratory

Blood laboratory analyses were carried out at LabPlus, ADHB laboratory. LabPlus ADHB is an accredited laboratory and observes the NZ Code of Laboratory Management Practice incorporating ISO 9000 Guide 25:119 and ISO 9002:1987, issues (up-dates in regulatory standards) 12-14 at the Greenlane/National Women’s Hospital site and issues 18-24 at the Grafton site in 2003. The Grafton site laboratory was used for immediate routine and safety tests such as haematology, and primary outcome markers for the Diet&Health-Weight Loss and Novel CVD Markers study including all biochemistry. LabPlus also stored frozen batched laboratory samples, for later analysis with the follow-up 6m sample. FPG and HbA1c were stored in -80°C freezers at the Greenlane Outpatients Clinic and Hospital site, and urate, LFT, sβCaro sVitA and sVitE were stored at the Grafton site for later analysis.

Sample Management

Sample Collection

All blood samples were collected by venous phlebotomy by staff at the HNU from fasted participants. For a few (6) unsuccessful venepunctures, participants were sent to the Auckland City Hospital blood collection rooms to have their blood drawn.

Sample Sorting

Samples for immediate transport to the analysing laboratory and/or same day analysis were kept in their blood tubes at room temperature for up to 3hr until delivery to the LabPlus Laboratory. These samples were FBC in the EDTA tubes, serum tubes for immediate biochemistry and fluoride oxalate containing the FPG samples.

Sample Preparation; Centrifuging, Pipetting and Storage

Serum tubes of whole blood were left in racks on the bench to clot for 30-90min after collection prior to being centrifuged. A Heraeus Christ™ Labofuge GL (Hanau, Germany) centrifuge was set at 3000rpm for 15min. Whole blood was separated into a supernatant of serum, leaving the waste blood cells at the bottom. After centrifuging, the supernatant was pipetted into 2mL screw cap tubes, double checked by other staff and put into temporary storage, 4-6 wk, in a Westinghouse™ model FJ383, -20°C freezer (Westinghouse Electric Company, Risley, U.K.), then transferred into a -80°C Sanyo™ freezer (Oosaka, Japan).
### 2.4.4.1 Laboratory Sample Analyses

The analytes are shown in Table 2-2.

#### Table 2-2. Laboratory Test Collection and Analytes

<table>
<thead>
<tr>
<th>Test group</th>
<th>Test analytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum Full Lipid Profile</td>
<td>TC, HDL-C, LDL-C (calculated), TC:HDL ratio (calculated).</td>
</tr>
<tr>
<td>Serum Biochemistry</td>
<td>FPG</td>
</tr>
<tr>
<td>Serum Fat Soluble Vitamins</td>
<td>sVitD, βCaro, sVitA, VitE, Prothrombin time (PT) International Normalised Ratio (INR) surrogate for VitK. INR (VitK)</td>
</tr>
<tr>
<td>Faecal Triglyceride</td>
<td>TG</td>
</tr>
</tbody>
</table>

1Safety tests 2Tests were analysed immediately. 3Sample stored frozen at -80°C and analysed at a later date. 4sVitD was batched analysed every two weeks. TC total cholesterol; HDL-C high density lipoprotein cholesterol; TG triglyceride; LDL-C low density lipoprotein cholesterol; FPG fasting plasma glucose; s serum; VitD vitamin D; βCaro beta-carotene; VitA/E/K vitamins A/E/K.

### 2.4.4.2 Biochemical Analytes

#### Lipids

Serum lipids (TC, HDL-C and TG) were measured using enzymatic colorimetric tests on a Roche/Hitachi 917/MODULAR P (Mannheim, Germany). LDL-C was calculated using the Friedewald equation at LabPlus, ADHB, Auckland.

Friedewald equation: In mmol/L: LDL-C = TC – HDL-C – (TG/2.2). Note: This method is limited by <12hr fasting, and if TG >2.5-4.5mmol/L accuracy is limited. At >4.0mmol/L the equation was not used in this study.

#### Fasting Plasma Glucose

FPG was analysed enzymatic using colorimetric tests on a Roche/Hitachi 917/MODULAR P autoanalyser (Mannheim, Germany).

#### Fat Soluble Vitamins

The methods of analysis are detailed in Section 2.6.2 below.

### 2.4.5 Fibre and Faecal Fat Loss Mechanism Substudy

The Fibre and Faecal Fat Loss Mechanism (Fibre&Fat Loss) substudy was part of the Diet&Health-Weight Loss trial. The time line is shown in Table 2-3.
2.4.5.1 Method

Fifty-one participants who were already recruited for the Diet&Health-Weight Loss trial were asked to join the Fibre&Fat Loss substudy. They filled in the extra consent part of the Participant ICF. Participants who entered Fibre&Fat Loss substudy were enrolled in a serial manner until 25 were randomised to the chitosan treatment group and 26 into the placebo group. Three day faecal collections were made by all participants for subsequent fat content analysis.

Table 2-3. Faecal Collection Time Line

<table>
<thead>
<tr>
<th>Baseline minus</th>
<th>Baseline</th>
<th>Baseline to 6m</th>
<th>6m minus 3d</th>
<th>6m</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline -2wk</td>
<td>3d</td>
<td>Submit Faecal</td>
<td>6m</td>
<td>3d</td>
</tr>
<tr>
<td>Registration</td>
<td>Faecal</td>
<td>Collection</td>
<td>chitosan/placebo</td>
<td>Faecal</td>
</tr>
<tr>
<td></td>
<td>Collection</td>
<td>Randomisation</td>
<td>Intervention</td>
<td>Collection</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Finish</td>
</tr>
</tbody>
</table>

2.4.5.2 Procedures

On the screening visit (-2wk) and the second to last visit at 5m, the consented participants were given a large thick paper bag with 6 plastic collection trays to collect all faecal production for 3d. They were also given spatulae to direct each faecal collection into thick double plastic bags.

2.4.5.3 Eligibility Criteria

*Inclusion Criteria*

The first 51 participants to agree to the faecal collection were randomised with 25 to treatment code B, and 26 allocated to code A.

*Exclusion Criteria*

Participants could not take laxatives or cholestyramine/cholestipol\(^1\), both of which could affect lipid/sterol losses and hence interfere with measurements.

---

\(^1\) Cholestyramine/Cholestipol, prescribed for serum cholesterol lowering, are resins which bind bile acids and hence affect enterohepatic circulation, prevent reabsorption and lead to an increased GI excretion
**Laboratory Work**

Research staff thawed and combined the 3d faecal collect under a fume hood, and rapidly sent the sample to LabPlus laboratory.

**Analyses**

A typical clinical procedure for analysis of faecal fat, as for documenting or monitoring treatment for steatorrhoea, was performed. LabPlus Greenlane staff used a 3-step process of saponification of fats, extraction of FFA, and determination of total FFA\(^{507}\).

**2.4.6 Demographic, Health Status and Lifestyle Questionnaire Analyses**

For the Novel CVD Marker study demographic, medical, medication, lifestyle (social habits, diet, PA) and QoL, and eating attitudes questionnaires were administered at baseline and 6m.

**2.4.6.1 Demographic, Lifestyle, Quality of Life and Eating Attitude Questionnaires - Analytical Methods**

**Demographics**

Ethnicity grouping was performed as per the NZ Statistical Standard for Ethnicity 2005\(^{508}\).

**Diet**

A standard 24Hr Food Recall record was administered (Appendix 1, Questionnaires) at baseline and 6m, with a detailed instruction booklet on food portion size estimates, recipe recording and other qualitative information. Data was entered into FoodWorks\(^{\text{TM}}\) v2.1 (2002 New Jersey, USA), a commercial computer programme that calculates the nutrient content of the food items, modified for foods available in NZ.

**Physical Activity**

The validated questionnaire Life In NZ (LINZ)-PA\(^{502,503}\) assessed reported PA over the previous month. Appended were 3 questions on sedentariness, modified from a non-validated sedentariness questionnaire published by Egger et al\(^{509}\). As the reported level of PA was low with little sports activity, it was elected to selectively analyse general modest
PA and intense PA indicators from the validated questionnaire, after scoring (Appendix 1 Questionnaires).

**Short Form-36 Question Health Related Quality of Life Survey**

The 36 item SF-36 was coded and analysed as per the validation articles. The SF-36 was analysed with a specific macro-programme, adapted to NZ norms, and used on licence from the SF-36 licence holder (QualityMetric, Ingenix UnitedHealth Group, USA). Higher scores were the most normal, or least impaired. The normative value of the component score was 50.

The scoring system for the SF-36 can be generated by two methods and both were used. (1) Two summary scores can be derived from the SF-36: the physical component summary and the mental component summary, and (2) subscale scores for physical functioning, role limitations due to physical problems, bodily pain, general health perceptions, vitality, social functioning, role-limitations due to emotional problems, and mental health (Appendix 1. Questionnaires).

Of note, the questionnaires were inadvertently printed with an error in each of two questions. Before the use of the SF-36 raw data, for question 8 (BP2), which had been incorrectly coded into 6 categories, 1 was subtracted off the score for all those with a score of 2 or more so that the first 2 options were combined and the remaining given the score as on the questionnaire. For Q10 (SF2) one was subtracted off the score for those coded as 2-6 to result in the score on the questionnaire. For those who had ticked the first category (coded as 1) they were given a score of 5 if SF1 (Q6) was missing or 1, 4 if SF1 = 2 and 3 if SF1 = 3. This correction was made by the statistician used for the multivariable analyses of the SF-36. The CTRU statisticians stated that they used a similar method.

**Obesity Specific Quality of Life**

The scores were summed into 4 groups, as per the validation instructions then were collapsed into 2 groups; (1) Physical state: ‘I have trouble squatting’, ‘I cannot sit down in a very low armchair’, ‘I walk as little as possible’, ‘I have to stop to catch my breath after walking several hundred meters’, ‘I have trouble climbing’, ‘people say I am not very athletic’, ‘people often say that I am not agile’ (2) Psychological State ‘I lack energy’, ‘I don’t move around much’ ‘I feel attacked when people talk about my weight’,
‘I feel ill at ease’. Higher scores were the most normal, or least impaired. A percentage score for each question, and then section was calculated. The reference for the use of this tool was vague, and was not used for the RCT (Appendix 1, Questionnaires).

**Eating Attitudes Test – 12 Question**

The EAT-12 was used. The question sets were divided into 3 subscale groups of 4 questions: (1) Diet: ‘I am preoccupied with a desire to be thinner’, ‘I engage in dieting behaviour’, ‘I feel uncomfortable eating sweets’, ‘I think about burning up calories when I exercise’ (2) Bulimia: ‘I vomit after I have eaten’, ‘I have gone on eating binges where I feel that I might not be able to stop’, ‘I give too much thought to food’, ‘I feel that food controls my life’ (3) Oral Control: ‘I take longer than others to eat meals’, ‘I cut my food into small pieces’, ‘other people think I am too thin’, ‘I feel that others pressure me to eat’. For each question, a score of 1 to 6 could be made, where 6 is ‘none’ or ‘never’. The cut points for scoring were <15 for the Diet subscore, <20 for the Bulimia subscore and <22 Oral Control subscore (Appendix 1, Questionnaires).

**2.4.6.2 Adverse Events Protocols**

Participants could be excluded for medical reasons including AE or protocol violations. Good Clinical Practice and guidelines, common terminology criteria for adverse events and the Ethics Committee severe adverse events (SAE) forms were used for grading and reporting AE and SAE as summarised the Adverse Events case report form (CRF) (Appendix 2). The coding, severity and relationship to treatment for AE and SAE were included on the AE form. AE included laboratory, physical or psychological abnormalities. They were recorded at every visit, and all reviewed for regular audit. SAE were faxed to the Auckland Ethics Committee and to the CTRU data safety monitoring board (DSMB). A HNU mobile phone was available for participants to make contact with research staff and the author was contactable via a mobile to take medical or emergency calls from the research staff.

**2.5 Dietary Fibre and Lifestyle for Health – Novel Cardiovascular Disease Markers**

The Diet&Health-Novel CVD Marker study involved all previously measured anthropometry, BP, lipids, FPG, and the addition of the analyses of stored blood samples.
These were the Novel CVD Markers, composed of CRSHaem&Biochem, and Adpn and Adpn30, from the study participants at baseline and 6m. Refer to Section 2.4.

2.5.1 Design, Population, Eligibility, Procedures, Anthropometry

The design of the Novel CVD Markers study was as for the Diet&Health study. Refer to Section 2.4.2.

Cardio-metabolic Medications

Cardio-metabolic medications were divided into: (1) BP lowering or hypotensive medication: centrally acting beta-blockers, angiotensin I converting enzyme (ACE) inhibitors, calcium channel blockers, thiazide diuretics, angiotensin II receptor blockers (ARB); (2) serum lipid normalising hypolipideamic medications: statins, fibrates and niacin; and (3) plasma glucose lowering or hypoglycaemic (diabetic) medications; biguanide (metformin) insulin sensitisers and sulphonyl urea secretogogues.

Inflammatory Medications and Inflammatory-Effects Medications

Two groups were formed: anti-inflammatory classes and groups of medication and medication with anti-inflammatory effects. The anti-inflammatory classes comprised the non-steroidal anti-inflammatory (NSAID) medications and the Cyclooxygenase-2 (COX2) inhibitors, and steroidal anti-inflammatory medications.

Medication that was classed as having an anti-inflammatory effects included topical (skin, eye, nasopharyngeal) and inhaled anti-inflammatory medication, for example: NSAID’s, a leukotriene receptor antagonist, and steroids, were included. Other medication groups with anti-inflammatory effects included were the anti-inflammatory tetracycline, nitroimidazole and macrolide antibiotic groups. Statins and fibrates are also reported to have anti-inflammatory effects and can reduce CRP\(^{515,516}\), so were added to the anti-inflammatory effects group. The anti-inflammatory \(\omega-3\) FA’s, DHA and EPA (fish oil, flaxseed oil) were included as was the VitA analogue (isotretinoin).

Supplements

Supplement doses were not clear for all preparations as participants did not always know what brand they were taking. It was not always possible to trace the dose after the study was complete. Thus the types of vitamin were ascertained and whether it was taken at
baseline and/or at 6m. Multivitamins were generally expected to contain the common vitamins unless they were specified as some other. The percentage of participants who were recorded as taking supplements at baseline and 6m was calculated.

**Classification of Inflammatory Illnesses**

Chronic inflammatory diseases recorded were: (1) Autoimmune: rheumatoid arthritis, systemic lupus erythematosus (2) Allergic: chronic sinusitis/hay fever (3) Infective: hepatitis, ulcers >1m; (4) Neoplastic: malignant tumours, metastases, or cancer not in remission. Excluded as chronic diseases were: premalignant cervical and basal cell dysplasias.

Infectious illnesses at the time of laboratory testing, at baseline or 6m, were included as acute diseases that would increase inflammatory markers. The group comprised: viral infections (colds and acute coughs that had associated fevers, influenza, upper respiratory tract infection, gastroenteritides, diarrhoea and vomiting), bacterial (pneumonia, naso-oro-pharyngeal, skin infections, acute toothache), wounds that were fresh or still discharging and acute gout.

**2.5.2 Novel Cardiovascular Disease Risk Markers - Clinical Routine Screening Biochemistry and Haematology**

**Study Design**

This Novel CVD Risk Markers-CRSHAem&Biochem study was part of the Diet&Health study. For methods refer to Section 2.2.2.

**Laboratory**

**2.5.2.1 Laboratory Analyses**

The laboratory analytes for this study comprised the CRSHAem&Biochem. The tests are shown in Table 2-4.

The metabolic and inflammatory, or metaflammatory, haematological markers were leukocytes; neutrophils, lymphocytes, monocytes and eosinophils and the aggregation factor; ESR. These laboratory tests were required to be measured on the same day as being taken.
Table 2-4. Laboratory Test Collection and Analytes

<table>
<thead>
<tr>
<th>Test group</th>
<th>Test Analytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Full Lipid Profile</td>
<td>TC; HDL-C; LDL-C (calculated); TC/HDL (calculated).</td>
</tr>
<tr>
<td>Biochemistry</td>
<td>FPG; LFT: bilirubin; AlkPhos; ALT; AST; γGT; total protein, albumin, globulin</td>
</tr>
<tr>
<td>1 Haematology or FBC</td>
<td>Leukocytes or white blood cells (WBC): neutrophils; lymphocytes; monocytes;</td>
</tr>
<tr>
<td></td>
<td>eosinophils; basophils; ESR</td>
</tr>
</tbody>
</table>

1Tests analysed immediately. 2Stored frozen for later batch analysis. 3Frozen, analysed within 2wk. CRSHaem&Biochem Clinical Routine Screening Haematology and Biochemistry TC total cholesterol; HDL-C high density lipoprotein cholesterol; LDL-C low density lipoprotein cholesterol; FPG fasted plasma glucose; LFT liver function tests; AlkPhos alkaline phosphatase; ALT alanine transferase; AST aspartate transferase; γGT gamma glutamyl transferase; HbA1c haemoglobin A1c; FBC full blood count; RBC red blood cells; Hb haemoglobin; Hct haematocrit; MCV mean corpuscular volume; MCHC mean cell Hb concentration; RBCW RBC width; ESR erythrocyte sedimentation rate

The meta-inflammatory biochemical markers were HbA1c, urate, ferritin, LFT; bilirubin, AlkPhos, ALT, AST, γGT, acute phase serum or liver-derived proteins, total protein, albumin, globulin and CRP. Serum for biochemistry, was spun and prepared for storage as detailed above, Section 2.4.4.1. Frozen samples were sent to LabPlus.

Haemoglobin A1c

Blood was drawn into an EDTA blood collection tube and sent to LabPlus within 3hr. An aliquot was removed by LabPlus, frozen and stored for later analysis of HbA1c. The sample was later thawed and was analysed at by affinity chromatography with a Primus CLC385 (Kansas City, USA) instrument calibrated to a National Glycohaemoglobin Standardization Program (NGSP), based in the USA. NGSP results are standardized to glycated haemoglobin test results so that clinical laboratory results are comparable to those reported in the Diabetes Control and Complications Trial517.

Urate

Urate was analysed by enzymatic colorimetric testing on a Roche/Hitachi 917/MODULAR (Mannheim, Germany) analyser.

Ferritin

Ferritin was analysed by immune-turbidimetric test on Roche/Hitachi 917/MODULAR (Mannheim, Germany) analysers.
C-Reactive Protein

The Tina-Quant CRP (Latex) high sensitive immune-turbidimetric assay was used in the in vitro quantitative determination of CRP in human serum on Roche Diagnostics Tina-Quant (Mannheim, Germany) automated clinical chemistry analysers.

Full Blood Count

Blood was drawn into an EDTA tube and sent to LabPlus within 3hr of collection. The FBC was performed on SYSMEX XE 2100 (Kobe, Japan) analysers.

Erythrocyte Sedimentation Rate

At LabPlus a modified Westergren method in addition to an automated method was used. The modified Westergren uses 50ul saline mixed with 250ul well mixed blood at room temperature of between 18- 25°C. A dilution was made in a small plastic test tube and a dispette placed into blood/saline mixture and drawn up using an adapted syringe until blood soaked into cotton plug. It was timed for 1hr and read.

2.6 Novel Cardiovascular Disease Protective Markers

2.6.1 Adiponectin, Adiponectin and Oligomers: Novel Cardiovascular Disease Protective Markers

Study Design

The Novel CVD Protective Marker – Adpn_{250} and Adpn_{30} and Oligomer study was part of the Diet&Health study. For Methods refer to Section 2.2.2 for study and Section 2.4.4 laboratory details.

Adiponectin Full Baseline Analysis – Adiponectin_{250}

The blood samples were drawn as detailed previously, Section 2.4.4. Samples were stored frozen. One microtube per person was reserved for baseline Adpn_{250}. Dr Yu Wang developed the following Adpn sandwich Enzyme-linked immunosorbent assay (ELISA) method at the University of Auckland, School of Biological Sciences laboratory. A His-tagged recombinant Adpn produced from an Eschericia coli antibody was mixed with Freund complete adjuvant, and then injected subcutaneously into rabbits, boosted twice with the same, and blood collected 1wk later. An ELISA was used for the evaluation of the resultant antisera.
Adiponectin and Adiponectin Oligomers – Adiponectin$_{30}$

Fifty samples were able to be analysed for Adpn oligomers, Adpn$_{30}$, as part of a collaboration with the laboratory of Drs Y Wang and A Xu in Hong-Kong. These collaborators had developed an assay to analyse the Adpn oligomers.

The procedure for selecting the 30 participants whose serum was to be used to analyse the total Adpn$_{30}$, HMW$_{30}$, MMW$_{30}$, and LMW$_{30}$ was devised by the author and supervisor in NZ. The method was based on selecting 30 participants post hoc for the extremes of weight (high or low), and MetS marker count (high or low), and to include participants with TIIDM. In addition 20 participants with baseline and 6m data were chosen from the 30 Adpn$_{30}$ participants for extreme change in weight (gain or loss), and change in MetS marker count (gain or loss) as shown in Table 2-5, to have their samples analysed. Thus 50 samples were selected for the Adpn oligomers (Adpn$_{30}$) substudy. Refer for details to Section 5.3.1.

Table 2-5. Criteria for Selecting the Adpn$_{30}$ Subset for Oligomer Analysis

<table>
<thead>
<tr>
<th>Parameter</th>
<th>n, Adpn$_{30}$ participants</th>
<th>Baseline</th>
<th>6m</th>
<th>TIIDM</th>
<th>IFG</th>
</tr>
</thead>
<tbody>
<tr>
<td>High BMI + No MetSMCt</td>
<td>3</td>
<td>3 W</td>
<td>2 W</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>High BMI + High MetSMCt</td>
<td>8</td>
<td>4 W, 4 M</td>
<td>2 W, 1 M</td>
<td>1 W, 1 M 0</td>
<td>1 M</td>
</tr>
<tr>
<td>High BMI + Low MetSMCt</td>
<td>2</td>
<td>2 M</td>
<td>2 M</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Low BMI + High MetSMCt</td>
<td>4</td>
<td>3 W, 1 M</td>
<td>1 M</td>
<td>1 M</td>
<td>0</td>
</tr>
<tr>
<td>Large Waist + Low MetSMCt</td>
<td>1</td>
<td>1 W</td>
<td>1 M</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>TIIDM (at Baseline)</td>
<td>2</td>
<td>2 W</td>
<td>1 W</td>
<td>2 W</td>
<td>0</td>
</tr>
<tr>
<td>Large MetSMCt Decr./6m</td>
<td>3</td>
<td>2 W, 1 M</td>
<td>2 W, 1 M</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Large Weight Loss/6m</td>
<td>4</td>
<td>4 W</td>
<td>4 W</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>MetSMCt Incr./6m</td>
<td>1</td>
<td>1 W</td>
<td>1 W</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Large Weight Incr./6m</td>
<td>2</td>
<td>2 W</td>
<td>2 W</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Total N</strong></td>
<td><strong>30</strong></td>
<td><strong>21 W, 9 M</strong></td>
<td><strong>14 W, 6 M</strong></td>
<td><strong>4 W, 2 M</strong></td>
<td><strong>1</strong></td>
</tr>
</tbody>
</table>

1Baseline only. Adpn$_{30}$ subset of 30 participants for whom total Adpn was re-analysed with different methods, time and place from Adpn$_{250}$, with the main aim to simultaneously analyse the associated 3 HMW, MMW & LMW. W women. M men; TIIDM type II diabetes mellitus; IFG impaired fasting glucose, Adpn Adiponectin, BMI body mass index, waist waist circumference HMW high molecular weight Adpn, MMW medium molecular weight Adpn, LMW low molecular weight Adpn, dechr. decrease; incr. increase; MetSMCt metabolic syndrome marker count.

Ethics Approval and Regulation for International Transport
Ethics Approval

Ethical permission was sought from the Northern X Committee and granted to have these 50 samples analysed in Hong Kong.

Importation Approval

Arrangements were made and the samples were air freighted, on dry ice, to Dr Yu Wang in Hong Kong.

2.6.1.1 Laboratory Analyses

Frozen stored serum samples were analysed for total Adpn (Adpn30) and 3, HMW30, MMW30 and LMW30, oligomers (Table 2-6).

Table 2-6. Laboratory Test Collection and Analytes

<table>
<thead>
<tr>
<th>Test group</th>
<th>Test analytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum Adipocytokine</td>
<td>Adpn250</td>
</tr>
<tr>
<td>Serum Adipocytokine oligomers</td>
<td>Adpn30, HMW30, MMW30, LMW30</td>
</tr>
</tbody>
</table>

1Sample stored frozen at -80°C and analysed at a later date.

The 3 oligomeric complexes of Adpn30 were separated using gel filtration chromatography. In summary, 10-30µl of human serum was diluted to 1ml with PBS, loaded onto an AKTA explorer fast protein liquid chromatography (FPLC) system, fractionated through a Hiload 16/60 Superdex 200 column (GE Healthcare, UK), and eluted with PBS at the flow rate of 1 ml/min. Each 1µl fraction was collected. The Adpn concentrations in each fraction were measured by ELISA to determine the percentage composition of each oligomeric isoform of Adpn per total Adpn.

The plasma levels of each oligomeric form of Adpn were calculated by multiplying the percentage of each oligomeric form with the plasma level of total Adpn. Results were reported by the laboratory as µg/ml and percentages.

2.6.2 Serum Vitamin D, Beta Carotene, Vitamin A and Vitamin E

Study Design

This study was also part of the Diet&Health -Novel CVD Protective Marker-sFSVitamin study, and methods have been covered previously. Refer to Section 2.4.2.
Laboratory Analyses

For laboratory collection details refer to Section 2.4.4 and Table 2-6.

Table 2-7. Laboratory Test Collection and Analytes: Fat Soluble Vitamins

<table>
<thead>
<tr>
<th>Test group</th>
<th>Test Analytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum Fat Soluble</td>
<td>$^{1}$sVitD, $^{2}$βCaro, $^{3}$sVitA, $^{2}$sVitE, $^{3}$Prothrombin time (PT) International Normalised Ratio (INR) surrogate for VitK. INR (VitK)</td>
</tr>
<tr>
<td>Vitamins</td>
<td>Ratio (INR) surrogate for VitK. INR (VitK)</td>
</tr>
</tbody>
</table>

$^{1}$Short term batching; analysed within 2wk. $^{2}$Stored frozen -80º and analysed at a later date. $^{3}$Tests were analysed immediately. βCaro serum beta–carotene; PT prothrombin time; sVitD serum Vitamin D; sVitA serum vitamin A; sVitE serum vitamin E The isocratic reversed-phase high performance liquid chromatography (HPLC) method was augmented by Dr G Woollard LabPlus, ADHB.

Serum Beta-Carotene, Vitamin A and Vitamin E

βCaro, sVitA (as retinol) and sVitE (as α-tocopherol) were measured simultaneously by isocratic reversed-phase HPLC using photodiode array detection, time-programmable wavelength changes, vortex spun to precipitate proteins and release vitamins from their binding proteins, extracted into twice the volume of n-hexane, evaporated to dryness with nitrogen in a warm water bath and finally, the sample was reconstituted in ethanol and injected into the chromatograph. Chromatography internal standards were retinyl acetate, for retinol, tocopherol acetate, for α-tocopherol, and echinenone, for βCaro.

Vitamin D

VitD3 was analysed using VitD25 pre-extraction with acetonitrile, double antibody radioimmunoassay (RIA) at LabPlus. The DiaSorin Inc Stillwater, MN, USA (formerly Incstar) 25 hydroxy vitD assay consisted of a two-step procedure, involving a rapid extraction of 25-OH-vitD and other hydroxylated metabolites from serum or plasma with acetonitrile. They were then assayed by using an equilibrium RIA procedure based on an antibody with specificity to 25 hydroxyvitD and tracer with antibody, were incubated for 90min at 20-25ºC. Phase separation was performed after a 20min incubation at 20-25ºC with a second antibody-precipitating complex, an addition buffer complex was added after incubation, and finally centrifugation performed to aid in reducing non-specific binding. Bio-pool controls were run from those expected extremes of sVitD.
**Vitamin K**

VitK (menaquinone, VitK) was not measured directly. A surrogate of VitK, the PT and its derived measure, the INR, which is the reported blood test, are measures of the extrinsic pathway of coagulation. INR was only used in multivariable analyses.

**Prothrombin Time**

Blood was drawn into a vacuutainer containing liquid sodium citrate and aliquotted correctly by filling to the mark on the tube. Citrate acts as an anticoagulant by binding the calcium in a sample. The blood was mixed, then centrifuged to separate blood cells from plasma which was analysed by LabPlus staff on an automated instrument, Dade Behring Berichrom BCS coagulation analyser (Marburg, Germany)™ at 37°C. Thromborel S (tissue factor from human placenta, ISI 1.09) and Pathrombin SL (Dade Behring, Marburg, Germany) were added, and the time the sample takes to clot was measured optically.
Chapter 3. The Dietary Fibre and Lifestyle for Health – Weight Loss trial: A Randomised Placebo-Controlled Weight Loss Model

3.1 Introduction

The Diet&Health-Weight Loss RCT was the model used to investigate the hypotheses of interest in the Diet&Health-Novel CVD Risk and Protective Marker studies. This model has been used throughout the thesis.

Weight loss is difficult to achieve using any method, but changing lifestyle to improve diet and increase PA is the cornerstone of any weight loss programme. Lifestyle changes which include decreasing dietary fat, increasing fruit and vegetable intake and improving physical fitness by increasing PA, have been shown to result in weight loss in highly controlled trials\textsuperscript{520}. Modest weight loss affords important risk marker reduction. In spite of many weight loss agents and medications specifically designed to aid weight loss, there continue to be significant safety and efficacy problems and few orthodox medications are registered for prescription or over-the-counter (OTC) sale\textsuperscript{521}.

Food supplements, herbal and ‘natural’ substances with ‘generally regarded as safe’ (GRAS) status have also been studied, although usually in trials with few participants for short periods, and with very modest weight loss\textsuperscript{522,523}. Chitosan is a dietary polysaccharide fibre as described in Chapter 1. The positive charge arising from deacetylation is thought to confer the added property of forming a gel by adsorbing negatively charged, ‘polar’ lipids. Animal studies and short human trials have suggested that weight loss and cholesterol lowering associated with chitosan ingestion, is caused by faecal lipid loss\textsuperscript{257,261,262} including cholesterol\textsuperscript{263,264}. Chitosan had not yet been studied with the rigor of a pharmaceutical trial. In the current study the effect of chitosan on weight loss and the underpinning mechanism of gastrointestinal lipid binding were investigated.
3.1.1 Hypotheses

**Dietary Fibre and Lifestyle for Health - Weight Loss trial**

The hypothesis for the Diet&Health-Weight Loss trial is that a putative lipid binding dietary fibre, chitosan, when compared with placebo, increases weight loss and improves CVD risk markers in overweight and obese participants over 6m within a model of best practice weight loss advice based on a low fat/high fruit and vegetable diet, and increased PA.

**Fibre and Faecal Fat Loss Mechanism Substudy**

The hypothesis for the Fibre&Fat Loss substudy is that the chitosan fibre, when compared with placebo, binds intestinal lipid forming a complex or gel, which is excreted, thereby resulting in greater faecal fat, and in turn greater weight loss and serum cholesterol lowering.

3.1.2 Aims

The aims of this study in 250 overweight and obese participants, with best practice dietary and PA advice, over 6m are:

**Primary Aim**

- To assess the difference between the chitosan group and the placebo group in weight loss

**Secondary Aim**

- To assess the differences between the chitosan group and the placebo group in other anthropometry: BMI, waist and %Fat, S/DBP, serum lipids: TC, LDL-C, TG, HDL-C and TC/HDL, and FPG

**Other Aims**

- To assess the differences between the chitosan group and the placebo group in dietary energy intake, PA, health-related QoL, SF-36, and EAT-12
- To establish a model of weight loss by employing dietary measures, including the chitosan, in order to investigate the relationship of MetS and Novel CVD Risk and Protective markers with waist change


**Fibre and Faecal Fat Loss Mechanism Substudy**

The aims of this study, where 51 participants provided a 3d faecal sample for TG analysis, are:

**Primary Aim**

- To investigate the difference in faecal fat loss between the chitosan subgroup and the placebo subgroup, thereby testing whether the mechanism of gastrointestinal lipid binding by chitosan and thence driving weight loss is plausible.

**Secondary Aim**

- To investigate the difference between the chitosan subgroup and the placebo subgroup sFSVitamins, which may decrease due to ingested FSVitamins dissolving in higher amounts of faecal fat excreted in the chitosan group.

**Outcomes**

The outcomes for the Diet&Health-Weight Loss trial are differences between the chitosan and placebo groups in:

*Primary Outcome:* Weight; *Secondary Outcomes:* (1) BMI, waist, %Fat (2) S/DBP (3) TC, LDL-C, TG, HDL-C, TC/HDL, and (4) FPG; *Other Outcomes:* Energy intake, PA, SF-36 and EAT-12 questionnaires scores.

The outcomes for the Fibre&Fat Loss substudy are differences between the chitosan and placebo groups in:

*Primary outcome:* Faecal fat loss; *Secondary Outcomes:* sFSVitamins

### 3.2 Methods

**Methods for the Dietary Fibre and Lifestyle for Health - Weight Loss Study**

For the methods of the Diet&Health-Weight Loss trial refer to Section 2.2.1

#### 3.2.1 Method Rationale & Summary

In brief, 250 obese and ambulatory women and men were randomised to a 6m weight loss double blind trial with a marine dietary fibre, chitosan or placebo, standardised advice on lifestyle (a prudent diet, modest physical activity and motivation). Individuals who answered the advertisements for the trial were screened by phone. Possible registrants
were interviewed, and if eligible gave informed written consent, were measured and started the two week run of single blind placebo that required 85% of capsules (4 x 3 times/d) to be consumed by count prior to randomisation. This method of assessing treatment or placebo capsule consumption compliance was observed at the 6 x monthly visits, where weight and waist measurements were performed, and written and verbal information given. Blood was drawn at for all tests at baseline and 6m, and for lipids at 3m. BIA body composition was measured at baseline, 3m and 6m. Questionnaires on lifestyle and eating attitudes were collected from participants at baseline and 6m.

Methods for the Fibre and Faecal Fat Loss Mechanism Substudy

For the methods of the Fibre&Fat Loss substudy refer to Section 2.2.1.1

Method Rational & Summary

At registration for the Diet&Health-Weight Loss trial, all possible participants for the Fibre&Fat Loss sub-study were screened for eligibility and consented to provide a baseline and 6m faecal sample. Fifty one participants started this substudy and brought their 3 day faecal sample for analysis on the day of randomisation, and participants remaining, at the last 6m visit.

3.2.2 Statistical Analyses

Study Power

The study sample size of 250 comprised 125 participants in each of two groups randomised to chitosan or placebo. For comparisons between 3g chitosan daily and placebo, the sample size of 250 participants provided 90% power (at the 5% level of significance) to detect a 2.5kg difference between each of the groups in weight loss from baseline to 6m, assuming a standard deviation (sd) of 6kg.524.

In a meta-analysis of randomised, controlled trials of chitosan, the mean difference in weight loss between the chitosan and placebo groups was 3.3kg.525. The sample size calculations were based on the planned number of follow-up visits (6) and the size of the correlation within the primary endpoint at each visit, and likely drop-out rate of 40%.

Since the trial had substantial statistical power overall, it had adequate power to reliably assess whether effects differ according to baseline characteristics of age, gender or BMI.
Randomisation

Study participants were randomised in a 1:1 ratio to receive chitosan or placebo capsules. Staff at the HNU dispensed the study medication under blinded conditions using a randomisation sequence generated by a computerised random-number generator with mixed block sizes to prevent discovery. There was no stratification by gender or other demographic variables. Treatment assignment codes were not available to the investigators, research staff or data entry staff at any point during the study and were held centrally by the Diet&Health-Weight Loss trial statistician, at the CTRU, University of Auckland.

Statistics

Observed data are untransformed recordings. Three analysis types were conducted: (1) the area under the curve (AUC) summary measure was employed to assess the response profile over time with the LOCF for any missing data, based on the intention to treat (ITT) approach, to ensure participants were analysed in the groups to which they were randomised (2) general linear mixed model, and (3) analysis of covariance (ANCOVA) LOCF was used for data collected at least twice, adjusting for baseline imbalance and for regression to the mean for normally distributed data, with Mann-Whitney tests used for non-normally distributed data.

All randomised participants were analysed as ITT. The group who attended the last visit were completers but may have missed visits (n=164), so data is only presented for the completer group who ‘attended all visits’ (n=146). The group who complied with attending all visits, returned capsule count and achieved a compliance of >85% were the ‘per protocol’ group (n=73). The AUC LOCF ITT was applied to all randomised participants for weight, BMI, waist, %Fat, S/DBP and lipid change. AUC LOCF was applied to the ‘attended all visits’ and ‘per protocol’ groups for weight, and ‘attended all visits’ group for BMI, waist, %Fat, S/DBP and lipid change.

General linear mixed model sensitivity testing was applied to all participants, and ‘attended all visits’ groups for weight, S/DBP and lipid change. ANCOVA was applied to weight, S/DBP and lipids, ‘attended all visits’ groups, as a sensitivity test and other laboratory tests and questionnaire data. All analyses were carried out using SAS v8.0
Chapter 3. The Dietary Fibre and Lifestyle for Health – Weight Loss Study

(SAS Institute Inc, Cary, NC, USA 2001) and p≤0.05 was used to determine statistical significance.

3.3 Results

All results are expressed as means and blood tests are levels or concentrations, unless otherwise notated.

3.3.1 Participant characteristics

Recruitment, retention and completion data for the study are shown Figure 3-1

Figure 3-1. Study Recruitment

Of the 432 individuals who registered to take part in the study, 182 withdrew or were excluded prior to randomisation. Of the 250 individuals randomised, 125 received chitosan and 125 received the placebo. Eighty-six participants dropped out during the
Chapter 3. The Dietary Fibre and Lifestyle for Health – Weight Loss Study

intervention period (42 in the chitosan group, 44 in the placebo group), and 164 (65.6%) completed the entire 6m (Figure 3-1).

Non-randomised individuals were similar to those randomised other than having a lower mean (sd) age 42(11.5)y vs. 48(11.7)y and a higher proportion of current smokers (19% vs. 9%) (p<0.05).

Baseline characteristics appear in Table 3-1, with all being the total group.

3.3.2 Primary Outcome

Body Weight

Observed data and LOCF ITT for weight is shown in Figure 3-2, with the number of participants attending at each monthly visit. In the AUC LOCF ITT analysis for the population, the chitosan group lost a mean (standard error of the mean (sem)) of 0.39(0.21)kg (p=0.03; 0.4%) and the placebo group experienced a net gain of 0.17(0.16)kg (p=1.36; 0.2%) with the difference being 0.56(0.26)kg (p=0.03) the during the 6m intervention (Figure 3-2 & Table 3-2). General linear mixed model sensitivity analyses performed on weight for the ITT group showed slightly greater changes, but at the same level of significance (Table 3-2).

The AUC analyses of weight when restricted to the subset of individuals who attended all study visits (n=146), and those who attended all visits and also maintained an average rate of compliance with treatment or placebo of >85% throughout the trial (n=73, the per protocol analysis participants), indicated that the mean (95% C.I.) difference between groups remained small; 0.9 (0.1, 1.7)kg (p=0.03) and 0.9 (-0.5, 2.2)kg (p=0.20) respectively. The general linear mixed model weight analysis of the completers gave a difference of 1.10 (0.10, 4.0)kg (p=0.02) between the chitosan and placebo (Table 3-2).

3.3.3 Secondary Outcomes

Body Mass Index

Observed data for BMI is shown in Figure 3-3. Using an AUC, LOCF, ITT analysis the difference in mean (sem) BMI between chitosan and placebo groups was 0.21(0.08)kg/m² (p=0.07). A borderline decrease in the chitosan group over the 6m was observed (Figure 3-3).
### Table 3-1. Baseline Characteristics: All, Chitosan vs. Placebo

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean(sd) %</th>
<th>Chitosan Group (n=125)</th>
<th>Placebo Group (n=125)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>47.7(11.7)</td>
<td>47.6(11.9)</td>
<td>48.8(11.5)</td>
</tr>
<tr>
<td>Gender, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-Women</td>
<td>206(82.4)</td>
<td>103(82.4)</td>
<td>103(82.4)</td>
</tr>
<tr>
<td>-Men</td>
<td>44(17.6)</td>
<td>22(17.6)</td>
<td>22(17.6)</td>
</tr>
<tr>
<td>Ethnicity, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-Maori</td>
<td>29(11.6)</td>
<td>14(11.2)</td>
<td>15(12.0)</td>
</tr>
<tr>
<td>-Samoan</td>
<td>5(2.2)</td>
<td>3(2.4)</td>
<td>2(1.6)</td>
</tr>
<tr>
<td>-Rarotongan</td>
<td>1(0.4)</td>
<td>1(0.8)</td>
<td>0</td>
</tr>
<tr>
<td>-Tongan</td>
<td>2(0.8)</td>
<td>0</td>
<td>2(1.6)</td>
</tr>
<tr>
<td>-Maori/Pacific</td>
<td>37(15.0)</td>
<td>18(14.4)</td>
<td>19(15.2)</td>
</tr>
<tr>
<td>-East Asian</td>
<td>1(0.4)</td>
<td>1(0.8)</td>
<td>0</td>
</tr>
<tr>
<td>-Indian</td>
<td>7(2.8)</td>
<td>3(2.4)</td>
<td>4(3.2)</td>
</tr>
<tr>
<td>-African</td>
<td>1(0.4)</td>
<td>1(0.8)</td>
<td>0</td>
</tr>
<tr>
<td>-European</td>
<td>212(84.8)</td>
<td>104(83.2)</td>
<td>108(86.4)</td>
</tr>
<tr>
<td>Reg. Cig. Smoker</td>
<td>20(8)</td>
<td>11(10.4)</td>
<td>9(8.0)</td>
</tr>
<tr>
<td>Ever Smoked</td>
<td>107(42.8)</td>
<td>56(45.0)</td>
<td>51(41.0)</td>
</tr>
<tr>
<td>Reg. Alc. Drinker</td>
<td>119(48.0)</td>
<td>62(50.0)</td>
<td>57(46.0)</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>97.4(16.2)</td>
<td>95.9(15.2)</td>
<td>98.9(17.1)</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>35.4(5.2)</td>
<td>34.8(5.1)</td>
<td>36.0(5.1)</td>
</tr>
<tr>
<td>Waist, cm</td>
<td>100.4(12.9)</td>
<td>99.6(11.9)</td>
<td>101.3(13.7)</td>
</tr>
<tr>
<td>%Fat, %</td>
<td>38.3(6.6)</td>
<td>37.8(6.8)</td>
<td>38.9(6.5)</td>
</tr>
<tr>
<td>SBP, mmHg</td>
<td>123.2(18.3)</td>
<td>122.6(17.7)</td>
<td>123.8(18.9)</td>
</tr>
<tr>
<td>DBP, mmHg</td>
<td>69.7(9.5)</td>
<td>69.4(9.4)</td>
<td>70.1(9.7)</td>
</tr>
<tr>
<td>TC, mmol/l</td>
<td>5.5(0.95)</td>
<td>5.6(1.0)</td>
<td>5.4(0.9)</td>
</tr>
<tr>
<td>LDL-C, mmol/L</td>
<td>3.4(0.81)</td>
<td>3.6(0.8)</td>
<td>3.3(0.8)</td>
</tr>
<tr>
<td>HDL-C, mmol/L</td>
<td>1.3(0.34)</td>
<td>1.3(0.3)</td>
<td>1.4(0.4)</td>
</tr>
<tr>
<td>TC/HDL</td>
<td>4.4(1.20)</td>
<td>4.6(1.2)</td>
<td>4.2(1.2)</td>
</tr>
<tr>
<td>TG, mmol/L</td>
<td>1.6(0.83)</td>
<td>1.6(0.8)</td>
<td>1.5(0.9)</td>
</tr>
<tr>
<td>FPG, mmol/l</td>
<td>5.3(1.38)</td>
<td>5.3(1.3)</td>
<td>5.4(1.4)</td>
</tr>
<tr>
<td>sVitD, nmol/L (n=243)</td>
<td>62.2(22.66)</td>
<td>64.9(25.2)</td>
<td>59.6(19.5)</td>
</tr>
<tr>
<td>sβCaro, μmol/L (n=238)</td>
<td>0.6(0.50)</td>
<td>0.7(0.5)</td>
<td>0.6(0.5)</td>
</tr>
<tr>
<td>sVita, μmol/L (n=239)</td>
<td>2.0(0.47)</td>
<td>2.0(0.5)</td>
<td>1.9(0.5)</td>
</tr>
<tr>
<td>sVitE, μg/L (n=239)</td>
<td>31.4(8.30)</td>
<td>31.7(8.5)</td>
<td>31.2(8.1)</td>
</tr>
<tr>
<td>INR (VitK) (n=232)</td>
<td>1.0(0.18)</td>
<td>1.0(0.2)</td>
<td>1.0(0.1)</td>
</tr>
<tr>
<td>Faecal fat, mmol/d (n=51)</td>
<td>13.4(7.38)</td>
<td>12.6(8.0)</td>
<td>14.3(6.8)</td>
</tr>
<tr>
<td>SF-36 – Phys., subscore</td>
<td>47.0(8.9)</td>
<td>47.9(6.2)</td>
<td>47.6(6.2)</td>
</tr>
<tr>
<td>SF-36 – Ment., subscore</td>
<td>46.2(10.9)</td>
<td>46.7(7.2)</td>
<td>47.7(7.2)</td>
</tr>
</tbody>
</table>

Faecal fat, normal range, 6-20 mmol or 0.5-5g/d (4 mmol=1g lipid)\(^{268,526}\); y year; Reg. regular; cig.cigarette; alc alcohol; BMI body mass index; %Fat body fat percentage; βCaro beta-carotene; S/DBP systolic/diastolic blood pressure; %Fat body fat percentage; Waist waist circumference; HDL-C High density lipoprotein-cholesterol; LDL-C Low density lipoprotein-cholesterol; TC/HDL Total/HDL cholesterol; TC Total cholesterol; TG Triglyceride; FPG Fasting plasma glucose; s seurm; Vitamin A; VitD Vitamin D; VitE Vitamin E; INR international normalised ratio; SF-36–Short Form 36 question Quality of Life ; SF-36-Phys physical component subscale (0 – 100); SF-36 Ment Short Form 36 mental component subscale (0 – 100).
Table 3-2. Change in Weight Sensitivity Analyses: Chitosan vs. Placebo

<table>
<thead>
<tr>
<th>Participants Analysis of Change</th>
<th>All Included</th>
<th>¹Attended All Visits Analysis</th>
<th>²Per Protocol Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean Change(sem)</td>
<td>Treatment Difference</td>
<td>p-value</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>Chitosan (n=125)</td>
<td>Placebo (n=125)</td>
<td>Treatment Difference</td>
</tr>
<tr>
<td>AUC (LOCF)</td>
<td>-0.39(0.21)</td>
<td>0.17(0.16)</td>
<td>0.56(0.26)</td>
</tr>
<tr>
<td>GLmM, C.I.</td>
<td>0.77(0.33)</td>
<td>(0.10, 1.40)</td>
<td>0.02</td>
</tr>
<tr>
<td>ANCOVA (LOCF), C.I.</td>
<td>1.07(0.41)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

¹AUC Area under the curve; ²ITT Intention to Treat Analysis; ³LOCF Last observation carried forward. Missing values are assigned last value carried forward from previous visit; ⁴GLmM general linear mixed model; ⁵95% C.I. confidence interval; ⁶ANCOVA Analyses of Co-variance; ⁷Includes only participants attending all study visits, n=146. ⁸Includes only participants who attended all study visits, maintaining ≥85% compliance throughout as measured by capsule counts, n=73.

Figure 3-2. Weight & Waist over 6m (A) Weight Observed (B) Weight LOCF, ITT & (C) Waist LOCF, ITT: Chitosan vs. Placebo
Waist and Body %Fat

As waist was measured at all visits and %Fat at baseline, 3m and 6m; waist and %Fat results are presented as AUC LOCF ITT analyses. Waist is shown in Figure 3-2.

Figure 3-3. BMI over 6m (A) Observed and (B) LOCF, ITT: Chitosan vs. Placebo

Waist also tended to decrease over the 6m; but the difference between groups was not significant as shown in Figure 3-2.

Systolic and Diastolic Blood Pressure

ITT analysis for S/DBP is depicted in Table 3–3. AUC changes in S/DBP are shown in Figure 3-4, and sensitivity analysis in Table 3-4. There was no significant difference between treatment groups for S/DBP in the AUC LOCF ITT analysis, although there was a borderline difference in the general linear mixed model analysis of SBP in the completers Table 3-4.
### Table 3-3. Change, Baseline to 6m BP & Lipids Sensitivity Analyses: Chitosan vs. Placebo

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean(sem)</th>
<th>95% C.L.</th>
<th>p-value</th>
<th>Mean(sem)</th>
<th>95% C.L.</th>
<th>p-value</th>
<th>Mean(sem)</th>
<th>95% C.L.</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>All Participants</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBP, mm/Hg</td>
<td>1.16(10.6)</td>
<td>-1.49, 3.81</td>
<td>0.39</td>
<td>2.27(1.31)</td>
<td>-0.30, 4.84</td>
<td>0.08</td>
<td>2.36(1.64)</td>
<td>-0.87, 5.59</td>
<td>0.15</td>
</tr>
<tr>
<td>DBP, mm/Hg</td>
<td>0.03(5.86)</td>
<td>-1.43, 1.49</td>
<td>0.97</td>
<td>0.51(0.7)</td>
<td>-0.87, 1.89</td>
<td>0.47</td>
<td>0.33(0.90)</td>
<td>-1.44, 2.10</td>
<td>0.72</td>
</tr>
<tr>
<td>TC, mmol/L</td>
<td>0.14(0.32)</td>
<td>0.06, 0.22</td>
<td>&lt;0.01</td>
<td>0.20(0.07)</td>
<td>0.06, 0.34</td>
<td>&lt;0.01</td>
<td>0.15(0.06)</td>
<td>0.019, 0.27</td>
<td>0.02</td>
</tr>
<tr>
<td>LDL-C, mmol/L</td>
<td>0.12(0.36)</td>
<td>0.05, 0.20</td>
<td>0.01</td>
<td>0.19(0.06)</td>
<td>0.06, 0.31</td>
<td>&lt;0.01</td>
<td>0.13(0.06)</td>
<td>0.01, 0.24</td>
<td>0.03</td>
</tr>
<tr>
<td>HDL-C, mmol/L</td>
<td>0.01(0.1)</td>
<td>-0.03, 0.02</td>
<td>0.60</td>
<td>-0.01(0.02)</td>
<td>-0.05, 0.04</td>
<td>0.69</td>
<td>-0.02(0.02)</td>
<td>-0.056, 0.02</td>
<td>0.37</td>
</tr>
<tr>
<td>TC/HDL</td>
<td>0.15(0.36)</td>
<td>0.06, 0.24</td>
<td>&lt;0.01</td>
<td>0.18(0.08)</td>
<td>0.03, 0.33</td>
<td>0.02</td>
<td>0.15(0.07)</td>
<td>0.021, 0.29</td>
<td>0.02</td>
</tr>
<tr>
<td>TG, mmol/L</td>
<td>0.06(0.37)</td>
<td>-0.03, 0.15</td>
<td>0.21</td>
<td>0.09(0.08)</td>
<td>-0.06, 0.24</td>
<td>0.24</td>
<td>0.09(0.07)</td>
<td>-0.047, 0.22</td>
<td>0.21</td>
</tr>
</tbody>
</table>

| SBP, mm/Hg     | 2.58(1.85) | -1.07, 6.237 | 0.16    | 3.26(1.63) | -0.46, 6.49 | 0.05   |
| DBP, mm/Hg     | 2.08(0.98) | 0.139, 4.01 | 0.04    | 1.70(0.84) | 0.04, 3.36 | 0.05   |
| TC, mmol/L     | 0.18(0.06) | 0.059, 0.03 | <0.01  | 0.21(0.07) | 0.06, 0.36 | 0.01   |
| LDL-C, mmol/L  | 0.20(0.05) | 0.089, 0.03 | <0.01  | 0.22(0.07) | 0.08, 0.35 | <0.01  |
| HDL-C, mmol/L  | -0.01(0.02) | -0.05, 0.03 | 0.57 | -0.01(0.02) | -0.06, 0.04 | 0.69    |
| TC/HDL         | 0.13(0.06) | 0.014, 0.252 | 0.03  | 0.17(0.08) | 0.01, 0.32 | 0.04   |
| TG, mmol/L     | 0.07(0.06) | -0.046, 5.0 | 0.22    | 0.07(0.07) | -0.04, 0.25 | 0.17   |

1AUC Area under the curve; 2LOCF Last observation carried forward; 3ITT Intention to Treat Analysis; 4GLmM general linear mixed model; 5ANCOVA Analyses of co-variance; S/DBP systolic/diastolic blood pressure; TC total cholesterol; LDL-C low density lipoprotein cholesterol; HDL-C high density lipoprotein cholesterol; TG triglyceride; 6Includes only participants who attended all study visits. Missing values are assigned last value carried forward from previous visit.
Table 3-4. Secondary & other Outcomes - Anthropometry, BP & Laboratory Analytes over 6m: Chitosan vs. Placebo

<table>
<thead>
<tr>
<th></th>
<th>Chitosan</th>
<th>Placebo</th>
<th>Treatment Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Mean change (sem)</td>
<td>N</td>
</tr>
<tr>
<td><strong>AUC (LOCF) Anthropometry</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>125</td>
<td>-0.17(0.09)</td>
<td>125</td>
</tr>
<tr>
<td>Waist, cm</td>
<td>125</td>
<td>-0.57(0.30)</td>
<td>125</td>
</tr>
<tr>
<td>Fat %</td>
<td>121</td>
<td>-0.85(0.27)</td>
<td>118</td>
</tr>
<tr>
<td>SBP, mmHg</td>
<td>125</td>
<td>-2.71(0.92)</td>
<td>125</td>
</tr>
<tr>
<td>DBP, mmHg</td>
<td>125</td>
<td>-2.70(0.55)</td>
<td>125</td>
</tr>
<tr>
<td><strong>ANCOVA (LOCF) Laboratory Tests</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FPG, mmol/L</td>
<td>118</td>
<td>-0.14(0.05)</td>
<td>116</td>
</tr>
<tr>
<td>sVitD, µmol/L</td>
<td>122</td>
<td>-9.00(1.81)</td>
<td>121</td>
</tr>
<tr>
<td>sβCaro, µmol/L</td>
<td>120</td>
<td>-0.02(0.03)</td>
<td>118</td>
</tr>
<tr>
<td>sVitA, µmol/L</td>
<td>120</td>
<td>-0.04(0.02)</td>
<td>119</td>
</tr>
<tr>
<td>sVitE, µmol/L</td>
<td>120</td>
<td>-1.15(0.50)</td>
<td>119</td>
</tr>
<tr>
<td>INR(VitK), s</td>
<td>117</td>
<td>0.01(0.01)</td>
<td>115</td>
</tr>
<tr>
<td>Faecal fat, mmol/d</td>
<td>25</td>
<td>-0.08(1.53)</td>
<td>26</td>
</tr>
</tbody>
</table>

1 AUC Area under the curve; 2 LOCF Last observation carried forwards AUC summary measure was used to assess change for all endpoints measured at more than 2 time points; BMI body mass index; Waist waist circumference; %Fat body fat %; SBP systolic/diastolic blood pressure; 3 ANCOVA Analysis of Co-variance; FPG fasting plasma glucose; VitE vitamin E; VitA vitamin A; βCaro beta-carotene; VitD vitamin D; INR international normalised ratio. The anthropometry was analysed using AUC LOCF. Change in endpoints that were only measured at baseline and 6m (glucose, fat-soluble vitamins, and faecal fat) was assessed using ANCOVA. ANCOVA was used for laboratory tests.

Serum Lipids

In the AUC LOCF ITT analysis for TC, levels decreased by a mean(sem) of 0.13(0.03) mmol/L (2.3%) in the chitosan group during the 6m period versus a net gain of 0.01(0.03) mmol/l (0.2%) for the placebo group (Figure 3-5) and (Table 3-4), over the 6m intervention.

Figure 3-5. Serum Lipids over 6m (A) TC (B) LDL-C: Chitosan vs. Placebo (LOCF ITT)
The mean(sem) (95% C.I.) difference between treatment groups was therefore -0.14(0.32) (0.05, 0.22) mmol/L (p<0.01). Using an AUC LOCF ITT analysis for LDL-C, levels decreased with a mean(sem) (95% C.I.) difference between groups of 0.12(0.36) (0.05, 0.20) mmol/L (p=0.01), and TC/HDL decreased with mean(sem) (95% C.I.) of 0.15(0.36) (0.06, 0.24) mmol/L (p<0.01), but there were no significant differences between groups in HDL-C (p=0.5) or TG (p=0.2) (Table 3-3).

**Fasting Plasma Glucose**

FPG was measured at baseline and 6m. ANCOVA showed that the chitosan group had a mean FPG -0.21 (0.07, 0.34) mmol/L lower than placebo (p<0.01) (Table 3-4).

**Fat Soluble Vitamins**

There were no significant changes between treatment and placebo groups for the sFSVitamins over 6m (Figure 3-8).

### 3.3.4 Other measures

**Energy intake**

There were no differences between the groups in energy intake (p=0.79) over 6m.

**Physical Activity**

There were no differences between the groups in PA (p=0.60) over 6m

**Short Form-36 Question Quality of Life**

There were no differences between groups in the physical and mental component subscales of the SF-36 throughout the period of the trial, mean(sem) difference of 1.3(1.7) (p=0.5) and 1.3(1.4) (p=0.4) respectively, over 6m.

**Eating Attitudes Test-12 Question**

There were no differences in the EAT-12 in the dieting subscore, mean(sem) difference of 0.1(0.24) (p=0.7), bulimia subscore, -0.37(0.21) (p=0.1) and oral control subscore, 0.01(0.06) (p=0.9) over 6m
Compliance

Self-reported adherence as measured by capsule counts decreased only slightly over the 6m study period -4.5 (0.9)% in the chitosan group and -4.0 (0.8)% in the placebo group (p=0.6).

Adverse Events

There were a total of 10 SAE recorded over the study period: 6 in the placebo group and 4 in the chitosan group (p=0.53) (Table 3-5).

Table 3-5. Adverse Events by Treatment Group

<table>
<thead>
<tr>
<th>Adverse Event</th>
<th>Chitosan, n</th>
<th>Placebo, n</th>
<th>Relative Rate</th>
<th>95% C.I.</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serious events</td>
<td>4</td>
<td>6</td>
<td>0.67</td>
<td>(0.19, 2.36)</td>
<td>0.53</td>
</tr>
<tr>
<td>Non-serious events</td>
<td>109</td>
<td>108</td>
<td>1.01</td>
<td>(0.77, 1.32)</td>
<td>0.95</td>
</tr>
<tr>
<td>Gastrointestinal events – non-infectious</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>6</td>
<td>4</td>
<td>1.50</td>
<td>(0.42, 5.32)</td>
<td>0.53</td>
</tr>
<tr>
<td>Bloating</td>
<td>10</td>
<td>3</td>
<td>3.33</td>
<td>(0.92, 12.11)</td>
<td>0.07</td>
</tr>
<tr>
<td>Constipation</td>
<td>17</td>
<td>8</td>
<td>2.13</td>
<td>(0.92, 4.92)</td>
<td>0.08</td>
</tr>
<tr>
<td>Indigestion</td>
<td>3</td>
<td>2</td>
<td>1.50</td>
<td>(0.25, 8.98)</td>
<td>0.66</td>
</tr>
<tr>
<td>Diarrhoea (presumed non-infectious)</td>
<td>3</td>
<td>3</td>
<td>1.00</td>
<td>(0.20, 4.95)</td>
<td>1.00</td>
</tr>
<tr>
<td>Other digestive disorder</td>
<td>7</td>
<td>2</td>
<td>3.50</td>
<td>(0.73, 16.85)</td>
<td>0.12</td>
</tr>
<tr>
<td>Total</td>
<td>36</td>
<td>19</td>
<td>1.89</td>
<td>(1.09, 3.30)</td>
<td>0.02</td>
</tr>
<tr>
<td>Gastrointestinal – infectious</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diarrhoea (presumed infectious)</td>
<td>9</td>
<td>9</td>
<td>1.00</td>
<td>(0.40, 2.52)</td>
<td>1.00</td>
</tr>
<tr>
<td>Nausea/vomiting</td>
<td>12</td>
<td>10</td>
<td>1.20</td>
<td>(0.52, 2.78)</td>
<td>0.67</td>
</tr>
<tr>
<td>Total</td>
<td>17</td>
<td>18</td>
<td>0.94</td>
<td>(0.49, 1.83)</td>
<td>0.87</td>
</tr>
<tr>
<td>Other non-serious events</td>
<td>105</td>
<td>100</td>
<td>1.05</td>
<td>(0.80, 1.38)</td>
<td>0.73</td>
</tr>
</tbody>
</table>

1Denotes number of people who experienced one or more adverse event. 2Analyses compared number of people who experienced one or more adverse events. Participants may have reported more than one event and a total of 420 non-serious adverse events were reported in the chitosan group and 309 in the placebo group (p<0.01).

The SAE were defined as hospitalisations, with 3 in the chitosan group and 4 in the placebo group (p=0.71). Cancer incidence accounted for 1 event in the chitosan group and 3 events in the placebo group (p=0.34). There was 1 death in the placebo group. Of the non-SAE, 36 participants in the chitosan group and 19 in the placebo group reported non-infectious gastrointestinal side effects, defined as abdominal pain, bloating, constipation, indigestion, or non-infectious diarrhoea (p=0.02) (Table 3-5).

There was a significantly greater event number in the chitosan group. There were no significant differences between intervention groups in any other category of non-SAE.
3.4 Fibre and Faecal Fat Loss Mechanism Substudy

The Fibre&Fat Loss substudy was the part of the Diet&Health-Weight Loss trial designed to test the hypothesis that chitosan adsorbs TG, and the excretion or loss of both fibre and fat together, and prevention of cholesterol enterohepatic recycling, is the mechanism of action.

3.4.1 Method

Refer to Section 2.2

3.4.2 Laboratory Work

Refer to Section 2.2

3.4.2.1 Results

Twenty-nine participants completed both baseline and 6m follow up faecal collections. There were no significant differences in mean faecal fat excretion between the chitosan group and the placebo group at baseline. On analyses of faecal fat excretion involving only the 29 participants who provided both baseline and 6m samples, mean (C.I.) difference between the chitosan and placebo groups was 0.3 (-7.5, 8.2) mmol/d (p=0.9) (Figure 3-6).

There were no significant differences in sFSVitamin levels between the chitosan and placebo subgroups in the Fibre&Fat Loss substudy participants (Figure 3-7).

There was no relationship between change in faecal fat and change in weight or waist and each of the sFSVitamin changes in the 29 participants who provided samples at baseline and 6m (Figure 3-8).
### Table 3-6. Baseline Characteristics of the Fibre&Fat Loss Substudy Completers: n=29

<table>
<thead>
<tr>
<th></th>
<th>Chitosan, n=14</th>
<th>Placebo, n=15</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender, women:men</td>
<td>13:1</td>
<td>12:3</td>
</tr>
<tr>
<td>Age, y</td>
<td>48(10)</td>
<td>53(7)</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>98(14)</td>
<td>100(20)</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>36(6)</td>
<td>37(6)</td>
</tr>
<tr>
<td>Waist, cm</td>
<td>102(16)</td>
<td>103(12)</td>
</tr>
<tr>
<td>Faecal fat, mmol/d</td>
<td>12.6(6.3)</td>
<td>13.3(6.4)</td>
</tr>
</tbody>
</table>

1Approximately 1 g lipid = 4 mmol. sd standard deviation; y year; BMI body mass index

### Figure 3-6. Baseline, 6m & Change in Faecal Fat Excretion: Chitosan & Placebo, n=29

### Figure 3-7. Change in sFSVitamins: Fibre&Fat Loss Substudy, n=29
Figure 3-8. Change in Serum Fat Soluble Vitamins vs. Faecal Fat: Fibre&Fat Loss Substudy, n=29

s serum VitD vitamin D; βCaro beta-carotene; VitA vitamin A; VitE vitamin E;

3.5 Discussion

3.5.1 General Comments

This randomised placebo controlled trial showed that chitosan supplementation combined with lifestyle and dietary advice produced marginal but significantly greater weight loss than lifestyle and dietary advice alone, in overweight and obese individuals. In addition to the main analyses, a number of sensitivity analyses were performed all of which gave similar results.

The mean difference between groups in weight loss of just over half a kilogram achieved over the 6m study period was also accompanied by a significant improvement in risk factors associated with obesity, including fasting TC, LDL-C, and FPG in the chitosan group compared to the placebo group. This may indicate that the effects of even very modest negative energy balance may be under-appreciated and that weight increase prevention is important. The secular trend is for weight gain in many populations, and the overweight and obese tend to keep gaining weight or fat, until they are elderly and become sarcopenic. Although a minor weight loss overall, the fibre nature of chitosan may add to other studies where fibre generally confers health benefits, although studies are not all in agreement.
Excluded and Non Randomised Participants

The main reasons for the registrants, already a motivated group, failing to be randomised or dropping out of the study included the selection criteria of taking >85% of capsules daily, finding it difficult to get to appointments and disillusionment with the effort needed for so little weight loss. Those to whom this applied were significantly more likely to be younger and smokers, as shown in the results. A meta-interpretation of this finding was that Maori smoke more, as we found in this study, and die younger. A propos of this finding is that an even greater issue is that only two Maori men were even randomised, and neither finished, so in fact Maori men were the least represented as coming forward and this group has the greatest CVD risk and mortality. They were effectively unstudied. The perennial problem with lifestyle problems is the most in need are the least likely to have the education, motivation and physical resources to attend trials or even free health care. For these reasons a wider community responsibility for obesity related MetS may have to be taken, particularly with overall, long term food safety and security – which involves healthy food access, being made an easier choice. Government regulation of food processors and marketers may become important.

Comment on Results in View of the Previous Trial Results

A number of previous trials investigating the effect of chitosan on body weight and lipids have been published but results are conflicting. A meta-analysis of 5 Italian trials involving a total of 386 participants indicated a mean difference of 3.3kg weight loss between intervention and placebo groups. However, the trials included in the meta-analysis were not retrieved by searching electronic databases, but were obtained from a single manufacturer and published in a single journal over a 2y period. They used a similar study design of 28d trial duration, and an energy restricted diet. It is unclear if ITT analyses were employed, and no description is given to the composition, or dose of chitosan used. It is unclear if the individual patient populations may have overlapped. As sustained fat loss is thought to be the main aim of weight loss studies, short studies of 1m duration are not likely to be clinically important.

Trials examining the effect of chitosan have produced more variable results (Table 3-7). Some trials have reported a positive effect on body weight, or lipid levels, while others have reported no effect on either outcome. A Cochrane systematic review of research investigating the effect of chitosan on overweight and obesity conducted, was performed by Jull et al. in 2005 and included co-
authors of the current study\textsuperscript{530}. One other RCT report has appeared after the paper\textsuperscript{531} on the current study and this showed chitosan was associated with significantly greater weight loss than placebo and ‘self help’ methods, as assessed by weight and a DEXA index. This was only a 60d study of 150 participants (134 completers) in a treatment, placebo or ‘no treatment arm’ study design.

Table 3-7. Clinical Trials of Chitosan for Weight Loss or Cholesterol Lowering

<table>
<thead>
<tr>
<th>Positive studies: RCT 14 (11 positive, 4 negative)</th>
<th>Either weight and/or cholesterol reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Abelin 1999\textsuperscript{491} (2-12 wk, not peer reviewed)</td>
<td></td>
</tr>
<tr>
<td>2 Ventura 1996\textsuperscript{490}</td>
<td></td>
</tr>
<tr>
<td>3 Veneroni 1996\textsuperscript{497} (\textit{?} Related to Ventura 1996, not peer reviewed)</td>
<td></td>
</tr>
<tr>
<td>4 Girola 1996\textsuperscript{561} (low dose chitosan, short period)</td>
<td></td>
</tr>
<tr>
<td>5 Wuolijoki 1999\textsuperscript{563}</td>
<td></td>
</tr>
<tr>
<td>6 Zahorska-Markiewicz 2001\textsuperscript{262} (plus a very low energy diet (VLED))</td>
<td></td>
</tr>
<tr>
<td>7 Schiller 2001\textsuperscript{257} (plus VLED)</td>
<td></td>
</tr>
<tr>
<td>8 Gallaher 2002\textsuperscript{533} (low dose, mixture of chitosan + other ingredients)</td>
<td></td>
</tr>
<tr>
<td>9 Kaats 2006\textsuperscript{534} (mixture of chitosan + small amount other ingredients)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Cholesterol reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Maezaki 1993\textsuperscript{488} (2 wks)</td>
</tr>
<tr>
<td>2 Tai 2000\textsuperscript{256}</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Negative Studies: 4 (Incl. Diet&amp;Health-Weight Loss)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Ho 2001\textsuperscript{260}</td>
</tr>
<tr>
<td>2 Pittler 1999\textsuperscript{265} (low chitosan dose)</td>
</tr>
<tr>
<td>3 Gades &amp; Stern 2003\textsuperscript{267} (clinically insignificant)</td>
</tr>
</tbody>
</table>

An ANCOVA, but not LOCF ITT, analysis was performed. The ratio of completers was 40:33 in the treatment: placebo groups, the self help group was mentioned but methods and results not reported, and small amounts of other natural weight loss agents were present in the preparation\textsuperscript{534}. One possible explanation for the variability in results obtained in these trials of chitosan is that different types and compositions of chitosan were used in the various studies. The composition of chitosan used has not been well described in many of the trials but in the current study a $\beta$-chitosan derived from NZ squid pens was used, which was 75.5% deacetylated. Few trials of dietary supplements and natural remedies are carried out according to pharmaceutical industry standards. One report on a small trial did admit that the sponsors found that there was only 42\% not 71\% of chitosan in the capsules\textsuperscript{265}. However, the current trial was conducted according to international Good Clinical Research Practice guidelines and was the largest trial of chitosan to date with the largest number of follow-up visits and the most outcome measures. Importantly, ITT analyses
and several sensitivity analyses were used thus limiting the bias that inevitably results from restricting analysis to data from ‘completers’ only.

**Fibre and Faecal Fat Loss Mechanism Substudy**

The minor effect of chitosan on clinical outcomes in the current trial is supported by the results of the Fibre&Fat Loss substudy, which demonstrated that chitosan had no detectable effect on faecal fat excretion. Other trials that have examined the effect of chitosan on faecal fat excretion have also failed to find a significant effect of chitosan on faecal fat excretion\(^{268}\), although in one study of men there was an increased loss\(^{267}\). It is possible and plausible that chitosan might bind bile acids, as reported in animal studies\(^{267}\), thus explaining some effect on serum lipid levels. Faecal cholesterol and bile acids have not been measured in clinical trials. At the dose in the current study it seems unlikely that chitosan could have a large effect on weight loss. There was no effect on the sFSVitamin levels, and none would be expected if negligible fat was excreted.

**Comparison with Other Weight loss and Cholesterol Lowering Agents**

Reviews and meta-analyses show minimal efficacy of most OTC weight loss agents\(^{535}\) except ephedrine, now banned in most countries\(^{523}\). Medical or medication therapy of obesity rarely results in on going large weight losses in communities, although some individuals may lose significant amounts. The only registered long term weight loss medication on the market in westernised countries is the non systemic lipase inhibitor, orlistat. Compared to placebo, in a meta-analysis, orlistat reduced mean (95% C.I) weight by 2.9 (2.5, 3.2)kg\(^{536}\), but in the present study, in chitosan mean weight loss was by only 0.56 (0.04, 1.08)kg.

The most efficient agents for reducing cholesterol are the medical therapies. The 3-hydroxy-3-methyl-glutaryl-coA reductase (HMG coA reductase) inhibitors or statins are widely used, and exert most of their considerable cholesterol lowering effects on LDL-C, and lesser effects on TC (Table 3-8). Ezetimibe is specific cholesterol absorption inhibitor. Niacin and fibrates (with a peroxisome proliferator-activated receptor gamma (PPAR-\(\gamma\)) effect) are more useful for increasing HDL-C\(^{537}\). However, they all have significant AE. Recently statins have been implicated in having an inappropriate treatment target (LDL-C), increasing risk of TIIDM, and may not be effective for primary prevention of CVD\(^{531,538-540}\).
Table 3-8. Summary of % Weight Loss and Cholesterol Lowering Trials

| Weight Loss | Current Study Chitosan: loss of ~ 0.9% body weight compared with gain of 0.2% on placebo
|            | Orlistat: loss of ~ 9% body weight compared with 6% on placebo
|            | Sibutramine: loss of ~ 5-8% body weight compared with 1-4% on placebo (now deregistered)

| Cholesterol Reduction (mainly via LDL-C) | Current Study Chitosan: 2.5% reduction in total cholesterol (current study)
|                                        | Sterols/stanols: 10-20% reduction
|                                        | Soluble fibre: 10 – 17% reduction
|                                        | Bile acid sequestrants: 15-17% reduction
|                                        | Statins: 23+% reduction

Non digested or non absorbed dietary plant fibre in food is GRAS and healthy. There are claims that fibre is efficacious for weight gain prevention, weight loss and cholesterol normalising. Soluble fibre, β-glucans, found in oats and barley and active at 3g/d have been allowed by the Food and Drug Administration (FDA) as a cholesterol lowering agent. However, the evidence is derived mainly from observational study data, and the clinical interventions, especially where studies used enriched β-glucans, do not clearly show efficacy. Soluble fibre including pectin, guar gum and psyllium, are reported to have satiating properties in complementary and alternative medicine (CAM) studies. High dose fibre psyllium fibre, together with fruit and vegetable fibre may reduce weight and MetS.

Some non-absorbed bile acid sequestrants or ‘resins’ may have a positive charge and are thought to bind to negatively charged intestinal lipids and cholesterol, thus preventing absorption. The bile acid sequestrants, as exemplified by cholestyramine and cholestipol, exchange anions, such as chloride ions, for bile acids. By doing so, they bind bile acids and sequester them from enterohepatic circulation. They are an important, non systemic medical therapy for cholesterol lowering. The mechanisms of fibre and bile acid sequestrants could possibly apply to chitosan but the current trial could not test for this mechanism.

Toxicity: Chitosan and Other Weight Loss Agents

Chitosan is widely researched and utilized in both industrial and biomedical areas. In the thousands of articles on chitosan use in the MEDLINE database of medical literature (8114 in July 2011), chitosan has been GRAS. Chitosan complexes with carbon nanotubes, hydrogels or film coatings are used for medication delivery to the intestine.
modifying haemostasis\textsuperscript{555}, and many other uses. One very common area of research and application of chitosan complexes is to enhance entry of substances through the intestinal tight junctions, whereby polycations effect a reversible or irreversible loosening of mucosal tight junctions\textsuperscript{556-559}. As chitosan has been found to be a polycation that can lead to irreversible opening of the intestinal cell tight junctions, toxic effects could result, and some researchers have questioned the safety of long term uses of chitosan for weight loss and cholesterol reduction\textsuperscript{560-562}.

Obesity is often still seen as a failure to exert restraint in eating or increase PA\textsuperscript{563,564}. Treatment of obesity has been viewed as unnecessary for health, and optional, by some in the health profession, and therefore any risks with pharmacotherapy are viewed as posing more of a threat than the condition. However, obese individuals are often very distressed, will resort to risky practices and in fact the centrally obese do have health risks, as discussed in Chapter 1. Humans have complex energy balance and neurophysiology (addiction and satiety issues) in the current ‘obesogenic’ environment\textsuperscript{536}.

A brief review of toxicity in other weight loss medications is in order. Many older weight loss medications were seen to be prescribed by fringe health practitioners, were poorly regulated, often had serious metabolic and psycho-active adverse effects and were finally banned\textsuperscript{565}. There have been many weight loss studies on CAM and herbal medications and supplements for weight loss. CAM in the USA do not have to prove efficacy or safety, and as such are often marketed as natural, and GRAS\textsuperscript{566}. However, in certain circumstances it is clear that CAM are not safe at high doses\textsuperscript{567} including the well-studied ephedra\textsuperscript{568-570}.

Preparations may have accidental or purposeful toxic plant, microbe, biochemical, heavy metal or orthodox medication contamination\textsuperscript{567,571,572}. In contrast, more modern orthodox weight loss drugs were expected to have very few, and no serious, side effects, and reduce CVD risk. Even well trialled orthodox ‘designer’ medications such as dexfenfluramine in 1998, rimonabant in 2008, and sibutramine in 2010, all marketed for years, have been removed from medication schedules after post-marketing surveillance confirmed AE\textsuperscript{565,573-575}.

In spite of many studies showing marginal efficacy for weight loss and cholesterol reduction, and the above mentioned probable, but poorly demonstrated toxicity, chitosan is widely marketed and sold in weight loss preparations.
Lastly, the Diet&Health-Weight Loss trial appears to be a realistic model with which to test MetS in the community. In particular, the reality is that some individuals gain weight and some lose, as occurred in this study, and this is expected to change MetS and MetS markers.

3.6 Conclusion

In conclusion, this trial demonstrates that when chitosan was studied under rigorous conditions and with thorough analysis it only had a minimal clinical effect on weight loss or other measured outcomes in overweight and obese men and women taking a dose of 3g/d. No increase in faecal fat excretion was observed to support the putative mechanism of action of chitosan and treatment is associated with some minor gastrointestinal side effects. There may be intestinal toxicity with chitosan, so further work on this aspect, and on bile acid secretion as a mechanism should be investigated. If low toxicity agents improve weight loss at all, and particularly if other risk factors, including dyslipidaemia, are reduced, cheap minimally-modified agents could be considered as part of a weight loss strategy. In the interim, it seems appropriate to focus public attention on proven effective means of weight loss, and improved health such as improved nutrition, with large amounts of dietary fruit, vegetable and invertebrate fibre, including chitin and chitosan, and increased PA.
Chapter 4. Novel Cardiovascular Disease Risk Markers in the Metabolic Syndrome

4.1 Introduction

MetS has been shown in meta-analyses and in population studies to significantly increase the likelihood of CVD events in most ethnicities where it has been diagnosed\(^9,94\). The definition and development of NCEP-derived MetS was addressed throughout Chapter 1. Briefly, in the early 2000’s the simple NCEP\(^6\) MetS was welcomed by clinicians as being more useable than previous definitions. However, over the last 5-7y the more central obesity-oriented, and TIIDM and CVD risk preventative the definition became, the more some westernised clinicians doubted the concept.

The IDF MetS includes increased numbers of overweight individuals from (1) many different ethnicities, and (2) younger obese individuals with less immediate and more reversible CVD risk, and not yet in need of cardiometabolic medication. The IDF MetS, or the JIS MetS\(^5\), where any three of the markers outside the cut-points can be used, has waist cut-points experimentally shown to be appropriate to many ethnicities. The IDF MetS has become an important tool used by epidemiologists and researchers interested in the world-wide rise of TIIDM and CVD. Much, but not all, of the non-European world has embraced the IDF MetS\(^{132,133,146,576-581}\), especially in countries undergoing a rapid ‘nutrition transition’\(^{582}\). The IDF MetS populations were not meant to be the same as those defined by NCEP/ADA, and a limited overlap should probably have been expected\(^{583,136,137,584}\).

Recently the WHO issued an advisory against the use of MetS and its concept at all\(^133\). These authors’ critiques included that lowered waist cut points were seen as including too many individuals who did not need treatment whilst MetS does not appear to perform better than the sum of its parts in older individuals who have established atherosclerosis or have already experienced a CVD event\(^{585,586}\). The definitions have been widely compared\(^{583,136,137,584}\). In addition, these authors contend that MetS simplifies and dichotomises risk markers that comprise MetS, and that there is too much CVD risk
marker variation. In defence of MetS, TIIDM and CVD are also beset by these latter problems, and both conditions are just later stages of MetS\textsuperscript{133}.

One way of reducing the dichotomisation of MetS or no MetS, is to form a MetS index, a summary measure or count of the MetS markers; MetS marker count. Each MetS marker, if outside the cut-point, qualifies so an individual can have a count of 0-5 MetS markers. As with BMI, the various levels indicate categories of risk, and new studies, or reviewing previous studies, with this in mind may be useful. The most important reason for using the IDF MetS is as a starting point, or screen, that can classify CVD risk early, before end organ disease has occurred. Diagnosis of MetS in young obese and non European groups, who are at risk but are relatively unstudied, may be important. Thus, it is clear that no definitions of MetS predict CVD perfectly, which might be expected in a definition of a syndrome.

Sophisticated experimentation and modelling has shown that most historic ‘CVD risk factors’, including those used in MetS, are only markers\textsuperscript{587}. They do not appear to be causes of the profound metabolic dysfunction of the many cross-linked biochemical and nutrient systems\textsuperscript{588} seen in TIIDM, CVD and cancer. The basic causes are possibly becoming clearer. The WHO authors\textsuperscript{133} state that MetS should be neither used, nor even be further modified, and propose that new markers, such as those listed in the notes to the IDF MetS definition\textsuperscript{4}, be studied or used. Other markers derived from biological pathways that are likely to be associated with root causes of degenerative disease should be developed for use. However, none has been established as causative, and none is available for clinical use at the present time. Thus, until the real causative factors are found, it seems appropriate to use a modified IDF or JIS MetS as a screen, and other extant or new tests which are related to profound disturbances in MetS.

What is known is that in the obesity-related MetS many biochemical and metabolic processes are abnormal. As already indicated, there are classical CVD risk markers which focus on circulating energy molecules, lipids/lipoproteins and plasma glucose. Wherever there are energy nutrients in relative excess, especially in metabolically compromised organisms, oxidative stress is likely. The glycation protein HbA\textsubscript{1c}, a plasma glucose indicator and urate are established oxidant markers dyslipidaemia and endothelial stress. Furthermore, there is overwhelming data now that the obesity-related MetS is associated with metaflammation\textsuperscript{325}, a concept introduced in Chapter 1.
Chapter 4. Novel Cardiovascular Disease Risk Markers in the Metabolic Syndrome

The liver, the major general and energy metabolic organ involved in metaflammation, releases enzymes and acute phase biomarkers into the circulation. Important markers are thus the liver function tests, liver proteins, CPR and ferritin. Inflammatory white cells, leukocytes, employ oxidative processes\(^{589}\). The ESR is a combination measure of inflammatory plasma protein effects on the erythrocyte. Oxidative stress and metaflammation are frequently linked and co-induce each other\(^{36}\), although inflammation may not always occur in oxidative stress\(^{587}\). In MetS there are likely to be many associated oxidative and inflammatory markers, although most investigations only employ experimental markers.

A battery of laboratory CRSHAem&Biochem, as noted above, are often performed on patients for metabolic disease screening, such as for diabetes and dyslipidaemia. Screening and/or tracking infection, tumour growth and nutritional screening, such as for anaemia and vitamin insufficiency, are further examples often also included in the same group of tests. Often MetS patients will have had CRSHAem&Biochem testing, and results reported in their files, but the results are not reviewed together with patients’ MetS status. As MetS is so widespread, it is important that CRSHAem&Biochem are carefully investigated, with a focus on refining estimates of metabolic decompensation and hence improving CVD risk prediction in those with MetS.

4.1.1 Hypothesis

It was hypothesised that a battery of CRSHAem&Biochem, sensitive to inflammatory and oxidant conditions, would provide strong markers of CVD risk, both individually and as a group, and be related to a MetS index, MetS marker count. Furthermore, some of these markers would be expected to worsen and/or improve in parallel with changes in MetS. Thus CRSHAem&Biochem can be employed together with, and additional to, MetS, and hence better predict CVD and track change in risk of CVD.

4.1.2 Aims

The aims of the Diet&Health-Novel CVD Risk Marker study in 250 overweight and obese women and men enrolled in a 6m weight loss study are:
Chapter 4. Novel Cardiovascular Disease Risk Markers in the Metabolic Syndrome

**Primary Aims**

- To investigate the strength of cross sectional relationships between CRSHaem&Biochem and MetS markers, and MetS marker count, in order to determine the most useful markers to use either individually or together, to enhance MetS and hence CVD prediction.
- To investigate the strength of change between baseline and 6m in CRSHaem&Biochem and MetS markers, and MetS marker count, in order to determine those most useful markers used either individually or together, to enhance tracking change in MetS marker count and hence CVD prediction.

**Secondary Aims**

- To explore the variability of weight, BMI, waist and %Fat over the 6m intervention and compare changes between 0-3m and 3-6m periods of the study.
- To compare the measures of body fat estimates – weight, BMI, waist and %Fat.
- To assess the utility of the various definitions of MetS.
- To assess the demographic, health status, lifestyle, and QoL measures.

**4.2 Methods**

The methods for this Chapter are contained in the Diet&Health study. Refer to Section 2.2.2.

**4.2.1 Method Rationale & Summary**

In brief, in the current study of 250 obese and overweight women and men who attended a the Diet&Health-Novel CVD Risk markers and Diet&Health-Weight loss trial over 6m, had their anthropometry weight, height, waist, BP and %Fat collected, with weight, waist and BP collected monthly and %Fat at baseline, 3m and 6m. Anthropometry variability from the two 3m periods could be compared.

The laboratory tests collected at baseline and 6m were used to provide the HDL, TG and FPG, and together with waist and BP, which comprise the MetS and MetS marker count.
The utility of 3 NCEP related MetS definitions were assessed by cross-sectional and longitudinal change comparisons in women and men. In addition, the MetS marker count was the main outcome, in this study.

For this Diet&Health-Novel CVD Risk Markers study it was important to collect demographic and medical history questionnaire details, especially CVD and risk. Data from AE and medication report forms on change in medical status (illness and accident) and treatments indicative of a metaflammatory condition were used for analyses.

The CRSHAem/Biochem laboratory samples were specifically, clinical routine screening, diagnosing or tracking tests including, for 1) diabetes: plasma glucose, HbA1c; 2) gout and renal dysfunction: urate; 3) dyslipidaemia: plasma TG and HDL-C; 4) liver dysfunction such as steatohepatitis, cholestasis: liver function tests including the enzymes ALT, AST, γGT and bilirubin 5) bone turnover and intestinal long chain FA transporter: AlkPhos; 6) immune disorders (infection/infestation/parasitism, auto-immune disease, cancer: leukocytes, acute phase proteins such as CRP, ferritin, immunoglobulins and ESR were collected at baseline and 6m for the main Pearson r and mixed model analyses analysis.

Clinical Study Procedures
Refer to Section 2.4.2

4.2.2 Laboratory Procedures and Analyses
Refer to Section 2.4.4

4.2.3 Statistical Analyses

4.2.3.1 General Comments

For the cross sectional data simple (Pearson) correlation coefficients (r) and partial r were formed. For baseline multivariable analysis a general linear (regression) model was employed where MetS markers and marker count were the outcomes, and the CRSHAem&Biochem and demographic, health status, lifestyle and QoL data the explanatory variables. For the longitudinal analyses a general linear mixed model was fitted to the data. When interested in the effect of time dependent explanatory variables,
the effect of the variable was split into its mean over the 6m (overall mean) and the deviation from the mean (change) at each time point. For the ordinal outcome an ordinal logistic regression or generalised linear mixed model was fitted.

4.2.3.2 Demographic, Health Status and Lifestyle Questionnaires

Refer to Section 2.4.6

4.2.3.3 Anthropometry

Untransformed anthropometric values are shown for 3 time points: baseline, 3m and 6m.

4.2.3.4 Metabolic Syndrome Comparisons

Metabolic Syndrome Definitions

The 5 markers of MetS were the same for all NCEP-derived definitions, NCEP, NCEP/ADA and IDF/JIS. (Refer back to Table 1-1). Three or more of 5 markers were required to be present, except for IDF MetS where waist is required to be one of the markers. The markers were: waist, S/DBP, HDL-C, TG and FPG. If outside the cut points, and fulfilling the definition, the parameters were denoted as MetS markers. In this study, participant inclusion was identical in the IDF and JIS MetS definitions, and they have been denoted IDF/JIS. (Refer back to Table 1-1).

Metabolic Syndrome Marker Count Definition

A MetS index was formed by calculation of a MetS marker count for which the minimum was 0 (no abnormal markers), and the maximum was 5 (all markers abnormal). MetS marker count was used in addition to MetS per se, to reduce the inefficiency that can be introduced by using a dichotomised measure when it is in reality continuous. The individual markers remain dichotomised as they were required for the construction of a MetS marker count.

Metabolic Syndrome Definition Comparisons

Three NCEP-derived MetS definitions were compared using simple statistics. Refer back to Table 1-1.

IDF/JIS Metabolic Syndrome Relationships

The IDF/JIS MetS was the MetS definition used to explore relationships in this study.
Waist and IDF/JIS Metabolic Syndrome Relationships: Women and Men

To investigate the relationship of waist with the MetS marker count in women and men separately, the MetS marker count was recalculated excluding the MetS marker of waist, and the mean(sd) of waist(cm) within each category calculated.

Waist in Different Ethnicities: European/Other and Maori

To investigate the relationship of waist with the MetS marker count in European/Other and Maori ethnicities separately, the same method as above was employed.

Relationship of (1) Weight to Metabolic Syndrome and (2) the Effect of Metabolic Syndrome on Weight.

Effect of Weight on Metabolic Syndrome

In order to investigate whether MetS influenced the ability to lose weight, a general linear mixed model was fitted with the log of the weight for an individual at each of the 7 visits as the outcome and an autoregressive correlation structure assumed between time periods. Age, gender, having ever smoked, number of cigarettes smoked/d, education level (primary/secondary schooling or tertiary), treatment group (chitosan or placebo), and whether or not participants completed the study, were included as explanatory variables along with the baseline measures of the MetS markers. The interactions of treatment and completion with time, were included as well as the interactions of the MetS markers, and time as the question of interest was whether the change in weight over time differed depending on the baseline values of the MetS markers. A separate analysis was performed including the summary variable or MetS index, MetS marker count.

The effect of Metabolic Syndrome Markers and Count on Weight

In order to investigate whether the degree of weight change affected the degree of change of any of the individual MetS markers, separate regression analyses were run. Each analysis had a change in one of the MetS markers as the outcome, and age, gender, having ever smoked, number of cigarettes smoked/d, education level, treatment group, weight change, mean of baseline and final weight (to allow for a difference in amount of change at different initial weights), as explanatory variables. A quadratic weight change term was also included to see if there was a non linear relationship of weight change with change in MetS variables, but this term was removed if not significant.
An ordinal logistic regression was also run with the change in the MetS marker count as the outcome. The same explanatory variables were included as above, apart from age and having ever smoked, as their inclusion caused the proportional odds assumption not to hold.

**Change in the Short Form-36 Question Quality of Life Scores with Metabolic Syndrome**

Two general linear mixed model analyses were used to investigate the relationship of the SF-36 scores and their change over time with the MetS marker count at the 2 time points. Age, gender, regular cigarette smoking, education level, and treatment group were included as explanatory variables in both analyses. Along with these explanatory variables, in the first analysis (1a) the mean, and (1b) the change of the combined the SF-36 physical and mental component summary subscores, and in the second analyses (2a) the mean, and (2b) the change of the individual subscales were included. Refer to Chapter 2, Section 2.4.6.1

For the MetS marker count the SF-36 subscores were measured at each time point, and were split into their mean and their change. This enabled a separating out of the effect of a mean relationship with MetS marker count and how the change was related to changes in the MetS marker count.

**4.2.3.5 Clinical Routine Screening Haematology and Biochemistry, and Metabolic Syndrome**

**Classification**

The haematology markers of inflammation were the WBC, or leukocytes, comprising neutrophils, eosinophils, monocytes and lymphocytes. The aggregation factor of inflammation, ESR, was also included. The inflammatory biochemistry markers, the acute phase proteins, mostly synthesized in the liver, were the variably inflammation-sensitive serum proteins, albumin and globulin and the classic CRP or high-sensitivity CRP (hsCRP).

The biochemistry nominated for this study comprised an ‘oxidant’ group of markers, and were composed of glycated HbA1c, urate, ferritin and LFT. The latter comprised the liver
enzymes, AlkPhos, the 3 liver transferases; ALT, AST and γGT. Bilirubin was included as part of the LFT.

**Laboratory Data**

The CRSHaem&Biochem are presented using descriptive statistics. Laboratory, gender specific reference ranges are given in Appendix 3. Evaluable Numbers and Normal Ranges.

**Cross Sectional Correlations**

Pearson r coefficients were calculated to investigate the simple relationship between the CRSHaem&Biochem, IDF/JIS MetS markers and MetS marker count. In order to investigate independent associations, partial r coefficients of MetS marker variables and MetS marker count were formed with each of the CRSHaem&Biochem, adjusting for all other CRSHaem&Biochem. Further partial r analyses were run in the two groups, ‘oxidant’ and ‘inflammatory’ as detailed above. SAS v9.1 statistical software (Cary, NC, USA, 2006) was used for these analyses.

**Longitudinal Multivariable Relationships**

In order to investigate the relationship of within-participant change over time of the CRSHaem&Biochem with the individual MetS marker, a general linear mixed model was fitted. Separate analyses were run for each MetS marker with its repeated measures as the outcomes, and the CRSHaem&Biochem as explanatory variables. The effect of each CRSHaem&Biochem was split into two parts (1) the mean of the 2 measures at the 2 time points for the participant and (2) the change which was the deviation from this mean at that time point.

The explanatory variables included for each outcome variable were age, gender, education level, having ever smoked, regular cigarette smoker, number of cigarettes smoked/d, treatment group, drinking >14 alcohol units/wk, acute or chronic inflammatory illness, taking anti-inflammatory medication, and taking medication with an anti-inflammatory effects. (Refer to Section 2.4.6.1). The CRSHaem&Biochem explanatory variables were: neutrophils, eosinophils monocytes lymphocytes, ESR, HbA1c, urate, ferritin, bilirubin, AlkPhos, ALT, AST, γGT, albumin, globulin, and hsCRP.
To investigate the relationship of within-participant change in the MetS marker count with change in inflammatory markers, an ordinal logistic regression or generalised linear mixed model was used, treating the MetS marker count variable as ordinal. The same explanatory variables as above were used. One participant was removed from the analyses as her extreme measure for AST prevented the generalised linear mixed model converging. This person only had baseline measures and so was dropped from all analyses for consistency.

4.3 Results

4.3.1 Demographic, Health Status, Lifestyle and Quality of Life

For this Chapter it is important to note that different numbers of participants qualified for the various analyses. The full study had 250 participants (206 women; 44 men) enrolled, but only 164 (132 women; 32 men), attended the final visit at 6m. For the anthropometry analysis 147 participants (119 women; 28 men), had all data for weight, BMI, waist and %Fat for 3 time points. For MetS analyses: at baseline there were 234 participants (194 women; 40 men), of whom all had waist, S/DBP, HDL-C, TG and FPG data recorded at enrolment, and 147 (117 women; 30 men), who attended at 6m and whose data was collected at both time points.

4.3.1.1 Baseline Characteristics – Anthropometry and Questionnaire Data

The data recorded at baseline are shown in Table 4–1. Eighty per cent of the participants in this weight loss study were women, who in turn were, on average, younger than the men. Approximately 80% of both women and men were of European ethnicity. The women were more likely to be working full time, although educational level was equivalent. Women were on average less likely to smoke currently, or drink alcohol. There was a much higher rate of past smoking than current smoking in both women and men. Men had a higher mean(sd) weight 105.5(13.0)kg and waist 111.1(11.5)cm than women (weight 95.6(16.3)kg; waist 98.9(12.0)cm), but women had a higher BMI which was 35.7(5.3)kg/m², and %Fat 40.4(4.7), compared with the male participants whose BMI was 33.8(3.9)kg/m² and %Fat was 28.14 (5.0) (Table 4-1).
### Table 4-1. Baseline Characteristics

<table>
<thead>
<tr>
<th>Baseline measurements: mean(sd) or N (%)</th>
<th>All (N=250)</th>
<th>Women (N=206)</th>
<th>Men (N=44)</th>
</tr>
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<tbody>
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<td><strong>Age, y</strong></td>
<td>47.7(11.7)</td>
<td>46.9(11.5)</td>
<td>51.5(12.0)</td>
</tr>
<tr>
<td><strong>Ethnicity</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Maori (%)</td>
<td>29.0(11.6)</td>
<td>27.0(13.0)</td>
<td>2.0(4.5)</td>
</tr>
<tr>
<td>- Samoan (%)</td>
<td>5.0(2.0)</td>
<td>4.0(1.9)</td>
<td>1.0(2.3)</td>
</tr>
<tr>
<td>- Rarotongan (%)</td>
<td>1.0(0.5)</td>
<td>1.0(0.5)</td>
<td>0.0</td>
</tr>
<tr>
<td>- Tongan (%)</td>
<td>1.0(0.5)</td>
<td>0.0</td>
<td>1.0(2.3)</td>
</tr>
<tr>
<td>- Chinese (%)</td>
<td>1.0(0.5)</td>
<td>1.0(0.5)</td>
<td>0.0</td>
</tr>
<tr>
<td>- Indian (%)</td>
<td>7.0(2.8)</td>
<td>4.0(2.0)</td>
<td>3.0(6.8)</td>
</tr>
<tr>
<td>- Other (%)</td>
<td>2.0(0.8)</td>
<td>2.0(1.0)</td>
<td>0.0</td>
</tr>
<tr>
<td><strong>European (%)</strong></td>
<td>206.0(82.4)</td>
<td>169.0(82.0)</td>
<td>37.0(84.1)</td>
</tr>
<tr>
<td><strong>Education and Work</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Edu. Level &gt;Secondary (%)</td>
<td>116(46.4)</td>
<td>95(46.1)</td>
<td>21(47.7)</td>
</tr>
<tr>
<td>Full Time Employment (%)</td>
<td>106(42.4)</td>
<td>90(43.7)</td>
<td>16(36.4)</td>
</tr>
<tr>
<td><strong>Lifestyle: Habits</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ever Drank Alcohol (%)</td>
<td>154(61.8)</td>
<td>121(59.0)</td>
<td>33(75.0)</td>
</tr>
<tr>
<td>Reg. Alcohol Drinker (%)</td>
<td>122(48.8)</td>
<td>92(44.7)</td>
<td>30(68.2)</td>
</tr>
<tr>
<td>1 Alcohol units/wk (LS)</td>
<td>6.4(5.8)</td>
<td>5.4(4.3)</td>
<td>9.7(8.4)</td>
</tr>
<tr>
<td>≥ 14 alcohol/ U wk (%)</td>
<td>8(3.2)</td>
<td>3(1.5)</td>
<td>5(11.6)</td>
</tr>
<tr>
<td>Ever Smoked (%)</td>
<td>107(42.8)</td>
<td>85(41.3)</td>
<td>22(50.0)</td>
</tr>
<tr>
<td>Reg. cig. Smoker (%)</td>
<td>23(9.2)</td>
<td>18(8.7)</td>
<td>5(11.4)</td>
</tr>
<tr>
<td>No. Cig. Smoked/d</td>
<td>9.9(5.9)</td>
<td>10.3(5.3)</td>
<td>8.3(8.8)</td>
</tr>
<tr>
<td><strong>Anthropometry</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Height, cm</td>
<td>166.0(8.0)</td>
<td>163.5(6.3)</td>
<td>176.6(5.8)</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>97.4(16.2)</td>
<td>95.6(16.3)</td>
<td>105.5(13.0)</td>
</tr>
<tr>
<td>BMI, kg/m2</td>
<td>35.3(5.2)</td>
<td>35.7(5.3)</td>
<td>33.8(3.9)</td>
</tr>
<tr>
<td>Waist, cm</td>
<td>100.4(12.9)</td>
<td>98.9(12.0)</td>
<td>111.1(11.5)</td>
</tr>
<tr>
<td>Waist/height, cm/cm</td>
<td>0.606(0.074)</td>
<td>0.601(0.075)</td>
<td>0.630(0.069)</td>
</tr>
<tr>
<td>Body Fat, %</td>
<td>38.3(6.6)</td>
<td>40.4(4.7)</td>
<td>28.14(5.0)</td>
</tr>
<tr>
<td>SBP, mmHg</td>
<td>123.2(18.3)</td>
<td>121.3(17.8)</td>
<td>132.1(17.8)</td>
</tr>
<tr>
<td>DBP, mmHg</td>
<td>69.7(9.5)</td>
<td>68.5(8.9)</td>
<td>75.3(10.5)</td>
</tr>
<tr>
<td><strong>Medical Record</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 All Disease</td>
<td>339</td>
<td>250</td>
<td>89</td>
</tr>
<tr>
<td>Disease/Individual</td>
<td>1.4</td>
<td>1.2</td>
<td>2.0</td>
</tr>
<tr>
<td>2 Any Disease (%)</td>
<td>173(69.2)</td>
<td>134(65.1)</td>
<td>39(88.6)</td>
</tr>
<tr>
<td>2 All CVD risk or CVD</td>
<td>128</td>
<td>83</td>
<td>45</td>
</tr>
<tr>
<td>CVD risk or CVD/Individual</td>
<td>0.5</td>
<td>0.4</td>
<td>1.0</td>
</tr>
<tr>
<td>2 Any CVD risk or CVD (%)</td>
<td>89(35.7)</td>
<td>61.0(29.8)</td>
<td>28.0(63.6)</td>
</tr>
<tr>
<td>CV risk or CVD&gt;1 (%)</td>
<td>31(12.5)</td>
<td>20.0(9.8)</td>
<td>11.0(25.0)</td>
</tr>
<tr>
<td>Dyslipidaemia (%)</td>
<td>27.0(10.9)</td>
<td>19(9.3)</td>
<td>8.0(18.2)</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>56.0(22.5)</td>
<td>36.0(17.5)</td>
<td>20.0(45.5)</td>
</tr>
<tr>
<td>TIIDM (%)</td>
<td>14.0(5.6)</td>
<td>5.0(2.4)</td>
<td>9.0(20.5)</td>
</tr>
<tr>
<td>Shortness of Breath (%)</td>
<td>21.0(8.4)</td>
<td>17.0(8.30)</td>
<td>4.0(9.1)</td>
</tr>
<tr>
<td>CHD (%)</td>
<td>7.0(2.8)</td>
<td>4.0(2.0)</td>
<td>3.0(6.8)</td>
</tr>
<tr>
<td>Stroke (%)</td>
<td>3.0(1.2)</td>
<td>2.0(1.0)</td>
<td>1.0(2.3)</td>
</tr>
<tr>
<td>Depression (%)</td>
<td>25.0(10.0)</td>
<td>17.0(8.3)</td>
<td>8.0(18.2)</td>
</tr>
<tr>
<td>Sleep Apnoea (%)</td>
<td>5.0(2.0)</td>
<td>1.0(0.50)</td>
<td>4.0(9.1)</td>
</tr>
<tr>
<td>Gallbladder disease (%)</td>
<td>2.0(0.8)</td>
<td>2.0(1.0)</td>
<td>0.0</td>
</tr>
</tbody>
</table>
Chapter 4. Novel Cardiovascular Disease Risk Markers in the Metabolic Syndrome

Baseline measurements: mean(sd) or N (%) | All (N=250) | Women (N=206) | Men (N=44)
--- | --- | --- | ---
Hyperthyroid (%) | 2.0(0.8) | 2.0(1.0) | 1
Hypothyroid (%) | 5.0(2.0) | 4.0(2.0) | 4.0(2.3)
Asthma (%) | 38.0(15.2) | 34.0(16.5) | 4.0(9.1)
Back problem (%) | 54.0(21.6) | 46.0(22.3) | 8.0(18.2)
Cancer (%) | 8.0(3.2) | 6.0(2.9) | 2.0(4.5)
Other Disease (%) | 72.0(28.8) | 55.0(26.7) | 17.0(38.6)
Tot. inflam. Disease (%) | 32.0(12.8) | 26.0(12.6) | 6.0(13.6)
Chronic inflam. Disease (%) | 22.0(8.8) | 18.0(8.7) | 4.0(9.1)
Acute inflam. Disease (%) | 11.0(4.4) | 8.0(3.9) | 3.0(6.8)

Medication

Anti-inflam. Med Effects (%) | 48.0(19.2) | 32.0(15.5) | 16.0(36.4)
Anti-inflam. Med (%) | 40.0(16.0) | 27.0(13.1) | 13.0(29.6)
Hypolipidaemic Med. (%) | 21.0(8.4) | 12.0(5.8) | 9.0(20.5)
Hypotensive Med. (%) | 56.0(22.4) | 35.0(17.0) | 21.0(47.7)
Hypoglycaemic Med. (%) | 11.0(4.4) | 3.0(1.5) | 8.0(18.2)

24Hr Food Recall Nutrients

Energy Intake, MJ/d | 8.657(3.522) | 8.278(3.293) | 10.428(4.030)
Calories, Kcal/d | 2068(841) | 1978(786) | 2491(963)
Protein, g/d | 88.7(35.7) | 84.7(33.2) | 107.7(41.1)
Tot Fat, g/d | 85.8(46.8) | 81.2(43.3) | 106.9(56.3)
Saturated Fat, g/d | 34.6(21.7) | 32.5(20.2) | 44.1(25.8)
CHO, g/d | 226.9(102.7) | 222.8(98.5) | 246.4(120.0)
Tot. Sugars, g/d | 100.8(65.3) | 101.2(58.1) | 98.8(92.5)
Fibre, g/d | 20.4(9.0) | 20.3(8.8) | 20.9(10.0)

Physical Activity (PA)

TV Watching (h/wk) | 24.6(13.2) | 24.4(12.5) | 25.6(16.0)
Mild-Moderate PA (h/wk) | 9.8(7.4) | 9.9(7.3) | 9.6(8.0)
Vigorous PA (%) | 98.0(40) | 82.0(40) | 16.0(36)

Vigorous PA, mins/wk (n=94) | 123.9(117.0) | 125.9(118.2) | 113.44(114.0)

Quality of Life (QoL) and Attitudes

SF-36 Phys Subscore | 47.02(8.9) | 47.04(8.9) | 46.94(8.9)
SF-36 Ment Subscore | 46.18(10.9) | 46.21(11.0) | 46.03(10.8)
OSQoL Total Subscore | 47.2(16.8) | 47.4(17.0) | 46.5(15.8)
OSQoL % Phys Subscore | 42.8(19.5) | 42.6(19.2) | 44.0(20.6)
OSQoL % Ment Subscore | 50.9(17.4) | 51.4(17.7) | 48.6(16.1)
Eat-12 Dieting Subscore | 14.9(4.1) | 14.6(4.1) | 16.0(3.6)
Eat-12 Bulimia Subscore | 19.2(3.4) | 18.7(3.4) | 21.2(2.5)
Eat-12 Oral Control Subscore | 21.1(2.4) | 21.1(2.4) | 20.8(2.3)

1 unit Alcohol=10g in NZ. 2CVD cardiovascular disease A single person may have >1. 3For analyses methods of SF-36 QoL, OSQoL and EAT-12, refer to Section.2.4.6.1 and Error! Reference source not found. OSQoL, Obesity Specific QoL higher score=most normal, 5EAT-12 scoring. Low scores=most normal, least impaired. 6EAT-12 Eating Attitudes Test-12, received from n=148 -145, w=201-204, m=44. Y year; wk week; waist circumference; S/DBP systolic diastolic blood pressure; TIIDM type II diabetes mellitus; CHD coronary heart disease; inflam inflammatory; tot total; OSQoL, Obesity Specific QoL PA physical activity; 24 FR 24 Food Recall; phys physical; ment mental; psyc psychological Refer to Table 1-1.
Women had fewer CVD markers, and men averaged at least one CVD marker or had diagnosed CVD, and 25% of all participants had more than 1 risk marker of CVD. Hypertension was reported in >45% men, and 20% had TIIDM, but for women 18% were hypertensive, and only 2% had TIIDM. The cardio-metabolic medications reflected the spread of CVD. Men reported a higher intake of macronutrients and energy, except for sugar, whilst fibre was similar in both women and men. PA levels were similar in both women and men.

4.3.2 Anthropometry Variability

**Anthropometry at baseline, 3m and 6m**

Figure 4-1 & Figure 4-2 show the variation in anthropometric changes over 6m. In the 147 participants (117 women; 30 men) who had anthropometry recorded at 3 time points, the change pattern was highly variable between- and within-individuals, with many combinations of gain or loss over both 3m periods observed (Figure 4-1 & Figure 4-2). The anthropometric changes for the 164 participants, who completed the study and had all baseline and 6m measurements, were a mean(sd) weight loss of 1.2(3.9)kg, decrease in BMI of 0.41(1.41)kg/m², decrease in waist of 2.0(4.7)cm, decrease in waist/height ratio of 0.012(0.028), and %Fat of 0.14(3.0) (n=157).

Percentage weight and waist change correlated highly in men over the first 3m period (r=0.63; p<0.001), and over the second 3m period, 3 to 6m (r=0.53; p<0.001) (Figure 4-3). In contrast, the percentage weight to waist change in women during the first 3m showed a modest correlation (r=0.29; p=0.002), which increased greatly in the second 3m period (r=0.75; p<0.001) (Figure 4-3).

In Figure 4-3 the percentage weight to %Fat change shown is borderline for the first 3m in women and men, and for all (r=0.19; p=0.02), becoming significant for women and men after the second 3m period (r=0.27; p=0.004) and (r=0.40; p=0.03), respectively.

4.3.3 Metabolic Syndrome Comparisons

**Baseline Metabolic Syndrome Comparisons**

Baseline laboratory data for this Chapter are shown in Table 4-2 and MetS data in Figure 4-3.
Chapter 4. Novel Cardiovascular Disease Risk Markers in the Metabolic Syndrome

Figure 4-1. Weight & Waist of Individuals at Baseline, 3m & 6m: Women, n=117 & Men, n=30

Bl baseline, 3m 3 months, 6m 6 months
Figure 4-2. BMI & %Fat of Individuals at Baseline, 3m & 6m: Women, n=117 & Men, n=30

Bl baseline, 3m 3 months, 6m 6 months, BMI body mass index, %Fat body fat percentage.
There was a full set of baseline data for all MetS markers to calculate the baseline NCEP, NCEP/ADA and IDF/JIS MetS comparisons in 234/250 participants (Table 4-1 & Table 4-2).

In contrast, the percentage weight to waist change in women during the first 3m showed a modest correlation ($r=0.29; p=0.002$), which increased greatly in the second 3m period ($r=0.75; p<0.001$) (Figure 4-3).

In Figure 4-3 the percentage weight to %Fat change shown is borderline for the first 3m in women and men, and for all ($r=0.19; p=0.02$), becoming significant for women and men after the second 3m period ($r=0.27; p=0.004$) and ($r=0.40; p=0.03$), respectively.

Eight %Fat values were >25% different from baseline and 6m, and were not in keeping with the weight, waist and BMI. These values were excluded for this analysis, but it made no difference to the p-values whether these values were retained or not. W women; m men; weight body weight; Waist circumference waist; %Fat body fat percentage; m month.
### Table 4-2. Baseline MetS Marker & CRSHaem&Biochem

<table>
<thead>
<tr>
<th>Parameter</th>
<th>All, mean(sd)</th>
<th>Women, mean(sd)</th>
<th>Men, mean(sd)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC, x10^12/L</td>
<td>4.84(0.38)</td>
<td>4.76(0.33)</td>
<td>5.22(0.37)</td>
</tr>
<tr>
<td>Hb, g/L</td>
<td>142.1(11.5)</td>
<td>139.3(9.9)</td>
<td>155.2(9.6)</td>
</tr>
<tr>
<td>Hct, %</td>
<td>0.425(0.033)</td>
<td>0.417(0.028)</td>
<td>0.460(0.03)</td>
</tr>
<tr>
<td>MCV, fl</td>
<td>87.9(4.2)</td>
<td>87.8(4.3)</td>
<td>88.4(4.1)</td>
</tr>
<tr>
<td>MCHC, pg</td>
<td>29.4(1.7)</td>
<td>29.3(1.7)</td>
<td>29.8(1.6)</td>
</tr>
<tr>
<td>Leukocytes, x10^9/L</td>
<td>7.00(2.21)</td>
<td>6.98(2.31)</td>
<td>7.10(1.67)</td>
</tr>
<tr>
<td>Neutrophils, x10^9/L</td>
<td>4.2(1.50)</td>
<td>4.16(1.57)</td>
<td>4.4(1.40)</td>
</tr>
<tr>
<td>Basophils, x10^9/L</td>
<td>0.071(0.021)</td>
<td>0.070(0.020)</td>
<td>0.075(0.022)</td>
</tr>
<tr>
<td>Eosinophils, x10^9/L</td>
<td>0.21(0.13)</td>
<td>0.21(0.13)</td>
<td>0.22(0.10)</td>
</tr>
<tr>
<td>Monocytes, x10^9/L</td>
<td>0.34(0.10)</td>
<td>0.33(0.10)</td>
<td>0.39(0.09)</td>
</tr>
<tr>
<td>Lymphocytes, x10^9/L</td>
<td>2.25(0.93)</td>
<td>2.27(1.0)</td>
<td>2.2(0.50)</td>
</tr>
<tr>
<td>Platelets, x10^12/L</td>
<td>329.2(70.5)</td>
<td>336.1(65.4)</td>
<td>296.2(84.3)</td>
</tr>
<tr>
<td>ESR, mm/h</td>
<td>20.9(13.5)</td>
<td>21.8(13.1)</td>
<td>16.0(14.5)</td>
</tr>
<tr>
<td>TC, mmol/l</td>
<td>5.46(0.95)</td>
<td>5.50(0.92)</td>
<td>5.29(1.09)</td>
</tr>
<tr>
<td>LDL-C, mmol/l</td>
<td>3.43(0.81)</td>
<td>3.48(0.79)</td>
<td>3.20(0.87)</td>
</tr>
<tr>
<td>HDL-C, mmol/l</td>
<td>1.33(0.34)</td>
<td>1.37(0.34)</td>
<td>1.1(0.26)</td>
</tr>
<tr>
<td>TG, mmol/l</td>
<td>1.57(0.83)</td>
<td>1.44(0.70)</td>
<td>2.15(1.11)</td>
</tr>
<tr>
<td>TC/HDL</td>
<td>4.36(1.20)</td>
<td>4.24(1.13)</td>
<td>4.94(1.35)</td>
</tr>
<tr>
<td>FPG, mmol/l</td>
<td>5.32(1.38)</td>
<td>5.24(1.40)</td>
<td>5.70(1.21)</td>
</tr>
<tr>
<td>HbA1c, %</td>
<td>5.3(0.8)</td>
<td>5.2(0.8)</td>
<td>5.5(0.7)</td>
</tr>
<tr>
<td>Urate, mmol/L</td>
<td>0.36(0.07)</td>
<td>0.35(0.06)</td>
<td>0.44(0.08)</td>
</tr>
<tr>
<td>Ferritin, µg/L</td>
<td>227(211)</td>
<td>83.2(77.6)</td>
<td>182.6(119.0)</td>
</tr>
<tr>
<td>Bilirubin, µmol/L</td>
<td>8.5(5.10)</td>
<td>8.6(5.2)</td>
<td>7.8(4.4)</td>
</tr>
<tr>
<td>AlkPhos, U/L</td>
<td>77.0(24.4)</td>
<td>76.6(23.4)</td>
<td>78.9(28.6)</td>
</tr>
<tr>
<td>ALT, U/L</td>
<td>17.2(10.6)</td>
<td>16.2(10.1)</td>
<td>21.4(11.6)</td>
</tr>
<tr>
<td>AST, U/L</td>
<td>23.5(12.0)</td>
<td>23.0(13.0)</td>
<td>25.7(5.1)</td>
</tr>
<tr>
<td>γGT, U/L</td>
<td>29.8(25.8)</td>
<td>26.4(23.2)</td>
<td>45.6(30.9)</td>
</tr>
<tr>
<td>Albumin, g/L</td>
<td>42.9(2.0)</td>
<td>42.6(2.2)</td>
<td>44.4(2.1)</td>
</tr>
<tr>
<td>Globulin, g/L</td>
<td>28.3(3.3)</td>
<td>28.4(3.2)</td>
<td>28.1(3.9)</td>
</tr>
<tr>
<td>hsCRP, mg/L</td>
<td>5.3(5.53)</td>
<td>5.4(5.45)</td>
<td>4.7(5.9)</td>
</tr>
</tbody>
</table>

MetS metabolic syndrome; CRSHaem&Biochem clinical routine screening haematology and biochemistry; RBC red blood cells; Hb haemoglobin; Hct haematocrit; MCV mean cell volume; MCHC MCHb concentration; ESR erythrocyte sedimentation rate; TC total cholesterol; L/HDL-C low/high density lipoprotein-C; TG triglyceride; FPG fasting plasma glucose; AlkPhos alkaline phosphatase; ALT/AST alanine/aspartate transferase; γGT Gamma glutamyl transferase; hsCRP high sensitive C reactive protein.

### 4.3.3.1 Metabolic Syndrome Comparisons

#### Baseline Metabolic Syndrome Comparisons

Baseline laboratory data for this Chapter are shown in Table 4-2 and MetS data in Table 4-3.

There was a full set of baseline data for all MetS markers to calculate the baseline NCEP, NCEP/ADA and IDF/JIS MetS comparisons in 234/250 participants (Table 4-1 & Table 4-2).
At baseline, using the NCEP definition, 31% (73/234) of participants had MetS, which rose approximately 7 to 8% and 12% on using NCEP/ADA and IDF/JIS MetS definitions respectively (Figure 4-4). Older men had higher rates of MetS than women, with the lowest rates in young women, especially on using IDF/JIS MetS (Figure 4-4 & Table 4-4).

Hypoglycaemic or antidiabetic medication for raised plasma glucose and lipid-normalising medication for dyslipidaemia TG/HDL, also increased the IDF/JIS MetS diagnosis, but BP treatment did not (Table 4-4).

Table 4-3. Baseline, NCEP, NCEP/ADA & IDF/JIS MetS: Gender & Age Groups

<table>
<thead>
<tr>
<th>Baseline Participants</th>
<th>All N=234</th>
<th>Women n=194</th>
<th>Women &lt;50 y n=107</th>
<th>Women ≥50 y n=87</th>
<th>Men n=40</th>
<th>Men &lt;50 y n=18</th>
<th>Men ≥50 y n=22</th>
</tr>
</thead>
<tbody>
<tr>
<td>MetS, %</td>
<td>31.2</td>
<td>25.1</td>
<td>23.6</td>
<td>27.3</td>
<td>60.0</td>
<td>38.9</td>
<td>77.3</td>
</tr>
<tr>
<td>NCEP</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NCEP/ADA, %</td>
<td>38.5</td>
<td>32.5</td>
<td>29.0</td>
<td>36.8</td>
<td>67.5</td>
<td>44.4</td>
<td>86.4</td>
</tr>
<tr>
<td>IDF/JIS, %</td>
<td>42.3</td>
<td>35.3</td>
<td>33.0</td>
<td>40.0</td>
<td>72.5</td>
<td>55.6</td>
<td>86.4</td>
</tr>
</tbody>
</table>

1N total=234/250. Some blood tests were unable to be drawn. No MetS marker values were imputed. Refer to Table 1-1 for MetS and MetS marker definitions. 2 For gender/age group, subtraction of NCEP and NCEP/ADA from IDF/JIS MetS gives an average effect size difference of mean(sd) 12.0(2.2) % and 7.4(3.6) % respectively. IDF International Diabetes Federation, JIS Joint Interim Statement, NCEP National Cholesterol Education Panel, ADA American Diabetic Association, y year; MetS marker count metabolic syndrome marker count.

Figure 4-4. Baseline (A) NCEP, NCEP/ADA & IDF/JIS MetSMCt & MetS (B) IDF/JIS MetS in Age & Gender Groups, n=236
Table 4-4. Baseline, MetS Marker Frequency: NCEP, NCEP/ADA & IDF/JIS MetS

<table>
<thead>
<tr>
<th>Frequency of Abnormal MetS Marker</th>
<th>All (%)</th>
<th>Women (%)</th>
<th>Men (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$n=234$</td>
<td>$n=194$</td>
<td>$n=40$</td>
</tr>
<tr>
<td>Waist, cm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NCEP MetS</td>
<td>184 (78.6)</td>
<td>151.0 (77.8)</td>
<td>33.0 (82.5)</td>
</tr>
<tr>
<td>NCEP/ADA MetS</td>
<td>184 (78.6)</td>
<td>151.0 (77.8)</td>
<td>33.0 (82.5)</td>
</tr>
<tr>
<td>IDF/JIS MetS</td>
<td>223 (95.3)</td>
<td>184.0 (94.8)</td>
<td>39.0 (97.5)</td>
</tr>
<tr>
<td>SBP, mmHg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NCEP MetS</td>
<td>88 (37.6)</td>
<td>67.0 (34.5)</td>
<td>21.0 (52.5)</td>
</tr>
<tr>
<td>NCEP/ADA MetS</td>
<td>88 (37.6)</td>
<td>67.0 (34.5)</td>
<td>21.0 (52.5)</td>
</tr>
<tr>
<td>IDF/JIS MetS</td>
<td>88 (37.6)</td>
<td>67.0 (34.5)</td>
<td>21.0 (52.5)</td>
</tr>
<tr>
<td>DBP, mmHg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NCEP MetS</td>
<td>15 (6.4)</td>
<td>8.0 (4.1)</td>
<td>7.0 (17.5)</td>
</tr>
<tr>
<td>NCEP/ADA MetS</td>
<td>88 (37.6)</td>
<td>67.0 (34.5)</td>
<td>21.0 (52.5)</td>
</tr>
<tr>
<td>IDF/JIS MetS</td>
<td>88 (37.6)</td>
<td>67.0 (34.5)</td>
<td>21.0 (52.5)</td>
</tr>
<tr>
<td>HDL-C, mmol/L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NCEP MetS</td>
<td>94 (40.2)</td>
<td>80.0 (41.2)</td>
<td>14.0 (35.0)</td>
</tr>
<tr>
<td>NCEP/ADA MetS</td>
<td>104 (44.4)</td>
<td>85.0 (43.8)</td>
<td>19.0 (47.5)</td>
</tr>
<tr>
<td>IDF/JIS MetS</td>
<td>104 (44.4)</td>
<td>85.0 (43.8)</td>
<td>19.0 (47.5)</td>
</tr>
<tr>
<td>TG, mmol/L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NCEP MetS</td>
<td>79 (33.8)</td>
<td>56.0 (28.9)</td>
<td>23.0 (57.5)</td>
</tr>
<tr>
<td>NCEP/ADA MetS</td>
<td>88 (37.6)</td>
<td>61.0 (31.4)</td>
<td>27.0 (67.5)</td>
</tr>
<tr>
<td>IDF/JIS MetS</td>
<td>88 (37.6)</td>
<td>61.0 (31.4)</td>
<td>27.0 (67.5)</td>
</tr>
<tr>
<td>FPG, mmol/L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NCEP MetS</td>
<td>21 (9.0)</td>
<td>12.0 (6.2)</td>
<td>9.0 (22.5)</td>
</tr>
<tr>
<td>NCEP/ADA MetS</td>
<td>45 (19.2)</td>
<td>31.0 (16.0)</td>
<td>14.0 (35.0)</td>
</tr>
<tr>
<td>IDF/JIS MetS</td>
<td>45 (19.2)</td>
<td>31.0 (16.0)</td>
<td>14.0 (35.0)</td>
</tr>
</tbody>
</table>

Note: Some blood tests were unable to be drawn, and samples were unsuitable for testing. No MetS marker values were imputed. Refer to Table 1-1 for MetS and MetS marker definitions. NCEP National Cholesterol Education Panel; ADA American Diabetic Association; IDF International Diabetes Federation; JIS Joint Interim Statement; MetS Metabolic syndrome; y years

Over the 6m study duration, decreased MetS marker count in men was nearly 10 times more than that for women for the NCEP, but only 2 times more on using the IDF/JIS MetS. As participants lost or gained weight, MetS marker count changed.

Table 4-5 shows the percentage of participants who lost and gained MetS overall was highest for NCEP MetS.

There was a decrease of MetS marker count using all MetS definitions, but a gain in BP in women, a slight loss in SBP in men, and gain in HDL-C in both women and men (Table 4-6).

More variation of NCEP MetS loss or gain was shown when compared with IDF/JIS MetS, but the NCEP MetS numbers were low. IDF/JIS showed participants losing MetS more in line with 2/3rd of them losing weight and waist as shown in Figure 4-5.
### Table 4-5. 6m Final Visit NCEP, NCEP/ADA & IDF/JIS MetS: Gender & Age Groups

<table>
<thead>
<tr>
<th>Participants at 6m</th>
<th>All, N=147</th>
<th>Women, N=119</th>
<th>Women&lt; 50y, N=57</th>
<th>Women≥ 50y, N=62</th>
<th>Men, N=28</th>
<th>Men&lt; 50y, N=11</th>
<th>Men≥ 50 yr N=17</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All: Baseline</td>
<td>Women: baseline</td>
<td>Women&lt; 50y, N=57</td>
<td>Women≥ 50y, N=62</td>
<td>Men, N=28</td>
<td>Men&lt; 50y, N=11</td>
<td>Men≥ 50 yr N=17</td>
</tr>
<tr>
<td>MetS Disappearance/Baseline MetS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NCEP (%)</td>
<td>20/47(42.6)</td>
<td>11/31(35.5)</td>
<td>3/13(23.1)</td>
<td>8/18(44.4)</td>
<td>9/16(56.3)</td>
<td>3/4(75.0)</td>
<td>6/12(50.0)</td>
</tr>
<tr>
<td>NCEP/ADA (%)</td>
<td>17/56(30.4)</td>
<td>11/40(26.2)</td>
<td>6/16(37.5)</td>
<td>7/24(29.2)</td>
<td>6/19(31.6)</td>
<td>0</td>
<td>6/14(42.9)</td>
</tr>
<tr>
<td>IDF/JIS (%)</td>
<td>19/66(28.8)</td>
<td>13/45(28.9)</td>
<td>5/18(27.8)</td>
<td>8/27(29.6)</td>
<td>9/21(42.9)</td>
<td>1/7(14.3)</td>
<td>7/14(50.0)</td>
</tr>
</tbody>
</table>

MetS Acquisition/Baseline MetS

| NCEP (%) | 10/47(21.3) | 8/31(25.8) | 3/13(23.1) | 5/18(27.8) | 2/16(12.5) | 1/4(25.0) | 1/12(8.3) |
| NCEP/ADA (%) | 6/56(10.7) | 4/40(10.0) | 2/16(12.5) | 2/24(8.3) | 2/19(10.5) | 1/5(20.0) | 1/14(7.1) |
| IDF/JIS (%) | 10/66(15.2) | 7/45(15.6) | 3/18(16.7) | 4/27(14.8) | 3/21(14.3) | 1/7(14.3) | 2/14(14.3) |

6m 6 month; IDF International Diabetes Federation, 2009; NCEP National Cholesterol Education Panel; NCEP-ADA National Cholesterol Education Panel-American Diabetic Association; JIS Joint Interim Statement; y years.

### Table 4-6. Change, Baseline to 6m. MetS, Marker & MetS Marker Count: Women n=117, Men n=30

<table>
<thead>
<tr>
<th>MetS Marker or MetS Marker Count definition</th>
<th>All: Baseline</th>
<th>All: Change baseline to 6m</th>
<th>Women: baseline, MetS Marker Count (Mean±SD)</th>
<th>Women: Change baseline to 6m, MetS Marker Count (Mean±SD)</th>
<th>Men: baseline, MetS Marker Count (Mean±SD)</th>
<th>Men: Change baseline to 6m, MetS Marker Count (Mean±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Waist, cm</td>
<td>98.66(11.82)</td>
<td>-2.14(0.38)</td>
<td>96.00(10.6)</td>
<td>-2.11(0.42)</td>
<td>110.20(9.9)</td>
<td>-2.28(0.96)</td>
</tr>
<tr>
<td>SBP, mmHg</td>
<td>123.07(17.97)</td>
<td>0.96(1.17)</td>
<td>121.30(17.7)</td>
<td>1.37(1.23)</td>
<td>130.60(17.2)</td>
<td>-0.77(3.27)</td>
</tr>
<tr>
<td>DBP, mmHg</td>
<td>69.36(9.04)</td>
<td>0.55(0.67)</td>
<td>68.20(8.5)</td>
<td>0.54(0.69)</td>
<td>74.40(9.7)</td>
<td>0.59(1.93)</td>
</tr>
<tr>
<td>HDL-C, mmol/L</td>
<td>1.33(0.34)</td>
<td>0.06(0.02)</td>
<td>1.38(0.33)</td>
<td>0.07(0.02)</td>
<td>1.13(0.29)</td>
<td>0.04(0.03)</td>
</tr>
<tr>
<td>TG, mmol/L</td>
<td>1.57(0.82)</td>
<td>-0.06(0.05)</td>
<td>1.45(0.71)</td>
<td>-0.07(0.05)</td>
<td>2.08(1.06)</td>
<td>-0.02(0.13)</td>
</tr>
<tr>
<td>FPG, mmol/L</td>
<td>5.25(1.06)</td>
<td>-0.07(0.05)</td>
<td>5.16(1.06)</td>
<td>-0.07(0.06)</td>
<td>5.61(1.01)</td>
<td>-0.04(0.16)</td>
</tr>
<tr>
<td>NCEP MetS Marker Count</td>
<td>2.03(1.14)</td>
<td>-0.24(0.08)</td>
<td>1.91(1.15)</td>
<td>-0.23(0.08)</td>
<td>2.57(0.92)</td>
<td>-0.29(0.18)</td>
</tr>
<tr>
<td>NCEP/ADA MetS Marker Count</td>
<td>2.47(1.20)</td>
<td>-0.18(0.07)</td>
<td>2.08(1.26)</td>
<td>-0.20(0.08)</td>
<td>2.93(0.94)</td>
<td>-0.07(0.16)</td>
</tr>
<tr>
<td>IDF/JIS MetS Marker Count</td>
<td>2.24(1.25)</td>
<td>-0.23(0.07)</td>
<td>2.29(1.14)</td>
<td>-0.24(0.08)</td>
<td>3.25(1.14)</td>
<td>-0.18(0.18)</td>
</tr>
</tbody>
</table>

NCEP MetS National Cholesterol Education Panel metabolic syndrome; NCEP/ADA MetS National Cholesterol Education Panel-Revised metabolic syndrome; IDF/JIS International Diabetes Federation Joint Interim Statement; Waist waist circumference; S/DBP systolic/ diastolic blood pressure; HDL-C high density lipoprotein cholesterol; TG triglyceride; FPG fasting plasma glucose; MetS Marker Count.
Waist in Women and Men.

When the waist MetS marker was excluded from the count, whereby the count drops by one, the mean waist for those with no other MetS marker was 92cm in Women, and for men the mean waist was approximately 101cm. (Table 4-7). Thus for a larger waist men have fewer MetS marker.

Table 4-7. Baseline. MetS Marker Count, Excluding MetS Marker Waist by Gender

<table>
<thead>
<tr>
<th>MetSMCt Excluding Waist</th>
<th>Women, n=194</th>
<th>Men, n=40</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n (%)</td>
<td>Waist cm, mean(sd)</td>
</tr>
<tr>
<td>0</td>
<td>49(25)</td>
<td>92.6(11.1)</td>
</tr>
<tr>
<td>1</td>
<td>75(39)</td>
<td>97.5(11.9)</td>
</tr>
<tr>
<td>2</td>
<td>43(22)</td>
<td>102.8(10.30)</td>
</tr>
<tr>
<td>3</td>
<td>20(10)</td>
<td>100.8(9.1)</td>
</tr>
<tr>
<td>4</td>
<td>7(4)</td>
<td>107.3(19.4)</td>
</tr>
<tr>
<td>Sum</td>
<td>194(100)</td>
<td>40(100)</td>
</tr>
</tbody>
</table>

1MetSMCt Metabolic syndrome marker count, number of metabolic syndrome markers outside the International Diabetes Federation–defined MetS marker cut-off level; 2n total=234/250 as some blood tests were unable to be drawn or samples were unsuitable for testing and no MetS Marker values were imputed. Waist waist circumference; sd standard deviation

Waist in European and Maori.

In the same type of analysis, when the waist MetS marker was excluded from the count, whereby the count drops by one, the mean waist for those with no other MetS marker was 92cm in European/Other, and for Maori the mean waist was approximately 99cm (Table 4-8). For a larger waist Maori had fewer MetS markers.

Table 4-8. Baseline. MetS Marker Count, Excluding MetS Marker Waist by Ethnicity

<table>
<thead>
<tr>
<th>MetSMCt Excluding Waist</th>
<th>European n=201</th>
<th>Maori n=27</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n (%)</td>
<td>Waist cm, mean(sd)</td>
</tr>
<tr>
<td>0</td>
<td>45(22)</td>
<td>92.3(11.2)</td>
</tr>
<tr>
<td>1</td>
<td>71(35)</td>
<td>98.5(11.5)</td>
</tr>
<tr>
<td>2</td>
<td>45(22)</td>
<td>104.6(11.3)</td>
</tr>
<tr>
<td>3</td>
<td>28(14)</td>
<td>104.6(11.9)</td>
</tr>
<tr>
<td>4</td>
<td>12(6)</td>
<td>106.9(13.8)</td>
</tr>
<tr>
<td>Sum</td>
<td>201(100)</td>
<td>27(100)</td>
</tr>
</tbody>
</table>

1MetSMCt Metabolic syndrome marker count, number of metabolic syndrome markers outside the International Diabetes Federation–defined MetS marker cut-off level; 2n total=234/250 as some blood tests were unable to be drawn or samples were unsuitable for testing and no MetS marker values were imputed. sd standard deviation; waist waist circumference.

Of the 119 women and 28 men with MetS who completed the study, 63.9% lost weight during the intervention, with 14.8% and 3.4% losing ≥5% and ≥10% of body weight.
respectively, and 61.2% of all participants losing waist, and 26.8% and 4.7% losing ≥5% and ≥10% of their waist respectively. Details for each gender are shown in Figure 4-5.

Figure 4-5. Baseline to 6m Change, Weight & Waist in MetS: Women & Men

In Figure 4-6 Pearson r are shown between change in weight and change in waist (r=0.59, p <0.001), SBP (r=0.20, p=0.02) DBP (r=0.16, p=0.06), TG (r=0.21, p=0.01), FPG (r=0.28, p=0.007), and MetS marker count (r=0.25, p=0.002). They were not significant for HDL-C nor MetS. Gender differences are shown (Figure 4-6).

Effect of Weight on Metabolic Syndrome and Count, and the Effect of Metabolic Syndrome and Count on Weight

Effect of Weight on Metabolic Syndrome

There was strong evidence of a linear relationship of weight change with changes in waist (p<0.001), SBP (p=0.03), DBP (p=0.05) and FPG (p=0.001), but no indication that the relationship was quadratic (p>0.45). Weight change was related to neither TG nor HDL-C, on linear or quadratic analysis (p>0.45). (Note that one participant was excluded due to an extremely large weight loss and TG change that was very influential in the test for a quadratic effect. When this participant’s data were removed this effect was lost). A linear relationship of weight change with change in MetS marker count was demonstrated (p=0.03). However there was no evidence of a quadratic relationship (p=0.16).
Effect of Metabolic Syndrome on Weight

The only baseline MetS marker for which there was any indication of a relationship with the change in weight over time was SBP (p=0.06), in contrast to waist (p=0.16), DBP (p=0.37), HDL-C (p=0.75), TG (p=0.73) and FPG (p=0.26). In case an influence of one of these variables was being masked by their interdependence, the model was also run with each MetS marker separately, however there was still no indication of a relationship with weight change. The MetS marker count could not be shown to be related to weight change (p=0.22).

Quality of Life Short Form-36 Scores and Change with the Metabolic Syndrome

The following results included the physical and mental summary subscores. There was some weak indication that both the mean and the within-person change of the physical summary subscore was related to the MetS marker count (with ordinal odds ratio 1.5; p=0.08, and 1.2; p=0.10 respectively). There was no evidence of an effect of the mean, or change of mental summary subscore (p=0.94 and p=0.60 respectively). The only subscale that could be shown to be associated with MetS marker count was the mean of general health (p<0.001; ordinal odds ratio 2.0).
4.3.3.2 Clinical Routine Screening Haematology and Biochemistry and Metabolic Syndrome

The values for the laboratory metabolic, MetS marker and CRSHaem&Biochem tests were presented as the mean(sd) in Table 4-2. Abnormal findings included TC, LDL-C and TC/HDL-C being above their normal ranges. TG was outside the normal range, and MetS cut point in men. FPG was in the impaired range and above MetS cut point in men. Urate was borderline for women and above the normal range for men. HsCRP was above the normal range for both women and men (Table 4-2). Ranges for both genders in the normal population are shown in (Appendix 3).

Cross Sectional Correlations

The Pearson correlation coefficients (r) and the partial r which adjust for the other CRSHaem&Biochem, and show an independent relationship, are set out in Table 4-9. The table is configured with the Pearson r on the top of the three rows for each parameter. In the next row are the partial r of that group, which is either oxidant (Ox) or inflammatory (Infl), and the bottom row is the partial r correcting for all CRSHaem&Biochem.

The major findings were that HbA1c and urate were much more strongly correlated with the IDF MetS marker count on Pearson and partial r analysis (r=0.28-0.40; p<0.001), than the other CRSHaem&Biochem. HbA1c and urate showed Pearson r with all MetS marker except urate did not correlate with FPG (r=0.15-0.89; p=0.04-<0.001) and All, Partial (Table 4-9).

Correlation adjustments for the other CRSHaem&Biochem with waist (r=0.26-0.41; p=0.003-<0.001), and BP were significant (r=0.15-0.89; p=0.07-<0.001). AlkPhos maintained independent modest relationships with TG and BP, and showed a possible independent association with MetS marker count (r=0.14-0.15; p=0.06), after adjustment for the other CRSHaem&Biochem (Table 4-9).

A similarly sized independent relationship was demonstrated by albumin (r=0.19; p=0.04), with MetS marker count, while globulin (r=-0.14; p=0.07), showed a possible negative independent relationship with MetS marker count.
### Table 4-9. Baseline Clinical Routine Screening Haematology/Biochemistry vs. MetS Marker Count, Simple and Partial Correlations

<table>
<thead>
<tr>
<th>CRSHaem&amp; Biochem Type</th>
<th>Inflammatory measures</th>
<th>Waist cm</th>
<th>SBP mmHg</th>
<th>DBP mmHg</th>
<th>HDL-C mmol/L</th>
<th>TG mmol/L</th>
<th>FPG mmol/L</th>
<th>MetSMCt</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>( r )</td>
<td>( p \text{-value} )</td>
<td>( r )</td>
<td>( p \text{-value} )</td>
<td>( r )</td>
<td>( p \text{-value} )</td>
<td>( r )</td>
</tr>
<tr>
<td>Neutrophils, ( x10^9/L )</td>
<td>Pearson</td>
<td>0.14</td>
<td>0.03</td>
<td>0.03</td>
<td>0.69</td>
<td>-0.01</td>
<td>0.89</td>
<td>-0.25</td>
</tr>
<tr>
<td></td>
<td>Partial, Infl</td>
<td>-0.08</td>
<td>0.26</td>
<td>-0.01</td>
<td>0.91</td>
<td>0.00</td>
<td>0.98</td>
<td>-0.08</td>
</tr>
<tr>
<td></td>
<td>Partial, All</td>
<td>-0.12</td>
<td>0.14</td>
<td>-0.08</td>
<td>0.33</td>
<td>-0.08</td>
<td>0.33</td>
<td>-0.09</td>
</tr>
<tr>
<td>Eosinophils, ( x10^9/L )</td>
<td>Pearson</td>
<td>0.12</td>
<td>0.08</td>
<td>0.02</td>
<td>0.77</td>
<td>0.06</td>
<td>0.37</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>Partial, Infl</td>
<td>0.09</td>
<td>0.21</td>
<td>-0.01</td>
<td>0.86</td>
<td>0.02</td>
<td>0.76</td>
<td>-0.02</td>
</tr>
<tr>
<td></td>
<td>Partial, All</td>
<td>0.17</td>
<td>0.04</td>
<td>-0.04</td>
<td>0.60</td>
<td>-0.03</td>
<td>0.71</td>
<td>0.10</td>
</tr>
<tr>
<td>Monocytes, ( x10^9/L )</td>
<td>Pearson</td>
<td>0.13</td>
<td>0.06</td>
<td>0.12</td>
<td>0.08</td>
<td>0.03</td>
<td>0.65</td>
<td>-0.17</td>
</tr>
<tr>
<td></td>
<td>Partial, Infl</td>
<td>0.10</td>
<td>0.16</td>
<td>0.07</td>
<td>0.36</td>
<td>-0.03</td>
<td>0.63</td>
<td>-0.12</td>
</tr>
<tr>
<td></td>
<td>Partial, All</td>
<td>0.10</td>
<td>0.21</td>
<td>0.07</td>
<td>0.38</td>
<td>-0.04</td>
<td>0.58</td>
<td>-0.12</td>
</tr>
<tr>
<td>Lymphocytes, ( x10^9/L )</td>
<td>Pearson</td>
<td>0.18</td>
<td>0.01</td>
<td>-0.06</td>
<td>0.39</td>
<td>-0.09</td>
<td>0.20</td>
<td>-0.11</td>
</tr>
<tr>
<td></td>
<td>Partial, Infl</td>
<td>0.18</td>
<td>0.01</td>
<td>-0.04</td>
<td>0.55</td>
<td>-0.08</td>
<td>0.27</td>
<td>-0.02</td>
</tr>
<tr>
<td></td>
<td>Partial, All</td>
<td>0.14</td>
<td>0.08</td>
<td>-0.06</td>
<td>0.45</td>
<td>-0.08</td>
<td>0.29</td>
<td>-0.18</td>
</tr>
<tr>
<td>ESR, ( \text{mm/h} )</td>
<td>Pearson</td>
<td>0.10</td>
<td>0.12</td>
<td>-0.02</td>
<td>0.78</td>
<td>-0.03</td>
<td>0.64</td>
<td>-0.20</td>
</tr>
<tr>
<td></td>
<td>Partial, Infl</td>
<td>-0.11</td>
<td>0.12</td>
<td>-0.05</td>
<td>0.50</td>
<td>-0.02</td>
<td>0.81</td>
<td>-0.10</td>
</tr>
<tr>
<td></td>
<td>Partial, All</td>
<td>-0.13</td>
<td>0.10</td>
<td>-0.09</td>
<td>0.28</td>
<td>-0.06</td>
<td>0.46</td>
<td>-0.10</td>
</tr>
<tr>
<td>Albumin, ( \text{g/L} )</td>
<td>Pearson</td>
<td>-0.05</td>
<td>0.44</td>
<td>0.08</td>
<td>0.20</td>
<td>0.14</td>
<td>0.03</td>
<td>-0.01</td>
</tr>
<tr>
<td></td>
<td>Partial, Infl</td>
<td>-0.06</td>
<td>0.45</td>
<td>0.14</td>
<td>0.05</td>
<td>0.22</td>
<td>0.002</td>
<td>-0.07</td>
</tr>
<tr>
<td></td>
<td>Partial, All</td>
<td>-0.14</td>
<td>0.08</td>
<td>0.10</td>
<td>0.23</td>
<td>0.16</td>
<td>0.04</td>
<td>-0.05</td>
</tr>
<tr>
<td>Globulin, ( \text{g/L} )</td>
<td>Pearson</td>
<td>0.17</td>
<td>0.009</td>
<td>0.02</td>
<td>0.82</td>
<td>0.03</td>
<td>0.70</td>
<td>-0.17</td>
</tr>
<tr>
<td></td>
<td>Partial, Infl</td>
<td>0.17</td>
<td>0.02</td>
<td>0.07</td>
<td>0.36</td>
<td>0.11</td>
<td>0.14</td>
<td>-0.07</td>
</tr>
<tr>
<td></td>
<td>Partial, All</td>
<td>0.12</td>
<td>0.14</td>
<td>0.06</td>
<td>0.46</td>
<td>0.11</td>
<td>0.16</td>
<td>0.00</td>
</tr>
<tr>
<td>hsCRP, ( \text{mg/L} )</td>
<td>Pearson</td>
<td>0.25</td>
<td>0.001</td>
<td>0.05</td>
<td>0.41</td>
<td>-0.02</td>
<td>0.78</td>
<td>-0.14</td>
</tr>
<tr>
<td></td>
<td>Partial, Infl</td>
<td>0.27</td>
<td>0.001</td>
<td>0.13</td>
<td>0.08</td>
<td>0.03</td>
<td>0.69</td>
<td>-0.01</td>
</tr>
<tr>
<td></td>
<td>Partial, All</td>
<td>0.15</td>
<td>0.06</td>
<td>0.07</td>
<td>0.37</td>
<td>0.00</td>
<td>0.97</td>
<td>0.01</td>
</tr>
<tr>
<td>CRSHaem &amp; Biochem</td>
<td>Correlation Type</td>
<td>Waist cm</td>
<td>SBP mmHg</td>
<td>DBP mmHg</td>
<td>HDL-C mmol/L</td>
<td>TG mmol/L</td>
<td>FPG mmol/L</td>
<td>MetSMCt</td>
</tr>
<tr>
<td>-------------------</td>
<td>------------------</td>
<td>----------</td>
<td>----------</td>
<td>----------</td>
<td>--------------</td>
<td>-----------</td>
<td>------------</td>
<td>----------</td>
</tr>
<tr>
<td>Inflammatory measures</td>
<td></td>
<td>r</td>
<td>p-value</td>
<td>r</td>
<td>p-value</td>
<td>r</td>
<td>p-value</td>
<td>r</td>
</tr>
<tr>
<td>Oxidant Measures</td>
<td></td>
<td>0.37</td>
<td>&lt;0.001</td>
<td>0.22</td>
<td>0.001</td>
<td>0.15</td>
<td>0.03</td>
<td>-0.17</td>
</tr>
<tr>
<td>HbA1c, %</td>
<td>Pearson</td>
<td>0.32</td>
<td>&lt;0.001</td>
<td>0.17</td>
<td>0.02</td>
<td>0.13</td>
<td>0.07</td>
<td>-0.12</td>
</tr>
<tr>
<td>Partial, Oxi</td>
<td>Partial, All</td>
<td>0.26</td>
<td>0.0013</td>
<td>0.15</td>
<td>0.07</td>
<td>0.10</td>
<td>0.20</td>
<td>-0.12</td>
</tr>
<tr>
<td>Urate, mmol/L</td>
<td>Pearson</td>
<td>0.41</td>
<td>&lt;0.001</td>
<td>0.38</td>
<td>&lt;0.001</td>
<td>0.34</td>
<td>&lt;0.001</td>
<td>-0.23</td>
</tr>
<tr>
<td>Partial, Oxi</td>
<td>Partial, All</td>
<td>0.37</td>
<td>&lt;0.001</td>
<td>0.30</td>
<td>&lt;0.001</td>
<td>0.29</td>
<td>&lt;0.001</td>
<td>-0.22</td>
</tr>
<tr>
<td>Ferritin, μg/L</td>
<td>Pearson</td>
<td>0.41</td>
<td>&lt;0.001</td>
<td>0.30</td>
<td>&lt;0.001</td>
<td>0.33</td>
<td>&lt;0.001</td>
<td>-0.18</td>
</tr>
<tr>
<td>Partial, Oxi</td>
<td>Partial, All</td>
<td>0.22</td>
<td>&lt;0.001</td>
<td>0.17</td>
<td>0.008</td>
<td>0.15</td>
<td>0.02</td>
<td>-0.03</td>
</tr>
<tr>
<td>AlkPhos, U/L</td>
<td>Pearson</td>
<td>0.20</td>
<td>0.002</td>
<td>0.14</td>
<td>0.04</td>
<td>0.11</td>
<td>0.09</td>
<td>-0.07</td>
</tr>
<tr>
<td>Partial, Oxi</td>
<td>Partial, All</td>
<td>0.16</td>
<td>0.03</td>
<td>0.15</td>
<td>0.04</td>
<td>0.11</td>
<td>0.12</td>
<td>-0.08</td>
</tr>
<tr>
<td>ALT, U/L</td>
<td>Pearson</td>
<td>0.12</td>
<td>0.13</td>
<td>0.22</td>
<td>0.01</td>
<td>0.19</td>
<td>0.02</td>
<td>-0.04</td>
</tr>
<tr>
<td>Partial, Oxi</td>
<td>Partial, All</td>
<td>0.15</td>
<td>0.03</td>
<td>0.16</td>
<td>0.02</td>
<td>0.12</td>
<td>0.07</td>
<td>-0.14</td>
</tr>
<tr>
<td>AST, U/L</td>
<td>Pearson</td>
<td>0.08</td>
<td>0.35</td>
<td>0.08</td>
<td>0.31</td>
<td>0.11</td>
<td>0.17</td>
<td>-0.13</td>
</tr>
<tr>
<td>Partial, Oxi</td>
<td>Partial, All</td>
<td>0.16</td>
<td>0.02</td>
<td>0.13</td>
<td>0.05</td>
<td>0.05</td>
<td>0.45</td>
<td>-0.12</td>
</tr>
<tr>
<td>γGT, U/L</td>
<td>Pearson</td>
<td>-0.06</td>
<td>0.40</td>
<td>-0.04</td>
<td>0.54</td>
<td>-0.09</td>
<td>0.20</td>
<td>-0.03</td>
</tr>
<tr>
<td>Partial, Oxi</td>
<td>Partial, All</td>
<td>-0.09</td>
<td>0.26</td>
<td>-0.07</td>
<td>0.39</td>
<td>-0.12</td>
<td>0.13</td>
<td>-0.01</td>
</tr>
<tr>
<td>Partial, All</td>
<td>0.21</td>
<td>0.001</td>
<td>0.17</td>
<td>0.01</td>
<td>0.15</td>
<td>0.02</td>
<td>-0.02</td>
<td>0.74</td>
</tr>
</tbody>
</table>

Partial r are for each measure over and above the others in that group. Eg Partial, All is for both oxidant and inflammatory measures. Oxidant; Infl inflammatory; MetSMCt metabolic syndrome marker count. r correlation coefficient; Waist waist circumference; HDL-C high density lipoprotein cholesterol; TG triglyceride; FPG fasting plasma glucose; S/DBP systolic/diastolic blood pressure; HbA1c haemoglobinA1c; AlkPhos alkaline phosphatase; ALT alanine transferase; AST aspartate transferase; γGT gamma glutamyl transferase; ESR erythrocyte sedimentation rate; hsCRP high sensitive C reactive protein.
There were various modest Pearson r of the liver enzymes: AlkPhos, ALT, AST and γGT (r=0.11-0.21; p=0.09-<0.001), with TG; and ALT, AST and γGT (r=0.14-0.20; p=0.003-0.04) and acute phase proteins: CRP and ferritin (r=0.19-0.22; p=0.003-<0.001), and leukocytes: neutrophils (r=0.14-0.23; p=0.03), lymphocytes and eosinophils (r=0.14-0.17; p=0.01-0.08), with MetS marker, predominantly waist, and/or MetS marker count.

However, for these CRSHaem&Biochem, almost all relationships reduced considerably or disappeared after adjusting for the other CRSHaem&Biochem. CRP showed a possible weak independent relationship with waist (r=0.15; p=0.06). ALT and lymphocytes showed a possible negative independent relationship with HDL-C. Other minor relationships are shown in Table 4-9.

**Longitudinal Multivariable Relationships**

**Demographic, Health Status, and Lifestyle Relationships**

Age was highly significantly related to mean MetS marker count (p<0.001), and S/DBP (p=0.001), lipids: HDL-C and TG (p=0.02), but not waist or FPG. Female gender was negatively related to waist and HDL-C (p=0.001 for both) (Table 4-10). A higher education level (p=0.06), indicated a borderline likelihood of a negative relationship with MetS marker count, but not any MetS marker. Alcohol intake of ≥14 units, or ≥280g/wk showed a significant negative relationship with HDL-C (p=0.03), but a positive relationship with TG (p=0.01).

There was a likelihood that ever having smoked was directly related to waist (p=0.02). Numbers of cigarettes/d, however, was significant negatively related to waist (p=0.004) (Table 4-10). Whilst there was weak evidence that acute and/or chronic inflammatory disease was related directly to HDL-C, there was also a weak relationship with TG (p=0.07) No anti-inflammatory medication had effects on any MetS marker or MetS marker count.

Medication with anti-inflammatory effects were positively related to HDL-C (p=0.03) and negatively to FPG (p=0.01).

Chitosan was negatively related: to waist (p=0.03), to SPB (p=0.08, trend) and to FPG (p=0.05).
Chapter 4. Novel Cardiovascular Disease Risk Markers in the Metabolic Syndrome

Mean and Change with Metabolic Syndrome Markers and Count

There was strong evidence of association of the overall mean HbA1c (p=0.001), and urate (p=0.002), with waist, but less evidence for bilirubin (p=0.05), and evidence for overall mean hsCRP, lymphocytes, and eosinophils, all three weak, associating with waist. There was evidence for albumin, negative, associating with waist, but change in none of these variables, could be shown to be related to waist (Table 4-11 & Table 4-12).

There was strong evidence of associations of overall mean urate (p=0.001), and ALT (p=0.003), with SBP, and weaker evidence of mean ferritin (p=0.04), negatively and HbA1c (p=0.06) relating to SBP. Change in AST (p=0.04), CRP (p=0.05), bilirubin (p=0.001), negatively, urate (p=0.03), negatively and possibly γGT (p=0.07), were also associated with SBP (Table 4-11 & Table 4-12).

There was strong evidence of overall mean urate (p=0.001), and ALT (p=0.001), being related to DBP, and ferritin (p=0.003), and AST (p=0.04), both negatively being related to DBP. Change in bilirubin (p=0.05), and ferritin (p=0.03), both negatively, were associated with DBP, in addition (Table 4-11 & Table 4-12). There was evidence of mean γGT (p=0.01), and ESR (p=0.03), negatively, and possibly urate (p=0.07), negatively, relating to HDL-C, and also evidence of the change in albumin (p=0.04), neutrophils (p=0.05), and AlkPhos (p=0.09), weakly, being associated with change in HDL-C (Table 4-11 & Table 4-12).

There was evidence that the overall mean concentrations of AST (p=0.05), albumin (p=0.03), lymphocytes (p=0.04), and bilirubin (p=0.002), strong and negative, and AlkPhos (p=0.0), negative, were associated with TG level, while the change in γGT (p=0.03), and globulin (p=0.02), was also associated with change in TG (Table 4-11 & Table 4-12).

There was extremely strong evidence that HbA1c (p<0.001), was associated with FPG, and there was some evidence of overall mean concentrations of ferritin (p=0.06), and AST (p=0.09), weakly, and change in γGT (p=0.08), weakly and negatively, associated with change of FPG.
### Table 4-10. Relationships of MetS Markers and MetS Marker Count with Demographic, Health Status and Lifestyle Parameters

<table>
<thead>
<tr>
<th>Explanatory variables</th>
<th>Waist</th>
<th>SBP</th>
<th>DBP</th>
<th>HDL-C</th>
<th>TG</th>
<th>FPG</th>
<th>MetS MCt</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Explanatory variables</strong></td>
<td>p-value</td>
<td>p-value</td>
<td>p-value</td>
<td>p-value</td>
<td>p-value</td>
<td>p-value</td>
<td>p-value</td>
</tr>
<tr>
<td><strong>Demography, Lifestyle, Illness, Treatment</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, y</td>
<td>0.03</td>
<td>0.70</td>
<td>0.72</td>
<td>&lt;0.001</td>
<td>0.27</td>
<td>&lt;0.001</td>
<td>0.005</td>
</tr>
<tr>
<td>Gender (female)</td>
<td>-9.34</td>
<td>&lt;0.001</td>
<td>4.62</td>
<td>0.23</td>
<td>-0.34</td>
<td>0.86</td>
<td>0.30</td>
</tr>
<tr>
<td>Edu. Level &gt; 2nd school</td>
<td>-0.97</td>
<td>0.51</td>
<td>0.80</td>
<td>0.72</td>
<td>-0.06</td>
<td>0.96</td>
<td>-0.00</td>
</tr>
<tr>
<td>Alcohol, ≥ 14 Units/wk</td>
<td>-3.32</td>
<td>0.45</td>
<td>-5.49</td>
<td>0.41</td>
<td>-6.14</td>
<td>0.06</td>
<td>-0.28</td>
</tr>
<tr>
<td>Ever smoked cig.</td>
<td>3.84</td>
<td>0.02</td>
<td>1.89</td>
<td>0.42</td>
<td>0.29</td>
<td>0.80</td>
<td>-0.01</td>
</tr>
<tr>
<td>Number cig. smoked/d</td>
<td>-0.79</td>
<td>0.004</td>
<td>-0.41</td>
<td>0.31</td>
<td>-0.20</td>
<td>0.31</td>
<td>0.01</td>
</tr>
<tr>
<td>Inflam. disease acute/chronic</td>
<td>-0.96</td>
<td>0.46</td>
<td>2.02</td>
<td>0.46</td>
<td>2.01</td>
<td>0.16</td>
<td>0.08</td>
</tr>
<tr>
<td>Anti-inflam. Med.</td>
<td>1.00</td>
<td>0.56</td>
<td>1.24</td>
<td>0.67</td>
<td>0.28</td>
<td>0.85</td>
<td>0.09</td>
</tr>
<tr>
<td>Anti-inflam. Med. effects</td>
<td>-0.88</td>
<td>0.49</td>
<td>0.87</td>
<td>0.73</td>
<td>0.03</td>
<td>0.98</td>
<td>0.09</td>
</tr>
<tr>
<td>Chitosan treatment,</td>
<td>-3.28</td>
<td>0.03</td>
<td>-3.82</td>
<td>0.08</td>
<td>-0.88</td>
<td>0.40</td>
<td>-0.04</td>
</tr>
</tbody>
</table>

MetS metabolic syndrome; MetSMCt metabolic syndrome marker count; Waist waist circumference; S/DBP systolic/diastolic blood pressure; HDL-C high density lipoprotein cholesterol; TG triglyceride; FPG fasting plasma glucose; y years; wk week; d day; edu education; inflam inflammatory; med medication.
Table 4-11. Relationships of MetS Markers and MetS Marker Count vs. Clinical Routine Screening Haematology/Biochemistry – Inflammatory

<table>
<thead>
<tr>
<th>Explanatory variables</th>
<th>CRSHaem/Biochem</th>
<th>Waist p-value</th>
<th>SBP p-value</th>
<th>DBP p-value</th>
<th>HDL-C p-value</th>
<th>TG p-value</th>
<th>FPG p-value</th>
<th>δMetS Mct p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>m Neutrophils</td>
<td>0.37</td>
<td>0.63</td>
<td>-0.05</td>
<td>0.97</td>
<td>-0.03</td>
<td>0.95</td>
<td>-0.03</td>
<td>0.12</td>
</tr>
<tr>
<td>δ Neutrophils</td>
<td>-0.50</td>
<td>0.54</td>
<td>-3.09</td>
<td>0.14</td>
<td>-1.01</td>
<td>0.39</td>
<td>0.06</td>
<td>0.05</td>
</tr>
<tr>
<td>m Eosinophils</td>
<td>12.85</td>
<td>0.06</td>
<td>-5.75</td>
<td>0.58</td>
<td>-0.45</td>
<td>0.93</td>
<td>0.05</td>
<td>0.80</td>
</tr>
<tr>
<td>δ Eosinophils</td>
<td>10.35</td>
<td>0.19</td>
<td>5.20</td>
<td>0.79</td>
<td>-17.15</td>
<td>0.14</td>
<td>-0.42</td>
<td>0.14</td>
</tr>
<tr>
<td>m Monocytes</td>
<td>1.91</td>
<td>0.83</td>
<td>9.88</td>
<td>0.46</td>
<td>-4.83</td>
<td>0.46</td>
<td>-0.05</td>
<td>0.85</td>
</tr>
<tr>
<td>δ Monocytes</td>
<td>-0.29</td>
<td>0.97</td>
<td>10.70</td>
<td>0.63</td>
<td>-15.00</td>
<td>0.24</td>
<td>0.03</td>
<td>0.92</td>
</tr>
<tr>
<td>m Lymphocytes</td>
<td>2.90</td>
<td>0.06</td>
<td>-0.06</td>
<td>0.98</td>
<td>0.04</td>
<td>0.97</td>
<td>-0.04</td>
<td>0.34</td>
</tr>
<tr>
<td>δ Lymphocytes</td>
<td>0.41</td>
<td>0.81</td>
<td>1.01</td>
<td>0.82</td>
<td>1.92</td>
<td>0.46</td>
<td>0.04</td>
<td>0.55</td>
</tr>
<tr>
<td>m ESR</td>
<td>0.05</td>
<td>0.54</td>
<td>0.05</td>
<td>0.64</td>
<td>0.06</td>
<td>0.31</td>
<td>-0.005</td>
<td>0.03</td>
</tr>
<tr>
<td>δ ESR</td>
<td>-0.01</td>
<td>0.80</td>
<td>0.02</td>
<td>0.87</td>
<td>0.01</td>
<td>0.91</td>
<td>-0.001</td>
<td>0.97</td>
</tr>
<tr>
<td>m Albumin</td>
<td>-0.97</td>
<td>0.01</td>
<td>0.35</td>
<td>0.55</td>
<td>0.37</td>
<td>0.20</td>
<td>-0.01</td>
<td>0.63</td>
</tr>
<tr>
<td>δ Albumin</td>
<td>-0.06</td>
<td>0.88</td>
<td>0.62</td>
<td>0.55</td>
<td>1.26</td>
<td>0.03</td>
<td>0.03</td>
<td>0.04</td>
</tr>
<tr>
<td>m Globulin</td>
<td>0.22</td>
<td>0.44</td>
<td>0.19</td>
<td>0.65</td>
<td>0.24</td>
<td>0.26</td>
<td>-0.004</td>
<td>0.63</td>
</tr>
<tr>
<td>δ Globulin</td>
<td>-0.01</td>
<td>0.98</td>
<td>1.25</td>
<td>0.21</td>
<td>-0.26</td>
<td>0.65</td>
<td>-0.003</td>
<td>0.83</td>
</tr>
<tr>
<td>m hsCRP</td>
<td>0.37</td>
<td>0.06</td>
<td>0.01</td>
<td>0.98</td>
<td>-0.11</td>
<td>0.44</td>
<td>-0.003</td>
<td>0.57</td>
</tr>
<tr>
<td>δ hsCRP</td>
<td>0.14</td>
<td>0.40</td>
<td>0.85</td>
<td>0.05</td>
<td>0.23</td>
<td>0.37</td>
<td>-0.003</td>
<td>0.66</td>
</tr>
</tbody>
</table>

MetS metabolic syndrome; MetSM metabolic syndrome marker; MetSMCt metabolic syndrome marker count; Waist waist circumference; S/DBP systolic/diastolic blood pressure; HDL-C high density lipoprotein cholesterol; TG triglyceride; FPG fasting plasma glucose; m mean (overall level), δ change (deviation from the mean of the (2) repeated measures); ESR erythrocyte sedimentation rate; hsCRP high sensitive C reactive protein. In both the general linear mixed model and ordinal logistic regression (or generalised linear mixed mode) analysis method for MetSM and MetSMCt, the estimates were multiply transformed, so are not intuitively interpretable. m mean (mean of baseline and 6m values), δ change (deviation from the mean of the (2) repeated measures).
### Table 4-12. Relationships of MetS Markers and MetS Marker Count vs. Clinical Routine Screening Haematology/Biochemistry – Oxidant

<table>
<thead>
<tr>
<th>Explanatory variables</th>
<th>Waist</th>
<th>P-value</th>
<th>SBP</th>
<th>P-value</th>
<th>DBP</th>
<th>P-value</th>
<th>HDL-C</th>
<th>P-value</th>
<th>TG</th>
<th>P-value</th>
<th>FPG</th>
<th>P-value</th>
<th>1^\text{MetSMCt}</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CRSHaem/Biochem</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>m HbA1c</td>
<td>3.45</td>
<td>0.001</td>
<td>2.90</td>
<td>0.06</td>
<td>0.89</td>
<td>0.26</td>
<td>-0.04</td>
<td>0.22</td>
<td>0.08</td>
<td>0.29</td>
<td>1.55</td>
<td>&lt;0.001</td>
<td>1.47</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>δ HbA1c</td>
<td>-1.43</td>
<td>0.40</td>
<td>-3.09</td>
<td>0.50</td>
<td>-2.27</td>
<td>0.40</td>
<td>0.01</td>
<td>0.81</td>
<td>-0.05</td>
<td>0.79</td>
<td>0.13</td>
<td>0.53</td>
<td>0.21</td>
<td>0.17</td>
</tr>
<tr>
<td>m Urate</td>
<td>39.93</td>
<td>0.002</td>
<td>77.65</td>
<td>&lt;0.001</td>
<td>41.95</td>
<td>0.07</td>
<td>0.99</td>
<td>0.24</td>
<td>-0.54</td>
<td>0.49</td>
<td>0.92</td>
<td>0.03</td>
<td>0.17</td>
<td>0.32</td>
</tr>
<tr>
<td>δ Urate</td>
<td>-18.41</td>
<td>0.20</td>
<td>-83.09</td>
<td>0.03</td>
<td>-6.80</td>
<td>0.75</td>
<td>0.40</td>
<td>0.44</td>
<td>0.73</td>
<td>0.66</td>
<td>-0.18</td>
<td>0.91</td>
<td>-0.17</td>
<td>0.32</td>
</tr>
<tr>
<td>m Ferritin</td>
<td>-0.02</td>
<td>0.11</td>
<td>-0.03</td>
<td>0.04</td>
<td>-0.02</td>
<td>0.003</td>
<td>&lt;0.001</td>
<td>0.65</td>
<td>0.001</td>
<td>0.10</td>
<td>0.001</td>
<td>0.06</td>
<td>-0.44</td>
<td>0.12</td>
</tr>
<tr>
<td>δ Ferritin</td>
<td>0.02</td>
<td>0.28</td>
<td>-0.05</td>
<td>0.34</td>
<td>-0.06</td>
<td>0.03</td>
<td>&lt;0.001</td>
<td>0.27</td>
<td>0.001</td>
<td>0.53</td>
<td>&lt;0.001</td>
<td>0.72</td>
<td>0.27</td>
<td>0.12</td>
</tr>
<tr>
<td>m Uric acid</td>
<td>0.35</td>
<td>0.05</td>
<td>-0.15</td>
<td>0.58</td>
<td>-0.05</td>
<td>0.70</td>
<td>&lt;0.001</td>
<td>0.84</td>
<td>-0.04</td>
<td>0.002</td>
<td>-0.01</td>
<td>0.44</td>
<td>0.36</td>
<td>0.19</td>
</tr>
<tr>
<td>δ Uric acid</td>
<td>0.17</td>
<td>0.20</td>
<td>-1.14</td>
<td>0.001</td>
<td>-0.39</td>
<td>0.05</td>
<td>&lt;0.001</td>
<td>0.98</td>
<td>-0.01</td>
<td>0.48</td>
<td>0.003</td>
<td>0.85</td>
<td>-0.17</td>
<td>0.33</td>
</tr>
<tr>
<td>m AlkPhos</td>
<td>0.03</td>
<td>0.36</td>
<td>0.04</td>
<td>0.38</td>
<td>0.02</td>
<td>0.30</td>
<td>&lt;0.001</td>
<td>0.34</td>
<td>0.004</td>
<td>0.09</td>
<td>&lt;0.001</td>
<td>0.87</td>
<td>0.19</td>
<td>0.46</td>
</tr>
<tr>
<td>δ AlkPhos</td>
<td>-0.07</td>
<td>0.26</td>
<td>-0.14</td>
<td>0.38</td>
<td>0.12</td>
<td>0.20</td>
<td>0.004</td>
<td>0.09</td>
<td>-0.01</td>
<td>0.10</td>
<td>0.005</td>
<td>0.52</td>
<td>-0.23</td>
<td>0.15</td>
</tr>
<tr>
<td>m ALT</td>
<td>0.16</td>
<td>0.20</td>
<td>0.54</td>
<td>0.003</td>
<td>0.30</td>
<td>&lt;0.001</td>
<td>-0.004</td>
<td>0.23</td>
<td>-0.003</td>
<td>0.77</td>
<td>&lt;0.001</td>
<td>0.96</td>
<td>0.59</td>
<td>0.12</td>
</tr>
<tr>
<td>δ ALT</td>
<td>-0.04</td>
<td>0.66</td>
<td>0.06</td>
<td>0.78</td>
<td>0.05</td>
<td>0.70</td>
<td>-0.002</td>
<td>0.57</td>
<td>&lt;0.001</td>
<td>0.92</td>
<td>0.002</td>
<td>0.82</td>
<td>0.09</td>
<td>0.56</td>
</tr>
<tr>
<td>m AST</td>
<td>-0.14</td>
<td>0.16</td>
<td>-0.23</td>
<td>0.11</td>
<td>-0.15</td>
<td>0.04</td>
<td>&lt;0.001</td>
<td>0.88</td>
<td>0.01</td>
<td>0.05</td>
<td>0.01</td>
<td>0.09</td>
<td>-0.30</td>
<td>0.58</td>
</tr>
<tr>
<td>δ AST</td>
<td>0.05</td>
<td>0.67</td>
<td>0.64</td>
<td>0.04</td>
<td>-0.09</td>
<td>0.62</td>
<td>-0.005</td>
<td>0.29</td>
<td>0.02</td>
<td>0.14</td>
<td>0.01</td>
<td>0.70</td>
<td>0.34</td>
<td>0.11</td>
</tr>
<tr>
<td>m γGT</td>
<td>0.02</td>
<td>0.61</td>
<td>-0.01</td>
<td>0.78</td>
<td>-0.03</td>
<td>0.25</td>
<td>0.003</td>
<td>0.01</td>
<td>&lt;0.001</td>
<td>0.97</td>
<td>-0.003</td>
<td>0.11</td>
<td>-0.14</td>
<td>0.63</td>
</tr>
<tr>
<td>δ γGT</td>
<td>0.01</td>
<td>0.86</td>
<td>0.36</td>
<td>0.07</td>
<td>0.05</td>
<td>0.67</td>
<td>-0.002</td>
<td>0.46</td>
<td>0.02</td>
<td>0.03</td>
<td>&lt;0.002</td>
<td>0.08</td>
<td>0.38</td>
<td>0.06</td>
</tr>
</tbody>
</table>

δ: change (deviation from the mean of the (2) repeated measures) MetS: metabolic syndrome; MetSMCt: metabolic syndrome marker count; waist: waist circumference; S/DBP: systolic/diastolic blood pressure; HDL-C: high density lipoprotein cholesterol; TG: triglyceride; FPG: fasting plasma glucose; m: mean (overall level); HbA1c: Haemoglobin A1c; AlkPhos: alkaline phosphatase; ALT: alanine transferase; AST: aspartate transferase; γGT: gamma glutamyl transferase. 1^\text{In the generalised linear mixed model, ordinal logistic regression method for MetSMCt the estimates were multiply transformed, so were not intuitively interpretable.}
There was evidence that the mean concentrations of HbA₁c (p<0.001), urate (p=0.003), and ESR (p=0.06), and neutrophils (p=0.09), weakly, were positively associated with MetS marker count, while change in γGT (p=0.06), and ESR (p=0.09), both weak, were positively associated with change in MetS marker count (Table 4-11 & Table 4-12).

4.4 Discussion

Main Findings: HbA₁c and Urate, Cross-Sectionally; γGT and ESR, Longitudinally

The most notable findings in the present study of overweight and obese participants were that the oxidant markers, HbA₁c and urate, were more highly related to MetS or MetS marker count than the inflammatory markers, such as CRP and other acute phase proteins, albumin and globulin. The diabetes monitoring marker, HbA₁c, and gout marker, urate, were highly related to IDF/JIS MetS marker count on Pearson and partial r, and overall mean (baseline to 6m). The neutrophils were also highly correlated using Pearson r, but exhibited no partial r, and had a borderline relationship of overall mean with IDF/JIS MetS marker count.

With respect to the longitudinal change over the study, change in γGT and ESR showed a relationship of borderline significance to change in MetS marker count, however this change was over and above all the demographic and CRSHaem/Biochem relationships, and thus could be interpreted as important. This mixed oxidant, γGT, and inflammatory, ESR, result appears to show a merging of pathologies.

When the overall mean of HbA₁c, and urate were examined with the other MetS marker variables in the mixed model, they again proved to be highly significantly related to waist, HbA₁c to FPG and urate to BP, and modestly to most of the IDF/JIS MetS markers. Other relationships with change in γGT is also related to change in SBP, and negatively to change in TG and to FPG.

These relationships were much stronger than, and overwhelmed, the classic inflammatory markers, notably CRP; and the neutrophil overall mean of the baseline and 6m was highly significantly related to MetS marker count. In addition ESR, and neutrophils, the most oxidant of the leukocytes due to their oxidative burst activity on pathogen marker stimulation, also showed a relationship to MetS marker count. These results were
hypothesised but it was not certain whether inflammatory or oxidant markers would be more highly related.

The most important overall relationship was that of the change in $\gamma$GT and ESR being related to the change over the study in MetS marker count. The results of the analysis of change between baseline and 6m of the CRSHaem&Biochem and IDF/JIS MetS marker and MetS marker count were also not predicted. With the same explanatory variables included as for the mean, the change relationships shown were apparently weaker, but of different in quality. A weaker relationship is to be expected with the added time variable. These are important changes as they are tracking and changing with the altering metabolism associated with either weight gain and positive energy balance or weight loss and negative energy balance. $\gamma$GT and ESR are related to MetS marker count index and thus to MetS over and above the other CRSHaem&Biochem.

The implications are that these CRSHaem&Biochem are also likely markers that should be studied in larger clinical trials as they may give valuable information on human health over time, together with MetS or MetS marker count.

Other contenders that could be used to aid in the prediction of CVD risk may be the highly significantly negative changes in bilirubin with changes in S/DBP which were shown, and may relate to bilirubin being a potent antioxidant. The change in ferritin and albumin, which were negatively related to change in DBP, could be expected from albumin which can have antioxidant effects, but is more surprising from ferritin which is usually seen as pro-oxidant. However, Ferritin can have significant indirect antioxidant effects by absorbing large amounts of reactive iron $^{281}$. 

*Variable Anthropometry*

These results were produced in spite of the high variability of anthropometric measurements in this study whereby weight and waist changed in both positive and negative directions, and approximately 2/3 of participants lost a very modest amount of weight and waist by 6m. There was no change relationship with waist and any of the CRSHaem&Biochem
Demographic, Health Status, Lifestyle and Quality of Life

The above could be seen in the context of this population, which appears typical of NZ adults, although it must be noted that the participants were not chosen at random, and have particular characteristics that kept them in the study.

Age had by far the strongest effect on MetS marker count over and above all else, via BP and lipids with HDL-C having a negative effect. Interestingly, age was not related to either FPG or waist. Female gender, as was as expected, had a significant negative relationship on waist and HDL-C. Low education level was borderline significantly related to MetS marker count, but with no specific relationship to any MetS marker. It is known that individuals from lower socioeconomic groups are more likely to have higher intakes of processed food and less healthy diets.

Current cigarette smoking related to a lower waist, and smoking is still used to keep weight down. However, smoking cessation can lead to weight gain and may explain why past smoking related to a large waist. Greater than 2 standard drinks/d related to higher TG, probably through alcoholic related fatty liver. The trend of lower DBP with higher alcohol consumption was hard to explain, but BP did increase over the study. Metabolic medication with the well known hypoglycaemic, hypolipidaemic and hypotensive and possible anti-inflammatory and antioxidant effects were related negatively to FPG, with a trend to being related to TG and HDL-C, negatively. This would be expected of a medication designed to treat diabetes and dyslipidaemia. However, medication for hypertension appeared to make no difference, and BP in MetS is hard to control.

Anti-inflammatory drugs had no effects. Inflammatory disease was related to a positive trend in HDL-C and negatively to TG, but whether this was a medication effect was not known. Surprisingly, the chitosan treatment was negatively related to waist, SBP (trend) and to FPG, and thus its efficacy for MetS may be greater than intimated in Chapter 3. In the current study of weight loss, European, mid-aged, middle class women predominated. The men who were enrolled had significantly more CVD risk factors such as higher alcohol and saturated fat intake, reported and measured overweight, hypertension, TIIDM and sleep apnoea and medication prescriptions for BP, lipids and TIIDM than the women. The data show that the most common were obesity-related CVD or risk factors, particularly TIIDM. Men who are centrally overweight are rarely healthy. Maori women joined the study at rates that were roughly equivalent to the population.
proportion (14-15% in 2006\textsuperscript{597}), although Asians did not, probably due to entry criteria of BMI excluding smaller sized ethnic groups\textsuperscript{12}. PA may have been less than reported as over-reporting is common\textsuperscript{598} and there may have been under-reporting of television viewing\textsuperscript{599}. In this overweight and obese group the mean energy intake recorded is close to that predicted for a slim population at approximately 8MJ/d for women and 10MJ/d for men\textsuperscript{600}, as compared with the 10.5MJ/d and 14.2MJ/d in an overweight/obese group respectively (mean BMI 29-30kg/m\textsuperscript{2}), whose energy intake was observed and measured by doubly labelled water\textsuperscript{68}. Restrained obese women and men may under-report food intake by up to a third\textsuperscript{601-605}. Alcohol intake was notable in that it was infrequent and in low volumes in the women, but may have been at a problematic level for the men’s CVD risk profile.

**IDF/JIS Metabolic Syndrome**

The IDF/JIS MetS that was compared and finally chosen was shown to be appropriate. In this study, as predicted from the literature review in Chapter 1, groups of less centrally obese but older less healthy individuals were classified with MetS in addition to the younger centrally obese. Interestingly, however, Maori could have a very large waist before other MetS marker numbers increased. Maori are prone to raised urate, and early Type 2 Diabetes with obesity\textsuperscript{606}. More work needs to be undertaken on ascertaining a waist cut-point in Maori and Pacific peoples. MetS appears useful as a screen to which other markers can be added.

**Metabolic Syndrome Markers**

Waist relationships with the CRSHaem&Biochem were mostly stronger with the oxidant markers rather than inflammatory markers. CRP was highly correlated to waist and it has been shown to be secreted by adipose tissue in obesity\textsuperscript{382}. Surprisingly, in this population there were no change relationships with waist and MetS marker count. It may be that central obesity itself is a marker of a deeper issue, and this will be addressed in Chapter 6. S/DBP were related to oxidant markers, overall more strongly to urate and ALT, and negatively to ferritin, but with negative changes in bilirubin. Using this statistical mixed model, some negative changes can be seen if there are strong interactions between CRSHaem&Biochem, as are present with ALT, AST, \(\gamma\)GT, and possibly ferritin. Change in SBP was also related to CRP, the only inflammatory CRSHaem&Biochem.
With respect to the lipids, as HDL-C is related to inflammation, it could be expected that HDL-C would relate negatively to ESR and directly to bilirubin, and direct changes in albumin and neutrophils were seen. The direct changes in albumin and neutrophil relationship with HDL-C was not expected, and may relate to alteration of HDL-C in inflammation. TG were strongly negatively related to bilirubin, compared to HDL-C, but probably related by fat accumulation in the liver related to AST and negatively to AlkPhos, again probably negatively due to the close relationship of AST and AlkPhos in the liver, and the way the mixed model handles them. TG also related to albumin, on which it is carried and lymphocytes, with changes in γGT and globulin. The latter two CRSHAem&Biochem are probably changing with AST and albumin.

FPG was only related to oxidant liver proteins, ferritin and the transferases, in addition to HbA1c, also an oxidant. Overall, relationships with MetS marker count were probably the most important and again oxidants; HbA1c, urate, and neutrophils were related and ESR, a mixed oxidant/inflammatory marker was directly related to MetS marker count.

**Details on the Main Clinical Routine Screening Haematology and Biochemistry**

HbA1c is related to raised FPG over approximately 3-12wk preceding testing and AGE and their associated oxidative stress. NHANES data shows that HbA1c predicts FPG well into the normal range, showing a continuous relationship. Interestingly, beneficial mechanisms of the antidiabetic medication, metformin, may include serum antioxidant preserver and anti-LDL glycation activity rather than anti-inflammatory action.

The relationship of urate, hypertension and MetS has been controversial and often ignored. Whilst urate may act as a powerful antioxidant itself at normal levels in the serum, its synthesis requires catalysis by the potent pro-oxidant xanthine oxidoreductase (XOR). This enzyme is induced excessively in central obesity and is also a regulator of adipogenesis. XOR is known to lead to damaging per-oxynitrite production at the arterial endothelium and increased BP. Uric acid has a role in innate immunity, and especially the activation of dendritic cells and antigen presenting cells to endogenous antigens, and this includes tumour cells.

The mechanism of γGT becoming a sensitive oxidant marker may be that it catalyses reduced glutathione (GSH) conjugation with xenobiotics, and high concentrations of γGT
deplete GSH\textsuperscript{87}. Excess native oxidised and xenobiotic reactive oxygen species (ROS) produce chemicals, examples of which are homocysteine\textsuperscript{621} and highly fat soluble persistent organic pollutants\textsuperscript{87} which when ingested and/or inhaled, impose oxidative stress. γGT concentrations can increase within the normal weight range, prior to obvious central obesity, MetS and TIIDM, and are measurable using classic markers\textsuperscript{370,622-624}. Suggestions have already been made that liver enzymes, particularly γGT, be used to fine tune CVD risk prediction\textsuperscript{374}.

The acute phase proteins that impede erythrocyte sedimentation and increase ESR are usually inflammatory and include Ig’s, CRP, fibrin and glycol-oxidised plasma proteins which increase in number and activation\textsuperscript{378,625,626}. Additionally, as “enhanced inflammation-sensitive agents”\textsuperscript{627} such proteins may interact with the inflammatory cells, various leukocytes, and augment erythrocyte adhesion/ aggregation\textsuperscript{628-630}. The RBC deformability decreases and rheology increases in obesity and MetS and normalises with weight loss\textsuperscript{349,351}.

Recently, tissue AlkPhos has been isolated from pre-adipocytes and may form a significant proportion of total serum AlkPhos\textsuperscript{361}. Intestinal AlkPhos isoenzymes are involved in chylomicron formation, FA metabolism, influence CHO levels, and thus are more likely to have oxidant rather than inflammatory properties. Ferritin predicts TIIDM development and was associated with γGT in the European Prospective Investigation of Cancer (EPIC) study\textsuperscript{356}. The liver in obesity appears to suffer metabolic derangements of iron trafficking per se\textsuperscript{631}. However, it may be a protective protein, as a non enzymatic Phase II enzyme in healthy slim individuals\textsuperscript{281}. Bilirubin, reduced glutathione and possibly urate, have complementary antioxidant roles, all conferring protection on cells from oxidative stress. Interestingly, Gilbert syndrome is protective of CVD, and may be more protective than HDL-C\textsuperscript{632}.

Leukocyte count is increased in MetS and other CVD risk settings\textsuperscript{633} The link between CRP and leukocytes may be related to adipose tissue mass\textsuperscript{634}. The relationship of leukocytes to waist is thought by many to indicate that central adipose tissue drives the inflammatory aspect of Mets\textsuperscript{635,636} although other researchers have favoured the theory that insulin resistance drives weight gain\textsuperscript{637,638}. Others do not discount that the innate immune system itself could be a contributing, enabling or even a primary driving factor\textsuperscript{639,640}.
Neutrophils undergo reactions of an acute-phase immune response with increased myeloperoxidase activity and myeloperoxidase is pro-oxidant\textsuperscript{641-643}. Endothelial adhesion can be initiated by oxidative stress associated with oxidised LDL-C and hyperglycaemia. In turn endothelial oxidative stress\textsuperscript{644} raises BP\textsuperscript{645}, in conjunction with many other MetS-related dysfunctional pathways.

Monocytes, circulating in the blood, can transform into macrophages in tissue\textsuperscript{646} and activated, but functionally impaired, lipid-congested macrophages, foam cells and central adipocytes share, and can interchange\textsuperscript{326} many functional and morphological traits\textsuperscript{338}. Additionally, this activated adipose tissue seems to provide an environment where macrophages develop dynamic controlling actions over other immune cells. Lymphocyte T-cells accumulate in adipose tissue, along with macrophages and up-regulate cytokine production in obesity\textsuperscript{647}. T-cells may contain levels of human urotensin II a potent vasoconstrictor that may have a role in hypertension and atherosclerosis\textsuperscript{648}.

**Oxidative Stress and Metaflammation**

HbA\textsubscript{1c}, urate, γGT, ESR and the neutrophils the CRSHAem&Biochem most strongly correlated with MetS in the current study, appear to be directly or indirectly associated with oxidative stress. With a relative excess intake of energy, oxidative stress is thought to arise from disordered macronutrient and micronutrient metabolism, and high, but poorly controlled visceral energy flux, probably preceding insulin resistance and obesity\textsuperscript{304,649,650}. Lipid-stuffed, hypoxic\textsuperscript{651} and dysfunctional adipocytes\textsuperscript{652} may accidentally ‘mal-activate’ the inflammatory system after priming by MetS-associated oxidative stress.

Although the proportion of participants with IDF/JIS MetS was less than half in this study, the increased numbers of participants with higher IDF/JIS MetS marker count, compared with NCEP MetS marker count was due to the combination of lowered cut-off levels for waist and FPG and the inclusion of those on cardiometabolic treatment.

These results need corroboration. The utility of having chosen these CRSHAem&Biochem laboratory tests is that most of them are first line screening tests generally, as well as for MetS, TIIDM, CVD, gout and cancer. They are routinely performed in primary care, and if general practices belong to groups such as primary healthcare organisations, can be pooled centrally. In countries such as NZ there is a free, annual ‘Diabetes Get Checked’
programme to monitor diabetes treatment. All clinical anthropometry, laboratory test results and prescriptions are pooled from yearly checks. One region, Wellington, recently has reported their results spanning 5y in TIIDM general practice patients using a mixed model analysis\textsuperscript{653}. The ADA advocates such practice\textsuperscript{654}. All the tests reported in this chapter, except the bioimpedance which is not routinely measured, could be pooled and analysed. MetS index can easily be calculated from the 5 markers. In this manner the current study could be corroborated using large data sets of patients with central obesity and MetS.

Various methods could be considered for deducing which factor or index (formulated from more than one marker) could be used to assess and weight markers to add, when using MetS as a screening tool. Factor analysis has been used to assess new components for MetS, including sleep and diet as well as laboratory markers\textsuperscript{655-657}, however cannot be used for longitudinal data. Receiver operating curves for sensitively and specificity could also be considered for assessing the best markers to add to MetS, and have been used for LFT, diabetes screening and waist-height markers in MetS\textsuperscript{374,658,659}.

Limitations of this part of the study are that the participants were not randomly chosen to undertake a weight loss study, so they may have special characteristics that make these results not able to be extrapolated to the population at large, but this was understood from the outset. Hip circumference was not measured. Hip measurement may have helped assess who was in less metabolic danger. Comparisons of CRSHaem&Biochem with some experimental oxidant markers would have been useful, and other studies have looked at MetS and experimental oxidative stress markers\textsuperscript{463,660-664}.

**Conclusion**

This study showed that firstly that the CRSHaem&Biochem comprise markers very sensitive to the metabolic dysfunction in MetS, and secondly that these markers have known oxidant (and in lean individuals, antioxidant) properties and associations.

The current study adds that IDF/JIS MetS does include individuals with a wide range of CVD risk, and that MetS index devised, the MetS marker count, may extend the use of MetS concept. In participants in the current 6m weight loss study whose weight changed, the MetS marker count was related strongly, cross-sectionally, to a range of oxidant markers, especially HbA\textsubscript{1c}, urate, and sufficiently well over the study period with γGT
and ESR. This study showed strong associations, and some of these markers are already commonly casually used. HbA\textsubscript{1c} is now accepted as a screening test for TIIDM, and this could be extended to those with IFG. Advisory interpretations on fatty liver for raised LFT and ferritin, are already on laboratory test reports, in addition to tables of hsCRP guides to be used in conjunction with dyslipidaemia\textsuperscript{665}.

These markers should be researched formally in larger studies with hard CVD end points in future with a view to making stronger, more accurate CVD prediction guides, for use alongside the IDF/JIS MetS.
Chapter 5. Novel Cardiovascular Disease Protective Markers

5.1 Introduction

Modest central or upper body obesity is often the only sign of CVD risk, although this risk may not be understood by individuals affected\(^{666}\). Obesity is often an early symptom and sign, and is seen in younger adults\(^{667}\). Central obesity and CVD risk can be reversible, especially if attended to early\(^{668}\). In populations where overweight and obesity are increasing, primary preventative care could extend the concept of prevention, health maintenance and longevity.

Clinical markers are usually tested to see if measurements are becoming abnormal, rather than testing for beneficial markers to see where they lie in the normal range. There may be a place for introducing protective CVD marker tracing to minimise CVD risk, but also to optimise health. Patients’ health can proceed toward degenerative disease, remain the same over the short term, or there can be regression of early signs of disease and improved health and outlook. Appropriate markers need to be found to perform this preventive medicine function. Hitherto, preventative primary care has often been overtaken by OTC supplement, vitamin and nutraceutical products, with vigorous marketing, but often with less than sound science behind the health claims.

Adpn, as previously introduced in Section 1.5.2.1, is a complex adipocytokine which is almost exclusively synthesised in adipocytes, undergoes extensive post-translational modification, and circulates multimeric forms\(^{669}\). Adpn appears to be associated with protective markers, normal weight and fewer MetS associated problems\(^{434,670-673}\).

The gender dimorphism resulting in lower levels in men may reflect the generally greater CVD risk in men\(^{428,430,674-676}\), which could make Adpn a gender specific protective marker.

Adpn is one of few known cytokines whose levels decrease in central obesity, TIIDM, CVD risk, and CVD itself. HMW becomes proportionately less than total Adpn in obesity...
probably due to its complicated synthesis which appears to be dysfunctional in central adipocytes in obesity related MetS\textsuperscript{677}. Thus HMW may be linked more closely to weight gain and loss and change in MetS than total Adpn. Although Adpn is complex, it is worth investigating as a clinical marker, as it may be contributing to obesity and associate with change in MetS.

The second group of adipose tissue-related Novel CVD Protective Markers are the sFSVitamins, whose functions have been outlined in Chapter 1 Section 1.5.2.2.

As all 5 FSVitamins have been found to have profound cell organising and multiple metabolic effects\textsuperscript{468,475,678-682}, their testing in MetS is likely to be a very useful adjunct to MetS screening. Although the vitamins are required biochemicals, and are antioxidants, the sFSVitamins are not often tested in general medical care, either to check for sufficiency or deficiency, or antioxidant status. Even sVitD testing in those in institutionalised care, a high risk group, is discouraged due to cost, and supplements are now becoming routine\textsuperscript{665}. VitK is not usually tested directly in clinical practice, but a prothrombin time (PT) is often measured, and reported as the international normalised ratio (INR) in monitoring VitK blocking; an anticoagulation therapy.

It is not clearly known what aspects of CVD risk or protection testing the sFSVitamins may reflect, or how wide ranging their effects in obesity and MetS are. Clinical testing has been reserved for those thought to be deficient. As $\beta$Caro is only synthesised by plants, serum carotenoids tend to reflect protective high fruit and vegetable diets\textsuperscript{309,683,684}. Furthermore, women have higher fruit and vegetable intakes in many westernised populations\textsuperscript{684,685}. In addition, in women, as HDL-C transports $\beta$Caro, higher HDL-C is likely to reflect on s$\beta$Caro levels.

On the contrary, men in westernised countries, consume more energy and more of it from saturated fat\textsuperscript{684} and processed meat\textsuperscript{686}, and have higher rates of dyslipidaemia, CVD and cancer. Furthermore, increased CVD rates have been detected in men with raised sVitE\textsuperscript{687}. VitE is abundant in healthy seed and olive oil products\textsuperscript{688} but also processed meats where synthetic $\alpha$ and $\gamma$-tocopherols are added as antioxidants\textsuperscript{689,690}. $\gamma$-tocopherol has been linked with adverse health\textsuperscript{477,687}.

VitA, which is classically sourced from liver, and lesser amounts in meat, high fat diary and egg, is highly added to refined fried starch products such as grain and potato crisps.
Thus various analyses were run on Adpn, Adpn oligomers and the sFSVitamins to ascertain their relationships with other CVD risk and protective markers which were measured, and particularly with MetS markers and count.

### 5.1.1 Hypothesis

It is hypothesised that the Novel CVD Protective Markers comprising: (a) serum Adpn and its 3 oligomers, and (b) the sFSVitamins, sVitD, sβCaro, sVitA and sVitE, are strongly related to a number of other CVD protective markers drawn from an array of anthropometry, laboratory, demographic, medical status, lifestyle (social habits, diet and PA), QoL and eating attitude study parameters in women and men.

Change between baseline and 6m of HMW30, will change with similar array of study markers, as above.

Change in sβCaro in all and women could indicate that women change their fruit and vegetable intake over the study, and change in VitE in all and men could indicate that men change their processed fatty food intake. Thus these Novel CVD Protective Markers, together with MetS, could be used to predict or track protection from degenerative disease.

### 5.1.2 Aims

The aims of the Diet&Health-Novel CVD Protective Marker study in 250 overweight and obese women and men, and a substudy of 30 participants chosen on anthropometry and MetS status to have their Adpn oligomers analysed, enrolled in a 6m weight loss study are:

**Primary Aims**

- To assess baseline Adpn250 and Adpn30, and 6m and change in Adpn30 oligomers
- To explore relationships of Adpn250 with an array of demography, health status, anthropometry, laboratory, lifestyle, QoL and eating attitude study parameters in all participants and women and men separately.
- To explore relationships of change between baseline and 6m of one of the oligomers, HMW30, with an array of change in anthropometry and laboratory, in all participants, and women and men separately.
- To assess baseline, 6m and the change between baseline and 6m mean serum, dietary and supplemental spFSVitamins: spVitD, spβCaro, spVitA and spVitE.
• To explore relationships of each sFSVitamin with anthropometry, laboratory, demographic, medical status, lifestyle, QoL and eating attitude study parameters in all participants
• To explore relationships of change between baseline and 6m of sβCaro in all participants and sVitE in all participants with an array of change in anthropometry and laboratory parameters.

Secondary Aims
• To compare the magnitude of the effect of sFSVitamins combined.
• To explore relationships of change between baseline and 6m of sβCaro in women and sVitE in men with an array of change in anthropometry and laboratory parameters.
• To assess the impact of ethnicity and season on sVitD

5.2 Methods

For the methods for the Diet&Health-Novel CV Protective Markers refer to Section 2.2.2.

5.2.1 Method Rationale & Summary

Briefly, in order to explore the Novel CVD Protective Markers and their baseline and change correlations with various CVD risk and protective markers and MetS markers, 250 obese and overweight women and men were enrolled in the Diet&Health-Weight loss trial where they were encouraged to make lifestyle changes.

By attending monthly for anthropometry and for advice to make prudent dietary changes to reduce fatty food and increase fruit and vegetables, increase moderate physical activity, and use a paradigm to enhance motivation, taking daily chitosan or placebo, and completing a 24HrFood Recall, validated Life in NZ Physical Activity questionnaire and Eating Attitudes questionnaire and have blood tests at baseline and 6m, it was planned that the participants would lose significant amounts of weight and show markers of metabolic and nutritional improvement.

Baseline Adpn was taken in 250 participants, a subset of 30 participants was chosen post hoc for their extreme anthropometry and MetS, and change in these measurements. The marked sexual dimorphism seen in Adpn meant separate analyses in women and men were made.
Although only 24Hr Food Recalls were only performed twice, a detailed search through participants’ baseline and 6m medication records for vitamin supplements gave some idea of their FSVitamin intake.

Relationships of sβCaro with its HDL-C plasma carrier, and sVitE with the non-HDL-C lipid carriers were important, and investigated. As sVitD, which is not carried on lipids, but has its own plasma carrier VitD binding protein\(^{691}\), is highly seasonal, and ethnic dependant regression analyses were performed for this vitamin, but not change correlations.

The baseline analyses of the sFSVitamins were not separated into the 2 genders. As treatment trials of change in sβCaro and sVitE have been controversial change correlations of these 2 sFSVitamins with other laboratory and dietary CVD risk and protective markers were formed, and also change correlations of sβCaro in the women, who tend to have higher fruit and vegetable consumption patterns, were investigated. In a similar manner, change correlations of sVitE in men who tend to have higher consumption of processed fatty food with added tocopherol antioxidants were explored. As sVitA derives preformed from various foods, is synthesised from fβCaro, and spVitA limited to very low levels due to VitA toxicity, no specific change hypothesis was tested for sVitA.

### 5.2.2 Clinical Study Procedures

Refer to Section 2.4.2

### 5.2.3 Laboratory Procedures and Analyses

Refer to Section 2.4.4

The procedure for selecting the 30 participants whose serum was to be used to analyse the total Adpn\(_{30}\), HMW\(_{30}\), MMW\(_{30}\), and LMW\(_{30}\) is presented in Section 2.6.1, Table 2-5.
5.2.4 Statistical Analyses

5.2.4.1 Adiponectin and Oligomers

*Baseline, 6m and Change Over 6m for Adiponectin*$_{250}$ *and Adiponectin*$_{30}$ *& Oligomers*$_{30}$

Baseline, 6m and change over the 6m of Adpn$_{30}$ and oligomers (HMW$_{30}$, MMW$_{30}$ and LMW$_{30}$) in all participants and women and men alone, and the percentage of each Adpn$_{30}$ oligomer, %HMW$_{30}$, %MMW$_{30}$, and %LMW$_{30}$, is tabulated with simple statistics.

*General Linear Model for Adiponectin*$_{250}$ *with Demography, Medical Status, Lifestyle, QoL and Eating Attitude Study Parameters*

A general linear (regression) model was fitted with Adpn as the outcome, and age, gender, season, Maori/Pacific or other, regular cigarette smoker, alcoholic drinks/wk, CVD, disease, EAT-12 diet subscore, EAT-12 bulimia subscore, EAT-12 oral control subscore, OSQoL Mental subscore, OSQoL Physical subscore, moderate PA hr/wk, intense PA hr/wk and TV watching hr/wk as the explanatory variables.

Least square means (LSM) were estimated to represent the effect size. The analysis from which p values were produced was performed with the continuous variables entered in their continuous form but for production of the LSM the continuous variables were split in 2 with approximately 50% in each group.

*Simple Correlation Arrays with Baseline Adiponectin*$_{250}$ *in All, Women and Men*

A simple (Pearson) correlation array was formed with serum Adpn$_{250}$ in all participants, and women and men alone with anthropometric and laboratory study parameters. Fuller correlation arrays for Adpn$_{250}$ serum concentrations with study parameters appear in Appendix 4, Table A, where at least one out of the groups ‘all, women and men’ had a statistically significant relationship and/or higher correlation than r>0.11.

*Simple Correlation Arrays with Change in HMW*$_{30}$ *in All, Women and Men*

A simple (Pearson) correlation array was formed with change in HMW$_{30}$ serum concentrations in all participants, and women and men alone versus study parameters. For this analysis as the participant numbers were small, the correlation coefficient levels were important as they may be as high as for the full group, but not statistically significant.
Fuller correlation arrays for change in \( \text{HMW}_30 \) serum concentrations verses study parameters appear in Appendix 4, Table B. where at least one out of the groups ‘all participants, and women and men alone’ has a statistically significant correlation and/or higher correlation than \( r > 0.20 \), then all are tabulated for comparison.

### 5.2.4.2 The Fat Soluble Vitamins

Note. All analysis are for \( s\text{VitD}, s\beta\text{Caro}, s\text{VitA} \) and \( s\text{VitE} \), except one multivariable analysis which includes the INR (VitK), which will be specified.

**Baseline, 6m and Change Over 6m Vitamin D, Beta-Carotene, Vitamin A and Vitamin E**

Baseline, 6m and change over 6m in sFSVitamin concentrations, dietary intake, and percentage of participants taking supplements of FSVitamins in all participants, and women and men alone, is tabulated, with simple statistics.

**General Linear Model for Vitamin D, Beta-Carotene, Vitamin A and Vitamin E with Demography, Medical Status, Lifestyle, QoL and Eating Attitudes Study Parameters**

A general linear (regression) model was fitted for each sFSVitamin for the demographic, medical, lifestyle, QoL and eating attitude data exactly as for Adpn. A log transformation was used for the analysis with \( s\beta\text{Caro} \) as the outcome but the LSM are displayed back transformed. Regular smoking was used instead of the number of cigarettes smoked because of the large number of non smokers.

**Simple Correlation Arrays with Vitamin D, Beta-Carotene, Vitamin A and Vitamin E**

A simple (Pearson) correlation array was formed with baseline concentrations of the 4 sFSVitamins in all verses study parameters. Fuller correlation arrays appear in Appendix 2. If at least one out of the groups ‘sVitD, \( s\beta\text{Caro} \), sVitA and sVitE’ had a statistically significant values or \( r > 0.2 \) then all are tabulated for comparison.
Simple Correlation Arrays with Change in Beta-Carotene in All & Women, and Vitamin E in All & Men

A simple (Pearson) correlation array was formed with change in sβCaro in all participants and women and men alone, and change in sVitE concentrations in all participants and men, versus change in study parameters. Fuller correlation arrays appear in Appendix 2. Where at least one out of the groups ‘change in sβCaro in all and women, or change in sVitE in all and men, versus a study parameter’ had a statistically significant correlation and/or r>0.15 then all are tabulated for comparison.

Baseline: Investigation of Relationships of Metabolic Syndrome Marker Count with Vitamin D, Beta-Carotene, Vitamin A, Vitamin E and INR (Vitamin K) and Other Explanatory Variables

In order to investigate the relationship of vitamin levels with MetS marker count a generalised linear (ordinal logistic regression) model was run using baseline data with MetS marker count as the outcome and sVitD, sβCaro, sVitA, sVitE and INR (VitK) together with age, gender, HbA1c, urate, albumin, globulin and AlkPhos as explanatory variables.

Baseline to 6m: Investigation of Relationships of Metabolic Syndrome Marker Count with Vitamin D, Beta-Carotene, Vitamin A, Vitamin E and INR (Vitamin K) and Other Explanatory Variables

In order to investigate the how changes in sFSVitamins are related to changes in the MetS marker count, a generalised linear mixed model was fitted with MetS marker count counted as the ordinal outcome, and sVitD, sβCaro, sVitA, sVitE and INR (VitK), split into their overall mean (baseline to 6m) for a person and their change (difference from the mean) at each time point included as explanatory variable along with the overall mean and change of age, gender, chitosan treatment group, HbA1c, urate, albumin, globulin and AlkPhos. Person was included as a random effect.

Baseline: General Linear Model Vitamin D

As sVitD has additional environmental effects of season, a general linear (regression) model was used to investigate the baseline relationships of VitD with demographic data, anthropometry and laboratory tests. Multivariable regression was performed with sVitD as the outcome variable. Explanatory variables were age, gender, ethnicity, season, and
either BMI or waist as these were likely to be highly correlated. An analysis including both BMI and waist was also carried out to investigate if one variable contributed over and above the other.

Graphpad Prism 5 (V5.04 La Jolla, CA, USA) was used for the correlation arrays and Microsoft Excel 7 for the T-Tests. SAS v9.1 statistical software (Cary, NC, USA, 2006) was employed for general linear and generalised linear mixed model analyses.

5.3 Results

5.3.1 Adiponectin and Oligomers

Adpn is sexually dimorphic. Results are presented for women and men separately Table 5-1.

Table 5-1. Baseline, 6m & Change - Adpn250 & Adpn30: All, Women & Men

<table>
<thead>
<tr>
<th>Adpn</th>
<th>All, Baseline (sd)</th>
<th>Women, Baseline (sd)</th>
<th>Men, Baseline (sd)</th>
<th>All, 6m &amp; Change (sd)</th>
<th>Women, 6m &amp; Change (sd)</th>
<th>Men, 6m &amp; Change (sd)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adpn250, ug/ml</td>
<td>N=242</td>
<td>n=199</td>
<td>n=43</td>
<td>3.00(0.98)</td>
<td>3.08(1.02)</td>
<td>2.67(0.67)</td>
</tr>
<tr>
<td>Adpn30 &amp; oligomers</td>
<td>N=30</td>
<td>n=21</td>
<td>n=9</td>
<td>8.35(5.87)</td>
<td>9.47(6.50)</td>
<td>5.74(2.91)</td>
</tr>
<tr>
<td>δ Adpn30, μg/ml</td>
<td>8.36(4.90)</td>
<td>9.84(5.05)</td>
<td>4.93(2.17)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HMW30, μg/ml</td>
<td>3.80(3.18)</td>
<td>4.51(3.46)</td>
<td>2.14(1.49)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>δ HMW30, μg/ml</td>
<td>4.33(3.27)</td>
<td>5.42(3.33)</td>
<td>1.80(0.82)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MMW30, μg/ml</td>
<td>3.48(2.29)</td>
<td>3.79(2.59)</td>
<td>2.77(1.22)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>δ MMW30, μg/ml</td>
<td>3.00(1.51)</td>
<td>3.25(1.59)</td>
<td>2.40(1.23)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LMW30, μg/ml</td>
<td>1.07(0.76)</td>
<td>1.18(0.87)</td>
<td>0.82(0.33)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>δ LMW30, μg/ml</td>
<td>1.02(0.70)</td>
<td>1.16(0.79)</td>
<td>0.69(0.29)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>%HMW30</td>
<td>41.2 (11.0)</td>
<td>44.2(10.2)</td>
<td>34.3(9.8)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>δ %HMW30</td>
<td>48.30(11.57)</td>
<td>53.2(9.3)</td>
<td>36.9(7.8)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>%MMW30</td>
<td>45.1(8.8)</td>
<td>42.9(9.1)</td>
<td>50.1(5.9)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>δ %MMW30</td>
<td>39.2(10.0)</td>
<td>35.4(9.0)</td>
<td>48.1(6.1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>%LMW30</td>
<td>13.7(4.5)</td>
<td>12.9(4.2)</td>
<td>15.6(4.6)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>δ %LMW30</td>
<td>12.08(3.59)</td>
<td>11.1(3.3)</td>
<td>14.4(3.4)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1p<0.01, 2p<0.05, 3p<0.1. a. baseline difference between women and men b. 6mth difference between women and men, δ change; Adpn250 Adiponectin in 250 participants, baseline only; Adpn30 Adiponectin in 30 participants at baseline and 20 at 6m; HMW30 high molecular weight Adpn; MMW30 medium molecular weight Adpn; LMW30 low molecular weight Adpn; sd standard deviation. Note the change in concentrations and percentages is only calculated for the 20 completers, but the baseline values are from the thirty 30 enrolled participants.
The baseline mean serum concentrations in the full participant sample set, Adpn_{250}, were analysed and shown in Table 5-1. Women had significantly higher Adpn than men. In the lower part of Table 5-1, the baseline data of a subset of 30 participants for the Adpn_{30} analysis is shown. Refer to the selection criteria for the Adpn_{30} subset in Section 2.6.1 Table 2-5. Twenty participants had data at 6m to calculate the change in Adpn_{30}. The 3 MW Adpn_{30} oligomers are shown as concentrations and as percentages of Adpn_{30} and their change over 6m is shown in Table 5-1.

Women had a borderline significantly higher Adpn at baseline and 6m (p<0.1) (Table 5-1). Adpn_{30} increased over 6m in women, but decreased slightly, together with LMW_{30}, in men. HMW_{30} increased but MMW_{30} decreased over 6m, but not significantly. The %HMW increased, but both %MMW_{30} and %LMW_{30} decreased in women and men over the study period. Women had significantly increased %HMW and decreased %MMW over 6m (p<0.05). As shown in Figure 5-1, only in men did change in Adpn_{30}, and HMW_{30} (p=0.092 and p=0.056, respectively) show any evidence of negative correlation with change in MetS marker count.

Analyses of the demographic, health status, lifestyle, QoL and eating attitudes questionnaires are shown in Table 5-2.

Figure 5-1. Change in Adpn_{30} vs. Change in MetS Marker Count: Women & Men

MetS metabolic syndrome; Adpn_{30} adiponectin in the subgroup of 30 participants with extreme weight, Mets or change in these measures. HMW_{30} high molecular weight adiponectin, MMW_{30} medium molecular weight adiponectin, LMW_{30} low molecular weight adiponectin,
Table 5-2. General Linear Model. Baseline Adpn<sub>250</sub>: Demography, Lifestyle & Quality of Life

<table>
<thead>
<tr>
<th>Variable</th>
<th>LSM mg/dL (95% C.I.)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age y</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;50</td>
<td>2.41 (2.12, 2.71)</td>
<td>0.008</td>
</tr>
<tr>
<td>≥50</td>
<td>2.79 (2.45, 3.12)</td>
<td></td>
</tr>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Women</td>
<td>2.78 (2.50, 3.05)</td>
<td>0.04</td>
</tr>
<tr>
<td>Men</td>
<td>2.42 (2.05, 2.79)</td>
<td></td>
</tr>
<tr>
<td><strong>Season</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Summer</td>
<td>2.77 (2.46, 3.08)</td>
<td>0.01</td>
</tr>
<tr>
<td>Winter</td>
<td>2.43 (2.12, 2.74)</td>
<td></td>
</tr>
<tr>
<td><strong>Maori/Pacific Ethnicity</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>2.41 (2.12, 2.71)</td>
<td>0.21</td>
</tr>
<tr>
<td>No</td>
<td>2.79 (2.45, 3.12)</td>
<td></td>
</tr>
<tr>
<td><strong>Reg. Cig. Smoker</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>2.36 (1.91, 2.81)</td>
<td>0.008</td>
</tr>
<tr>
<td>No</td>
<td>2.84 (2.61, 3.06)</td>
<td></td>
</tr>
<tr>
<td><strong>Alcohol, Unit/wk</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>2.36 (1.91, 2.81)</td>
<td>0.58</td>
</tr>
<tr>
<td>&gt;0</td>
<td>2.84 (2.61, 3.06)</td>
<td></td>
</tr>
<tr>
<td><strong>CVD</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>2.65 (2.36, 2.94)</td>
<td>0.34</td>
</tr>
<tr>
<td>No</td>
<td>2.55 (2.19, 2.90)</td>
<td></td>
</tr>
<tr>
<td><strong>Disease</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>2.56 (2.21, 2.92)</td>
<td>0.77</td>
</tr>
<tr>
<td>No</td>
<td>2.63 (2.35, 2.92)</td>
<td></td>
</tr>
<tr>
<td><strong>Modest PA, hr/wk</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;20</td>
<td>2.54 (2.23, 2.84)</td>
<td>0.98</td>
</tr>
<tr>
<td>≥20</td>
<td>2.66 (2.34, 2.98)</td>
<td></td>
</tr>
<tr>
<td><strong>Intense PA, min/wk, n=95</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>2.52 (2.23, 2.81)</td>
<td>0.67</td>
</tr>
<tr>
<td>&gt;0</td>
<td>2.68 (2.35, 3.01)</td>
<td></td>
</tr>
<tr>
<td><strong>Watch TV, hr/wk</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤20</td>
<td>2.58 (2.27, 2.89)</td>
<td>0.96</td>
</tr>
<tr>
<td>&gt;20</td>
<td>2.62 (2.31, 2.93)</td>
<td></td>
</tr>
<tr>
<td><strong>OSQoL Ment., Subscore</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤15</td>
<td>2.64 (2.33, 2.95)</td>
<td>0.56</td>
</tr>
<tr>
<td>&gt;15</td>
<td>2.56 (2.24, 2.88)</td>
<td></td>
</tr>
<tr>
<td><strong>OSQoL Phys., Subscore</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤10</td>
<td>2.55 (2.23, 2.86)</td>
<td>0.81</td>
</tr>
<tr>
<td>&gt;10</td>
<td>2.65 (2.33, 2.97)</td>
<td></td>
</tr>
<tr>
<td><strong>EAT12 Diet, Subscore</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;15</td>
<td>2.61 (2.28, 2.84)</td>
<td>0.36</td>
</tr>
<tr>
<td>≥15</td>
<td>2.59 (2.29, 2.89)</td>
<td></td>
</tr>
<tr>
<td><strong>EAT12 Bulimia, Subscore</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;20</td>
<td>2.62 (2.28, 2.95)</td>
<td>0.94</td>
</tr>
<tr>
<td>≥20</td>
<td>2.58 (2.28, 2.88)</td>
<td></td>
</tr>
<tr>
<td><strong>EAT12 Oral, Subscore</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;22</td>
<td>2.44 (2.15, 2.72)</td>
<td>0.01</td>
</tr>
<tr>
<td>≥22</td>
<td>2.76 (2.43, 3.09)</td>
<td></td>
</tr>
</tbody>
</table>

Adpn was significantly higher with age (p=0.008), higher with being a non-smoker (p=0.008), being of the female gender (p=0.04), summer season (p=0.008), and EAT-12 oral control subscore (p=0.01) (Appendix 1. Questionnaire forms)

Shown in Table 5-3 are Adpn<sub>250</sub> correlations with anthropometric and laboratory study parameters.
Table 5-3. Baseline Adpn_{250} vs. Study Parameters - Correlations: All, Women & Men

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Adpn_{250}(n=250)</th>
<th>All, n=212-243</th>
<th>Women, n=176-199</th>
<th>Men, n=39-40</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pearson</td>
<td>p-value</td>
<td>Pearson</td>
<td>p-value</td>
</tr>
<tr>
<td><strong>Anthropometry</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight, kg</td>
<td>-0.30</td>
<td>&lt;0.001</td>
<td>-0.29</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Height, m</td>
<td>-0.32</td>
<td>&lt;0.001</td>
<td>-0.28</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>-0.15</td>
<td>0.02</td>
<td>-0.19</td>
<td>0.007</td>
</tr>
<tr>
<td>Waist, cm</td>
<td>-0.20</td>
<td>0.01</td>
<td>-0.17</td>
<td>0.02</td>
</tr>
<tr>
<td><strong>Haematology</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hct, %</td>
<td>0.18</td>
<td>0.01</td>
<td>0.17</td>
<td>0.02</td>
</tr>
<tr>
<td>Leukocytes, x10⁹</td>
<td>-0.16</td>
<td>0.02</td>
<td>-0.13</td>
<td>0.06</td>
</tr>
<tr>
<td>Neutrophils, x10⁹</td>
<td>-0.19</td>
<td>0.01</td>
<td>-0.15</td>
<td>0.05</td>
</tr>
<tr>
<td>Platelets, x10¹²</td>
<td>-0.13</td>
<td>0.05</td>
<td>-0.14</td>
<td>0.06</td>
</tr>
<tr>
<td>ESR, mm/hr</td>
<td>-0.13</td>
<td>0.04</td>
<td>-0.16</td>
<td>0.03</td>
</tr>
<tr>
<td><strong>Biochemistry</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TC, mmol/L</td>
<td>0.23</td>
<td>&lt;0.001</td>
<td>0.25</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HDL-C, mmol/L</td>
<td>0.40</td>
<td>&lt;0.001</td>
<td>0.38</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TC/HDL</td>
<td>-0.26</td>
<td>&lt;0.001</td>
<td>-0.23</td>
<td>0.001</td>
</tr>
<tr>
<td>Urate, mmol/L</td>
<td>-0.26</td>
<td>&lt;0.001</td>
<td>-0.19</td>
<td>0.007</td>
</tr>
<tr>
<td>hsCRP, mg/L</td>
<td>-0.15</td>
<td>0.02</td>
<td>-0.14</td>
<td>0.06</td>
</tr>
<tr>
<td><strong>Hormones/Cytokines</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insulin, μU/L</td>
<td>-0.25</td>
<td>&lt;0.001</td>
<td>-0.26</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HOMA-IR, mmol/uU/L</td>
<td>-0.20</td>
<td>0.01</td>
<td>-0.24</td>
<td>0.01</td>
</tr>
<tr>
<td>Adpn, mg/L</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Serum Fat Soluble Vitamins</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sVitD, ng/L</td>
<td>0.22</td>
<td>0.001</td>
<td>0.25</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>sβCaro, μg/L</td>
<td>0.30</td>
<td>&lt;0.001</td>
<td>0.28</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>sVitA, μg/L</td>
<td>0.06</td>
<td>0.33</td>
<td>0.14</td>
<td>0.05</td>
</tr>
<tr>
<td>sVitE, μg/L</td>
<td>0.13</td>
<td>0.05</td>
<td>0.18</td>
<td>0.01</td>
</tr>
<tr>
<td><strong>Diet</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>fVitE, mg/d</td>
<td>0.14</td>
<td>0.03</td>
<td>0.15</td>
<td>0.04</td>
</tr>
<tr>
<td><strong>Metabolic Syndrome</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IDF/JIS MetSMCt</td>
<td>-0.25</td>
<td>&lt;0.001</td>
<td>-0.25</td>
<td>0.001</td>
</tr>
<tr>
<td>IDF/JIS MetS</td>
<td>-0.23</td>
<td>&lt;0.001</td>
<td>-0.21</td>
<td>0.003</td>
</tr>
</tbody>
</table>

Both the r and p-values are of interest as there are different numbers of women and men. The values are presented within the tables, with the significant correlations given in the text.
For the correlations of Adpn$_{250}$, the whole group, all, are shown for completeness in but in the text the representative Adpn range for women and men alone are presented.

In women positive correlations of Adpn$_{250}$ were shown with: TC, HDL-C, sVitD, and sβCaro (all $p<0.006$), haematocrit and fVitE (both $p<0.04$) (Table 5-3).

In men positive correlations of Adpn$_{250}$ were shown with: haematocrit and HDL-C (both $p<0.02$) (Table 5-3).

In women, negative correlations of Adpn$_{250}$ were shown with weight, height, BMI, TC/HDL, urate, HOMA-IR, insulin, IDF/JIS MetS marker count IDF/JIS MetS (all $p<0.003$) and waist, neutrophils and ESR ($p<0.05$) (Table 5-3).

In men, negative correlations of Adpn$_{250}$ were shown with height, leukocytes, neutrophils, platelets, ESR, urate, hsCRP (all, $p<0.05$) (Table 5-3).

There were few participants in the change in HMW$_{30}$ part of the study, and men were more influential in this analysis. Correlations were often high, so the cut off for reporting in the text is $r\geq 0.3$ (Table 5-3). An expanded table appears in full in Appendix 4, Table D.

In women, positive change correlations of HMW$_{30}$ were shown with, bilirubin ($p=0.01$), and note high correlations that are not quite significant in Table 5-4, such as niacin.

In men, positive change correlations of HMW$_{30}$ that were significant or tended towards significance were shown with niacin ($p=0.01$) eosinophils ($p=0.09$) but note the high correlation levels with albumin, lymphocytes, sVitA, and bilirubin in (Table 5-4). In women, negative change correlations of HMW$_{30}$ were shown with BMI, %Fat, HbA$_{1c}$ and weight (all, $p<0.05$), and note the high correlations with haematocrit, monocytes, and note the correlation level with TC/HDL, leptin, and waist in (Table 5-4).

In men, negative change correlations of HMW$_{30}$ were shown with weight, BMI, waist, RBCW, leptin, thiamine (all; $p<0.05$) and note high correlation levels with TC/HDL, VitD, niacin, iron and with neutrophils, ferritin and sβCaro in (Table 5-4).
### Table 5-4. Change in HMW\textsubscript{30} vs. Study Parameters, Correlations: All, Women & Men

<table>
<thead>
<tr>
<th>Change in HMW\textsubscript{30} Parameter</th>
<th>All, Change in HMW\textsubscript{30} n=15-20</th>
<th>Women, Change in HMW\textsubscript{30} n=9-14</th>
<th>Men, Change in HMW\textsubscript{30} n=5-6</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Anthropometry</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>δ Weight, kg</td>
<td>-0.58 0.007</td>
<td>-0.53 0.05</td>
<td>-0.90 0.02</td>
</tr>
<tr>
<td>δ BMI, kg/m\textsuperscript{2}</td>
<td>-0.59 0.006</td>
<td>-0.55 0.04</td>
<td>-0.91 0.01</td>
</tr>
<tr>
<td>δ Waist, cm</td>
<td>-0.40 0.08</td>
<td>-0.27 0.35</td>
<td>-0.85 0.03</td>
</tr>
<tr>
<td>δ %Fat, %</td>
<td>-0.41 0.10</td>
<td>-0.57 0.04</td>
<td>0.13 0.83</td>
</tr>
<tr>
<td><strong>Haematology</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>δ Hct, %</td>
<td>-0.32 0.18</td>
<td>-0.53 0.06</td>
<td>0.13 0.81</td>
</tr>
<tr>
<td>δ RBCW, %</td>
<td>-0.20 0.42</td>
<td>-0.11 0.73</td>
<td>-0.86 0.03</td>
</tr>
<tr>
<td>δ Neutrophils, x10\textsuperscript{9}</td>
<td>-0.18 0.46</td>
<td>-0.13 0.69</td>
<td>-0.74 0.10</td>
</tr>
<tr>
<td>δ Eosinophils, x10\textsuperscript{9}</td>
<td>0.06 0.80</td>
<td>-0.14 0.66</td>
<td>0.74 0.09</td>
</tr>
<tr>
<td>δ Monocytes, x10\textsuperscript{9}</td>
<td>-0.45 0.06</td>
<td>-0.55 0.07</td>
<td>-0.24 0.65</td>
</tr>
<tr>
<td>δ Lymphocytes, x10\textsuperscript{9}</td>
<td>-0.39 0.11</td>
<td>-0.73 0.01</td>
<td>0.74 0.10</td>
</tr>
<tr>
<td><strong>Biochemistry</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>δ TC/HDL</td>
<td>-0.47 0.04</td>
<td>-0.43 0.13</td>
<td>-0.76 0.08</td>
</tr>
<tr>
<td>δ HbA\textsubscript{1c}, %</td>
<td>-0.58 0.03</td>
<td>-0.68 0.04</td>
<td>0.25 0.69</td>
</tr>
<tr>
<td>δ Ferritin, μg/L</td>
<td>-0.13 0.60</td>
<td>-0.03 0.93</td>
<td>-0.70 0.12</td>
</tr>
<tr>
<td>δ Bilirubin, μmol/L</td>
<td>0.68 0.003</td>
<td>0.71 0.01</td>
<td>0.57 0.32</td>
</tr>
<tr>
<td>δ Albumin, g/L</td>
<td>-0.08 0.74</td>
<td>-0.07 0.82</td>
<td>0.74 0.15</td>
</tr>
<tr>
<td><strong>Hormones/Cytokines</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>δ Leptin, pg/ml</td>
<td>-0.47 0.04</td>
<td>-0.45 0.11</td>
<td>-0.91 0.03</td>
</tr>
<tr>
<td><strong>Serum Fat Soluble Vitamins</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>δ sVitD, ng/L</td>
<td>0.10 0.66</td>
<td>0.33 0.25</td>
<td>-0.77 0.08</td>
</tr>
<tr>
<td>δ sβCaro, μg/L</td>
<td>-0.04 0.88</td>
<td>0.06 0.84</td>
<td>-0.69 0.13</td>
</tr>
<tr>
<td>δ sVitA, μg/L</td>
<td>0.24 0.33</td>
<td>0.10 0.74</td>
<td>0.70 0.12</td>
</tr>
<tr>
<td><strong>24Hr Food Recall Dietary Nutrients</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>δ Energy, J/d</td>
<td>0.35 0.14</td>
<td>0.30 0.33</td>
<td>0.63 0.18</td>
</tr>
<tr>
<td>δ Alcohol, mL/d</td>
<td></td>
<td></td>
<td>0.05 0.93</td>
</tr>
<tr>
<td>δ Protein, g/d</td>
<td>0.34 0.16</td>
<td>0.25 0.42</td>
<td>0.63 0.18</td>
</tr>
<tr>
<td>δ Tot Fat, g/d</td>
<td>0.34 0.16</td>
<td>0.25 0.42</td>
<td>0.53 0.28</td>
</tr>
<tr>
<td>δ CHO, g/d</td>
<td>0.11 0.66</td>
<td>0.17 0.59</td>
<td>-0.20 0.71</td>
</tr>
<tr>
<td>δ Fibre, g/d</td>
<td>0.20 0.41</td>
<td>0.30 0.33</td>
<td>-0.28 0.59</td>
</tr>
<tr>
<td>δ fVitD, μg/d</td>
<td>-0.17 0.50</td>
<td>-0.24 0.42</td>
<td>0.04 0.95</td>
</tr>
<tr>
<td>δ fβCaro, μg/d</td>
<td>-0.03 0.92</td>
<td>0.00 0.99</td>
<td>-0.16 0.80</td>
</tr>
<tr>
<td>δ Retinol Equivs., μg/d</td>
<td>0.09 0.71</td>
<td>0.10 0.75</td>
<td>0.34 0.51</td>
</tr>
<tr>
<td>δ fVitE, mg/d</td>
<td>-0.11 0.67</td>
<td>-0.20 0.51</td>
<td>0.14 0.79</td>
</tr>
<tr>
<td>δ fVitA, μg/d</td>
<td>0.00 1.00</td>
<td>0.02 0.94</td>
<td>0.05 0.93</td>
</tr>
<tr>
<td>δ Thiamine, μg/d</td>
<td>-0.19 0.45</td>
<td>-0.11 0.71</td>
<td>-0.82 0.05</td>
</tr>
<tr>
<td>δ Niacin, μg/d</td>
<td>0.25 0.29</td>
<td>0.54 0.06</td>
<td>-0.75 0.09</td>
</tr>
<tr>
<td>δ Niacin Equivs., μg/d</td>
<td>0.42 0.08</td>
<td>0.52 0.07</td>
<td>-0.49 0.33</td>
</tr>
<tr>
<td>δ Iron, mg/d</td>
<td>-0.01 0.98</td>
<td>0.32 0.29</td>
<td>-0.75 0.09</td>
</tr>
</tbody>
</table>

Refer to Appendix 4, Table B for expanded Table 5-4. δ delta or change; s serum; f food/dietary weight body weight; BMI body mass index; waist waist circumference; %Fat body fat percentage; Hct haematocrit; RBCW red blood cell width; TC/HDL total cholesterol / high density lipoprotein; HbA1c haemoglobin A1c; VitD vitamin D; βCaro beta carotene; VitA vitamin A; tot total; CHO carbohydrate; equivs equivalence; d day; g gram; J joule.
Table 5-5. Baseline Adpn$_{250}$ vs. Adpn$_{30}$ - Correlations: All, Women & Men

<table>
<thead>
<tr>
<th>OligoAdpn Baseline</th>
<th>Adpn$_{250}$ (n=250) Baseline</th>
<th>All N=30 p-value</th>
<th>Women N=21 p-value</th>
<th>Men N=9 p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adpn$_{30}$</td>
<td>0.59</td>
<td>0.001</td>
<td>0.54</td>
<td>0.87</td>
</tr>
<tr>
<td>%HMW$_{30}$</td>
<td>0.45</td>
<td>0.014</td>
<td>0.29</td>
<td>0.79</td>
</tr>
<tr>
<td>%MMW$_{30}$</td>
<td>-0.32</td>
<td>0.088</td>
<td>-0.17</td>
<td>0.474</td>
</tr>
<tr>
<td>%LMW$_{30}$</td>
<td>-0.46</td>
<td>0.010</td>
<td>-0.33</td>
<td>0.143</td>
</tr>
<tr>
<td>HMW$_{30}$</td>
<td>0.57</td>
<td>0.001</td>
<td>0.51</td>
<td>0.017</td>
</tr>
<tr>
<td>MMW$_{30}$</td>
<td>0.59</td>
<td>0.001</td>
<td>0.55</td>
<td>0.010</td>
</tr>
<tr>
<td>LMW$_{30}$</td>
<td>0.39</td>
<td>0.031</td>
<td>0.35</td>
<td>0.117</td>
</tr>
</tbody>
</table>

% percentage, Adpn Adiponectin, HMW high molecular weight Adpn, MMW medium molecular weight Adpn, LMW low molecular weight Adpn, sd standard deviation.

5.3.2 The Fat Soluble Vitamins

The sFSVitamin results for this section have not been corrected for serum lipids as the usual equation does not account for the different sFSVitamin carriers in obesity (Table 5-6).

Table 5-6. Serum Lipids vs Serum Fat Soluble Vitamins – Correlations

<table>
<thead>
<tr>
<th>sFSVitamin Lipid</th>
<th>sVitD, nmol/L n=243 Pearson r p-value</th>
<th>sβCaro, μmol/L, n=239 Pearson r p-value</th>
<th>sVitA, μmol/L, n=238 Pearson r p-value</th>
<th>sVitE, μmol/L, n=236 Pearson r p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC, mol/L</td>
<td>0.10</td>
<td>0.13</td>
<td>0.28</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HDL-C, mol/L</td>
<td>0.07</td>
<td>0.25</td>
<td>0.27</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LDL-C, mol/L</td>
<td>0.08</td>
<td>0.21</td>
<td>0.25</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TG, mol/L</td>
<td>0.03</td>
<td>0.69</td>
<td>-0.08</td>
<td>0.24</td>
</tr>
<tr>
<td>TC/HDL</td>
<td>0.01</td>
<td>0.87</td>
<td>-0.09</td>
<td>0.18</td>
</tr>
</tbody>
</table>

HDL-C high density lipoprotein-cholesterol, LDL-C low density lipoprotein-cholesterol, TC total cholesterol, TG triglyceride; s serum; VitD vitamin D; βCaro beta carotene, VitA vitamin A; VitE vitamin E;

sVitD related to none of the lipids (Table 5-6). SβCaro correlated with all lipid fractions (r=0.25-0.28; p<0.001) except TG and TC/HDL. SVitA and sVitE correlated highly and significantly with TG (r=0.41 & 0.45; p<0.001) and TC (r=0.30 & 0.62; p<0.001) (Table 5-6).

SVitE also correlated with LDL-C and TC/HDL (r=0.46 & 0.31; p<0.001). Neither SVitA nor sVitE correlated with HDL-C (Table 5-6).
The baseline, 6m and change data is depicted in Table 5-7. SVitD was similar in women and men, but decreased over the 6m, highly significantly in women (p<0.001) and significantly in men (p<0.05), as shown in Table 5-7. SβCaro was significantly higher in women at baseline and 6m (p<0.001).

SβCaro increased in women and decreased in men, non-significantly in both genders.

SVitA was significantly higher in men than women at baseline and 6m (p<0.001), and decreased insignificantly in women but slightly increased in men. SVitE was borderline higher in men than women at baseline and 6m (p<0.1), and decreased insignificantly in women and men (Table 5-7).

Men consumed more fVitA & fVitE, and both vitamins were reported increased, but not significantly, at 6m. In women, fVitA and fVitE consumed was less at 6m, but not significantly (Table 5-7).

There was a borderline higher percentage of women taking spVitD and spVitE at baseline than men (Table 5-7).

Demographic, health status, lifestyle, QoL and eating attitudes data is shown in Table 5-8.

Age was positively associated with sβCaro, sVitA and sVitE (all p<0.001). Female gender was positively associated with sβCaro, and negatively with sVitA (both p<0.001). Summer season was positively associated with sVitD (p<0.001), but negatively with sβCaro (p=0.036).

Maori/Pacific ethnicity was negatively associated with sVitA (p=0.004). Regular cigarette smoking was highly significantly negatively with sβCaro (p<0.001), with smokers having approximately

Alcohol consumption was positively related significantly to sVitD (p=0.036). CVD and disease were not significantly related to sFSVitamins, nor were the 3 EAT-12 subscores
except for a very weak possible positive relationship between the Eat-12 Oral Control and sVitD (p=0.1) (Table 5-8).

The OSQoL Physical subscore was negatively related to sβCaro (p=0.013), but no other OSQoL relationships were significant. Modest PA was negatively associated with sVitE but intense PA (n=95), and television watching were not associated with the sFSVitamins (Table 5-8).

half the sβCaro value (Table 5-8).

Baseline sVitD was positively correlated with haematocrit, sVitA, basophils and Adpn (all p≤0.01), and with, albumin and sβCaro (p≤0.05) (Table 5-9).

Baseline sVitD was negatively correlated with weight, height, BMI, waist and leptin (all p≤0.002) (Figure 5-2), HbA1c and INR (VitK) (both ≤0.02) (Table 5-9).

Baseline sβCaro was positively correlated with lipids as shown in Table 5-7 and with Adpn, fβCaro, fVitE, fVitC, riboflavin, folate, potassium, magnesium and dietary fibre (all p≤0.001), total sugars and fVitA equivalents (all ≤0.003), and sVitD and sVitE (both p≤0.02) (Table 5-9).

Baseline sβCaro was negatively correlated with weight, height, leukocytes, neutrophils, hsCRP and IDF/JIS MetS marker count (all p≤0.001) and with BMI, waist (), %Fat, HbA1c, urate, NCEP MetS marker count (all p≤0.01) (Table 5-9).

Baseline sVitA was positively correlated with lipids as shown in Table 5-7 and SBP, Hb, haematocrit, MCV, MCHC, urate, ferritin, γGT, albumin, sVitD, sVitE and NCEP MetS marker count (all p≤0.001) and with basophils, ALT, DBP, and IDF/JIS MetS marker count (all p≤0.01). Baseline sVitA was negatively correlated with BMI (p<0.001) and height (p=0.05), Table 5-9).

Baseline sVitE was positively correlated with lipids as shown in Table 5-7 and SBP, DBP, ferritin, γGT, sVitA IDF/JIS MetS marker count, NCEP MetS marker count (all p<0.001), height, Hb, urate, albumin, haematocrit (all p<0.01), MCHC and βCaro (all p≤0.05) (Table 5-9). Baseline sVitE was negatively correlated with weight, BMI, Adpn30 (all p≤0.05) (Table 5-9).
Table 5-7. Baseline, 6m & Change – Serum, Dietary, & Numbers Taking Supplemental FSVitamins: All, Women & Men

<table>
<thead>
<tr>
<th>Fat Soluble Vitamin</th>
<th>All, Baseline</th>
<th>Women, Baseline</th>
<th>Men, Baseline</th>
<th>All, 6m or Change</th>
<th>Women, 6m or Change</th>
<th>Men, 6m or Change</th>
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</thead>
<tbody>
<tr>
<td>Serum</td>
<td>Mean (sd)</td>
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<tr>
<td>sVitD, nmol/L</td>
<td>238-243</td>
<td>197-200</td>
<td>41-43</td>
<td>154-162</td>
<td>124-131</td>
<td>30-32</td>
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<tr>
<td>δ sVitD, nmol/L</td>
<td>-13.66(23.4)</td>
<td>-14.61(22.7)</td>
<td>-9.60(26.3)</td>
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<td></td>
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<tr>
<td>βCaro, μmol/L</td>
<td>0.67(0.51)</td>
<td>0.45(0.40)</td>
<td>0.66(0.50)</td>
<td>0.73(0.53)</td>
<td>0.39(0.29)</td>
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<tr>
<td>sVitA, μmol/L</td>
<td>1.9(0.44)</td>
<td>2.27(0.50)</td>
<td>1.970.53</td>
<td>1.88(0.51)</td>
<td>2.29(0.49)</td>
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<tr>
<td>sVitE, μmol/L</td>
<td>30.9(7.9)</td>
<td>33.8(9.9)</td>
<td>31.0(48.83)</td>
<td>30.4(79.1)</td>
<td>33.3(7.5)</td>
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<table>
<thead>
<tr>
<th>Dietary Mean 24 Food Recall Nutrients (sd)</th>
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<tr>
<td>fVitD, μg</td>
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<td>2.4(2.6)</td>
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<tr>
<td>δ fVitD, μg</td>
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<td>-0.6(3.8)</td>
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<tr>
<td>βCaro, μg</td>
</tr>
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<td>4111(6989)</td>
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<tr>
<td>δ βCaro, μg</td>
</tr>
<tr>
<td>-309(5066)</td>
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<tr>
<td>VitA, μg</td>
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<tr>
<td>587(3004)</td>
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<tr>
<td>δ VitA, μg</td>
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<td>-79(5529)</td>
</tr>
<tr>
<td>VitE, μg</td>
</tr>
<tr>
<td>10.6(5.5)</td>
</tr>
<tr>
<td>δ VitE, μg</td>
</tr>
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<td>0.3(5.7)</td>
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<th>Supplemental n(%)</th>
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<tr>
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<tr>
<td>33(13.2)</td>
</tr>
<tr>
<td>spβCaro</td>
</tr>
<tr>
<td>26(10.4)</td>
</tr>
<tr>
<td>spVitA</td>
</tr>
<tr>
<td>33(13.2)</td>
</tr>
<tr>
<td>spVitE</td>
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<td>37(15.8)</td>
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</table>

1p<0.001, 2p<0.01, 3p<0.05, 4p<0.1, 5baseline difference between women and men 66m difference between women and men 7δ change, sd standard deviation; s serum; f dietary/food; sp supplemental VitD, vitamin D; βCaro beta-carotene; VitA, vitamin A; VitE vitamin E. 8Note the problem with only having 2 x 24hr Food recalls resulted in the wide sd for example in βCaro intake reports ranging from 58-95,000μg/d and fVitA 47-47,000 μg/d at baseline and βCaro ranging from 43-22,000μg/d and fVitA from 6-50,500μg/d at 6m, in different participants.

Women and men consumed similar amounts of fVitD with women significantly decreasing fVitD over the study (Table 5-7). Women consumed more fβCaro at baseline, but fβCaro intake dropped in both women and men, but not significantly.
### Table 5-8. Demographic, Health Status, Lifestyle, Quality of Life and Eating Attitudes with Serum Fat Soluble Vitamins

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Category</th>
<th>sFSVitamins</th>
<th>sVitD</th>
<th>p-value</th>
<th>LSM, μmol/L</th>
<th>95% C.I.</th>
<th>sβCaro</th>
<th>sVitA</th>
<th>p-value</th>
<th>LSM, μmol/L</th>
<th>95% C.I.</th>
<th>sVitE</th>
<th>p-value</th>
<th>LSM, μmol/L</th>
<th>95% C.I.</th>
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<td>&lt;0.001</td>
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<td>(26.1, 31.4)</td>
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<td>0.27</td>
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<td>Gender</td>
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<td>0.04</td>
<td>1.88</td>
<td>1.73, 2.03</td>
<td>0.35</td>
<td>30.4</td>
<td>(27.6, 33.2)</td>
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<td>Winter</td>
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<td>0.27</td>
<td>2.04</td>
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<td>2.01</td>
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<td>1.87</td>
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<td>U/wk</td>
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<td>0.32</td>
<td>0.25</td>
<td>1.93</td>
<td>1.77</td>
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<td>1.78</td>
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<td>Bulimia</td>
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<td>LSM, nmol/L</td>
<td>p-value</td>
<td>LSM, μmol/L</td>
<td>95% C.I.</td>
<td>p-value</td>
<td>LSM, μmol/L</td>
<td>95% C.I.</td>
<td>p-value</td>
<td>LSM, μmol/L</td>
<td>95% C.I.</td>
<td>p-value</td>
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<td>0.29 (0.22, 0.36)</td>
<td>1.91</td>
<td>(1.76, 2.07)</td>
<td>30.6</td>
<td>(27.7, 33.4)</td>
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<tr>
<td></td>
<td>≤10</td>
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<td>0.34</td>
<td>0.33 (0.26, 0.42)</td>
<td>0.12</td>
<td>30.2</td>
<td>(27.4, 33.0)</td>
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<td>OSQol Phys. Subscore</td>
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<td>0.29 (0.23, 0.37)</td>
<td>1.85</td>
<td>(1.69, 2.00)</td>
<td>30.8</td>
<td>(27.9, 33.6)</td>
<td></td>
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<tr>
<td></td>
<td>&lt;20</td>
<td>55.6</td>
<td>0.30</td>
<td>0.30 (0.24, 0.38)</td>
<td>0.43</td>
<td>1.92</td>
<td>(1.77, 2.06)</td>
<td>0.71</td>
<td>30.8</td>
<td>(28.1, 33.5)</td>
<td>0.05</td>
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<td></td>
<td></td>
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<tr>
<td></td>
<td>≥20</td>
<td>57.8</td>
<td>0.32 (0.25, 0.40)</td>
<td>1.89</td>
<td>(1.74, 2.04)</td>
<td>30.2</td>
<td>(27.3, 33.0)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Intense PA min/ wk</td>
<td>&lt;20</td>
<td>55.7</td>
<td>0.69</td>
<td>0.69 (0.52, 0.86)</td>
<td>0.80</td>
<td>1.93</td>
<td>(1.79, 2.07)</td>
<td>0.44</td>
<td>31.8</td>
<td>(29.2, 34.4)</td>
<td>0.21</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td>≥20</td>
<td>57.6</td>
<td>0.32 (0.25, 0.40)</td>
<td>1.90</td>
<td>(1.75, 2.05)</td>
<td>30.8</td>
<td>(28.0, 33.6)</td>
<td></td>
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</table>

sd standard deviation; LSM least square mean; C.I. confidence interval, sVitD vitamin D; sβCaro beta carotene; sVitA vitamin A; sVitE vitamin E; s serum; reg.cig.smoker regular cigarette smoker; CVD cardiovascular disease y year; wk week; d day; hr hour, min minute; EAT-12 eating attitudes questionnaire – 12 question; EAT-12 Diet/Bulimia/Oral diet/bulimia/oral control subgroups; OSQoL obesity specific quality of life; mod moderate; PA physical activity; TV television; phys physical; ment mental;
Table 5-9. Baseline Serum Fat Soluble Vitamins vs. Study Parameters

<table>
<thead>
<tr>
<th>Parameters</th>
<th>sFSVitamin</th>
<th>sVitD, n=238-243</th>
<th>sβCaro, n=236-238</th>
<th>sVitA, n=236-238</th>
<th>sVitE, n=237-239</th>
</tr>
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<td></td>
<td>Pearson</td>
<td>p-value</td>
<td>Pearson</td>
<td>Pearson</td>
<td>Pearson</td>
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<tr>
<td>Anthropometry</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight, kg</td>
<td>-0.22</td>
<td>0.001</td>
<td>-0.36</td>
<td>&lt;0.001</td>
<td>-0.11</td>
</tr>
<tr>
<td>Height, m</td>
<td>-0.26</td>
<td>&lt;0.001</td>
<td>-0.18</td>
<td>0.006</td>
<td>-0.13</td>
</tr>
<tr>
<td>BMI, kg/m2</td>
<td>-0.20</td>
<td>0.002</td>
<td>-0.29</td>
<td>&lt;0.001</td>
<td>-0.24</td>
</tr>
<tr>
<td>Waist, cm</td>
<td>-0.14</td>
<td>0.03</td>
<td>-0.36</td>
<td>&lt;0.001</td>
<td>0.03</td>
</tr>
<tr>
<td>%Fat, %</td>
<td>-0.09</td>
<td>0.27</td>
<td>-0.22</td>
<td>0.009</td>
<td>-0.08</td>
</tr>
<tr>
<td>SBP, mmHg</td>
<td>-0.09</td>
<td>0.14</td>
<td>0.22</td>
<td>0.001</td>
<td>0.22</td>
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<td>DBP, mmHg</td>
<td>0.03</td>
<td>0.66</td>
<td>-0.08</td>
<td>0.22</td>
<td>0.22</td>
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<td>Haematology</td>
<td></td>
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<td></td>
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<tr>
<td>Hb, g/L</td>
<td>0.04</td>
<td>0.58</td>
<td>-0.01</td>
<td>0.85</td>
<td>0.29</td>
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<tr>
<td>Hct, %</td>
<td>0.23</td>
<td>&lt;0.001</td>
<td>0.02</td>
<td>0.79</td>
<td>0.28</td>
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<tr>
<td>MCV, fL</td>
<td>0.03</td>
<td>0.61</td>
<td>0.35</td>
<td>0.21</td>
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<tr>
<td>MCHC, fL/L</td>
<td>0.12</td>
<td>0.07</td>
<td>0.27</td>
<td>0.27</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Leukocytes, x109</td>
<td>0.00</td>
<td>1.00</td>
<td>-0.29</td>
<td>&lt;0.001</td>
<td>0.01</td>
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<tr>
<td>Neutrophils, x109</td>
<td>0.01</td>
<td>0.91</td>
<td>-0.28</td>
<td>&lt;0.001</td>
<td>-0.03</td>
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<tr>
<td>Basophils, x109 (n=164-166)</td>
<td>0.20</td>
<td>0.009</td>
<td>-0.11</td>
<td>0.16</td>
<td>0.23</td>
</tr>
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<td>Biochemistry</td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>HbA1c, %</td>
<td>-0.17</td>
<td>0.01</td>
<td>-0.19</td>
<td>0.006</td>
<td>0.01</td>
</tr>
<tr>
<td>Urate, mmol/L</td>
<td>0.02</td>
<td>0.79</td>
<td>-0.18</td>
<td>0.005</td>
<td>0.33</td>
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<tr>
<td>Ferritin, μg/L</td>
<td>0.07</td>
<td>0.27</td>
<td>-0.01</td>
<td>0.84</td>
<td>0.34</td>
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<tr>
<td>ALT, U/L</td>
<td>0.01</td>
<td>0.94</td>
<td>-0.07</td>
<td>0.31</td>
<td>0.21</td>
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<tr>
<td>γGT, U/T</td>
<td>0.00</td>
<td>0.95</td>
<td>-0.04</td>
<td>0.59</td>
<td>0.25</td>
</tr>
<tr>
<td>Albumin, g/L</td>
<td>0.15</td>
<td>0.02</td>
<td>0.02</td>
<td>0.81</td>
<td>0.34</td>
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<tr>
<td>hsCRP, mg/L</td>
<td>0.07</td>
<td>0.30</td>
<td>-0.28</td>
<td>&lt;0.001</td>
<td>-0.03</td>
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<tr>
<td>Metabolic Syndrome</td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>IDF/JIS MetS McCl</td>
<td>-0.10</td>
<td>0.11</td>
<td>-0.23</td>
<td>0.001</td>
<td>0.16</td>
</tr>
<tr>
<td>NCEP MetSM McCl</td>
<td>0.04</td>
<td>0.59</td>
<td>-0.22</td>
<td>0.001</td>
<td>0.27</td>
</tr>
<tr>
<td>Serum Fat Soluble Vitamins</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sVitD, ng/L</td>
<td>Perfect line</td>
<td>0.15</td>
<td>0.02</td>
<td>0.33</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>sβCaro, μg/L</td>
<td>0.15</td>
<td>0.02</td>
<td>Perfect line</td>
<td>0.12</td>
<td>0.08</td>
</tr>
<tr>
<td>sVitA, μg/L</td>
<td>0.33</td>
<td>&lt;0.001</td>
<td>0.12</td>
<td>0.08</td>
<td>Perfect line</td>
</tr>
<tr>
<td>sVitE, μg /L</td>
<td>0.06</td>
<td>0.36</td>
<td>0.16</td>
<td>0.02</td>
<td>0.46</td>
</tr>
<tr>
<td>INR (VitK)</td>
<td>-0.16</td>
<td>0.02</td>
<td>0.05</td>
<td>0.41</td>
<td>0.00</td>
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<td>Hormones/Cytokines</td>
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<tr>
<td>Insulin, μIU/L</td>
<td>-0.09</td>
<td>0.15</td>
<td>-0.22</td>
<td>0.001</td>
<td>0.03</td>
</tr>
<tr>
<td>Adp250, mg/L</td>
<td>0.22</td>
<td>0.001</td>
<td>0.30</td>
<td>&lt;0.001</td>
<td>0.06</td>
</tr>
<tr>
<td>LMW30, mg/mL</td>
<td>0.20</td>
<td>0.28</td>
<td>0.22</td>
<td>0.21</td>
<td>-0.34</td>
</tr>
<tr>
<td>%HMW30 %</td>
<td>-0.01</td>
<td>0.97</td>
<td>0.14</td>
<td>0.43</td>
<td>0.12</td>
</tr>
<tr>
<td>%MMW30 %</td>
<td>0.06</td>
<td>0.74</td>
<td>-0.21</td>
<td>0.24</td>
<td>-0.03</td>
</tr>
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</table>
## Chapter 5. Novel Cardiovascular Disease Protective Markers

### Parameters

<table>
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<tr>
<th>Parameters</th>
<th>24Hr Food Recall Nutrients</th>
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<th></th>
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<tr>
<td></td>
<td>Pearson r</td>
<td>p-value</td>
<td>Pearson r</td>
<td>p-value</td>
<td>Pearson r</td>
</tr>
<tr>
<td>Leptin, pg/ml</td>
<td>-0.27</td>
<td>&lt;0.001</td>
<td>0.17</td>
<td>0.31</td>
<td>-0.20</td>
</tr>
<tr>
<td>24Hr Food Recall Nutrients</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fibre g/d</td>
<td>0.02</td>
<td>0.72</td>
<td>0.25</td>
<td>&lt;0.001</td>
<td>0.00</td>
</tr>
<tr>
<td>Tot Sugars g/d</td>
<td>0.09</td>
<td>0.17</td>
<td>0.20</td>
<td>0.002</td>
<td>0.05</td>
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<tr>
<td>fVitE, mg/d</td>
<td>0.05</td>
<td>0.46</td>
<td>0.21</td>
<td>&lt;0.001</td>
<td>0.02</td>
</tr>
<tr>
<td>β-Caro, mg/d</td>
<td>0.04</td>
<td>0.50</td>
<td>0.30</td>
<td>&lt;0.001</td>
<td>0.07</td>
</tr>
<tr>
<td>Tot fVitA, EQUIV.</td>
<td>-0.01</td>
<td>0.91</td>
<td>0.20</td>
<td>0.003</td>
<td>0.08</td>
</tr>
<tr>
<td>VitC, mg/d</td>
<td>0.08</td>
<td>0.21</td>
<td>0.30</td>
<td>&lt;0.001</td>
<td>-0.06</td>
</tr>
<tr>
<td>Riboflavin, mg/d</td>
<td>0.01</td>
<td>0.83</td>
<td>0.25</td>
<td>&lt;0.001</td>
<td>0.01</td>
</tr>
<tr>
<td>Folate, mg/d</td>
<td>0.04</td>
<td>0.58</td>
<td>0.23</td>
<td>&lt;0.001</td>
<td>0.04</td>
</tr>
<tr>
<td>Potassium, mg/d</td>
<td>0.08</td>
<td>0.24</td>
<td>0.32</td>
<td>&lt;0.001</td>
<td>-0.01</td>
</tr>
<tr>
<td>Magnesium, mg/d</td>
<td>0.09</td>
<td>0.16</td>
<td>0.24</td>
<td>&lt;0.001</td>
<td>-0.01</td>
</tr>
</tbody>
</table>

Refer to Appendix 4 Table C for expanded Table 5-9

Weight body weight; BMI body mass index; waist waist circumference; %Fat body fat percentage; SBP systolic blood pressure; DBP diastolic blood pressure; Hb haemoglobin; Hct haematocrit; MCV mean cell volume; MCHC mean cell haemoglobin concentration; HbA1c haemoglobin; ALT alanine transferase; γGT gamma glutamyl transferase; hsCRP high sensitive C reactive protein; IDF International Diabetes Federation; JIS Joint interim statement; MetSMCt metabolic syndrome marker count; NCEP National Cholesterol Education Programme; VitD vitamin D; βCaro beta carotene; s serum; VitA vitamin A; VitE vitamin E; INR VitK Internationalised Normalised Ratio vitamin K; Adpn adiponectin; LMW low molecular weight; HMW high molecular weight; MMW medium molecular weight; tot total; d day; g gram.

### Figure 5-2. Baseline sFSVitamin vs. Waist

![Figure 5-2](image-url)

s serum; sVitD vitamin D; sβCaro beta carotene; sVitA vitamin A; sVitE vitamin E
Chapter 5. Novel Cardiovascular Disease Protective Markers

Change Correlations: Serum Beta Carotene in All and Women and Serum Vitamin E in All and Men

As explained in the introduction change correlations were only formed for sβCaro, in all and women, and for sVitE in all and men and are presented in Table 5-10.

Table 5-10. Change in sβCaro in All & Women, & Change in sVitE in All & Men vs. Study Parameters

<table>
<thead>
<tr>
<th>Change in Parameter</th>
<th>Change in sFSVitamin</th>
<th>(\delta) sβCaro All, (n=145-155)</th>
<th>(\delta) sβCaro Women, (n=120-125)</th>
<th>(\delta) sVitE All, (n=144-154)</th>
<th>(\delta) sVitE Men, (n=28-30)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pearson r</td>
<td>p-value</td>
<td>Pearson r</td>
<td>p-value</td>
<td>Pearson r</td>
</tr>
<tr>
<td><strong>Anthropometry</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(\delta ) Weight, kg</td>
<td>-0.17</td>
<td>0.04</td>
<td>-0.19</td>
<td>0.04</td>
<td>0.09</td>
</tr>
<tr>
<td>(\delta ) BMI, kg/m(^2)</td>
<td>-0.17</td>
<td>0.03</td>
<td>-0.19</td>
<td>0.04</td>
<td>0.09</td>
</tr>
<tr>
<td>(\delta ) Waist, cm</td>
<td>-0.14</td>
<td>0.09</td>
<td>-0.16</td>
<td>0.07</td>
<td>0.13</td>
</tr>
<tr>
<td>(\delta ) SBP mmHg</td>
<td>-0.11</td>
<td>0.20</td>
<td>-0.17</td>
<td>0.05</td>
<td>0.14</td>
</tr>
<tr>
<td>(\delta ) DBP mmHg</td>
<td>0.04</td>
<td>0.63</td>
<td>0.02</td>
<td>0.79</td>
<td>0.28</td>
</tr>
<tr>
<td><strong>Haematology</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(\delta ) RBC x 10(^{12})</td>
<td>0.07</td>
<td>0.41</td>
<td>0.10</td>
<td>0.27</td>
<td>0.18</td>
</tr>
<tr>
<td>(\delta ) Hb g/L</td>
<td>0.15</td>
<td>0.08</td>
<td>0.21</td>
<td>0.02</td>
<td>0.17</td>
</tr>
<tr>
<td>(\delta ) Hct, %</td>
<td>0.12</td>
<td>0.15</td>
<td>0.17</td>
<td>0.06</td>
<td>0.12</td>
</tr>
<tr>
<td>(\delta ) MCHC L/L</td>
<td>0.15</td>
<td>0.07</td>
<td>0.18</td>
<td>0.05</td>
<td>-0.02</td>
</tr>
<tr>
<td>(\delta ) Leukocytes, x10(^9)</td>
<td>-0.21</td>
<td>0.01</td>
<td>-0.15</td>
<td>0.11</td>
<td>-0.07</td>
</tr>
<tr>
<td>(\delta ) Eosinophils, x10(^9)</td>
<td>-0.14</td>
<td>0.21</td>
<td>-0.12</td>
<td>0.35</td>
<td>-0.18</td>
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<tr>
<td>(\delta ) Monocytes, x10(^9)</td>
<td>-0.05</td>
<td>0.61</td>
<td>0.02</td>
<td>0.85</td>
<td>-0.22</td>
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<td>(\delta ) Lymphocytes, x10(^9)</td>
<td>-0.30</td>
<td>0.001</td>
<td>-0.21</td>
<td>0.03</td>
<td>0.02</td>
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<tr>
<td><strong>Biochemistry</strong></td>
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<tr>
<td>(\delta ) TC, mmol/L</td>
<td>0.21</td>
<td>0.009</td>
<td>0.22</td>
<td>0.01</td>
<td>0.49</td>
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<tr>
<td>(\delta ) LDL-C, mmol/L</td>
<td>0.21</td>
<td>0.01</td>
<td>0.22</td>
<td>0.01</td>
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<tr>
<td>(\delta ) TG, mmol/L</td>
<td>0.04</td>
<td>0.59</td>
<td>0.02</td>
<td>0.86</td>
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<tr>
<td>(\delta ) TC/HDL</td>
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<td>0.03</td>
<td>0.13</td>
<td>0.16</td>
<td>0.42</td>
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<td>(\delta ) Bilirubin, μmol/L</td>
<td>0.15</td>
<td>0.08</td>
<td>0.13</td>
<td>0.15</td>
<td>0.14</td>
</tr>
<tr>
<td>(\delta ) ALT, U/L</td>
<td>0.06</td>
<td>0.43</td>
<td>0.03</td>
<td>0.76</td>
<td>0.19</td>
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<tr>
<td>(\delta ) γGT, U/L</td>
<td>-0.03</td>
<td>0.75</td>
<td>-0.04</td>
<td>0.69</td>
<td>0.22</td>
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<td>(\delta ) Tot Protein, g/L</td>
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<td>0.08</td>
<td>-0.17</td>
<td>0.07</td>
<td>0.14</td>
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<td>(\delta ) Albumin, g/L</td>
<td>-0.11</td>
<td>0.20</td>
<td>-0.14</td>
<td>0.12</td>
<td>0.14</td>
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<tr>
<td>(\delta ) Globulin, g/L</td>
<td>-0.18</td>
<td>0.03</td>
<td>-0.18</td>
<td>0.04</td>
<td>0.11</td>
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<tr>
<td><strong>Metabolic Syndrome</strong></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>(\delta ) IDF/JIS MetSMCt</td>
<td>-0.11</td>
<td>0.18</td>
<td>-0.17</td>
<td>0.07</td>
<td>0.17</td>
</tr>
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<td>(\delta ) IDF MetS</td>
<td>-0.06</td>
<td>0.51</td>
<td>-0.12</td>
<td>0.18</td>
<td>0.14</td>
</tr>
<tr>
<td><strong>Serum Fat Soluble Vitamins</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(\delta ) sβCaro, μg/L</td>
<td>-</td>
<td>-</td>
<td>0.21</td>
<td>0.008</td>
<td>0.27</td>
</tr>
<tr>
<td>(\delta ) sVitA, μg/L</td>
<td>0.12</td>
<td>0.16</td>
<td>0.16</td>
<td>0.07</td>
<td>0.30</td>
</tr>
</tbody>
</table>

166
Previous diet and serum lipid relationships and intervention studies have led to hypotheses relevant to the current enquiry.

Positive change correlations in all of sβCaro were seen with change of TC, sVitE, Riboflavin, niacin and copper (all p≤0.009) and with TC/HDL and LDL-C (p≤0.05) (Table 5–10).
Positive change correlations in women of sβCaro were seen with change of riboflavin (p=0.004) and with change in TC, LDL-C, Hb, fVitE, niacin and copper (all p≤0.02) (Table 5–10).

Change in βCaro in all negatively correlated with change in lymphocytes (p=0.001) and in weight, BMI, leukocytes and globulin (all ≤0.03) (Table 5–10).

Change in βCaro in women negatively correlated with change in weight, BMI, lymphocytes and in globulin (all p≤0.05) (Table 5–10).

Change in sVitE in all positively correlated with change in DPB, TC, LDL-C, TG, TC/HDL (all p<0.001), and in γGT, dietary protein, dietary phosphorus and in sβCaro (all p≤0.008) and in sVitA, RBC, ALT, Hb and in dietary: energy, calcium, total fat, riboflavin, niacin equivalents, MUFA, potassium, zinc and iron (all p≤0.05) (Table 5–10).

Change in sVitE in men positively correlated with change in DPB, TG and in TC/HDL (all p<0.001), and in TC, dietary: protein, potassium, zinc and phosphorus (all p≤0.007) and in SBP, ALT, sVitA, and in dietary: energy, total fat, saturated fat, fibre, niacin equivalents, calcium, and iron (all p≤0.05) (Table 5–10).

Change in sVitE in all negatively correlated with change in monocytes (p=0.01) alone, and there were no negative change relationships in men (Table 5–10).

In the baseline ordinal regression, shown in Table 5-11, HbA1c (p=0.003), urate (p<0.001), sVitE (p=0.03) and age (p=0.02), were significantly positively related to MetS marker count. SβCaro (p<0.001), was highly significantly negatively related to MetS marker count (Table 5-11).
Table 5-11. Baseline Serum Fat Soluble Vitamin vs. Metabolic Syndrome Marker Count – Ordinal Logistic Regression

<table>
<thead>
<tr>
<th>Relationship to the outcome, Metabolic Syndrome Marker Count</th>
<th>Analysis of Maximum Likelihood Estimates</th>
<th>Odds Ratio Estimates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Explanatory Parameter or Effect</td>
<td>Estimate</td>
<td>Standard Error</td>
</tr>
<tr>
<td>Gender, F</td>
<td>-0.14</td>
<td>0.22</td>
</tr>
<tr>
<td>Age, y</td>
<td>0.03</td>
<td>0.01</td>
</tr>
<tr>
<td>sVitD, nmol/L</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>sβCaro, μmol/L</td>
<td>-0.90</td>
<td>0.28</td>
</tr>
<tr>
<td>sVitA, μmol/L</td>
<td>-0.00</td>
<td>0.38</td>
</tr>
<tr>
<td>sVitE, μmol/L</td>
<td>0.04</td>
<td>0.02</td>
</tr>
<tr>
<td>INR (VitK)</td>
<td>0.67</td>
<td>0.74</td>
</tr>
<tr>
<td>HbA1c %</td>
<td>1.03</td>
<td>0.23</td>
</tr>
<tr>
<td>Urate, mmol/L</td>
<td>6.31</td>
<td>2.12</td>
</tr>
<tr>
<td>Albumin, IU</td>
<td>0.02</td>
<td>0.06</td>
</tr>
<tr>
<td>Globulin, IU</td>
<td>-0.04</td>
<td>0.04</td>
</tr>
<tr>
<td>AlkPhos, IU</td>
<td>0.0073</td>
<td>0.00549</td>
</tr>
</tbody>
</table>

The signs of the estimates indicate the direction of the relationship such that explanatory variable is negatively related to the outcome, MetSMCt. The odds ratio is < 1 if there is a positive relationship of the explanatory variable to the outcome. βCaro beta carotene, AlkPhos alkaline phosphatase s serum; VitA vitamin A, VitD vitamin D, VitE vitamin E, MetSMCt metabolic syndrome marker count.
A generalised (ordinal regression) mixed model was fitted to the sFSVitamins with the MetS marker count, over 2 time points, baseline and 6m. For sVitD there was no evidence of a relationship with its mean for the two time points (p=0.64) and MetS marker count, but there was evidence of a positive relationship of change (p=0.02) in sVitD with MetS marker count.

There was evidence of a negative relationship of the overall mean (baseline to 6m) of sβCaro (p=0.02) with MetS marker count and also possibly a negative relationship with its change (p=0.08). No relationship could be demonstrated for the overall mean (baseline to 6m) (p=0.99) of or change (p=0.32) in sVitA with MetS marker count. There was strong evidence of a positive relationship in the mean of sVitE with MetS marker count (p=.004) and a possible positive relationship of the change in sVitE (p=0.09).

With respect to sVitD a general linear (regression) modelling was formed to include season and ethnicity. It showed an estimated decrease of 0.74nmol/L (p=0.002), in sVitD per 1kg/m² increase in BMI with the total model explaining 22% of the variation in sVitD levels and BMI explaining 3% of the variation. On replacing BMI with waist, there was a decrease of 0.29nmol/L (p=0.01), in sVitD per 1cm increase in waist, with the total model explaining 21% of the variation in sVitD and waist explaining 3%. There was strong evidence of the association of sVitD with ethnicity (p<0.001), and season (p<0.001). When both BMI and waist were included neither could be demonstrated to contribute over and above the other (p=0.25 and p=0.67 respectively), nor could an association of sVitD with gender (p=0.52), or age (p=0.52), be shown.

5.4 Discussion

Overall, the findings in this part of the study were that the sFSVitamins had almost opposite effects; sβCaro was a protective marker and sVitE acted as a strong risk marker. The hypothesis that all FSVitamins, including sVitA and sVitE would be lower in the disturbed metabolism of MetS, was true for sβCaro and sVitD, but not for sVitA and sVitE, whose levels were higher in MetS.

The Adpn oligomers showed increased sexual dimorphism, with Adpn being more pro-oxidant, probably via MMW, were clearly shown to have very different and complex relationships with obesity-related MetS, each other and other CVD risk and protective
markers. Not all were shown to have protective relationships, which meant that the protective aspects of Adpn were not complete.

Firstly, and most importantly, sβCaro was shown in this analysis to perform better than expected, in that it correlated with a wide range of parameters that were all beneficial, or sβCaro correlated negatively with CVD risk markers or adverse markers, such as leukocytes. The same relationships were maintained with change correlations of sβCaro and when other strong CRSHaem/Biochem explanatory markers such as urate and HbA1c were added in the mixed model sβCaro was still highly significantly negatively related to MetS marker count.

In addition, sVitD had fewer numbers of associations but they were positive for protective factors and negative for risk factors.

Surprisingly, sVitA and sVitE were the opposite; they could have functioned as potent risk markers for MetS with multiple and overall positive relationships with MetS marker count and many other MetS-related markers. On change correlations sVitE correlated with dietary markers of a high fat, high meat diet. SVitE maintained a strong negative change relationship with MetS marker count when the other sFSVitamins and other strong CRSHaem/Biochem explanatory markers such as urate and HbA1c were included in the mixed model. Both sVitA and sVitE tended to disprove part of the hypothesis, as they showed no signs of being protective markers.

Adpn showed mixed relationships with MetS and other study parameters correlating with some pro-oxidant appearing markers – such as age and AlkPhos, yet relationships with female gender, HDL-C being antioxidant. Adpn in men was more pro-oxidant.

SβCaro was positively correlated with almost all major CVD health markers Adpn, HDL-C, and many healthy food nutrients such as fibre. The adverse marker correlations were to CVD risk. They fall into groups of inflammatory markers containing and oxidant or metaflammatory markers and external oxidant stressors, namely cigarette smoking, and lastly obese anthropometry. βCaro is mostly derived from vegetables and fruit and is a marker of their dietary consumption. Fruit and vegetable foods have extensive health promotion properties, including mental health and Mediterranean diets have been well shown to protect health. Some dietary intake may be from βCaro as
red/orange/yellow food colouring, although phytoxanthines are highly used as yellow colorants, since added βCaro might be considered a risk in some individuals\textsuperscript{694}. The only anomalies may appear to be the significant positive correlation of sβCaro to lipids TC, and LDL-C in addition to HDL-C.

βCaro is carried on the latter two lipoproteins\textsuperscript{470}, and notably βCaro was not related to TG. Another negative appearing relationship was that sβCaro was related to age. Perhaps as individual’s age they make an effort to eat more vegetables, or this older cohort always ate more than the young obese\textsuperscript{695}. Overall serum βCaro decreased in women over the study, which may have been due to a decrease in sugar based fruit.

The βCaro-derived asymmetric carotenals, some of which can inhibit the nuclear receptors Retinoid X (ROX) and PPARs, are interesting as they may antagonise βCaro stimulatory actions\textsuperscript{468,475}. Note that the PPARs are downstream signalling molecules of Adpn. βCaro appears to be promising as a CVD protective marker to use for tracking MetS and CVD risk.

In the current study there were fewer relationships of sVitD than the other sFSVitamins with CVD protective markers. sVitD was negatively related to obesity-related anthropometric markers of weight, height, BMI, waist, in addition to leptin and HbA\textsubscript{1c}. The negative relationship of sVitD to HbA\textsubscript{1c} and waist, in the present study, adds weight to sVitD as opposing MetS and insulin resistance. Central obesity is not healthy for bone\textsuperscript{696}. VitD, via its receptor which is present in insulin-producing beta-islet cells, is known to be a potent regulator of cell proliferation and differentiation\textsuperscript{697}. SVitD was related to haematocrit, which relates to oxygen carriage, and physical fitness. Albumin, a carrier of lipids\textsuperscript{390} is similar to VitDBP, and coincidental reductions in both are detrimental to physical capability\textsuperscript{698}. VitD has effects on muscle and the obese are often relatively physically unfit, sarcopaenic and have excessive fat within muscle\textsuperscript{699,700}. Interestingly sVitD levels predict weight loss success\textsuperscript{701,702}.

It is not known if low sVitD in obesity is a storage issue and the VitD is sequestered in the large adipose tissue depots, or whether obese individuals are less likely to sunbathe or be exposed to the sun. Finally, obesity may interfere with VitD metabolism, but mechanisms are uncertain\textsuperscript{449,703}. 
The inter-relatedness of the sFSVitamins includes sVitD which was positively correlated with sβCaro, sVitE in addition to Adpn. All have profound metabolic activities which may link with obesity via the immune system and leukocytes\textsuperscript{704-706}. Basophils are probably involved as part of the skin immune system and skin barrier protection from infection and autoimmune problems, as is sVitD\textsuperscript{678}. The lack of dietary correlations of sVitD possibly indicates its dependence on skin photosynthesis and hormone-type actions. Alcohol was a surprise link to sVitD, but again both can be beneficial markers. VitD may have a complex antioxidant effect on some chemicals associated with smoking\textsuperscript{707}.

Low sVitD in obesity appears to cause metabolic destabilisation\textsuperscript{451,708,709}. Interestingly, VitD action is usually assumed to be related to 1,25 hydroxyVitD, but the (serum 25 hydroxy) sVitD itself may be active in TIIDM and other CVD risk\textsuperscript{710}. The relationship between the two forms, taking into account parathyroid hormone, VitDBP and its receptor, is complex.

In the current study, sVitD was highly influenced by season and ethnicity, likely via skin pigment, as both factors interfere with skin solar photosynthesis of VitD\textsuperscript{711}. These factors overwhelmed the effects of obesity. Waist and weight explained only a few % of the variability in this group. In fact, in the mixed model sVitD was positively correlated MetS marker count. This may be explained by participants having more MetS at the study start in late in summer after the greatest sun exposure of the year, and skin VitD photosynthesis. There was a large drop in sVitD over the 6m as most participants enrolled at the end of summer, thus mainly proceeding with the study through winter.

Therapeutic studies of spVitD have usually been positive, particularly on bone, although recently both calcium with and without supplemental VitD have shown increased calcific arteriosclerosis and myocardial infarction\textsuperscript{712}. The current study and literature point to sVitD having a positive influence in obesity and MetS. sVitD may be a useful protective marker in MetS.

Historically, high blood levels of the antioxidant fat and water soluble vitamins have been associated with health, and have been associated with a healthy diet\textsuperscript{684,713}. In the current study both sVitA and sVitE were negatively correlated to weight and BMI and the sVitA and sVitE concentrations were higher in men.
Many researchers correct for lipids, but as shown in the present study all sFSVitamins correlated differently and differentially with each fraction. That they are transported on the CVD risk lipids may explain both the raised sVitA and sVitE, and their mimicking of CVD risk. SVitE, especially, associates with both LDL-C and TG. Further their correlations with TG and BP, and other CRShaem/Biochem: urate, ferritin, and liver enzymes, likely partly explain why SVitE and sVitA correlated MetS marker count and MetS.

Another issue with sVitA and sVitE is that they are highly added to deep fried starches such as potato crisps and processed meats respectively to prevent fat oxidisation and rancidity. γ-tocopherol, a VitE vitamer, is probably the largest component of tocopherols in the diet, and it has its own biology\textsuperscript{714,715}. Thus, even without dietary supplements higher levels of serum α- and γ- tocopherol are occurring\textsuperscript{716}. γ-tocopherol is associated with worse food choices and may be less healthy than α-tocopherol\textsuperscript{714}.

There are an increasing number of reports that indicate that obesity is associated with raised levels of sVitE and sVitA\textsuperscript{717,718}. It has been suggested that high levels of sVitE may be a CVD risk marker and diabetogenic\textsuperscript{716,719}. As noted previously vitamin interventions for CVD and cancer prevention have been disappointing\textsuperscript{162,720-722}. High dose vitamin intervention studies have been tested for disease prevention or amelioration, and generally there has been no benefit, and where there has been benefit such as telomere preservation has been shown, a concurrent healthy diet often cannot be excluded\textsuperscript{504,723,724}.

Both VitA and VitE have antioxidant effects but they may be pro-oxidants in some circumstances, and many small pro-oxidant molecules stimulate antioxidant response systems, which will be addressed in Chapter 6.Oxidative stress may result in the excess mobilisation and consumption of blood and stored antioxidant FSVitamins\textsuperscript{695}, and the related, low grade systemic metaflammation may impinge on functions and activities\textsuperscript{476}.

The present study does not answer why sVitA and sVitE act as pro-oxidant markers, or whether VitA and VitE action at the cell is normal and antioxidant. The hypothesis for these two vitamins appears incorrect, and more work is required to resolve mechanisms and other unknowns.
Chapter 6. Causes of Obesity and Metabolic Syndrome

**Adiponectin**

There is still some doubt about the primary function of Adpn in health. Adpn appears to be associated with protective markers, normal weight and fewer MetS associate problems, and in the current study Adpn correlated with female gender, HDL-C, and negatively with the markers of weight, BMI and waist, inflammation and oxidant markers.

The sexual dimorphism was marked in this study as expected, with negative correlations of Adpn with anthropometry were shown in women, but not men except for height. Insulin and HOMA showed a negative correlation in women but this was lost in men. Surprisingly, in men there was no negative correlation of Adpn with MetS.

Adpn responded as predicted to a large extent, and serum levels increased in women over the study with weight loss. However, Adpn had a proportionately higher MMW and lower HMW levels, and overall Adpn decreased in men. Even so HMW and % HMW increased in both women and men, and MMW and % MMW decreased in both groups, with similar effect sizes. The other difference between the genders was that change in MetS correlated with Adpn and oligomers in men, except for MMW, but not women in the small Adpn group.

Importantly, Adpn has a number of links with a high micronutrient diet, typified by large volumes and variety of fruit, vegetables and nuts, low refined starches and modest meat content, somewhat irrespective of energy content, and the Mediterranean diet. Other nutrient connections include resveratrol, from ground nuts, grapes and other seeds, which affects multimerisation. This may be the reason for the linkage of Adpn with the sFSVitamins, especially which may be the marker of a high fruit and vegetable diet, and some of these markers may interact with Adpn. However, only dietary fVitE, which can also derive from nuts, correlated with Adpn in this study.

Adpn associates with other positive health behaviours including physical activity.

Adpn was negatively correlated with raised %Fat in women, TG, hyperinsulinaemia and HOMA in the current study, and prior studies show Adpn predicts lower %Fat, TG and
Adiponectin (Adpn) release from adipocytes may be affected by large over full central adipocytes. Mechanisms for low Adpn in obesity may involve mitochondrial and ER stress, whose function is linked to Adpn synthesis in adipocytes. As obesity resolves Adpn synthesis and function return to normal.

Recently Adpn has been reported to stimulate mitochondrial biogenesis, prevent apoptosis and enhance longevity. In this study Maori and Pacific individuals had consistently lower levels of Adpn. They also typically smoked 1.6 x more than the European. Socioeconomic factors of affluence, food quality and smoking rates affect obesity rates rather than ethnic differences. More alcohol appeared to be healthier for men by Adpn levels. Alcohol may compete with Adpn in the liver and may augment beneficial effects.

When there is a reason for inflammation such as in infection, in individuals with normal fat mass, Adpn increases and becomes pro-inflammatory. Chronic inflammatory disease, although an immune disorder, is probably not a central adipocyte problem. As such, Type I DM and Graves' disease in slim individuals is related to higher Adpn, as presumably Adpn becomes proinflammatory.

Age correlated with Adpn, probably as more chronic inflammation is occurring due to other senescence processes. The accumulation of inflammatory ‘oxidative stress’ in age, especially as weight and even fat levels drop, may also explain the raised Adpn in age. Furthermore, those with ‘age-accelerated CVD’ appear less able to raise an antioxidant response as ‘degenescence’ overtakes cellular functions.

The HMW was shown to be higher in women than men in this study, and this is well known, and MMW was lower. Increased testosterone suppresses HMW production in men and may be the reason for both lower levels in men and the greater proportion of MMW. The women in the study, who were over all healthier, had increased HMW and lowered MMW & LMW, with HMW increasing, and MMW and LMW, decreasing over time. In obesity, HMW formation is the most compromised and proportionately HMW is reduced, so its peripheral effects are diminished, although the brain still receives input into appetite controls from MMW and LMW.
The disulfide bond-A oxidoreductase-like protein (DsbA-L) that multimerises the Adpn monomers to the HMW is also decreased in obesity. The 3 oligomers may be acting with different functions. HMW may mediate some pro-inflammatory effects in T2DM and exercise, yet LMW may inhibit lipopolysaccharide (LPS) and be negatively related to prooxidant myeloperoxidase. The production of oligomers may also be differentially affected by hyperinsulinaemia.

Adpn receptors, Adipo R1& 2 also very complex, are increasingly found to have new protective functions, including those of antiapoptosis, longevity, and have other nutritional ligands. The phytoalexins include osmotins (dehydration defence chemicals) which stimulate the AdipR’s.

The consistently high relationship with HDL-C warrants comment. HDL-C and Adpn are known to be related in normal health and confer protection from CVD. HDL-C is a collector/carryer of lipids, taking them to the liver for oxidation or other metabolic functions. Adpn controls the efflux of lipids from HDL-C and adipocytes and sensitizes the liver and muscle to lipid uptake. At the arterial endothelium higher levels of Adpn limit foam cell formation, by increasing lipid efflux as monocyte/macrophages have the same lineage as adipocytes and share functions.

It appears that HMW can be the most pro-inflammatory oligomer, and is highly related to HDL-C. In intensive care hospital populations with high glucose and TC, HDL-C is often low. However, in some highly infected subsistence populations with low HDL-C, there is little atherosclerosis. There is no definitive research showing raising HDL-C with pharmaceuticals, as opposed to diet and exercise, reduces CVD.

There were negative and positive correlations with leukocytes and Adpn, in the current study, with men having higher leukocyte activity. Adipose tissue hypoxia may contribute to inflammatory monocytes migrating to this tissue, and setting up inflammatory communication with neutrophils and lymphocytes. When the men’s HMW increased over time there was a marked decrease in the white cells in the current study.

Urate negatively correlated with Adpn in both women and men indicating that Adpn is sensitive to oxidant stress, but it did not change with HMW change. AlkPhos is usually negatively related to obesity but had positive correlations with Adpn. CRP is now known
to be secreted by central adipose tissue and may be a product of adipose tissue mass\textsuperscript{380,382}. Positive correlations of Adpn with bilirubin should be helpful as this haem metabolite has recently been shown to be an antioxidant, antiatherogenic and positively associated with good health\textsuperscript{358,632}.

Overall Adpn in women was correlated with other beneficial markers, except smoking and then the relationship was negative when the number of cigarettes was correlated. The changes in HMW in women were less beneficial.

As Adpn is such a complex molecule with complex functions it is hard to interpret how useful it could be. However, obesity-related MetS is complex and it may be that once more is known about Adpn’s functions, especially the separate oligomers, it may yet hold potential as an adjuvant marker for MetS. At present, it is inadequately understood and its use could well become confusing.

Although mentioned, change data for the sFSVitamins was limited to the two serum vitamins where CVD treatment interventions have been performed. Added sampling of the change in sVitE in men was performed for sVitE as the literature has shown relationship with CVD in men with high levels of sVitE.

There were significant limitations to the current study.

As the Adpn and sFSVitamin studies were hypotheses generating, the usual limitation of widespread correlations increasing the risk of false positives or type I errors still applies. It is expected that the findings will be corroborated or not in further studies. Only the Adpn\textsubscript{250} baseline was available for assessment, and gave unexpectedly low mean values.

The Adpn\textsubscript{30} oligomers analysed were selected or ‘hand picked’ post hoc from participants who exhibited extremes in weight, MetS and in change in these parameters. The number of participants was very small, especially in the men who completed the study. As the range in the variables in this group was chosen to be high the correlations were high, as expected and planned.

A major limitation was a 24Hr Food Recall instrument recording just one day of food intake at baseline and 6m visit. Extremes are encountered, as there is no regression to the
mean, which makes their use limited. The range of βCaro reported was extremely wide at 40 to 95,000μg. In this type of study 3d Food Recall, recorded 3 times, as well as a Food Frequency Questionnaire could have been administered.765

The physical activity questions were condensed, and the sedentary questions were not validated and proved to be insensitive. Highly biased reporting was suspected in the food energy. In the obese, under or misreporting of high energy food is more common than in leaner individuals and over reporting of healthy food types, and physical activity which are perceived as healthy can occur.598,602,766-768. However, the findings of specific correlations were expected and borne out by the literature.

The positive aspects of the study were that using a wide array allowed the separation of general tendencies of the markers. The findings have shown that sβCaro is by far the most widely correlated and predictable CVD protective marker and that sVitD was also consistently associated with positive markers in CVD.

SvitA & sVitE remain important, as are all vitamins, but they appear to be trapped within putative causes of obesity and MetS: (1) being carried on adversely raised serum lipids and (2) consumed as modified fried industrial fat antioxidants in baked starch foods232-236.

Lastly, Adpn, reveals itself as being very complex multimeric adipokine with complex receptor signalling in the literature. In this study its relationships appeared unpredictable. The lack of complete knowledge about its normal function in health, and its abnormal function in obesity render it an unreliable CVD protective marker in MetS at this time.

Conclusion

In conclusion, the study achieved its objectives of studying a number of putative protective CVD markers. SvitA, sVitE, and Adpn were eliminated, for the time being as Novel CVD Protective markers, owing to either having CVD risk properties or as having an unpredictable combination of risk and protective correlations. Much more study of the mechanisms of VitA, VitE and Adpn is needed.

The study was successful in indicating testing sβCaro and sVitD should be considered, possibly as part of an index, as CVD protective markers for use with obesity-related MetS.
Gaining adequate sun exposure for dermal VitD photosynthesis to suit latitude, skin pigment and lifestyle, but minimise solar skin damage is also probably prudent advice, but is controversial in pale skinned populations in some areas.

As βCaro has been available in food for as long as primates have been evolving, and is well studied, the standard advice of eating a high fruit and vegetable and fish diet as part of a healthy lifestyle can still be given.
Chapter 6. Causes of Obesity and Metabolic Syndrome

6.1 Introduction

6.1.1 Scope

In this chapter a unifying hypothesis on causes of obesity and related MetS is proposed, with reference to a multidisciplinary literature. The hypothesis attempts to explain what is occurring at the level of the individual, and also why MetS is developing in large numbers in populations of humans, in the current era.

6.1.2 The Need for a New Theory on Causes of Obesity

There has been a failure to prevent up to nearly a third\textsuperscript{769} of some westernised populations from developing obesity and its comorbidity of MetS. MetS contributes to the degenerative diseases, as previously outlined\textsuperscript{248,770-772} and treatment has also largely been unsuccessful. The need for a unifying hypothesis on causes is therefore reviewed.

In the existing main-stream paradigms on researching and managing obesity, certain assumptions have been made that have set current working hypotheses on a trajectory that has limited further interpretations of the problem and research needed. A more developed understanding of deep seated causes may indicate new management approaches, or at least an abandonment of unhelpful treatments, and a cautious approach to taking up new treatments\textsuperscript{773}.

The first assumption that may be problematic is the literal interpretation of the first law of thermodynamics. The first law of thermodynamics states that energy can be neither created nor destroyed so the assumption is that fat gain = energy in - energy out (PA) x moderators\textsuperscript{774}. The moderators in this scheme appear to be basal metabolic rate.

Individuals who are obese are assumed to eat excess of all foods. It is likely that this group has the largest imbalance. Energy dense food is over consumed, by a large margin,
compared with fruit and vegetables, which are often severely neglected and under consumed, along with their vitamins and micronutrients.

As all of the vitamins have been discovered, the types of foods where they are found are well known, vitamin supplement use has become widespread in wealthy countries\textsuperscript{775,776}, food fortification is common\textsuperscript{777,778}, thus it is often assumed that vitamin insufficiency does not occur. Dietary studies relating to obesity and TIIDM are therefore performed where food energy alone is adapted. Manipulating macronutrients in weight loss studies is the main concern\textsuperscript{779} and controlling or lowering plasma glucose and HbA\textsubscript{1c} is the major outcome in TIIDM trials\textsuperscript{780}. Once the assumption that pure energy balance, rather than nutrient balance, is the main issue, then future research follows along these lines, and gradually filters into clinical management.

‘Pick the Tick’ is a Australasian dietary guide; ‘These foods have met the National Heart Foundation’s criteria for lower fat or preferred fat choices, but may still be high in sugar and energy’\textsuperscript{781}. Food micronutrients are rarely considered by obesity researchers.

However, biology is about manipulation and control of energy reactions with catalysts and antioxidants, and a myriad of other acquired or synthesised biochemical based processes. In large mammals this is likely to be a complex process, and humans have some extra issues to deal with, as will be discussed. This approach may explain why it appears surprising to many obesity researchers that the Mediterranean diet can augment health, decreasing MetS, stabilising or even decreasing weight and waist. This diet includes a high content of plant food nutrients, but may contain 40\% energy from fat and oil, and even with added energy from nuts appears beneficial\textsuperscript{240,248,713,782,783}.

Another assumption is that individuals just have to change their behaviours, and that they are in control of their environment and food intake; that diet is just a matter of ‘personal choice’\textsuperscript{784}. Blame for lack of moral restraint, gluttony and sloth, is still common\textsuperscript{50,785}. Ridicule and stigma of obese individuals is profound, and associated with significant psychological morbidity, not least of which are the eating disorders\textsuperscript{784,786,787}. Obese individuals themselves often feel guilt, distaste and despair, mirroring what others may think\textsuperscript{788-790}. Presently, as society has not been able to control the rise of obesity, either in less affluent countries or in children\textsuperscript{791}, the profound issues of the interaction of humans and the westernised food pattern, is starting to be taken more seriously.
6.1.3 Background to Theory Development

As obesity appears to have influences arising from basic biology to socioeconomic factors, it appears a new approach is needed. One new approach is to include an overview from a more distant vantage point (evolution), that nevertheless incorporates concurrent, congruent and plausible biological (biochemical), and sociological (behavioural) factors. Thus there are two broad areas. The first is the biological aspect of obesity, including energy balance and nutrition, and the other is the psychosocial area of why humans produce and eat the food they do. Both of these areas are likely to be informed by studies of human evolution (Figure 6-1).

Figure 6-1. A Version of Human Phylogeny

Slightly adapted from Carroll 2003 \textsuperscript{792} Myr million years
Of particular interest is the rapid evolution of humans (Figure 6-1), human brain development, the effect on energy balance of encephalisation, and particularly other associated co-adaptations. The current Chapter is about exploring the likely co-adaptations proposed as arising in response to supplying a large brain with extra energy. Hominins separated from a last common ancestor with the other hominoids, the great apes, about 7 million y ago (Figure 6-1).

Humans have been consuming highly varied omnivorous diets, derived from a frugivorous diet of a primate ancestor about 20 million y ago. Hominins had been becoming omnivorous over the last 7 million y and by 2.5 million y ago, they were shown to be relying on higher fat and energy animal-based food.

However, fruit and vegetable foods were always important for general nutrition. Even before the shift to animal products, the diet, with fruit and oil seeds, has been described as a high quality or a high energy diet as shown in Figure 6-2. The importance of energy manipulation became especially relevant to hominins, as they developed a unique metabolic state due to the development of a large, energy demanding brain.

**Figure 6-2. Relative Brain Size & High Dietary Quality, or High Energy Diets, in Primates**
Encephalisation in the early human, a medium-sized mammal, meant that the human brain drew a relatively high proportion of the body’s energy. The human brain is moderately, but constantly, metabolically active\textsuperscript{793,794,796-799}. 

6.2 Aims

In this theoretical Chapter the overall aims are to develop a unifying hypothesis of obesity and MetS.

The specific aims are to:-

- Review and critique past theories on the topic of obesity related MetS
- Explore the multidisciplinary literature on the evolution of humans, including the effect of brain enlargement on nutrition and health
- Investigate
  - 1) Appetite science, the modern mesolimbic functions of the human brain, and energy dense food addiction, and
  - 2) Basic metabolic biochemistry of human nutrition, relevant disease pressures, and the employment of phytochemical modulation, or situation specific and modest adjustments, in human metabolism.

The above are related to past and current living conditions and environments.

Further aims are to:-

- Suggest further investigations on preparing an energy budget which takes micronutrients into account.
- Propose study designs for future clinical studies to test micronutrient dense whole-food diets in obesity and MetS.

6.3 Methods

This chapter does not have experimental data to present. A multitopic, multidisciplinary broad-based literature search is undertaken whilst the composite unifying theory on obesity related MetS is constructed. Importantly, the search cannot be a single topic, multidatabase review\textsuperscript{800}, or a systematic review, as the premises of much of what has gone before, are questioned.

The search is presented in a narrative style, an accepted method for this purpose\textsuperscript{801}. 

185
Chapter 6. Causes of Obesity and Metabolic Syndrome

The concept of proximal or distal is introduced. It is defined here because sociology, philosophy, general biology and genetics produce different and contested meanings. Proximal causes contribute to what individuals actually do such as inventing technologies when there is food scarcity, such as digging with tools for underground tubers to increase consumption of energy dense foods, and which thence affect other factors, for example body weight and shape. They often have behavioural aspects and individuals can differ in the population. Distal causes are those whose influence, such as genetic endowment, has already affected the population, and derives from the past. Socioeconomic or physical and environmental constraints, such as desertification and ice ages, are also distal causes. Both causes overlap and leap-frog as genetic/epigenetic changes become embedded in response to the environment.

Secondly, new technologies in archaeological investigation have become very sophisticated. Genetic and epigenetic methods are now used, along with mathematical modelling. Thus genomic and metabolomic high through-put microarray computer assisted analysis and mathematical modelling have brought new knowledge into the two major areas that are relevant; namely human evolutionary studies, and biochemistry. Metabolomics is ‘the systematic study of the unique chemical fingerprints that specific cellular processes leave behind’. Nutrigenomics is the study of how nutrients influence the genome.

Possibly surprisingly, the same areas of technology-assisted study also shed light on the neurochemistry of food related behaviours. Human archaeology and biochemistry can converge in genomic archaeology, both of which inform the new hypothesis.

This investigation, therefore, commences with an examination of initial conditions. In this thesis initial conditions are taken as time periods in evolution where unique human attributes that relate to energy metabolism were becoming established. As the brain enlarged, new adaptations were required by allometry to support this organ’s increased energy requirements. Allometry is a size to shape/metabolic rate/longevity relationship of one part of the body as it grows with respect to the rest of the body. Various energy expensive-tissue trade-off theories have been proposed by other researchers, which relate to brain energy, will be considered.
Chapter 6. Causes of Obesity and Metabolic Syndrome

The first principles of the biochemistry of energy metabolism are explored. These first principles are examined to determine if humans were employing different pathways of energy management from other animals.

6.4 Influences from Thesis Study on the Unifying Hypothesis

The study for this thesis confirmed reports in the literature that the pathology of oxidative stress is highly likely to be present in obesity-related MetS, as seen in degenerative disease. Oxidative stress either antedates or co-induces inflammation or metaflammation, of which inflammation may be of lesser influence (Table 6-1 & Table 6-2) summarise an interpretation of the study results in the light of the association with MetS of the strongly oxidant HbA1c and urate and γGT which predicts and tracks MetS.

To a lesser extent, ESR and neutrophil inflammatory stress markers, probably with oxidant properties in addition, had both cross sectional and longitudinal relationships. Bilirubin may protect from oxidant related hypertension. There was possible protection from oxidant stress by sβCaro or associated dietary microconstituents or micronutrients, as is also shown in the literature, and sVitD, and the factors that increase sVitD.

6.4.1 The Unifying Hypothesis on Causes of Obesity and Metabolic Syndrome

The hypothesis is expounded early in this Chapter. As it comprises two main parts, with the modern day corollary, it is instructive to have it in mind as the evidence is investigated in the following sections.

Firstly, appetite control, especially in hedonic mesolimbic pathways in the brain, which relates to energy dense food acquisition, became very well developed.

The dopamine dependant reward/motivation system became a very strong mechanism to ensure maximum energy uptake and intake, by driving smart energy dense food procurement methods, and subsequently the invention of high energy food cultivating, processing, storage technologies, transport, and marketing technologies.
Table 6-1. Estimate of General Oxidant and Inflammatory Properties of Laboratory Markers in the Diet&Health-Novel CVD Marker Study

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Antioxidant</th>
<th>Pro-oxidant</th>
<th>Anti-inflammatory</th>
<th>Pro-inflammatory</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adpn</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>- (+)</td>
<td>Can be proinflammatory. Raised with age and inflammatory conditions in the current study(^{739})</td>
</tr>
<tr>
<td>sVitD</td>
<td>?+</td>
<td>-</td>
<td>(-) ?</td>
<td>- (+)</td>
<td>Now thought to prevent infection, autoimmune disease and cancer(^{816,710})</td>
</tr>
<tr>
<td>sβCaro</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>Marker of high fruit and vegetable diet(^{817})</td>
</tr>
<tr>
<td>sVitA</td>
<td>(-+)</td>
<td>+</td>
<td>-</td>
<td>+/-</td>
<td>Is an antioxidant, vital for vision, retinoid X receptor. Recent studies show may be pro-oxidant(^{716,817,818})</td>
</tr>
<tr>
<td>sVitE</td>
<td>(-+)</td>
<td>+</td>
<td>-</td>
<td>+/-</td>
<td>Known antioxidant, recent studies show can be pro-oxidant. Intervention studies failed(^{716,818}).</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>Pro-oxidant as secretes H(_2)O(_2) to kill pathogens(^{739})</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>-</td>
<td>+/-</td>
<td>(-)</td>
<td>(+)</td>
<td>Known allergy marker.</td>
</tr>
<tr>
<td>Basophils</td>
<td>?</td>
<td>+/-</td>
<td></td>
<td></td>
<td>Related to histamine producing mast cells in inflammation, become foam cells(^{819})</td>
</tr>
<tr>
<td>Monocytes</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>Sensing and coordination of leukocytes in inflammation, become foam cells(^{819})</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>T-cell natural killer cells, may be pro-oxidant</td>
</tr>
<tr>
<td>ESR</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>ESR an effect marker, not an item</td>
</tr>
<tr>
<td>Albumin</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>Can be anti or pro-oxidant(^{820})</td>
</tr>
<tr>
<td>Globulin</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>Known inflammatory marker, due to immunoglobulins</td>
</tr>
<tr>
<td>HsCRP</td>
<td>?</td>
<td>?</td>
<td>-</td>
<td>+</td>
<td>Synthesised in adipose tissue and liver</td>
</tr>
<tr>
<td>HbA(_1c)</td>
<td>-</td>
<td>++</td>
<td>-</td>
<td>+</td>
<td>Glycated protein, oxidant</td>
</tr>
<tr>
<td>Urate</td>
<td>-</td>
<td>++</td>
<td>-</td>
<td>++</td>
<td>Known antioxidant but oxidant in MetS</td>
</tr>
<tr>
<td>Ferritin</td>
<td>-</td>
<td>+</td>
<td>(-)</td>
<td>(+)</td>
<td>Known oxidant, but antioxidant effects in healthy individuals(^{821})</td>
</tr>
<tr>
<td>Bilirubin,</td>
<td>+</td>
<td>-</td>
<td>?</td>
<td>?</td>
<td>Recently found to be antioxidant(^{821,822})</td>
</tr>
<tr>
<td>AlkPhos</td>
<td>-</td>
<td>+</td>
<td>?</td>
<td>?</td>
<td>Weak oxidant, lipid relationships</td>
</tr>
<tr>
<td>ALT</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>Oxidant – in glucose metabolism</td>
</tr>
<tr>
<td>AST</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>Oxidant – in glucose and lipid metabolism</td>
</tr>
<tr>
<td>γGT</td>
<td>-</td>
<td>+</td>
<td>?</td>
<td>?</td>
<td>Oxidant - can be antioxidant in lean healthy individuals, but predicts obesity &amp; MetS(^{71,73})</td>
</tr>
</tbody>
</table>

Key. A significant positive relationship with waist = +/- inflammatory or pro-oxidant. A negative relationship with waist = +/- anti-inflammatory or antioxidant. A significant positive relationship with Waist and negative relationship with HDL-C = + inflammatory and ? pro-oxidant. A significant negative relationship with Waist and positive relationship with HDL-C = + anti-inflammatory and ? antioxidant. A significant positive relationship with waist and TG and/or FPG and/or BP = + oxidant and ? inflammatory. A significant negative relationship with Waist and/or FPG and/or BP = + antioxidant and ? anti-inflammatory. No relationships is left blank. Well known properties not shown in this study have (brackets). Adpn adiponectin; ESR erythrocytes sedimentation rate; HbA\(_1c\) haemoglobin A\(_1c\); AlkPhos alkaline phosphatase; ALT alanine transferase; AST aspartate; γGT Gamma glutamyl transferase; tot total; hsCRP high sensitive C-reactive protein; MetSMet metabolic syndrome marker count; Mets metabolic syndrome; s serum; VitD vitamin D; βCaro beta carotene; VitA vitamin A; VitE vitamin E
Table 6-2. Patterns of Association with CVD Risk or Protective Markers by Gender

<table>
<thead>
<tr>
<th>Parameter</th>
<th>All</th>
<th>Women</th>
<th>Men</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adpn</td>
<td>Mostly protective</td>
<td>Almost all protective</td>
<td>Equal balance between</td>
<td>With more research Adpn and oligomers may be able to predict risk in</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>protective/risk Balance</td>
<td>women and men separately</td>
</tr>
<tr>
<td>Change in HMW&lt;sub&gt;30&lt;/sub&gt;</td>
<td>Many mostly protective</td>
<td>Almost all protective</td>
<td></td>
<td></td>
</tr>
<tr>
<td>sVitD</td>
<td>Fewer, All protective</td>
<td></td>
<td></td>
<td>Good CVD protective marker, but few correlations</td>
</tr>
<tr>
<td>sβCaro</td>
<td>Many, and all CVD</td>
<td>-</td>
<td>-</td>
<td>Good CVD protective marker, many correlations.</td>
</tr>
<tr>
<td>Change in sβCaro</td>
<td>Many, and all</td>
<td>Many, and all assoc. with CVD</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>sVitA</td>
<td>Many, and most increase CVD risk</td>
<td>-</td>
<td>-</td>
<td>Antioxidants VitA and VitE acted as oxidant risk markers. More work is needed to understand why these Vitamins are associated with CVD risk not protection&lt;sup&gt;168,825&lt;/sup&gt;</td>
</tr>
<tr>
<td>sVitE</td>
<td>Many, and most increase CVD risk</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Change in sVitE</td>
<td>Many, and most</td>
<td>-</td>
<td>Increase in VitE risk for CVD</td>
<td></td>
</tr>
</tbody>
</table>

Adpn, adiponectin; HMW<sub>30</sub>, high molecular weight Adpn; s serum, VitD vitamin D; βCaro beta carotene; VitA vitamin A; VitE vitamin E

Secondly, the human body has become highly efficient in its energy metabolism by exploiting a wide variety of whole-food, mainly phytochemical, micronutrients as energy modulators. The postulated ‘super-efficient’ phytochemical antioxidant system, probably the Kelch ECH associating protein 1-nuclear factor-erythroid 2-related factor 2-antioxidant response element (Keap1/NRF2/ARE or NRF2) system based<sup>824</sup>, may protect cells so efficiently that they potentially function for long periods of time. Longevity could also allow slow brain growth and development in the infant, allowing ample energy for daily use. In addition, a long lifespan for humans meant that culture and technologies could be developed and passed to the next generations.

6.4.1.1 Unifying Hypothesis Corollary: Evolutionary Adaptations Outwitted?

The corollary of the unifying hypothesis is that it evolved to serve forager ancestors well. Evidence shows forager hominins to be taller, more muscular, more robust and with less signs of infection. Note that there are few, if any foragers, currently living in arable land with extant large game, although a few hardened subsistence survivors exist<sup>825,826</sup>. 

189
The era of farming technologies dates from about 10,000 y ago. Energy dense, storable and transportable, starch foodstuffs increased to 50-70% of the diet. The diminishment of variety was extreme. The diet has allowed adequate survival for humans to breed and spread around the world. However, there was a health cost to the loss of a widely varied, whole-food diet. There is evidence that many, but not all, agricultural populations became stunted; even brain size reduced. Lack of micronutrients was the probable cause.

City dwellers especially became more disease, particularly infection, prone. Examples include poor immunity to acute food-borne illnesses, wound, respiratory and puerperal infection. The lack of sVitD predisposes humans to chronic infections of tuberculosis, leprosy and syphilis. Poor nutrition and infection probably also exerted epigenetic, if not genetic, selection.

The mean height of pre-19th Century European women was approximately 155cm and of men, 166cm, with men losing more highly nutritionally demanding, lean tissue. Improved sanitation and antibiotics has decreased infection and increased infant survival.

The ‘green revolution’ of chemical and machinery assisted energy food crop farming, last century, is testament to the, possibly short term, success of agricultural technology. Human longevity, now a major research area, did increase.

However non communicable degenerative CVD increased, declined to a degree, but may be increasing again with the increase in obesity, the likely latest micronutrient deficiency syndrome. One public health publication warned of this scenario 60y ago. The current wave of industrial farming has surpassed itself, possibly as the energy yield per capita has been so massive.

On the other hand, are the volumes of ever more addictive nature of the refined processed energy dense foodstuffs. The addictive tendencies, excessive motivation and reward seeking, that drive this type of eating pattern, leaves many individuals – populations - dependant on the current westernised food type. Neglect of adequate, large amounts of healthy whole-foods is currently the norm. The well-developed human reward/motivation
system has probably shaped current food production and trade environments. Overweight and some metabolic problems in middle age are now typical of many populations in many countries. Obesity has become a human specific public health problem.

Whole-food micronutrients are no longer consumed in quantities enough to allow efficient energy oxidation and protect cells from excess oxidants. Oxidative stress and lipo-glycotoxicity, including glycated protein and oxidised lipoprotein damage results from non-metabolised toxic lipid, glycogen and glucose accumulating in the circulation and ectopically in cells.

Other environmental, fat soluable toxicants may also contribute to metabolic disruption in cells into which excess lipid has spilled. Long lived human cells degenerate, and adipose tissue may become hypoxic, apoptotic and metaflammation becomes chronic. Damage to the endothelium occurs and ischaemic infarction is likely in the heart and brain. Infection and cell dysplasia, and cancer risk increases.

### 6.5 Current Theories on Causes of Obesity and Metabolic Syndrome

The literature in various disciplines revealed a number of theories on energy management processes with respect to obesity and MetS in humans. Significant areas have not been resolved. Obesity rates may be stabilising in some sectors in some countries but they are rarely falling.

**Considerations on the Causes of Obesity**

The causes of obesity discussed fall into both categories of proximal and distal, and many current studies of gene-environment effects are looking at both concurrently.

By using BMI in many human epidemiology and interventional trials, adipose tissue distribution is eclipsed. Favourable, but large, peripheral subcutaneous adiposity, sometimes seen in European women is not differentiated from, for example, thick-waisted Asian men, who can be at considerable risk.

It is important to give due weight to cohorts and sub populations studied in rapid epidemics. The decline in CVD since the late 1950s was and is much less steep among...
people from low socio-economic cohorts, ethnic minority women and those otherwise disadvantaged\textsuperscript{173,851,852}

### 6.5.1 Putative Contributors to Obesity

The paper by McAllister et al\textsuperscript{78} has 10 extra mainly proximal theories on obesity causation, in addition to the usual two; excess dietary energy intake and lack of exercise. These authors do not discount these two likely causes but expand on them. Some of the more novel causes of obesity reviewed are ‘microorganisms, epigenetics, increasing maternal age, greater fecundity among people with higher adiposity, assortative mating, sleep debt, endocrine disruptors, pharmaceutical iatrogenesis, reduction in variability of ambient temperatures, and intrauterine and intergenerational effects’. The current Chapter will consider some of these theories.

The authors of the McAllister et al\textsuperscript{78} \textsuperscript{853} paper made an effort to look at \textit{in vitro} work, animal work and clinical studies for each topic.

### Environmental Toxicants - Endocrine Disrupters and Psychotropic Medication

There is a large amount of data on man-made human toxicants in the environment. Many pollutants and medications may cause metabolic disruption that increases risks of both central obesity and MetS\textsuperscript{87,854,855}.

With the unifying hypothesis in mind it is important to consider that environmental pollution may have been impacting on CVD type diseases. Both CVD and environmental pollution saw widespread increases from last few decades of the 19\textsuperscript{th} Century, which continued without abatement until the mid-20\textsuperscript{th} Century. Obesity was increasing, but at a lower rate. The huge uptake in tobacco smoking, a toxic, oxidant, certainly was shown to be a health risk from the 1930s onwards\textsuperscript{184,856}.

Persistent organic pollutants, endocrine disrupters \textsuperscript{857,858}, and iatrogenic causes have been covered in the McAllister paper\textsuperscript{78}. They have been, and are implicated in MetS, obesity \textsuperscript{85,859-861} and, for many years, cancers\textsuperscript{861-865}.
If there is a refined energy dense food environment present at the same time as an increase in these persistent pollutants, detoxification may be impeded by deficient general nutrition\textsuperscript{86,824}.

**Microbes and Central Obesity Related Metabolic Syndrome**

Micro-organisms can contribute to both increases or decreases in obesity\textsuperscript{78,346}. Early ideas of true infection as a cause is now thought unlikely\textsuperscript{866-869} as CVD inflammation is metaflammatoiry. Some animals infected with AD-36 adenoviruses acquire increased fat mass\textsuperscript{870} with metaflammation\textsuperscript{811}. Infection of adipocytes by Chaga’s disease and leukocytes by immunodeficiency virus (HIV) cause a peripheral lipodystrophy and central obesity with variably severe MetS\textsuperscript{871}. Leukocytes and adipocytes share a common lineage, and cross functionality can be induced\textsuperscript{872}.

**Intestinal Microbes**

The intestinal milieu changed over the last few thousand years as milk ferments, or yogurt, from domesticated herbivores became part of some human diets\textsuperscript{873}.

Probiotics containing certain exogenous, often dairy-derived, Lactobacilli microbes can establish in the intestine and are credited with beneficial effects\textsuperscript{873-876}. Plant fibre content and type, and other food constituents have altered\textsuperscript{632}, and with it the intestinal microbiota. The genetic background of ethnic groups may alter the intestinal microbiota as some groups have had significant gut pathogen stress, and high risks of infant mortality from diarrhoea such as the Indian Asians and Europeans. Wells\textsuperscript{877} theorises that certain peoples had different requirements for immediate energy release, thus central obesity allows large and rapid energy outflows to immune cells and processes in the intestine.

The intestinal microbiota in obese and MetS individuals is different from that of the lean and fit\textsuperscript{878}. Firmicutes, depending on ethniticy\textsuperscript{879}, are the more favourable groups, but less numerous in obesity. The microbiota derive energy from CHO, especially fibre (fermentation), protein (putrefaction), and lipids. The short chain lipid butyrate by-product appears beneficial\textsuperscript{880}. However, westernised food is typified by highly refined, fried or baked protein, starch/sugars, and lipid mixes which can form AGE/ALES in Malaird reactions, which continue in the bowel.
Fermentation of these products by certain microbe groups to amines and phenols may be variably toxic and/or carcinogenic to the colonic epithelium. If absorbed systemically, the kidney may be negatively affected, in as seen in diabetes. Interestingly, extra nutrients may aid pre- and pro-biotic activity. Invertebrate chitin and cellulose plant fibre is usually ingested with other nutrients in addition to the fermentable moiety.

Lastly, the microbiota acquired as the infant passes through the cervico-vaginal canal and the perineum during birth, and colostrum breast milk, including stem adipogenic cells, are ideal first exposures that help prevent autoimmune diseases, which are more common in those with MetS.

**Sleep deprivation**

Sleep deprivation is one of many physiological/psychological stressors that induce metabolic derangements that, as with sleep apnoea, are more MetS related than body fat related. Many genes including the newly understood clock genes participating in circadian rhythm control are related to energy management, including appetite, digestion and metabolism. They also have high levels of genetic structural variations relating the organism closely to the changes in the environment. Sleep hygiene is now advised for health.

**Reproduction**

In the McAllister et al paper the discussion on fecundity and age at first birth and relationships to BMI-defined obesity was reviewed. In the past, more robust, well-nourished women would be expected to successfully bear children. Some constitutively slim women, who do not have anorexia nervosa, may have bone and other fragilities.

Currently, obesity-related PCOS, and consequent infection proneness, may increase rates of gonorrhoea, chlamydia, herpes, syphilis and HIV, all contributing sub-fertility of obese women, with increased foetal loss and neonatal infection. First births are associated with increased intrauterine pressures and smaller babies. Older mothers and their ova accumulate oxidative damage.

**Intergenerational Effects**

The intrauterine and intergenerational effects section of McAllister et al’s paper was wide-ranging. Bottle formula feeding, with poorly or never established breast feeding, in
human neonates is associated with later obesity, MetS, early degenerative disease\textsuperscript{853,898-901}, and various other suboptimal outcomes such as possible cognitive deficits\textsuperscript{902}.

Studies in omnivorous rat maternal diets, and their manipulations, may show some comparable aspects with diet types of pregnant humans, producing various embryo and foetal effects. One study of a true human cafeteria diet in rats showed worse metabolic profiling than animals fed high fat but nutrient sufficient chow based diets\textsuperscript{903,904}. The proposed unifying hypothesis, however, indicates less extrapolation should be made from laboratory animals to humans due to brain dynamics, and the adaptations discussed.

**Maternal Capital**

This theory is basically another way of saying that growth retarded babies skimp on lean tissue – including the organs - but make sure that they have enough energy reserves to survive, postnatally\textsuperscript{898}.

**Protein Leverage Hypothesis**

The protein leverage hypothesis separates macronutrients into protein and the energy macronutrients, CHO and fat\textsuperscript{482}. These authors’ work shows that humans tend to eat enough protein, if it is present in the food supply, but probably over eat CHO energy if it is not well balanced\textsuperscript{482,905 906-909}.

**Macronutrients and Micronutrient Combinations**

Various other authors have criticised the current dietary patterns as deficient in non energy, nutrients\textsuperscript{248,270,910-912}. Note that protein and fats and oils are often in combinations of high micronutrient containing foods, as for example dairy and oil seeds. Oils can be refined but still contain a mix of lipids\textsuperscript{913}. Only CHO based foods such as sugar beet and grains are commonly refined to pure starch and sugars, almost devoid of minerals and other micronutrients. Refer to \textbf{Section 1.4.2}.

**Critique of Theories**

Above are various historical factors thought to contribute to obesity. None of them is likely to explain why individuals and/or society set up systems to support unhealthy eating patterns, and the discussion on excess dietary energy does not explain the extent of degenerative change seen in MetS.
6.6 Evolution of the Human Brain

Humans have evolved rapidly, and became *H. sapiens* by approximately 200,000 y ago (200 kilo years ago, 200kya) ([Figure 6-1](#)). The striking features were the development of the combination of a proportionately very large brain and prehensile upper limbs, freed from the task of locomotion, both of which allowed technological development. The human palaeontology literature is vast.

Rather under-recognised in the nutrition and medical world are the significant energy requirements of the human brain and the co-adaptations required to supply this over-large organ with sufficient resources\(^{794,914}\). Maintaining a vast association cortex of expansive dendritic arbours and long-range projecting axons is thought to be particularly energy expensive\(^{915}\). Brain metabolism in a lean human adult requires ≈ 23\% of the body’s basal metabolic rate (BMR), as opposed to 3-8\% in many other mammals\(^{830,915,916}\). The allometry of brain to body was altering beyond sustainability\(^{917}\). Humans are required to stay on the same metabolic to mass ratio line as other animals; commonly known as the mouse to elephant curve or Kleiber curve. Although there is controversy about exact equations and applicability\(^{918,919}\), this log-linear curve is similar for all animals. Small mammals have small reservoirs of fat energy with high metabolic rates, and large mammals the converse. More fuel had to be found to supply the brain by two possible mechanisms: increased energy 1) conservation or 2) uptake from the environment.

### 6.6.1 Theories of Increased Energy Conservation Adaptations to Encephalisation

**Expensive Tissue Energy Trade-off Theories**

Traditional theories of energy provision for the brain include the ‘expensive tissue energy trade-off’ hypotheses\(^{914,920}\). The human intestine is the organ most commonly proposed as the trade-off. This organ has become smaller and less energy demanding, compared to the large complex foregut ruminants whose bacteria synthesise all required nutrients from low energy grasses, but flexible in the types of food that it can digest. The omnivorous pig has a smaller intestine, with similarities to the human; fermentation occurs in the caeco-colon\(^{921}\). The intestinal-brain energy trade off theory however may be overrated as other primate intestinal mass is nearly, but not quite, proportional\(^{795}\).
Humans are generally under-muscled compared to other mammals and primates. It is proposed that, in effect, this is a once removed ‘expensive tissue trade-off’ as in this case high metabolic rate-muscle is traded for high energy content but low metabolic rate-adipose tissue. Human babies are almost unique in being born with significant levels of subcutaneous fat. These adipose tissue depots are thought to be reserves which allow the provision of continuous energy to supply the brain.

**Omnivory and Dietary Energy**

Omnivory, arising at some stage in early *H. erectus*, the species before *H. Sapiens*, was important, allowing greater variety in the diet. Archaeological evidence shows the post-canine molar grinding surface changed, tooth size decreased along with mandible and maxillary robustness for supporting large masticator muscles. Energy intake was maximised from animal protein and fat, and there is evidence that diets derived up to 35% protein, and >50% of energy from scavenged carrion and hunted animal brain and liver, particularly. These sources supplied the long chain ω-3 fatty acids EPA and DHA, a good supply of which is probably needed for optimal human brain development.

As ω-3 fatty acids and other nutrients such as iodine were required for brain tissue, some authors posit a period of rapid brain evolution using shore and water based (sea, lake or river) resources, including fish, but others do not support that postulate. Most high energy food sources were ripe fruit with fruit sugars, vegetables such as squash containing starches, nuts and seed providing oils, and animal products including brain and marrow which were high in fat. Of note, starches were a rare source of energy in the past as they often occurred as very tough, stringy, dirty roots, that yielded a low proportion of energy, especially if not cooked.

**Slow Growth and Development**

Humans, usually singleton infants, grow and develop slowly for their size, possibly to supply the brain with constant energy and to allow the acquisition of social and survival skills. Theories abound on menopause in humans with explanations including the grandmother hypothesis, the mother hypothesis and others. Mathematical modelling supports the development of the menopause to allow later and shorter breeding periods in daughters, rather than longevity.
Technologies

Technologies such as stone tools have been recorded as in use from at least 2.5 million y ago\textsuperscript{930}, and were used for acquiring hard-to-extract foods such as underground tubers\textsuperscript{931}. Cooking practices, probably commencing 800,000 y ago, released extra energy from food by gelatinising starch, softening meat and killing pathogens\textsuperscript{828,932}. Technologies slowly advanced until, relatively rapidly, certain human groups started farming\textsuperscript{480,933}.

6.7 Increased Energy Uptake from Environment via Energy Dense Food Reward System - First Part of the Unifying Hypothesis

In this section evidence and information will be presented that support the idea of increased neural mechanisms to drive the acquisition of energy dense food.

Modern Food Energy Environments

Current human-modified food environments may relate to the human brain’s preference for constant, large supplies of glucose\textsuperscript{798} and maladaptive feed-forward effects of chronic hyperinsulinaemia and leptin resistance\textsuperscript{564}. The salivary amylase copy number increased starch digestion in the mouth for the populations who became dependant on tuber or grain starch\textsuperscript{828}.

6.7.1.1 Evolution of Epigenetic and Genetic Brain Control Mechanisms

Of great interest to the current hypothesis generation are the many genetic and epigenetic processes that occur in the brain, especially the frontal cortex. On reflection, a fast evolving brain, with new energy requirements, but long lived cells, would be advantaged to exploit DNA regulatory responses to the environment.

Epigenetic changes are molecular modifications to DNA and chromatin. DNA methylation, chromatin packaging alterations of DNA by post-translational histone modifications, regulation by non-coding ribonucleic acid (RNA), such as microRNAs, and mechanisms that control the higher-level organization of chromatin within the nucleus, are all called marks\textsuperscript{934}. MicroRNAs can activate gene expression in non-dividing cells such as neurons. Unlike in other tissues the human brain depends on active
epigenetic processes to allow for ‘adaptive plasticity’ but also well-coordinated extremely complex patterning and this is a lifelong, continuous function\textsuperscript{841}. Thus the brain can adjust to and accommodate rapidly changing and variable environmental challenges. Any disruption of epigenetic modifiers and/or epigenetic dysregulation may have severe consequences as seen with mental retardation and complex psychiatric disease\textsuperscript{841}.

In addition to the epigenetic variations depending on environmental influences, DNA genetic variability between individuals is extremely wide. In the brain many genetic structural or sequence variations\textsuperscript{935} and processes are employed as regulatory mechanisms. DNA structural variations include many single nucleotide polymorphisms (SNP), variable number tandem repeats (VNTR) of DNA sequence, and gene copy number variations (CNV) where additional or missing chromosomal segments occur\textsuperscript{841,889}. VNTR are more common in the forebrain, a rapidly evolved enlarged section of the human brain cortex\textsuperscript{841,891}. SNP, VNTR and CNV often occur together in genes in certain brain regions and can increase the information content, and variation of the genome, in individuals.

Of note, in humans, CNVs are significantly overrepresented in telomere-proximal regions\textsuperscript{841,889}. Whilst these numbers and types of sequence variations in populations usually confer plasticity to exploit new environments, they also risk dysfunctionality. Of special interest to the current argument is that vast inter-individual behavioural phenotypes will be manifest.

\subsection*{6.7.1.2 The Mesolimbic System and Appetite Control}

The changes for the expansion of the physical brain included making provision for 1) biochemical pathways for more energy to be readily available, 2) the development of the neurotransmitter chemistry to enhance motivation of behaviours to procure more energy and lastly 3) neuronal connections to plan and coordinate strategies for active procurement in time and space.

These three attributes are those of the mesolimbic/mesocortical (mesolimbic) system, the dopamine based reward and motivation system that spans the emotional midbrain and the decision making frontal cortex (\textbf{Figure 6-3}).
The mesolimbic system involves the accumulation of expanded ‘coils’ of varicose axons for dopamine, serotonin, and acetylcholine, and which may represent episodes of plasticity and synaptic reorganisation\textsuperscript{923,936}. These morphological features suggest enhanced facilitation of intracortical processing in the prefrontal cortex by neuromodulatory systems\textsuperscript{923}. The large lateral cerebella hemisphere of hominoids is thought to function in the planning of complex motor patterns, sensory discrimination, attention shifting, and procedural learning\textsuperscript{923}.

Figure 6-3. Reward Pathways - Hypothalamic Nuclei to Frontal Cortex

This enlarged part of the cerebellum probably developed with pressure to perform and be aware of the body in 3-dimentional space, and to coordinate visio-spatial mapping skills when earlier primates were arboreal feeders, balancing fairly large bodies while collecting food at the tips of branches. This area of the cerebellum also appears to relate to tracking seasonal resource availability. In humans, it functions as a neuroanatomical base for refining motor behaviour and then supporting other higher-order cognitive abilities. Processing and coordination of spatial and temporal complex procedures are emphasised in humans. Thus, this is evidence for the development of a magnified, highly organised, neural ‘self-reward system’.
Energy dense foods also stimulate the release of ‘self-addictive’ opiates found in central appetite control pathways, encouraging repeat acquisition\textsuperscript{938-940}. Natural cannabinoids, the endocannabinoids, are involved in the motivation for food seeking and hedonic behaviour involving high-energy food\textsuperscript{941}. In rats, orosensory stimulation by fat ingestion induces intestinal endocannabinoids release, the neural effects of which are mediated by dopamine\textsuperscript{942}.

6.7.1.3 Genetic and Epigenetic Dopamine Control

Dopamine is the neurotransmitter involved in the mesolimbic system responsible for the motivation to act, with or without pleasure, and for the planning and physical coordination of that action. Pleasure requires other neurotransmitters, characteristically, secreted opiates. Dopamine, may be under various rapidly evolving genomic and epigenomic controls in humans. Patterns of genetic and epigenetic regulation change in the face of environmental pressure. Such processes occur in rapidly evolving organisms, and can result in response to altering environmental pressures throughout the individual’s lifetime.

Dopamine, a highly conserved neurotransmitter itself, mediates core, yet seemingly diverse, functions such as locomotion, cognition and motivation, and coordinates behaviour, the reasons for which have been discussed. Any dysfunction in dopaminergic signalling action can lead to neurological disease such as Parkinson’s, neuropsychiatric disease including schizophrenia, attention deficit/hyperactivity disorders (ADHD) and addiction\textsuperscript{841}. Two receptors, D1 & D2, also carry polymorphisms\textsuperscript{943,944}.

**Dopamine Transporter**

The control of dopamine resides largely within its transporter, dopamine transporter (DAT). DAT recycles extracellular dopamine from the synaptic cleft, returning it to the presynaptic terminal. DAT exerts control on dopamine expression. DAT expression is highly dynamic so as the dopamine signalling increases, DAT increases and when dopamine release is low, DAT decreases. The range and variation change over the lifespan\textsuperscript{841}.

Some of the genomic and epigenomic systems coding for, and controlling, DAT are unique to humans. Influences on DAT include environmental factors, palatable food reward motivation\textsuperscript{797,945}, certain pharmaceuticals and other salient stimuli\textsuperscript{797,841,946,947}.
Even in the same individual with region-specific DAT expression, in the same brain region there is significant highly dynamic variability in DAT expression, and more so than in serotonin. DAT control needs to be stable enough to perform routine tasks and adaptable enough to function in an unpredictable environment\textsuperscript{841}.

A comprehensive combination of modelling and computer programme analyses of preconstructed databases was run by the authors of this paper to examine a large variety of genomic and epigenomic mechanisms, which seem to regulate the activity of DAT\textsuperscript{841}.

This one transporter employs nearly all the previously mentioned genetic and epigenetic modes discussed above. Such processes endowed humans with such strong drives that they have developed technologies to find, and later farm, process, refine, store and trade energy dense food. Note also, that whilst dopamine control is crucial to the unifying hypothesis, it is but one pathway in a complex organism.

**Energy Uptake Enhancements in the Brain**

Amino acid changes arose after some of the electron transport chain genes underwent positive selection. These changes allowed greater functional efficiency of the mitochondrial aerobic metabolism pathways to ensure that highly active cells such as neurons were supplied with energy substrates\textsuperscript{948}.

Gene copy number was doubled for glutamate dehydrogenase (GDH). The hominoid isoform of this GDH gene, in the astrocyte, is activated during high glutamate flux\textsuperscript{949}. This increased capacity for glutamate metabolism, and thence energy throughput, was a likely adaptation to enable relatively high levels of excitatory neurotransmission required by hominoid brains\textsuperscript{923}.

Of note, newly discovered orexin/hypocretin, or desire-to-eat hormones, facilitate glutamate-mediated responses, and are necessary for glutamate dependent, long-term potentiation in certain dopamine neurons. Thus orexin/hypocretin neurons play an important role in reward processing\textsuperscript{950}. 
Appetite and Feeding

The type of neurons involved in appetite stimulatory (orexigenic) or inhibitory (anorexigenic) pathways show nearly all can be involved in all substance addictions, via the mesolimbic dopamine pathway. Highly refined energy dense food may have been the first human addiction. However, human appetite had often been classified into homeostatic eating and hedonic eating, and addiction has not been seriously considered until recently.

Appetite Mechanisms–Homeostasis to Addiction

Homeostatic mechanisms are those that sense basic hunger, coordinating brain memories of nutritious, safe, non-toxic food objects and how to procure them, and ensure that the body has the correct amount. Hedonic eating is where there is pleasure in the food. The cortical part of the mesolimbic system is important for assessing decisions on what is sensed. Energy sensing and awareness of levels of satiation are still present in hedonic eating (Figure 6-3).

In the last few years the concepts of ‘liking’ and ‘wanting’ have been extended to the area of food addiction. Liking relates to pleasure, such as felt on exposure to opiates, whether endogenous or exogenous. Wanting implies a motivation for something that catches attention or has incentive salience, in the environment, such as pleasure and acquisition which excites a feeling of reward.

Note that in addiction, often liking is minimally present, or has been lost. In addition, the reward may not feel good, but just allows a sense of relief, in the same way as those with obsessive disorders find relief but not pleasure on completing the compulsion to wash hands, for example. This is dopamine reward hypofunction.

In the case of refined energy dense foodstuffs, for many individuals, especially those who binge, either in obesity or bulimia, enjoyment is often no longer present. Whether permanent or long term brain changes occur in binge eating and obesity, as in drug addiction, is not known.

Food becomes a problem in all ways – refined energy dense food is compulsively sought and consumed, healthy food neglected and there is transient or minimal pleasure in eating.
Food addiction was proposed many years ago, but at that stage neuronal and dopamine pathways were only suspected to exist, and clearly not all food was addictive.\textsuperscript{953,957,958}

\textbf{Refined Energy Dense Food Addiction - Psychology and Neuropsychiatry}

It is instructive to review some of the characteristics of addictions. For those not addicted to food or not obese, it is not easy to understand that most of the criteria of addictions apply to obese individuals and binge eaters, and need to be taken into account when treating. As neurotransmitter functional changes are profound in addictions, neuropsychiatry developments have become important to the medical management. Opiate blockers are increasingly being used for alcoholism, and their use in obesity is being reviewed by the FDA.\textsuperscript{959} Interestingly, bromocriptine, a dopamine agonist, is licensed in some countries for prescription in TIIDM as it reduces plasma glucose, but the mechanism is not known.\textsuperscript{960}

There are many addiction definitions, and currently they cover gambling, computer games and other similar past times, as well as substance abuse.

According to the Diagnostic and Statistical Manual Version 4\textsuperscript{961}, for a person to be considered dependent on, or addicted to, any given substance, at least three of the following seven criteria must be met at any time within a given year: 1) tolerance; more substance is needed for the same effect, 2) withdrawal, 3) taking a larger amount of the substance or taking the substance for a longer period than was intended, 4) experiencing a persistent desire [craving] for the substance or an inability to reduce or control its use, 5) spending much time seeking or consuming the substance or recovering from its effects, 6) use of the substance interfering with important activities [healthy eating], and 7) use of the substance continuing despite known adverse consequences [obesity, MetS, TIIDM, CVD and also in this section resorting to antisocial, guilt provoking or clandestine behaviours].\textsuperscript{961} If either criterion 1 or 2 is met, then physiological dependence is diagnosed.

These criteria are met for sugar, in animal studies\textsuperscript{962} and as noted, the rat reward orosensory effects of fat are also brain dopamine mediated via gut endocannabinoids.\textsuperscript{942}

However, human substance dependence (or addiction) can be diagnosed using entirely behavioural criteria. There are elegant studies performed in rats, which are omnivorous,
showing that dopamine reward hypofunction or dysfunction\textsuperscript{953} develops only in those animals which have had long term access to palatable, energy dense foods. Once obesity develops, compulsive energy-dense food eating becomes established. However, if these rats have been eating a continuous supply of energy dense cafeteria type food for many wk (>40d) and subsequent access was restricted, only modest overeating occurred. They ate as much as they did during the 40d cafeteria diet and were very hard to deter from this monotonous, compulsive, behaviour, even with aversive training\textsuperscript{797}.

Clinical addiction studies also show long lasting and obesity-related changes can occur in the mesolimbic pathway. In a clinical study, the near-total mesolimbic pathway was activated in neurological functional magnetic resonance imaging when obese women were shown pictures of palatable energy-rich food. In contrast, in the normal weight control women, only one part of the pathway, the dorsum striatum classically associated with addiction, was activated. Interestingly, when normal weight women looked at low energy food pictures their left lateral orbitofronal cortex activated more vigorously, and they had less subcortical emotional activation\textsuperscript{963}. The orbitofrontal cortex is part of the emotion and reward limbic pathway, but it also functions as a centre of cognitive decision making. One could speculate that the normal weight women were able to associate the visual input via the orbitofrontal cortex and the left lateral cortex and together with the prior memory/knowledge of safety and utility of the low energy food, make an analytical assessment and informed decision less hampered by a contrary, emotional and hedonic response\textsuperscript{963}.

In another study normal weight restrained women and men could reduce ‘wanting’ perceived unhealthy, delicious food verses healthier less appetising food, more than unrestrained participants\textsuperscript{963}. Thus, learning before obesity develops may be able to occur and reinforce efforts to prevent weight gain.

**Craving and Cues**

Opinions about whether only highly palatable, energy dense foods are addictive or whether all foods can be, is still divided\textsuperscript{964,965}. Humans can learn to crave behaviours, events or unpalatable foods, of all types. Notably, humans can learn to crave punishment and starvation in conditions such as anorexia\textsuperscript{966}. Some authors maintain that humans can be cued to crave any foods\textsuperscript{964,965}.
In many cultures, where food is perhaps limited or seasonal, seemingly strange items are consumed, such as bird’s nest soup, and can become a delicacy. Repeated exposure to certain foods, especially if there is very strong cueing and emotional investment, leads to acceptability, which in time becomes a preference. As long as the taste organs, of which some are in the intestine and do not have a clear taste sensation\(^{967}\), can sense nutrients without toxins, humans can become accustomed to a wide variety of types of foods. Unlike with addictive drugs, there is a solid homeostatic pathway for food, and energy dense food and drug comparison experiments in rats show that they can opt for food over cocaine\(^{954}\).

In contrast, there is a report stating that mammals have been seeking and procuring psychoactive foods, as have hominins, for thousands of years. This behaviour has been hypothesised to enhance positivity and fitness seeking\(^{968}\). However, the self-reward and motivation to procure energy dense food appears to be a much more likely reason for a well-developed mesolimbic system in humans, which, unfortunately, in the current environment, can become dysfunctional.

Some authors state that care needs to be taken about labelling food as addictive, as all organisms need to acquire nutrients\(^{964}\). Much of the CHO is particularly highly bred grains and tubers for starches and sugars. Such foodstuffs, new in evolutionary terms, are made even more attractive by adding extra well known, highly palatable flavours, eye catching packaging, advertising of convenience and easy availability. Individuals are ‘cued in’ by advertising with other desirable items/lifestyle situations.

Thus, the current westernised food environment may be a result of these adaptations for making plenty of energy available to the human brain. With the great power, adaptability but variability of human motivation systems, it seems that when coupled with privation, social or physical stress, and the tendency for positivity seeking in adversity or with stress\(^{968}\), the limbic system overreacts and addiction can occur in many individuals.

**The First Part of the Unifying Hypothesis in Light of Evidence Presented**

Evidence has been presented to support the first part of the unifying hypothesis; that is a highly-honed, robust, recently evolved neural self-reward/motivation system for procuring and consuming energy dense food has developed in humans to augment energy requirements of the brain.
The mesolimbic/corticolimbic systems may have contributed to the current energy dense food environment, but it may have over shot its design and become dysfunctional with the extremely refined food, allowing addiction. Many individuals lose appetite control, and binge or consume large amounts of mainly energy dense food, to the extent that they become centrally obese with MetS. Other individuals make behavioural adaptations such as bulimia, which is effectively defined in the EAT-12 in Section 2.4.6.1.

This is the ‘self-addiction hypothesis’. The self-addiction hypothesis is not new. What is new is linking it clearly to the high brain requirement for energy. Strengthening the hypothesis is the revelation, by genomic advances in the last 5y, that the human pattern of addiction is related to rapid, and probably current, biochemical and physical brain evolution.

Typical of addictions is the neglect of other parts of the individual’s life; in this case the neglect or distaste for healthy or less stimulating types of foods, and the increasing avoidance of them. These foods are the whole-foods, replete with fibre and essential and useful micronutrients. This depletion leads to the second part of the hypothesis. Humans preferentially seek high-energy food whilst at the same time requiring a wider variety and volume of many preformed micronutrients than come from current processed foodstuffs.

In summary, the human brain expanded, and with is expansion and increased energy requirement, the mesolimbic part of the brain also developed to serve itself. Homeostatic food consumption for energy, as seen in all animals, expanded into hedonic eating, where intense pleasure was associated with energy dense food. However, in humans the mesolimbic/corticolimbic system developed further and the link between pleasure in eating energy rich food, was reinforced with a reward system linked to a very high motivation component, allowing a degree of perseveration that in a permissive environment becomes addiction.

Thus human encephalisation is hypothesised to be a major driver in devising technologies and engineering social systems to supply themselves with ever-increasing amounts of refined energy dense foods or foodstuffs. In true addictive manner, self control is limited and there is little regard for costs to health or the environment.
6.8 Micronutrient Deficiency in Central Obesity – Second Part of the Unifying Hypothesis

For this section, basic and comparative physiology is reviewed with respect to normal and abnormal energy management. Oxidative stress and metaflammation will be related to obesity and degenerative disease. Small oxygen or nitrogen molecules, termed reactive oxygen and nitrogen species (RONS), or free radicals will be discussed. Examples of energy controlling and modulating pathways and the use of micronutrients in the human diet will be explored. Micronutrient influences related to reducing RONS and possibly increasing longevity in humans will be addressed. A mathematical model proposal is suggested to fit to the findings.

6.8.1 Basic Principles in Energy Metabolism

Energy Reactions

Basic reactions of glycogenolyis/glycolysis and fat oxidation, citric acid and fatty acid oxidation cycle basic energy cycles are conserved from bacteria. However, as already noted, increasing copy number of key pathway enzymes can increase enzyme activity828.

6.8.1.1 Reactive Oxygen and Nitrogen Species

One area of control in energy-related reactions is management of extra energy that is transferred to molecules, which may or may not be part of the reaction, but whose atoms become very unstable. Undirected or unchannelled energy transfer from RONS, as defined above, can damage complex structures. These free radicals have an extra energetic electron which is easily transferred to other molecules, disrupting their structure and ability to perform their appropriate reactions. In normal physiology most animals have both the production of RONS, and management, well controlled.

Sometimes controls are contained in the nature of the reaction. There is some evidence that control of RONS lengthens survival662,843,969-973. That is not proven in humans, and is part of the unifying hypothesis. Land tortoises, very low metabolic rate animals are long lived, and birds decrease mitochondrial RONS production, living longer than mammals of a similar size969,974,975.
Reactive Oxygen and Nitrogen Species in Mammals - Signalling and Repair.

In most active mammals, many reactions are by definition variable in energy use and energy supply. Normal oxidative processes can signal energy use and repair. Mitohormetic (mito = cell replication, hormesis = small useful amounts) RONS are important for signalling that repairs and restitution are required. Post PA, upregulation of repair to microdamage in muscle fibres and protein reconstitution are required. The endothelium, the largest body organ, relies on shear stress to activate endothelial nitric oxide production and other cytoprotective mechanisms.

It is likely that the whole body’s maintenance and repair systems are transiently increased post-exercise, which is the great benefit of PA. Of interest, high dose antioxidant supplements may abrogate this normal and healthy effect in athletes. Note there are also repair adaptive responses where small amounts of damage prepare the body for larger insults.

High RONS fluxes occur normally post-prandially, as CHO & protein are secreted into the portal vein blood, or lipids into the aorta via the lymph thoracic duct. They are transported to the liver which processes the energy and other nutrients for immediate release into the blood stream and organ use, or stores excess for delayed use. For example, during starvation, ketone bodies acetoacetate and β-hydroxybutyrate are synthesised for use in obligate glucose-using organs, such as the brain. The liver also processes micronutrients for various reactions, including antioxidant and cytoprotective functions. Hepatic detoxification of xenobiotics, which are foreign man-made or natural or native chemicals not normally expected to be found in the body, is another energy requiring liver function. These irregular processes produce variable amounts of RONS and if there are excess free radicals oxidative stress ensues.

Immune activation requires high level oxidative processes. It is important that infection and injury activate immediate and longer term innate and adaptive immune responses. This is the classic inflammatory response of redness, swelling, heat and pain.

These processes often require intense energy mobilization, as in sepsis, and much energy for tissue repair in injury. In managing infection leukocytes, especially neutrophils as discussed, form, and release, oxidant molecules such as hydrogen peroxide, proteolytic...
biochemicals and cytotoxins, with which to destroy microbial pathogens. The body’s thermostat is altered to increase the body’s temperature; another energy demanding function. When the urgent need abates, high levels of oxidation are no longer required and resolution is complete.

An efficient return to normal lower metabolic rates, oxidation and inflammatory deactivation, with high levels of immune readiness, should be effected.

### 6.8.1.2 Oxidative Stress and Central Obesity

It is surprising that obesity, characterised by a large depot of oxidisable lipid energy, has not been the target of clinical studies of oxidative stress until recently. Publications on inflammation in obesity increased after CRP was associated with CVD. Mitochondrial oxidation and stress has recently been shown to be increased in early stage obesity.

Macronutrients, mainly as lipid stored in adipose tissue, but also as circulating lipid and glucose, and liver and muscle lipid and glycogen, could be expected to exert secondary oxidative stress. These macronutrients can spontaneously form glycoxidation/lipoxidation products with protein and other biochemicals.

In contrast, researchers of treatments for the degenerative diseases of cancer and renal failure have looked long and hard at the antioxidants, such as genistein and epigallocatechin gallate sometimes to use with other plant derived agents such as tamoxifen, a taxol antioestrogen anticancer plant derived chemotherapy. In the last decade or so CVD has been associated with oxidative stress. Unsuccessful antioxidant vitamin intervention trials have been discussed.

In central obesity-related MetS, oxidative stress, whether a cause or an effect, is perhaps the most basic pathology which occurs. The following is known but not usually framed from an oxidative stress perspective. Note that oxidative stress, in western diet fed animals, has been shown to precede insulin resistance and obesity. Energy overload in obesity also releases inflammatory cytokines that stimulate mitochondrial RONS production via ceramide formation.

An inflammatory link has been proposed whereby in central adipose tissue sufficient increases in angiogenesis fail, impairing oxygen supply and causing hypoxia. With an increased rate of cellular stress and damage, programmed cell death or apoptosis
Monocyte chemoattractant protein-1 (MCP-1) is elaborated by adipocytes. Circulating monocytes enter adipose tissue, becoming macrophages, and neutrophils are also recruited; the metaflammatory reaction is initiated (Figure 6-4).

In addition to the mitochondria, the rough endoplasmic reticulum (RER) where proteins are assembled, and smooth ER where steroid, lipids and CHO are processed, appear to become involved. It is likely that oxidative stress activates metaflammation by a number of pathways. Insulin resistance is also linked, especially in hepatocytes and inactive muscle.

Oxidative Stress-Induced Degenerative Change in Metabolic Syndrome

The processes in chronic oxidative stress, metaflammation, degeneration and organ failure, form a well-known degenerative pattern. As soon as damage is inflicted, blood vessels leak serum and clotting proteins, and leukocytes from the blood invade the site. After pathogens are killed, debris is engulfed by leukocytes. Fibrin, fine temporary resorbable strands that allow tissue regeneration, is secreted. As the swelling recedes, replication of local stem cells of site-appropriate cells occurs. Restitution and/or repair should occur and the fibrin reabsorbed, resulting in minimal permanent collagen scarring. Chronic immune activation has a prolonged and intermittent destructive phase, with damaged cells undergoing apoptosis and/or even unplanned cell death, necroptosis, and cytokines and myeloperoxidase are constantly active, so ongoing repair is also intermittent or often deferred.

Attempts at repair are abnormal. Fibrin is replaced with permanent collagen fibrosis rather than restitution with tissue structure.

The liver is a clear example of stages of fibrinosis followed by fibrosis that proceeds to cirrhosis, with tight bands of collagen strangling islands of hepatocytes, decreasing function. Constant inflammatory stress increases rates of cell disruption, followed by dysplasia and cancer. Degenerative change in and under the arterial endothelium and vessel wall smooth muscle was described in Section 1.3.3. Chronic injury sites can also calcify, as at the arterial endothelium, or even ossify.
Chapter 6. Causes of Obesity and Metabolic Syndrome

Figure 6-4. Excess Lipid in Adipocytes: Endoplasmic Reticulum & Mitochondrial Stress

‘Lean’ fat cells versus ‘Fat’ fat cells. Functional small metabolically active adipocytes are master regulators of glucose homeostasis. They have high numbers of insulin and GLUT-4 receptors on their cell surface. Normal central adipocytes are designed to work with a modest amount of TG droplets as an energy source to be used as required. However, when surplus TG stored in central adipocytes surpasses their critical mass of expansion, the cells become a source of adipocytokines such as TNF-α, IL-6 and MCP-1. In addition, the lipid overload initiates ER and mitochondrial stress/RO(N)S generation. More adipocytokines are produced and activate JNK, initiating insulin resistance and decreased glucose uptake, in adipose tissue, and via adipocytokine cross talk, liver and muscle insulin resistance. Large, inflamed adipocytes have higher rates of apoptosis, attract macrophages which become adipophages. The eliciting of more cytokine release perpetuates a vicious cycle of cytokine release. GLUT-4 Glucose transporter type 4; TNF-α tissue necrosis factor-α; IL-6 interleukin-6; MCP-1 macrophage chemoattractant protein-1; RO(N)S reactive oxygen (and nitrogen) species; TG triglyceride; ER endoplasmic reticulum; ser serine; tyr tyrosine; IRS-1 insulin receptor substrate-1; JNK c-Jun N-terminal kinases. Graphic from Mitta 2008204.

ER stress can result in misfolded and oxidised proteins811,988. The kidney is a site of oxidised proteins such as amylin that thicken the basement membrane and prevent filtration989. Brain tissue degeneration is also associated with mitochondrial and ER oxidative dysfunction and amyloid plaque complexes which may form the basis of proteotoxic damage to neurons, glia and synapse maintainence990-992.

6.8.1.3 Methods of Dealing with Reactive Oxygen and Nitrogen Species

One of the major controls of oxidative stress is the buffering of reactions by antioxidant cofactors. These cofactor complexes temporarily react with the RONS, allowing the energy to be dissipated through the complex. These complexes are antioxidants, although
probably they should be called oxidation control buffers or redox modulatory agents.\textsuperscript{993,994}

**Cofactors and Vitamins**

Many enzymes are catalysts, which allow reactions to proceed along routes, with less energy required at each stage than without catalysis. These enzymes are proteins, but to complete the task a vast array of cofactors is employed. These cofactors are of many and varied types, some being formed in vivo but many being a required part of the diet. They may be inorganic minerals, especially metals, salts or mixes or organic protein, lipid and CHO complexes. Cofactor bonds vary from tight covalent links, ionic bonds and a large number that depend on the spatial orientation and subtle ionic overall charges of a cleft with its ligand.

There are loose associations where any of a certain group of molecules provides adequate function. These cofactors are generally not consumed by reactions. They need constant reconstitution rather than replacement. They or their constituents are often referred to as the micronutrients. The constituents of these cofactors that are obligatorily derived from the diet are the vitamins.

**Antioxidants and Antioxidant Vitamins**

Antioxidants are an amorphous group of biochemicals, with many being synthesised by the organism. In humans particularly, a number are required in the diet, and thus designated antioxidant vitamins. The balance of well-known vitamins, including those which are fat soluble, alters with central obesity as shown in Figure 6-5.

**Autophagy**

Ideally, it is hypothesised, antioxidants maintain cells and there is minimal wear and tear. Autophagy is a limited cell organelle recycling scheme. Old and damaged proteins and organelles, including mitochondria and ER, are engulfed by autophagosomes, thence fused with lysosomes to dismantle and reuse constituents.\textsuperscript{995-997} As shown in the right part of Figure 6-6, autophagy may help prevent cells dying through apoptosis or prevent them replicating without adequate and normal controls, thus stalling neoplastia. However, as with so many other processes, autophagy in obesity appears faulty.\textsuperscript{998,999} Autophagy is stimulated by starvation, as many cells, including adipocytes, appear able to economise
on disused cell organelles by recycling and reusing nutrients when food is in short supply\textsuperscript{1000}.

**Figure 6-5. Antioxidant Vitamins in Central Obesity**

Figure 6-5 removed due to copyright reasons.

**Telomeres and Telomerase**

Some short lived somatic cells have certain cell machinery called telomeres that allow replication before they become too damaged to function. Telomeres are repeating parts of DNA that stabilise and anchor the tip of the chromosome to the nuclear membrane. Telomerase is an enzyme that maintains or even elongates the telomere, but in humans most mature somatic cells have limited amounts of telomerase.

Stem cells frequently reform glandular epithelium that has high secretary activity as in breast, prostate and intestinal cells. If there is plenty of telomerase the cells can replicate faithfully for many cycles. These cells may or may not have performed repeated bouts of autophagy first but finally apoptose and are sloughed off.

\textit{AOX antioxidant; SOD superoxide dismutase; GPX glutathione peroxidase; CAT catalase; TAS total antioxidant status; FRAP ferric acid reducing potential; HMW high molecular weight; ROS reactive oxygen species. Graphic adapted from Vincent 2005\textsuperscript{463}
It is hypothesised that the best protection for long-lived cells with minimal telomerase content is constant oxidant sensing, signalling and prevention of excess reactive oxygen and nitrogen species (RONS). Phytochemicals modulate antioxidant activity, providing continuous, cytoprotection, damage prevention and repair mechanisms. If damage does slowly accumulate a periodic form of repair is autophagy, a regulated process for the removal of damaged proteins and organelles. Autophagy occurs under basal conditions and is stimulated by starvation, although it is not clear if it is just energy, or total dietary deprivation. This process is mediated via sirtuins, but appears faulty in obesity, a condition of relative lack of phytochemicals and of excess oxidative pressure. The removal of damaged cellular components, especially damaged mitochondria, might decrease the level of RONS, which in turn might reduce genomic instability (cancer initiation) or forestall cellular senescence (apoptosis). Oncogenes in general block, and tumour suppressors stimulate, the process. Adequate types and concentrations of phytochemicals to modulate oxidative stress may be the first cell mechanism, and autophagy is a second tier process. Such mechanisms might allow moderate increases in autophagy to reduce the incidence of cancer and prolong lifespan. Adapted from Virgili 2007 & Finkel 2007.

Short, aged telomeres may represent damage which autophagy should clear, and are more common in obesity. Some normal somatic cells are not expected to replicate often and are depleted in telomerase. Other long lived cells do not have telomerase at all.

Longevity and aging

Longevity is how long-lived an organism is, whereas aging or senescence is the process of cells becoming altered, usually with reduced reserve and function. Organs and organ systems have many processes to maintain function including prevention of cells becoming stressed and malfunctioning, repair, recycling cell parts, destruction and replacement, as previously mentioned. Each species tends to have a life expectancy related to its members’ interdependent mass and metabolic rate, and a dynamic energy budget model.
can be a useful framework to study this\textsuperscript{1002}. Empirical evidence indicates a strong relationship between the acceleration of embryo growth rate and the acceleration of ageing-related mortality in mammals.

More rapid embryo growth produces lower-quality adult individuals, and consequently reduced longevity\textsuperscript{1003}. Development, including development of reproductive maturity, is not the same as physical growth. From allostERIC comparisons humans should have a potential life of 37-40y, rather than the potentially 110+ years\textsuperscript{975}. It appears there are processes in human development and maintenance that allow a potentially much longer lifespan.

\textit{Antioxidant Protection of Long-Lived Cells – Phytochemicals}

Long lived cells are expected to have a high degree of antioxidant protection and if they are part of a long lived homeotherm with a reasonably high metabolic rate the antioxidant protection required may need to be particularly strong. A scheme is shown for these combined roles, with phytochemicals modulating them (Figure 6-6).

It is hypothesised in this thesis that modulation of energy metabolism by phytochemicals has a number of results. Keeping the cells in good condition, by cytoprotective antioxidant mechanisms defers autophagy. Apoptosis in long lived cells is prevented. Replication is carefully controlled in high turnover cells of skin, intestine and endocrine and exocrine glands, and neoplastic change is abrogated. Functions of phytochemicals appear to include antioxidant effects in addition to ‘signalling’ both in the glycolysis and lipolysis pathways.

The vitamins are part of these phytochemical groups and are indispensable to normal metabolism, but a multitude of phytochemicals from many different classes have variable levels of control over basic biochemical pathways. The types of modulation are often in inhibiting energy pathways, buffering oxidants, or having antioxidant effects, and modulating the initiation or cessation of inflammatory processes (Figure 6-6).

\textit{Unusual Metabolic Pathways in Hominoids}

It is worth mentioning a couple of unusual cases of antioxidant metabolism in humans, as it would seem that both relate to energy metabolism and balance. Energetic cost or energy saving has not been calculated.
Vitamin C Metabolism

VitC, ascorbate, is a vitamin, derived from c-glucuronate and L-gulonate sugars, and is an antioxidant. Inactivation of the enzyme catalysing the last step prior to ascorbate formation occurred at least twice during hominoid evolution. It appears that during evolution there was plenty of vegetation supplying VitC in the hominoid diet obviating its synthesis. VitC can be converted into a metabolisable sugar via the pentose pathway\textsuperscript{1004}, possibly as can ascorbate catabolism, via l-erythrulose\textsuperscript{1005}. ‘Inefficient’ recycling of the ascorbate conserves this vitamin. Furthermore ascorbate may replace another major intracellular antioxidant, glutathione, in some instances, or help its maintenance. VitC is an important antioxidant that interacts with other antioxidants. The available metabolisable sugars may save energy by not making VitC, but whether this is energetically significant in saving energy for the brain, would need to be modelled.

Urate Metabolism

The loss of uricase in the higher order primates results in raised levels of urate. Urate may aid survival during water stress, and raise blood pressure\textsuperscript{1005}. Urate can be pro-oxidant in obesity, infection or dysplasia, probably augmented by the action of the enzyme xanthine oxido-reductase (XOR). Urate has antioxidant functions and aids in restoration of VitC. It may have specific neurobiology effects, probably on foraging behaviour in some animals, during the seasonal cycles of more or less food availability, promoting fat storage via insulin resistance before winter\textsuperscript{1005}

6.8.1.4 Phytochemicals in Human Diets

More work is being done on secondary phytochemicals which have evolved in plants, some of which appear to be synthesized for specific effects on herbivores, although probably not specifically for humans\textsuperscript{1006,1007}.

Most human groups have had access to varieties and species of the main large plant food genera on eating their typical fruits, vegetables, herbs and spices, so human metabolism has developed uses for some, or probably many, and there is evidence of synergistic action\textsuperscript{310,312,467} in phytochemical groups. The phytonutrients aid general health, including immune modulation where cell replication is up-regulated in infection, and cytoprotection in cardiovascular and brain metabolism\textsuperscript{1006,1008}.
Numerous groups of phytochemicals modulate reactions in humans and many of the reactions are where large energy fluxes occur, as mentioned previously, post-prandially, on physical activity or with acute infection. Many have been discovered to be anti-inflammatory where experimental evidence shows antimicrobial and antidysplastic properties\textsuperscript{1009}.

**Phytoalexin modulation of energy pathways**

There are many microconstituents in fruits and seeds. Phytoalexins are pro- or re-active plant defence chemicals that may be either constantly present or inducible in damaged plants\textsuperscript{1010}. Useful phytochemicals in humans include a large number of phytoalexins. Older varieties of fruit and vegetables still synthesise large amounts of phytoalexins, as they are without human applied or engineered defences. Apple juice coarsely squeezed from old varieties may contain 4,000 useful phytochemicals\textsuperscript{1011}.

Osmotins confer salt and dehydration tolerance to, and antimicrobial protection for, a wide range of plants. Polyphenolic compounds, terpenes and other groups such as chitinases keep yeasts at bay, especially in ripe damaged fruit such as grapes. Should the yeasts proceed to ferment the fruit sugars, and an alcoholic beverage form, these phytoalexins are additional useful phytochemicals to liquer. Also, being reactive defence chemicals, phytoalexins could be apt phytochemicals to co-opt into energy modulatory functions.

**Figure 6-7. Phytoalexins**

![Diagram of phytoalexins and their effects]

These chemicals are strongly represented in health claims as nutraceuticals but are also heavily represented as anticancer adjuvants.
The effect of these phytochemicals on key pathways that operate in obesity and MetS will be addressed below.

**Insulin Action and Phytochemical Modulation**

There are a number of phytochemicals that modulate the insulin receptor complex. Phytochemicals of groups from green tea, hops, and specifically phyto-oestrogens resveratrol and quercetin\textsuperscript{1012-1015}, stimulate glucose uptake via glycogen synthase kinase and phosphatidylinositol-3\textsuperscript{303,1012-1014}. The brain has non-insulin dependent glucose uptake.

**Oxidative and Inflammatory Processes with Nuclear Factor kappa-Light-Chain-Enhancer of Activated B Cells (NFκB)**

TNF-α and RONS are released from hypoxic adipose tissue tissue, within which the adipocytes are overstuffed with lipid\textsuperscript{986}, and both stimulate NFκB, a transcription regulator of inflammatory processes. This NFκB proinflammatory pathway is one of a number of systems set for maximal engagement but is inhibited, in this case by the inhibitor kappa B kinase (IKK) part of the complex or by displacement of NFκB inhibitor (IκB).

A number of diterpenoid phytochemicals are involved in modulating the inhibitors\textsuperscript{1016}. The failure of the innate and adaptive immune system is thought to be at the heart of cancer initiation, ‘proliferation, deregulation of apoptosis, angiogenesis, invasion and metastasis’\textsuperscript{1017}. Metabolically influenced mutations as opposed to inherited mutations are becoming more seriously investigated\textsuperscript{1018}.

**Adiponectin, Phytoalexins and Energy Management**

Resveratrol, a berry/ground nut produced, antifungal, to be reviewed below, appears to up-regulate HMW production via DsbA-L, suppressing the phosphoinositide-dependent kinase-1/serine/threonine kinases (PDK1/Akt) signalling pathway, and activating another signalling pathway adenosine monophosphate activated protein kinase (AMPK)\textsuperscript{588}.

AdipoR1 and 2 have at least one phytoalexin ligand, osmotins, a member of the large pathogenesis-related -5 protein family\textsuperscript{758}. One could hypothesise that if native Adpn were in low concentrations, a diet plentiful in osmotins may allow activation of liver insulin sensing PPAR pathway, and/or the muscle AMPK signalling pathway.
**Sirtuins**

Sirtuins, for example silent mating type information regulation 2 homologue 1 (SIR2), proteins\(^{1019}\) are a highly conserved family of nicotinamide adenine dinucleotide (NAD\(^+\))-dependent energy sensing, protein deacetylases. Sirtuins have various roles as providing critical cellular responses for maintenance of homeostatic balance in metabolism, stress resistance, and increasing life span\(^{1020}\).

SIRT2, a silencing agent, functions as a cell metabolic activity sensing agent that altered gene transcription, initiated DNA repair and recombination and increased lifespan\(^{1019}\). Its function was later clarified as linking chromatin silencing to cellular reduction-oxidation (redox) status\(^{1020,1021}\). SIRT6 may be a master regulator of glucose metabolism\(^{1021}\).

**Resveratrol, Other Polyphenols and Phytochemicals – Protective Effects**

The sirtuins have been of interest as they are stimulated by some phytoalexin polyphenols, of which resveratrol, in high concentrations in grapes and wine has been well studied. Resveratrol is reported to have anti-inflammatory, antiatherogenic, cardioprotective effects, in addition to exhibiting antiproliferative, chemopreventive and anticancer properties\(^{1019,1020,1022-1024}\).

Resveratrol extends the lifespan of rodent animal models via sirtuin activation\(^{1025}\). Most, but not all, of the studies show benefits of resveratrol, although it may be the combination with other phytochemicals, the polyphenols; *trans*-resveratrol, pterostilbene and quercetin, and the polyamine; spermidine, that confer a greater advantage to health\(^{588,1025-1029}\). They synergistically restore levels of the endogenous antioxidant reduced glutathione (GSH)\(^{1030}\) and stimulate autophagy\(^{1025}\).

Resveratrol mimics energy restriction\(^{1021,1031}\). Resveratrol has been shown to reverse MetS and increase longevity, which agrees with microarray analysis revealing that resveratrol opposed the effects of a high energy diet in 144 out of 153 significantly altered genes, most of which are involved in the ageing process and metabolism\(^{1032}\). An increase in mitochondrial biogenesis and enhanced oxidative phosphorylation were shown by resveratrol\(^{1019}\). Human pre-adipocyte differentiation was shown to be augmented by resveratrol in *in vitro* studies, but was dose dependent\(^{1033}\).
Much of the discussion above indicates that resveratrol activates sirtuins by low energy sensing. This brings up an important point of whether it is pure energy restriction engendering improvement in mitochondrial action. Most animal studies restrict energy in healthy laboratory animals on healthy soya based diets. In restricting energy in animals with oxidative stress, an amyotrophic lateral sclerosis model, lifespan was shortened.\textsuperscript{662,1034} It may be proportionate total nutrition restriction that stimulates sirtuins. This same argument applies to autophagy.

**Other Phytochemical effects**

Different quantities of phytochemicals and their presence in different metabolic milieu have different effects.\textsuperscript{1006-1008}

Chitin and chitosan, although from insect and crustacean exoskeletons when used in this study, are present in fungi. It is possible that even they are having more than just a stool bulking effect, depending on concentrations and mixtures of other phytochemicals. There is recent work on phytochemicals having direct and indirect effects on neuronal activities and brain process control, via various mechanisms.\textsuperscript{1007}

Cancer research particularly shows phytochemicals as frequently having hormone-like, often oestrogen/breast, but also androgen/prostate modulation effects or having cell replication regulation effects.\textsuperscript{1035-1038}

Sometimes when phytochemicals act as ligands for hormone receptors, it is the concentration, often very small, that determines the type of action, and in fact many phytochemicals have low bioavailability; where higher blood or body concentrations are toxic the gut purposely controls absorption and digestion.\textsuperscript{1030,1039,1040}

Genistein, a flavanoid phyto-oestrogen derived from soya stimulates oestrogen receptor-β which activates cell nuclear machinery via NF-κB subunit 50 to stimulate antioxidant effects, and longer life in females.\textsuperscript{843} Genistein (and daidzein) via PPARs can inhibit adipogenesis,\textsuperscript{1041} and TNFα, platelet aggregation\textsuperscript{843,1042}. Thus Many phytochemicals are not well absorbed but perform functions differently at different doses.\textsuperscript{1015}

*Aspergillus sojae,* which is used for soya sauce fermentation,\textsuperscript{903} induces the soya plant to produce glyceollins. Glyceollins have been shown to have various biological activities
including antifungal, antiestrogenic, antidiabetic, and anticancer activities. Glyceollins induced phase 2 antioxidants/antitoxicants nicotinamide adenine dinucleotide phosphate NAD(P)H:quinone oxidoreductase (NQO1) activity in a dose-dependent manner in mouse hepatoma cells and increased the expression of some other representative phase 2 antioxidant enzymes, such as haem oxygenase (in the bilirubin pathways) 1, γ-glutamylecysteine synthase, and glutathione reductase, by promoting nuclear translocation of the nuclear factor erythroid 2-related factor-2 (NRF2)903.

The introduction of the NRF2 pathway is very apposite. Thus far in the discussion, plant phytochemicals, especially the plant defence chemicals from a number of different classes, appear to be active in rather non-specific modulatory ways. This does not answer the question of why so many effects appear to be antioxidant.

6.8.1.5 NRF2-Mediated Indirect and Direct Homeostatic Control System

A ubiquitous complex antioxidant activator hub denoted NRF2, already introduced, is present and active in humans. There are 3 other NFRs but they are developmental or tissue specific, with specific subtypes NFR1 & 3 functioning in the liver antioxidant system. NFR2 works in conjunction with an actin binding KeaP1 and antioxidant ARE, as mentioned (Figure 6-8).

The cell is provided with interleaving levels of inducible defence and protection from a number of groups. The inducible aspect suggests that this is an efficient system. There are different groups of inducers which stimulate the NRF2 action and these are direct, indirect and bifunctional, the latter a mix of both direct and indirect (Figure 6-8). Then there are the types or Phases 1-III of reactors that perform the appropriate cytoprotective functions, including antioxidation (Figure 6-8).

On entry to the direct, indirect and/or bifunctional small molecules inducers cause KeaP1/NRF2/ARE to signal appropriate transcription of many Phase1-III reactors eliciting antioxidant, antixenobiotic or antiforeign particle, detoxifying, cell protection and repair mechanisms (Figure 6-8)1043,1044. Phase III reactions are usually those through which conjugated xenobiotics are transported out of the cell and excreted1045.
Interrelations of direct, indirect and bifunctional antioxidants. Cellular protection against oxidative stress involves two types of small-molecule antioxidants: (i) direct, redox active, are consumed during their antioxidant functions, need to be regenerated, are short-lived, and may evoke pro-oxidant effects; and (ii) indirect, may or may not be redox active, function through induction of cytoprotective proteins that act catalytically, are not consumed, have long half-lives, are unlikely to evoke pro-oxidant effects. Some antioxidants are both direct and indirect and can be designated as bifunctional. Some direct antioxidants are also required for the catalytic functions of cytoprotective proteins. Many cytoprotective proteins in turn participate in the synthesis and/or regeneration of direct antioxidants. Note most are phytochemicals. Figure from Dinkova-Kostova & Talalay 2008.

Phase I-II general antioxidant – type reactions may or may not be interrelated. The cytoprotective phase II enzymes are comprised of the classical conjugating enzymes; again the familiar glutathione transferases and uridine diphosphate-glucuronosyl transferases. Other proteins which are usually classified as antioxidant enzymes, examples of which are haem oxygenase 1 (bilirubin pathway enzyme) and catalase, are included in this group. Interestingly, other significantly protective, non-enzyme proteins also act as phase II cytoprotectors and include ferritin and thioredoxin. Phase II reactions may not be related to those of phase I (Figure 6-8).

The direct antioxidants are usually low molecular-weight compounds, such as the well-recognised vitamins: VitC, VitE, VitK in addition to bilirubin, some of which have been the subject of the study in this thesis, and glutathione, lipoic acid and ubiquinol.
Direct antioxidants undergo the classic redox reactions and scavenge RONS (Figure 6-8). They can be consumed intravascularly during their action and, of note, can become pro-oxidant

The indirect antioxidants are highly involved in deactivating xenobiotics and toxins. Some quinone inducers are not adequately detoxified and are associated with carcinogenesis

There is some overlap in these reactions and processes, and those with dual functions have been termed bifunctional antioxidants as shown in Figure 6-8.

The bifunctional antioxidants can play a dual protective role behaving both as direct antioxidants and as indirect antioxidants through induction of cytoprotective proteins. These tend to be Michael acceptors. The Michael reaction concept is where organic moieties with certain carbon, oxygen and hydrogen bond configurations have electrons which are moved into different positions to enable C-C bonds, with a base temporarily taking a proton and returning it once the electrons are stably repositioned

The most notable point of the powerful bifunctional antioxidants is that they are often phytochemicals (Figure 6-8). Examples are resveratrol from grape seeds, skins and peanuts, isothiocyanate from cruciferous vegetables, catechins from green tea, and curcumin from turmeric. Again note that they are usually mild pro-oxidant inducers.

This bears crucially on previous antioxidant studies and brings to the fore the problems of interventions of high dose pure synthetic antioxidant and or vitamin therapies to prevent degenerative disease such as atherosclerosis, diabetes and cancer. Giving high dose vitamin therapy or phytochemical monotherapy has been shown to worsen the pro-oxidant conditions just mentioned. This is likely to be due to these agents being mildly pro-oxidant, thus in large doses they have an overall oxidant effect (Figure 6-8).

The biology of using small, useful but controlled amounts of an agent, that is toxic in large amounts, to produce signals for remedial action is the concept of hormesis and adaptive response as previously introduced, with respect to exercise and aging. RONS stimulate signalling to initiate restitution. Thus small molecules that are mildly oxidant stimulate antioxidant cytoprotection via the Keap1/NRF2/ARE system.
Currently, the Keap1/NRF2/ARE-dependent cytoprotective system is of interest in cancer research. The whole system protects against degenerative change and may be pivotal in obesity MetS and CVD (Figure 6-8). Already thousands of phytochemicals have been studied for their benefits, which are almost all for degenerative disease prevention, treatment or amelioration. Myriads of phyto- and some zoo- chemicals are part of most traditional whole-food diets.

**Second Part of Hypothesis - High Requirement for Phytochemicals to Modulate Multiple Antioxidant/Cytoprotective Pathways**

Humans have been ingesting a wide variety of phytochemicals in food, including herbs and spices and herbal medicaments for thousands of years. They had learnt, by and large, what foods were poisonous, with bitter taste playing a large part. The liver, which hosts 3 NRFs, is a powerhouse of detoxifying and antioxidant activity, and the omnivorous human diet has been well supplied with many phytochemical groups (Table 6-3).

<table>
<thead>
<tr>
<th>Diet Attribute</th>
<th>Typical Forager (past)</th>
<th>Typical Westernised (current)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) Glycaemic load</td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>2) Fatty acid composition</td>
<td>Low ω-6/ω-3, 1-2:1</td>
<td>High ω-6/ω-3, 8-10:1</td>
</tr>
<tr>
<td>3) Macronutrient composition</td>
<td>Lower CHO/Higher Pro/Higher fat</td>
<td>High CHO/Mod Pro/High fat</td>
</tr>
<tr>
<td>4) Micronutrient density</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>5) Acid-base balance</td>
<td>Alkaline</td>
<td>Acidic</td>
</tr>
<tr>
<td>6) Sodium-potassium ratio</td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>7) Fibre content</td>
<td>High</td>
<td>Low</td>
</tr>
</tbody>
</table>

Table adapted from Cordain 2005

It is theorised that this ample supply of, and experience with, phytochemicals has enabled humans to have an extremely efficient inducible antioxidant cell protection system. This is in addition to energy and inflammatory transcription phytochemical modulatory systems, which have allowed energy sparing for the brain and produced an added bonus of healthy, long lived cells and overall longevity. Limiting the energy dense, addictive foods or foodstuffs and returning to a high volume, micronutrient dense diet of low processed whole-foods may prevent or ameliorate MetS (Table 6-3).
6.8.2 The Unifying Hypothesis and the Current Westernised Environment

The unifying hypothesis then is summarised; as the human brain increased in size, co-adaptations arose serving, to increase energy uptake from the environment, and to enhance energy oxidation system efficiencies associated with phytochemical antioxidant and cytoprotective systems (Figure 6-9).

Note that the unifying hypothesis is a composite of, and subsumes or depends on, many others, but seeks to ensure that the two parts together are necessary complements.

Firstly, the newly expanded mesolimbic system of the human brain includes a well-developed self-reward and motivation system, both of which ensure the drive to attain extra energy for the brain is maximised.

Note that this hypothesis also explains why humans have moulded their environment the way they have. Farming practices, processing and storage of energy dense food, which is then marketed with strong cues, is a product of this self-reward/motivation system.

At the individual level the dopamine-based limbic system can malfunction in the face of greater chronic, excesses of the energy dense food, than it evolved to manage, and become an addictive system.

This is likely when there is minimal tempering from homeostatic, and nutrient dense, whole-food. The second part of the hypothesis is a response to economise on the body’s use of energy. As large quantities and varieties of plant chemical comprised a major part of the diet, it became energy efficient to utilise these phytochemicals as antioxidant buffers/complex cytoprotectors via the NRF2 system, to maintain long lived cells.

If there is a failure in provision of these phytochemicals, in other words humans do not consume enough, degenerative diseases develop much earlier in life, reducing productivity and lifespan. The development and ramifications of MetS have been described throughout this thesis.
Factors determining food intake and energy balance in the energy restrictive micronutrient replete (or forager and modern environments. The availability of nutrients (internal milieu) is detected by a plethora of distributed sensors and controls food intake directly through classical hypothalamic-brainstem pathways and indirectly through modulation of food reward processes in the meso- and cortico-limbic structures. Low macronutrient availability produces very strong sensitization of cognitive and hedonic mechanisms enabling procurement and ingestion of energy dense food as well as generating high reward and satisfaction. In the past plenty of micronutrients always came with the macronutrients. This system evolved to enhance adequate energy supply, partly for the brain, in restrictive environments requiring a high physical activity level. The modern environment and lifestyle are characterized by high macronutrient availability, abundant food cues and high food palatability all enhancing food intake either directly or through the same meso- and cortico-limbic systems. There is neglect of very low micronutrient variety and volume. In addition, the built environment, sedentary lifestyle and low procurement costs lead to decreased physical activity and, in turn, increased macronutrient availability. Obesity develops in prone individuals who either efficiently translate exaggerated hedonic, cognitive and/or emotional pressure exerted by the modern environment and lifestyle into increased eating, or in individuals in whom energy repletion signals are not able to suppress hedonic eating, or both. As obesity is rarely welcomed by any sufferer it is likely that most obese are out of control and have an addictive pattern of food craving and eating. There is no data on micronutrient or vitamin craving. (NB. Micronutrient craving is unlikely to be the (main) cause of pica\textsuperscript{1049}. Schematic adapted from Zheng 2009\textsuperscript{1050}

Both parts of the hypothesis would need addressing in any treatment paradigm. Current patterns of food production and foodstuff processing will not change unless regulation for healthy food is enacted – addicted individuals and addicted societies need help to change.

In addition and related, but only briefly touched on, is stress hormone release, prominent in sleep deprivation.
6.8.2.1 A Whole-Food Diet Prescription Derived from the Unifying Hypothesis and Consequences

Clinical and public health obesity management plans abound in diet prescriptions. Most well-known are the Food Pyramids\textsuperscript{1051}. Recently devised and published is a therapeutic food plan for MetS with each point portrayed with an accompanying researched rationale\textsuperscript{1052}. It has many useful features such as advice to include ample fruit and vegetables, eat mainly whole-foods, and decrease sugar and artificial sweeteners. However, the advocating of other concepts such as controlling portions of whole grains, choosing low glycaemic index foods, consuming almost no animal fat and mindfulness eating attitudes, are not proven to work reliably in the population\textsuperscript{1053}.

The current unifying hypothesis diet prescription needs more study as discussed below. It is reminiscent of a Palaeolithic diet\textsuperscript{288}, but humans have adapted since those times, and the main thrust is to eat nutrient dense, low processed foods.

Whole-foods could include some dairy along with their enhanced fermented microbe (probiotic) nutrients. Lightly cooked, free range unprocessed meats have reduced levels of burnt carbon, heterocyclic amines, nitrite additives and other potential carcinogens\textsuperscript{879,1054,1055}. Other reasons for including the latter two foods is that dairy is a highly nutritious food designed to support a young mammal, and free range or non-grain fed meat/egg again is a high quality protein source with complex fats, and both have high mineral levels\textsuperscript{231,1056}. So saying bovine, nor other ruminant dairy, is not an ideal food for a human infant.

The current hypothesis indicates that a prudent approach may be to nearly totally exclude grain and potato tuber derived starches and sugars in most older individuals, and all obese individuals with MetS. Modern wholegrain and potato skin products, as discussed, are still too highly bred as dense starch foodstuff, too depleted in micronutrients, contain ω-6 oils and are too addictive to fit into a whole-food diet for weight maintenance and CVD protection.

This strategy would strongly elevate the micronutrient/macronutrient ratio in these groups. Restricting high starch and sugar and other processed foods, back to a very few ‘set date’ festivities per year tends to make these energy dense foods too occasional to
hold strong addictive cues. However, it is important, to help with restricting these foods by long-term antiaddiction management techniques, possibly including medication.

The most controversial aspect, and the one that needs energetics modelling as will be introduced in the next section, is that it is expected that whole-food raw fruit and vegetables will be unrestricted or an increased volume encouraged. High energy whole-foods are permitted. There is no need for energy counting initially, and probably never, as whole-foods tend to restrict themselves. Abstinence of processed food is expected to get easier with time and with experience preparing and consuming a whole-food diet\(^{1057}\). The higher fruit and vegetable fibre content also introduces food energy dilution. It is unknown whether once the body is micronutrient-replete overall cravings reduce.

The unifying hypothesis proposes that, once the centrally obese body is absorbing plenty of food micronutrients, its metabolism will mobilise fat stores and maximise energy use to upkeep long lived cells, so extra energy will be gainfully employed in cell repair, and weight loss should ensue.

A further corollary is that micronutrient dense, healthy plant and animal whole-foods, which have vigorous microbe defence systems, are less likely to transmit food borne pathogens, decreasing chances of immunologically healthy consumers acquiring, or succumbing to pathogens in prepared food\(^{346}\).

Finally, another consequence is the change required in food production. Although there is much resistance to change in industrial farming practices, public health concerns may force a review of current agribusinesses and industrial food processing practices\(^{1058-1061}\).

### 6.9 Future Directions

As the unifying hypothesis is theoretical thus far, a discussion follows on:-

1) performing clinical whole-food studies to test (A) proof of concept by change in metabolic, oxidative, nutritional, genetic and metabolomic laboratory markers and anthropometric markers and indexes, and (B) acceptability, adherence and response to antiaddiction therapies and possibly medication, and

2) Initiating plans for mathematical modelling of the energetics in human models on different diets in differing circumstances, including obesity.
6.9.1 Sketch Plan for Whole-Food Diet Clinical Studies

6.9.1.1 Introduction

Obese individuals generally eat a high energy diet and may not acquire an adequate proportion of micronutrients in their diets, and thus many obese individuals develop MetS. Whole-food diets may supply micronutrients which had hitherto been insufficient in their diets. Palaeolithic diets have been studied in small, uncontrolled groups\(^{288,1062,1063}\). The whole-food that is proposed to be studied requires that grain and potato based foods be excluded, but dairy, pulses and access to olive oil remain. This is a main difference with the modern Mediterranean diet, where bread (usually whole grain), pasta and potato is eaten. The hypotheses have been introduced.

6.9.1.2 Community Diet Study A) A 6m Randomised, Parallel, Adherence and Health Change Study

**Aims A)** The aims are to conduct a randomised parallel community weight and MetS management study in overweight and obese individuals. Participants will be randomised to join one of 3 x 6m options: a current best practice healthy diet, a guided Mediterranean diet or a strict low refined CHO, whole-food only diet. An antiaddiction medication RCT may be conducted at a later date to ascertain effects on reducing addiction to energy dense food, in each diet.

**Methods A)** Overweight and obese participants will be randomised to one of three diet groups. They will visit monthly for anthropometry, bio-impedance testing, and complete 3d Food Recall records and Food Frequency, PA, QoL and readiness to change, eating disorders and attitudes questionnaires. Baseline, 3m and 6m blood tests will include safety CRSHaem&Biochem, metabolic, nutritional\(^{42}\), various new oxidative stress\(^{295}\) & inflammatory\(^{296}\) marker, genetic, epigenetic and metabolomic analyses.

6.9.1.3 Residential Diet Study - Proof of Whole-Food Diet/ Health Concept

**Aim B)** To conduct a randomised 12wk residential study with totally controlled and measured whole food or a Mediterranean *ad libitum* diet, with a typical *ad libitum* NZ diet as 3wk ‘wash in’ period before each test diet.
Methods B)

Overweight and obese participants will live in the HNU for 12 weeks on the test diets for 3wk each and both diets will be preceded by 3wk of a typical NZ western diet. Participants will be randomised to either a modern Mediterranean diet or a whole-food diet for 3wk, and each diet will be preceded by a 3wk ‘wash in’ period, to measure various parameters that change with different diets, and treatment regimens.

A typical NZ *ad libitum* westernised diet to be defined from nutrition surveys \(^685\) will be food such as toast and jam or cereals for breakfast, ham and cheese sandwiches for lunch, meat and three vegetables for dinner. Participants choose from a buffet-for-one breakfast and dinner meal times. The buffet food is measured before and the remains after consumption. Participants take packed measured lunches and snacks to work, which they have chosen from a buffet for that particular diet. The food will be a totally controlled and measured *ad libitum* diet, although the packed lunch consumption and the returned remains recording will be taken on trust.

Weight and waist measurements will be taken daily. Blood testing, for similar tests as for the Adherence study, will be performed before and after the 3wk ‘wash in’ and every two days during the test diets. Daily visual analogue scale tests will be used for food effects such as change in hunger, and food properties of palatability and acceptability. Eating attitudes will be assessed at baseline, after the wash in and after the 3wk. Many other details will need addressing.

### 6.10  Modelling Energy Budgets with Incorporation of Food Micronutrients for Lean and Obese Humans

#### 6.10.1.1 Background

The human body is generally over-fat compared with other terrestrial mammals, thus keeping a brain energy buffer. One human body morphology, usually female, easily stores extra fat, preferentially in the lower body subcutaneous adipose tissue depot. This may be a buffer of energy for gestation and lactation, which is now a buffer for two brains. Men tend to only have a central adipose, short term buffer, and they have a large muscle energy reserve.
To recap in terms of energy management, the human body needs to organise enough energy and micronutrient availability for its large demanding brain, and there are probably two, at least, unusual processes for this function.

Humans have strong neural reward systems, the mesolimbic system, for making vigorous efforts at succeeding in acquiring energy dense food.

Humans also employ a wide range and volume of environmentally available and recognisable food micronutrients via the NRF2 system, as an energy modulator/toxin manager for sparing use of energy in the cell and oxidative stress control. Slow growth and development also spared energy for the demanding brain, but required some irreplaceable cells to be maintained and conserved for decades, which in turn allowed longevity and cultural transmission.

However, the large brain and prehensile forelimbs also gave rise to technology, and an early and persevering technological aim has been to increase energy-dense food acquisition.

Unfortunately, humans appear to have limited capacity for detecting micronutrient deficiency, which has become a problem. The current westernised diet, designed to be energy dense, does not supply enough micronutrients for normal energy (fat) oxidation, let alone if there is a large surplus of energy, in addition.

Thus fat accumulates over and above adipocyte capacity, and is toxic when deposited ectopically; in the liver, heart, pancreas and many other tissues. There is a coincident general failing of protection of large organ systems prone to damage such as the endothelium, and poor control of replication such as in immune cells and endocrine tissue, which allows infection and tumour growth. All are part of the continuum of degenerative change.

Mathematical modelling of nutraceuticals and biomarkers in health and disease is occurring, but the energy component needs integrating\textsuperscript{1064-1067}.
6.10.1.2 Hypothesis

The initial hypothesis, to be developed and tested in future studies, is that whole-food micronutrient action can be mathematically modelled to show both economic cell energy use and oxidative stress management by functional elements modulating energy employment in cell maintenance. Thus, a high micronutrient, whole-food diet, irrespective of modest excesses in energy intake, will prevent central adipose tissue accretion and associated MetS.

To complete the model a total ecological human dynamic energy budget framework may need to be put in place.

6.10.1.3 Questions for review

1) What is the utility of previous energy balance equations in humans using energy containing mass relationship to food energy input and energy expenditure on a number of levels with or without moderators?
2) What are the population groups (over evolution, in recent human technologically advanced environments, over developmental periods) for which it is useful to model body composition in order to develop an understanding of possible energy metabolic processes and storage and fat distribution morphology in humans?
3) How can the energy compartments be handled?
4) Can a basic science, ‘bottom up’ energy use paradigm be constructed along the line of micronutrients being rate-limiting or rate altering and thus introducing energy inefficiency?
5) Can energy fluxes be modelled in antioxidant/energy management systems with the micronutrients factored in?
6) Is an overall mathematically modelled energy budget framework is needed?

6.10.1.4 Aim

The aim is to put forward hypotheses and ideas in order to start planning for the construction of human specific mathematical models of energy use, in the context of a micronutrient-replete diet, in order to work toward understanding obesity related metabolic syndrome in humans.
A very brief overview of problems with previous energy balance models for weight loss will be made, showing the necessity for a review of the energetics in obese humans.

A basic science ‘bottom up’ approach could start with examining the actual metabolic energy reactions, electing the micronutrient-replete scenario as optimal and making an estimate of the reduced efficiency with micronutrient insufficiency.

An existing NRF2 model will be examined.

The relevant energy compartments in the modern obese human can be compared with other hominoids/ancestral humans in order to understand environmental effects on body energetics with respect to obesity.

**Previous Energy Balance Equations in Obese Humans**

Energy balance models have been developed for various reasons. Early examples, previously mentioned, include energy estimates to sustain manual workers for the highest labour output and, latterly, to estimate energy deficits required to for weight loss in obesity.

The oft quoted first law of thermodynamics of energy conservation is rightly a starting point: Energy Balance is expected to be equivalent to Energy Input – Energy Output. The Energy (Fat) Equilibrium = [Energy in (fat) – Energy out (PA)] x physiological adjustments or moderators\(^{774}\) does not factor in other nutrients and, of course, PA is a small proportion of energy output even in fit, active humans. Some perceived problems with the energy balance equations in humans have been addressed frequently, with ever more adjustments made to accommodate the many variables, as has been thought necessary\(^{1068}\). However, the models have not been very successful at predicting weight loss, possibly due to issues such as tissue mass change and energy utilisation change being mismatched\(^{1068}\), let alone knowing exactly what humans under study in the community actually do with regard to food intake or physical activity\(^{1069}\). In much obesity research this basic equation is firmly established and energy alone has become the currency of weight loss, and by (questionable) extension, health\(^{774,1068-1072}\).
However, there have been hints, indirectly noted, that other food (micro) nutrient lack\textsuperscript{1073} or presence\textsuperscript{1074} might be having some influence on fat gain. Furthermore, even large fat losses do not necessarily lead to reduced mortality, or MetS reversal, especially if CVD or risk factors are well established\textsuperscript{1075}.

In summary, energy balance equations have struggled to explain weight or fat loss or gain adequately. None has factored in micronutrient effects on energy flux.

**Archetypal Primate to Obese Human**

It would seem to be important to develop models which include a progression of comparisons of energy balance leading to current day obese humans, the subject of this thesis.

To aid in this project, a progression of comparisons of an obese modern human, a normal weight modern (including growth and development of a human; neonate to adult), to a human forager, to same body sized hominoid and archetypal primates in order to try to ascertain as many human specific factors as possible will be suggested (Table 6-4).

The first set was the primate series of three hominoids; a small lesser ape, a larger great ape and a forager human. The small frugivorous primate is to approximate the last common ancestor (Table 6-4). An extant primate, *Hylobagtes lar*, the gibbon was chosen for this model. The model to approximate human size was chosen from the great ape, *Pongo pygmeus*, the orang-utan (Table 6-4).

The second set is all adult *Homo sapiens*; the early forager human, the current normal weight human and the obese human. The forager human is leaner, with less fat, than a current day adult.

The last set was to show an extreme aspect of human evolution, the normal fat baby and the normal adult and the abnormal obese adult (Table 6-4).

The modern human is presumed to inhabit the current westernised lifestyle environment, and the foragers and other primates are presumed to be eating diets as found during evolutionary times, or in the wild habitat.
Table 6-4. Energy Content Body Compartments of Female Hominoids

<table>
<thead>
<tr>
<th>Female Hominoids</th>
<th>Homo sapiens</th>
<th>Pongo pymaeus</th>
<th>Hylobates lar</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parameter</td>
<td>Modern Obese</td>
<td>Modern Slim</td>
<td>Past/Current Forager</td>
</tr>
<tr>
<td>Age, y</td>
<td>43.16</td>
<td>31.67</td>
<td>25.00</td>
</tr>
<tr>
<td>Height, cm</td>
<td>164.96</td>
<td>161.67</td>
<td>150.00</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>124.08</td>
<td>59.27</td>
<td>50.00</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>45.60</td>
<td>22.68</td>
<td>22.22</td>
</tr>
<tr>
<td>Brain Weight, kg</td>
<td>1.360</td>
<td>1.360</td>
<td>1.360</td>
</tr>
<tr>
<td>Brain:Body Weight</td>
<td>1.10%</td>
<td>2.29%</td>
<td>2.72%</td>
</tr>
<tr>
<td>Brain:Body Lipid</td>
<td>0.129%</td>
<td>0.930%</td>
<td>1.248%</td>
</tr>
<tr>
<td>Est. Fat, kg</td>
<td>62.04</td>
<td>17.25</td>
<td>10.00</td>
</tr>
<tr>
<td>Est. Prot., kg</td>
<td>11.33</td>
<td>9.84</td>
<td>8.30</td>
</tr>
<tr>
<td>Est. CHO, kg</td>
<td>0.47</td>
<td>0.28</td>
<td>0.29</td>
</tr>
<tr>
<td>Fat/Weight, %</td>
<td>9.1</td>
<td>16.6</td>
<td>16.6</td>
</tr>
<tr>
<td>Protein/Weight, %</td>
<td>9.1</td>
<td>16.6</td>
<td>16.6</td>
</tr>
<tr>
<td>CHO/Weight, %</td>
<td>0.38</td>
<td>0.47</td>
<td>0.59</td>
</tr>
<tr>
<td>Total Fat EN, MJ</td>
<td>2364.14</td>
<td>637.68</td>
<td>380.00</td>
</tr>
<tr>
<td>Total Pro EN, MJ</td>
<td>192.59</td>
<td>167.25</td>
<td>141.10</td>
</tr>
<tr>
<td>Total CHO EN, MJ</td>
<td>7.94</td>
<td>4.74</td>
<td>4.98</td>
</tr>
<tr>
<td>Total EN in body, MJ</td>
<td><strong>2564.67</strong></td>
<td><strong>809.67</strong></td>
<td><strong>526.08</strong></td>
</tr>
<tr>
<td>REE, Kcal/day</td>
<td>1827.95</td>
<td>1180.23</td>
<td>1100.00</td>
</tr>
<tr>
<td>REE, MJ/day</td>
<td>7.66</td>
<td>4.95</td>
<td>4.61</td>
</tr>
<tr>
<td>REE, x 1.33 MJ/day</td>
<td>10.19</td>
<td>6.58</td>
<td>6.13</td>
</tr>
<tr>
<td>REE, x 1.5 MJ/day</td>
<td>11.49</td>
<td>7.42</td>
<td>6.91</td>
</tr>
<tr>
<td>REE, x 2 MJ/day</td>
<td>15.32</td>
<td>9.89</td>
<td>9.22</td>
</tr>
</tbody>
</table>


Energy Content Body Compartment Modelling

**Body and Energy Composition Table**

To extend the evolutionary enquiry in this thesis, a preliminary estimate was made, from the literature, of the body composition of three intersecting or overlapping sets, as
detailed above, and in Table 6-4. The body compartments were tissue type rather than physical sites, and the brain indexes included to show the evolutionary changes. Females have been chosen, as the data was more available. Data were sourced from many references, as appended to the table in the subtitle (Table 6-4). Some values are best estimates.

The human adult brain is very large compared with the other hominoids. To underline the extreme physiology of normal humans, data presented show that the brain of the human infant constitutes a very large proportion of its body, and requires an ever higher proportion of its energy. At birth the human infant brain consumes 87% of its energy even an active 5y old directs more than $2/5^{th}$ (44%) of its energy to its brain$^{793}$. For the compartment modelling the brain lipid was added to the fat compartment, even though this lipid cannot be mobilised.

The energy contained in an obese person’s body can be 2.5 times that in a normal weight person. The multiples of resting energy expenditure (REE) indicate possible excess energy intake scenarios (Table 6-4). From this point mathematical modelling could be commenced.

*Energy Content Body Compartment Mathematical Systems Modelling*

This part of the model could start with a simple energy per body mass as a concentration such that Energy (J) / Biomass (Kg) = Concentration (J/Kg). The Concentration will be subdivided into compartments. Numerous conditions apply but 3 will be addressed so that a simple configuration can be modelled. They are outlined as follow.

1) Energy is spread between compartments in 2 forms a) basically structural /functional which is usually conserved and, b) more or less mobilisable as a short running energy source or a longer term reserve.

For the current argument each compartment will just be allocated a concentration, and later constants can be used to allow for % mobilisable

2) The form of energy is in 3 compartments: CHO, fat, and protein.

The magnitude of CHO energy is small (Table 6-4) so will be subsumed into the lipid compartment mobilisable concentration, as shown in Figure 6-11.

3) The compartments for this model are taken as discrete or independent, although there are numerous structural and functional molecular overlaps. Lipoproteins, glycoproteins, lipopolysaccharides will be allotted to Concentration_pro (Figure 6-11).
Thus, a two-compartment energy per mass model (Figure 6-11) could be a starting point.

**Figure 6-10. Two Compartment Model – Growth or Increase in Biomass**

<table>
<thead>
<tr>
<th>Compartment Total Body</th>
<th>Compartment Lipid</th>
<th>Compartment Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Change over time (y) in Total Body Energy (J)/Biomass (Kg) = Concentration ( \text{TotBod}(t) )</td>
<td>Change over time (y) in Lipid Energy (J)/Biomass (Kg) = Concentration ( \text{Lip}(t) )</td>
<td>Change over time (y) in Protein Energy (J)/Biomass (Kg) = Concentration ( \text{Pro}(t) )</td>
</tr>
</tbody>
</table>

For total body growth, where there is a limit in adulthood, a logistic growth equation could be used where \( G \) is specific growth, \( C \) concentration, \( t \) time and \( dC/dt \) is the rate of change of \( C \) with respect to \( t \)

\[
G = \frac{1}{C_{\text{TotBod}} \cdot dC_{\text{TotBod}}/dt} = rC_{\text{TotBod}} = r(1-C_{\text{TotBod}}/K)
\]

where \( r \) is the specific growth rate at low concentrations (ie. not yet limited by space, resources etc.) and \( 1-C/K \) is the logistic term\(^{1086} \) which when \( C = K \), growth stops.

Thus for this model of growth
\[ \frac{dC_{TotBod}}{dt} = \frac{dC_{Lip}}{dt} + \frac{dC_{Pro}}{dt} \]

\[ = r_{Lip} \left( 1 - \frac{C_{Lip}}{K_{Lip}} \right) + r_{Pro} \left( 1 - \frac{C_{Pro}}{K_{Pro}} \right) \]

where the \( r \)’s are the specific growth rate at low concentrations and are different for each compartment.

This equation will not be investigated further at this stage, as it is immediately apparent that many other factors need to be taken into account, and worked on in future. Normal growth will have different rate limiting factors from expansion of extra lipid stores.

However, it is into this \( r \) term that the effect of the micronutrients will affect the system. It is expected that the \( r \) (micronutrients) \( Pro \) will increase where by more structural and functional protein will be synthesised for lean tissue growth and that increased functional molecules will employ (excess) energy for maintenance and repair. The corollary will be \( r \) (micronutrients) \( Lip \) will maintain adequate reserves but excess energy will transfer to \( r \) (micronutrients) \( Pro \).

**Modelling Macronutrient Plus Micronutrient Oxidation Energy Expenditure**

It would seem that the well-known examples of enzymes in biological systems which catalyse reactions to manipulate energy pathways, in order to control and use energy in smaller aliquots, are accepted in energy balance equations.

So one could hypothesize that a metabolic pathway could be modelled in terms of, for example, setting a pathway at 75% of optimal because a micronutrient, which happens to be a cofactor in a rate-limiting reaction, is deficient, and then examine likely energy flows as a result.

At this point, it is envisaged, that food micronutrient factors should be considered with these other factors. Here the equations become difficult, and extra interdisciplinary input is required.

It is likely that with the non-essential micronutrients, the system is not so clear cut. For example, insufficiency of various similar members of a phytonutrient group which tends to work synergistically with others in other groups that commonly ‘travel’ together, have
important modulatory effects depending on a number of variables, may act through being a Michael acceptor in the NRF2 system. Variable energy fluxes speculatively giving a range of 75-90% of optimal are more likely. Thus, the lack of consuming certain plant groups will be estimated. In these instances many combinations may need running in large computer models, which by the time various genetic and epigenetic influences will also be altering the model, render ball park-estimations of food micronutrient effects on energy stores acceptable. As shown in Table 6-4, there is a large amount of fat mass difference between an obese human and a fit slim one.

However, various parts of the system are being researched and modelled at very basic energy flux levels. Following is work undertaken on energy of the actual micronutrient reactions within the above mentioned NRF2 system.

**Mathematical Modelling at the Chemical Reaction Level**

The antioxidant/antitoxicant NRF2 management pathways and system, previously discussed, has been modelled. This has been performed as a multicompartment ‘engine’ and could be a first step in calculating the energy flux through the system, as depicted in Figure 6-10.

The NRF2 system is mainly an antioxidant signalling and amplification, as well as a detoxifying, system. The Michael and other similar reactions in organic chemistry are generally well studied for their energy efficiencies, and have been studied in the NRF2 system, as shown in Figure 6-10. These reactions mainly relate to slightly higher energy unstable molecules or complexes having their electrons rearranged and the slight difference in energy channelled to signalling transcription of high level antioxidants.

Once the mathematical modelling parameters are put in place computer programmes can be run to follow the energy flux of the reactions. There are computer programmes for calculating the energy of inducers, the xenosensor-controller interaction with the Keap1/NRF2 as they dock. The actions of uncoupling protein activity may change when individuals are well fed a whole-food diet.
Figure 6-11. An Engineering View of the Nrf2-Mediated Redox Homeostatic Control System

The system comprises several functional modules routinely found in man-made control devices—transducer/xenosensor, controller, actuator, and plant—which are linked in tandem through feedback and feedforward loops. The inner feedback loop contains two pathways which are not separately illustrated here: a fast loop through increasing the activities of redox-sensitive antioxidant enzymes, and a slow loop through increasing the stabilization of antioxidant messenger ribonucleic acids (mRNAs). The two dashed loops represent positive autoregulation of nuclear factor erythroid 2-related factor-2 (Nrf-2) and transcription factor V-musculoaponeurotic fibrosarcoma oncogene homolog (Maf) genes. Constitutive and other signals represent inputs to the controller other than NRF2, such as those mediated by activator protein 1 (AP-1), nuclear factor-kappaB (NF-κB), and other redox-sensitive signals, etc. Nuclear receptors acting as xenosensors/inducers include aryl hydrocarbon receptor (AhR) pregnane X receptor (PXR) constitutive androstane receptor (CAR). Actuators: glutamate cysteine ligase (GCL), glutathione peroxidase (GPx), catalase (CAT), glutathione peroxidase (GPx) superoxide dismutase (SOD), glutathione reductase (GR), glutathione S-transferase (GST), UDP-glucuronoyl transferase (UGT). reduced glutathione (GSH) /oxidized glutathione (GSSG), nicotinamide adenine dinucleotide phosphate:H2 (NADPH2)/nicotinamide adenine dinucleotide phosphate (NADP) ROS reactive oxygen species, ROOH; lipid peroxides, X reactive electrophiles. Keap1/NRF2/ARE Kelch ECH associating protein 1/nuclear factor-E2-related factor 2/antioxidant response element. Graphic and adapted caption from Zhang 2010^1048.

Energy Flow Mathematical Modelling Framework - Dynamic Energy Budget

As alluded to there are a myriad extrinsic and intrinsic factors at work in energy management of organisms. Humans have a number of special conditions, some evolutionary and some brought about by their technologies, but also many in common with other organisms that need consideration. In order to keep in mind these factors, the
above-considered aspects of human energy management could be worked into an energy flow mathematical modelling framework, and a Dynamic Energy Budget may be appropriate.

Mathematical modelling has been strongly developed in evolutionary biology and ecology and should be able to be adapted for humans, human obesity and energy metabolic dysfunction.

The Dynamic Energy Budget\textsuperscript{1087-1089} is both an individual and community energy budget model that looks at life forms though life stages in a real environment, taking into account diet, diet changes, environmental toxicants and many more factors. It has recently been modified to deal with extra body reserves. The DEB depends on setting up the mathematical systems analysis model appropriately. It is envisaged that this model would provide the overall framework.

As yet this dynamic energy budget is not developed for a large homeotherm within an unusual energy use pattern in ideal nutritional environment, let alone one in an environment which is recently extensively altered from the period of most of its unusual evolution, even if this organism was the activator of the change.

**Summary**

In summary, for this section, it is hoped that in future, an idea of the magnitude of cellular energy processing in humans who consume plentiful food micronutrients can be estimated. It will possibly start with the above energy compartment modelling, general energy fluxes through known living systems, combined with the NRF2 micronutrient-employing system of energy processing, all within the broad DEB framework.

Models of smaller, then larger primates, and then examples of humans in various stages of life, all with their known habitus and morphology in their different food environments, will be employed.

It is hoped that the above modelling, probably bringing in other factors, will shed light on the mismanagement of energy in a micronutrient depleted, energy-overloaded diet, and will partly explain human obesity. A healthy, nutrient-replete whole food diet may help in
the prevention of excess central fat and/or its loss in the already obese, somewhat independently of the pure energy content of the food supply.

### 6.11 Discussion

The reasons for formulating a new unifying hypothesis arose from observations that obesity-related MetS has become epidemic, but also that only a few researchers or clinicians appeared to be delving deeply into the causes, perhaps as relinquishing accepted current practices is difficult.

The new science of obesity management was making assumptions about obesity that may or may not take into account CVD risk scenarios. Concurrently, historical research and treatment of TIIDM and CVD was not really addressing the relationship to the newly increasing rates of obesity. There were assumptions based on medical traditions and societal attitudes to obesity.

Plentiful micronutrients and a high micro-/macro- nutrient ratio probably contributes very significantly to human health, in terms of healthy longevity. However, humans are resilient and manage to survive and breed on fairly meagre overall rations. However, if the micronutrient deficiencies are severe, less successful reproduction and offspring survival occurs, and humans suffer stunting, infection, and life tends to be short. However, in the current westernised environment the massive energy overload and micronutrient undersupply may be damaging health and also reducing fertility via central obesity – TIIDM - polycystic ovary syndrome - dysmetabolism, and male hypogonadism in the breeding years.

A sensible recourse was to initiate an inquiry from an initial conditions and basic biochemistry. Happily, there was a rich, thorough resource of human archaeology using modern techniques, and many theories of energetics and nutritional relationships to sift through.

There was also an exhaustive set of research data on phytochemical properties and relationships with cancer, which was very portable to MetS as MetS is a risk for cancer, in addition to CVD.
Furthermore, there was also some very advanced biochemical and ‘omics’ work linking behaviour, and neural studies on appetite control and addiction. Thus, reflections on the unique development of the human brain and its energetics consequences precipitated thoughts of adaptive sequelae.

The exploration proceeded, ideas were formulated, investigation continued to establish likelihood, and more ideas were generated until the hypothesis appeared tenable.

Research is needed to increase the likelihood that the composite unifying hypothesis, on causes and possible amelioration of central obesity-related metabolic syndrome, is a working paradigm. It will need multidisciplinary teams that are open to new ideas.

The first new idea is that human metabolism has important differences from that of other closely related animals – and that increased encephalisation is responsible for much that is interesting, but brings some energetics challenges as well.

The clinical studies may need to abandon the RCT reductionist single item testing, and instead work in a more ecological manner, which is more accommodating of less exact dietary pattern analysis, and that food-effect testing uses systems that can analyse synergistic effects of micronutrients\textsuperscript{1,1065,1066}. Mathematically tested models of epidemiological data are also helping to bring new data to the table\textsuperscript{482}.

Modelling of energy use systems will require mathematical, dynamic modelling, with large arrays of metabolites – metabolomics and an understanding that micronutrients can act like hormones in some concentrations and cytokines at others, that they can alter between anti- and pro-oxidant and inflammatory or metaflammatory in different situations. In addition, genomics may need to deal with the idea psychological stressors can push epigenetic/genetic selection. In addition, epigenetic environmental responses in the still-evolving human brain should be expected, and not be a surprise.

Whether mathematical modelling of micronutrient effects will show large or easily perceptible effects on human energy balance, central adipose tissue accretion and largely reverse early MetS is yet to be seen.
There will be problems with the unifying hypothesis proposed. Not least will be that it challenges beliefs and treatment practices, including medical and nutritional therapy. In an obesogenic environment the hypothesised diet will still be hard to implement in the current environment.

The research supported the main tenets of the hypothesis, which is borne out by evolution, to a level that further study, as suggested, is warranted.

The unifying hypothesis dietary advice is congruent enough with current therapies to consider using in some patient groups for whom other theoretical treatments have not worked. The dietary advice is unlikely to do harm, but is likely be helpful. Various antiaddiction drugs are in phase III trials of obesity, and the FDA is exercising due caution in wanting longer term studies before registering them.
Chapter 7. Discussion and Conclusion

7.1 Discussion

The aim of this thesis was twofold: firstly to develop a model of best practice weight loss through which to investigate MetS related Novel CVD Risk and Protective Markers in overweight and obese women and men and, secondly to develop a unifying hypothesis on causes of obesity-related MetS. Both aims were fulfilled although the study sub-hypotheses were disproven in some cases.

An over-riding theme in this thesis is an investigation into the most profound issue affecting or causing obesity and MetS, both risks for the most common non-communicable causes of death\textsuperscript{165}.

Initially, the concept of metaflammation was hypothesised to be the main issue to study, and in the design of the CVD Risk Markers study the analysis was corrected for acute and chronic inflammatory disease, inflammation modifying medication. ESR and CRP were included as CRSHaem&Biochem.

Early in the analysis it became clear that, it was the oxidant markers, particularly Hb\textsubscript{A1c} and urate which were more highly correlated on Pearson and partial $r$ analysis, and whose mean in the mixed model analyses was related to the MetS marker count over and above the other explanatory CRSHaem&Biochem variables, including CRP, for example. Moreover, the oxidant markers were highly related to most of the other MetS markers, with Hb\textsubscript{A1c} most highly related to FPG, and urate to BP. Note also that the recently discovered antioxidant marker, bilirubin, was negatively related to BP. Hb\textsubscript{A1c} and urate were quite complementary and they are both of interest.

Firstly, glycated proteins are oxidant, and such is Hb\textsubscript{A1c}. Recently, Hb\textsubscript{A1c} has been recognised as a T1IDM screening and diagnostic marker in its own right by the ADA\textsuperscript{143}, which means that it will be frequently tested and on the medical records of millions of obese persons. There have already been studies by NHANES looking at Hb\textsubscript{A1c} T1IDM screening and prediction\textsuperscript{610,1091}. Prospective tracking using Hb\textsubscript{A1c} for hard endpoints such as CVD events and degenerative related disease mortality will be very important.
Urate is a cellular oxidant\textsuperscript{1092,1093} and although used for gout screening, was also very strongly related to MetS. That Kylin\textsuperscript{96} in 1923 included hyperuricaemia in the first version of MetS gives an indication of its prominence. Serum urate levels are raised in obesity\textsuperscript{94,101,1005,1094} but in normal weight individuals serum urate has antioxidant effects. The oxidant XO that catalyses urate’s high level in obesity has an effective antioxidant inhibitor, allopurinol, which significantly lowers urate but, unfortunately, it is only used for florid gout. Part of the reluctance to acknowledge urate as a marker may be that treating oxidative stress has not been thought possible, although it is now known that metformin, the insulin sensitisier, has antioxidant properties\textsuperscript{1095,1096}.

Although, age was the most influential factor overall in the longitudinal study, that γGT, the liver enzyme, was borderline significantly positively related to MetS marker count in a general mixed model over and above the other the demographic, lifestyle and the other CRSHam&Biochem, makes γGT potentially the most important marker. HbA1c and urate, which were included as other explanatory variables of the 6m study were strongly associated with MetS. This meant that γGT was able to act as marker over time to track MetS change as weight was lost, or in fact gained, over the 6m.

γGT was also correlated with waist, BP, TG & MetS marker count at baseline. γGT is becoming recognised as a strong predictor of MetS and CVD and even obesity in some studies\textsuperscript{175,380,1097-1099}. The biology of γGT probably explains why it is useful marker, already in clinical terms. As a transferase, γGT catalyses reduced glutathione, depleting glutathione depending on high cellular native, such as homocysteine, and xenobiotic, toxicants\textsuperscript{87}. Glutathione participates in the NRF2 system. In fact one research group suggests that persistent organic pollutants via γGT actually precipitate TIDM\textsuperscript{87}. The current study adds that γGT’s strong positive association with MetS marker count may well be able to be used to track patients’ progress towards better health or CVD risk.

The other liver enzymes ALT and AST are usually positively associated with benign NAFLD and NASH, respectively, but in this study they only had modest BP, TG and FPG relationships, with ALT also associating with mean DBP. Interestingly, in this study, their association with MetS marker count was significantly less than γGT. ESR, the inflammatory marker, was the other borderline CRSHam&Biochem that positively related to MetS marker count on mixed modelling over 6m.
The ESR may be affected by both inflammatory and oxidant plasma markers. The autoimmune diseases, for which ESR is classically used as a monitoring tool, are in fact pro-oxidant states\textsuperscript{1100}. The most oxidant of the leukocytes, the neutrophils, were also associated with the overall mean (baseline to 6m) in the mixed model MetS marker count.

The above CRSHaem&Biochem are tests for a generalised multi-metabolic condition. Different markers are useful both cross-sectionally and longitudinally, thus the CRSHaem&Biochem should be investigated further.

Centralised anonymously pooled results of some or all of these markers from many patients belonging to large healthcare maintenance type organisations can be matched with future health outcomes such as TIIDM incidence, CVD events (such as proven MI) and CVD mortality. CVD events and CVD are examples of hard events which is ultimately what MetS is used to try to predict and prevent. This could be effected by the method mentioned previously, and used in the NZ ‘Diabetes Get Checked’ treatment monitoring analysis\textsuperscript{653}. Using this method key markers, and their values, are tracked over time and entered into a mixed modelling analysis, as was used in the current study. Mathematical modelling has been employed using metabolic markers\textsuperscript{1101}. Results from local community data can be compared with individual patient changes and predictions made for future health.

When looking at the CVD Protective markers it is notable that a different method was used from the CRSHaem&Biochem analysis. These markers do not have a history of use as screening markers, although there is data relating them to CVD protection. Thus the hypothesis for their study was that they were expected to be related to known protective characteristics or protective markers with respect to CVD in the study group.

In spite of searching for clear information of the role of Adpn is in normal physiology this information is not known. This is important as serum Adpn, the adipocytokine investigated, is higher in normal weight individuals, but higher still in those who are lean with autoimmune disease, and lowest in obesity\textsuperscript{750,1102}. Studies show variable situation-specific Adpn levels, complicated by the oligomers with different functions, and sexual dimorphism\textsuperscript{430,1103}. Adpn and receptors have relationships with phytonutrients has been shown\textsuperscript{434,728}, and indicated in this study, with its relationship with s\betaCaro and fVitE.
A wide array of simple Pearson correlations was performed for the laboratory tests, which were generally normally distributed data. The baseline Adpn\textsubscript{250} data was mostly correlated with healthy, non inflammatory markers in women, but in this group of older men there were a number of positive correlations, as if the age of the men and their increased number of CVD and inflammatory diseases were associating with proinflammatory functions of Adpn.

HMW is the fraction that is most diminished in obesity due to problems with formation in overfull adipocytes, and therefore should recover the most in weight loss\textsuperscript{743}. It is also the most insulin sensitising and anti-inflammatory\textsuperscript{740,741,743}. The HMW was the only fraction that increased in both genders, and appeared to be an appropriate choice for change correlations.

The Adpn\textsubscript{30} was the small study with both oligomer and follow up data available. The 30 participants were selected post hoc as having the most extreme baseline data with respect to weight and waist and extreme changes over the 6m. The Adpn\textsubscript{30} change data was only performed in the HMW\textsubscript{30} group of 14 women and 6 men completers. The change correlations for the HMW\textsubscript{30} were very high and with a number of positive study parameters associated with health. As the HMW\textsubscript{30} increased, weight and MetS, other negative study parameter markers decreased, in women and men.

One could speculate that normally Adpn is present in adipose tissue to respond to sudden increased energy requirements, such as during intense PA, and acute infections, and releases itself and large quantities of lipid particularly for muscle and liver uptake. Once the stimulus is gone Adpn again becomes anti-inflammatory and maintains insulin sensitivity. This function is damaged in overlarge central adipocytes in obesity and may contribute to MetS\textsuperscript{743}. HMW should perhaps be investigated as a marker first rather than total Adpn, although it function is most altered in central obesity. Thus Adpn is not a useful clinical marker at this stage, as it is too complex and possibly malfunctioning. A recent study showed that Adpn did not add further information on taking other clinical factors into account\textsuperscript{1104}.

With respect to the sFSVitamins, it is important to note that lipid standardization was not performed on purpose\textsuperscript{441}. Correction of lipid levels of the sFSVitamins may need
reviewing or another approach taken in obese populations. In addition, the community laboratories do not report corrected levels. It is not clear whether the raw data indicate vitamin activity or not. In the current study the pervasive adverse associations of sVitA and sVitE with CVD risk may need thorough testing in an obese, dyslipidaemia population, with good diet and supplement data, with and without lipid standardisation.

Reporting on sβCaro was straightforward in comparison to the other CVD Protective Markers. It was clearly negatively related to MetS marker count in women and men. The adverse markers correlations were with CVD risk, inflammatory and oxidant markers, and TC and LDL-C. LDL-C and HDL-C are its plasma carriers so it was positively related to both. Age relationships were negative, too, which were thought to be that the older participants were returning to the fruit and vegetable diets of their youth. As a marker of probable whole-food diets, it is the best marker to use from the study. Interestingly, this was the only novel marker to relate to a lot of food nutrients, mostly healthy plant derived, including fibre. However, some nutrients were typically from animal foods, such as iron, magnesium and phosphorous.

SVitD did not relate to many study parameters, but any were positive markers, such as Adpn, sβCaro and sVitE. SVitD was negatively related to HbA1c, leptin and waist. Interestingly sVitD levels predict weight loss success. It is possible that low sVitD in obesity is partly a storage issue and the sVitD is sequestered in the large adipose tissue depots, as well as a serious metabolic issue. SVitD reduces chronic infection, especially of skin for example, although this does not seem to be a pro-inflammatory effect. SVitD has effects on insulin action, and may have a cytoprotective effect in the face of chemicals associated with smoking.

Although, in this study, sVitD related far more to issues of skin sun exposure and skin pigment and VitD photosynthesis, as NZ does not have VitD food fortification, increasing amounts of evidence show VitD to have profound metabolic functions. This indicates that VitD function is likely to be disrupted by MetS. Thus, hypovitaminosis D is likely to impede metabolic recovery from MetS. As central obesity is not healthy for bone or muscle, it was expected that there would be more negative correlations with low PA.
parameters, and this may have been due to inadequate PA data collection. Overall, however, sVitD should be a useful protective marker in MetS.

Historically, high blood levels of the antioxidant fat and water soluble vitamins have been associated with health, and have been associated with a healthy diet. In the current study the sVitA and sVitE concentrations were higher in men. Both sVitA and sVitE were negatively correlated to weight and BMI but otherwise they appear to be CVD risk markers, not protective. VitE, especially, negatively correlated with both TG and LDL-C. SVitE and sVitA correlated with urate, ferritin, and liver enzymes BP, CVD, MetS marker count and MetS. There are an increasing number of reports that indicate that obesity, as a CVD risk marker is associated with raised levels of sVitE and sVitA, and that these sFSVitamins diabetogenic.

However, it may be the fat-soaked, starchy foods and fatty meats, which have VitA and VitE, respectively, added as antioxidants may, in turn, become pro-oxidant, which is the problem. How and whether lipid standardization should occur with the sFSVitamins, more work is required to ascertain if they are diabetogenic, and certainly the issues need to be resolved before they can be markers.

All of the above FSVitamins have been problematic when given as mono or multivitamin (high dose) supplements. VitA can be toxic at high dose, and high dose VitE and βCaro have had probable pro-oxidant effects in cancer and heart failure studies, and VitD and calcium has increased atherosclerosis.

This brings the closing discussion to the unifying hypothesis of causes on obesity and MetS, and the second part of the unifying hypothesis on micronutrients; however the initial cause of desire to consume energy dense food will be addressed first.

The first part of the hypothesis on causes of obesity and MetS was a counter- or co-adaptation for increasing energy for the expanding brain. The evidence has been reviewed showing that an expanded mesolimbic reward/motivation system in humans has developed. It responds rapidly, by genetic and epigenetic regulation, to cues to acquire very energy dense food. It appears to be still evolving.

However, this same human reward/motivation system has also driven humans to develop agriculture to farm and process a hitherto unrecognised rich sources of CHO energy. The
industry has developed so fast and been so complete that the reward/motivation system has become overwhelmed, and become dysfunctional in many humans. This leads to a refined food addiction, neglect of cell- and life-preserving food micronutrients, inability to oxidise the excess macronutrient efficiently and safely, and oxidant and metaflammmatory stress; obesity and the beginning of degenerative disease or MetS.

A reversion to a whole-food diet would seem very prudent, but a great deal of effort will need to be made to help individuals and populations overcome their addiction to industrialised, over-palatable refined food. Two combination medications containing, naloxone, an opiate blocker already used for alcohol addiction, are under review by the FDA at present, but need more clinical testing\textsuperscript{959}. Phentermine, an old, short term medication for weight loss has monoamine, and probably, some dopaminergic effects\textsuperscript{1105}. Lastly bromocriptine, a dopamine agonist is on the market in some countries as a hypoglycaemic\textsuperscript{960}. The medications, if used, would need to accompany compassionate, thorough, up-to-date addiction counselling.

The second part of the hypothesis explains how as part of an adaptation to provide more energy to the expanding brain, the human body evolved to make particular use of many, mainly reactive plant defence, chemicals as very efficient, direct antioxidants and/or inducers of cell protection. This may be via the NRF2 system. Also these phytochemicals are often modulators of energy or inflammatory processes.

This hypothesis of phytochemical induced antioxidant long lived cytoprotection also explains why humans can live many decades longer than other mammals of comparable size. It has since been found that many of these phytochemicals, of which few of the possible millions have been studied, are often mild pro-oxidants that stimulate the cytoprotective effects.

This does not bode well for mono or multivitamin or nutraceutical therapy. It does make sense of why a high volume diet of fruit and vegetables, and low volume of refined energy may be a very suitable diet for humans.

This returns us to the first part of the thesis study; the RCT of a natural fibre, chitosan. With the demise of a number of orthodox weight loss medications in the last decade or so, natural fibre based agents may be of some use.
Chapter 7. Discussion and Conclusion

The chitosan did show a clinically mild effect on both weight and MetS markers, including waist in the mixed model. Although this fibre needs more mechanistic safety studies, as it is a cation, before the dose could be raised, a recent study$^{252}$ showed that a mild natural fibre, psyllium at high dose together with a high fruit and vegetable diet resulted in weight loss and metabolic improvement.

A high fruit and vegetable diet is already advised, but in order to use an ad libitum low processed but medium energy whole diet prescription in obese individuals it is advisable that 1) highly controlled proof of concept and longer adherence whole-food diet studies, with ‘omics’ analyses, are conducted and 2) studies in dynamic energy budget mathematical modelling are undertaken.

7.2 Conclusion

Finally, in obese individuals it is worth using the IDF/JIS MetS as a screen. From the current study it was shown that MetS in central obesity, is related to important known metabolic, pro-oxidant and coincident metaflammation markers.

The main new findings of the study were that the greatest issue in MetS is the pro-oxidant state, partly metabolic and partly due to the large amount of non-oxidised lipid present, and how extensive its degenerative effects are even early in the syndrome.

Indications from the widespread and profound effects of βCaro and VitD, or conditions for which they are markers, such as high fruit and vegetable food, appropriate sun exposure and probably PA, are that lifestyle change to these conditions are likely to bring remedial change.

The unifying hypothesis investigation may be plausible. It brings together some new (and ancient) information, unique to humans, to bear on the biological and psychosocial issue of obesity and MetS. Thus, via: powerful new technologies, genetic archaeology, and ‘omics’ studies, together with other obesity-related MetS research, the current study, and the further energy budget mathematical modelling and the clinical whole-food diet studies suggested, a way forward is seen.
Clinically, for the normal weight, overweight and obese patient who wants to regain and/or preserve their health, screening anthropometry and MetS marker laboratory tests should be taken, and MetS status calculated. CRSHaem&Biochem, sβCaro and sVitD testing should be performed to fine tune CVD degenerative risk prognosis or protective diagnosis and for monitoring.

A simple plan for whole-food consumption and management should be discussed, and the reasons why it is important for health, given. Consideration should be given to possible high dose fibre and antiaddiction medication prescriptions, with ongoing support and antiaddiction counselling, as needed.
Appendices

Appendix 1. Questionnaire Forms

24-Hour Dietary Recall Record

Patient Identifiers

<table>
<thead>
<tr>
<th>Patient</th>
<th>Registration number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Date of birth</td>
<td></td>
</tr>
<tr>
<td>Initials</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Assessment type (tick one only)</th>
<th>Assessment date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>0.1</td>
</tr>
<tr>
<td>6 months</td>
<td>0.2</td>
</tr>
</tbody>
</table>

Q1: 24-hour Dietary Recall

Day of the week *please circle one*

Mon Tues Wed Thurs Fri Sat Sun

INSTRUCTIONS – Read this first

I. Read the booklet ‘Guide to completing your 24-hour Dietary Recall’ before beginning to fill out this 24-hour Dietary Recall.

II. Note down all the food and drink you consumed yesterday in the ‘memory list’ form on page 2; listing the foods in this manner should help you to remember clearly. Copy these foods across into the *24-hour Dietary Recall form* (on page 3 and 4 enclosed) and fill in the details as described below.

III. In the columns on the following pages write the name, brand name (if known), method by which the food was prepared (where applicable) and the amount of the food *YOU* ate in cups, grams, ml etc.

- Please record the recipes of any home-cooked/home-prepared meals and baking that you ate on page 5 of this form.
- Don’t forget to include spreads, dressings and mayonnaise, oils used in cooking, and snacks such as potato chips, nuts, muffins or snacks.
- Please refer to the booklet ‘Guide to completing your 24-hour Dietary Recall’ pages 5-8 if you are having difficulty estimating portion size or amount.
- If you have any queries please contact Dr Fiona Leahy or Jane Easton on Ph (09) 630 3744.
Appendices

Appendix 1. Questionnaire Forms
24-Hour Dietary Recall Record (Cont.)

<table>
<thead>
<tr>
<th>Patient Identifiers</th>
<th>Registration number:</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1.2 Date of birth</td>
<td>0.3.2</td>
</tr>
<tr>
<td>0.2.2 Initials</td>
<td></td>
</tr>
<tr>
<td>0.4.2 Assessment type (tick one only)</td>
<td>Assessment date:</td>
</tr>
<tr>
<td>☐ Baseline</td>
<td>0.5.2</td>
</tr>
<tr>
<td>☐ 6 months</td>
<td>30 20</td>
</tr>
</tbody>
</table>

Please complete this questionnaire and bring with you to your next visit
‘Memory list’ form

Write from memory all the foods and drinks you remember eating and drinking yesterday. No
details need be given here, just the basic name of the food e.g. milk, cornflakes, bread, potato.
This memory list is intended to ‘jog’ your memory.

<table>
<thead>
<tr>
<th>Meal/Eating occasion</th>
<th>Foods</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breakfast</td>
<td></td>
</tr>
<tr>
<td>Morning snacks</td>
<td></td>
</tr>
<tr>
<td>Lunch</td>
<td></td>
</tr>
<tr>
<td>Afternoon snacks</td>
<td></td>
</tr>
<tr>
<td>Dinner</td>
<td></td>
</tr>
<tr>
<td>After-dinner snacks</td>
<td></td>
</tr>
</tbody>
</table>

If you have any queries, please contact Jane Easton or Fiona Leshey on (03) 639 3744
Appendix 1. Questionnaire Forms.

24-Hour Dietary Recall (Cont.)

<table>
<thead>
<tr>
<th>Meal/ Eating occasion</th>
<th>Food type</th>
<th>Brand name (if known)</th>
<th>Preparation/ Cooking method</th>
<th>Estimated amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>e.g. Breakfast</td>
<td>Chicken eggs, Camomile oil, Bread, white, hollandaise sauce, Camomile oil</td>
<td>AMCO Quality Bakers, maggi sachet, Camomile oil</td>
<td>Fried in camomile oil, Used for frying, Toasted, Added to 1 cup hot water</td>
<td>2 x large, 1 Tbsp, 2 x toast slice, ¾ of a cup prepared</td>
</tr>
<tr>
<td>Breakfast</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Morning snacks</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lunch</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Did you remember (please tick)?

- spreads & dressings
- quick snacks
- drinks such as milkshakes, smoothies, hot chocolate (or other hot drinks)
- oils used in cooking
- potato chips and peanuts, etc

If any of these foods were forgotten please go back and write them in now.

If you have any queries, please contact Jane Easton or Fiona Leasty on (03) 639 3744
## 24-Hour Dietary Recall Form

<table>
<thead>
<tr>
<th>Meal/ Eating occasion</th>
<th>Food type (if known)</th>
<th>Preparations/ Cooking method</th>
<th>Estimated amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Afternoon snacks</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dinner</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>After-dinner snacks</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Did you remember (please tick)?

- ☐ spreads & dressings
- ☐ quick snacks
- ☐ drinks such as milkshakes, smoothies, hot chocolate (or other hot drinks)
- ☐ oils used in cooking
- ☐ potato chips and peanuts, etc

If any of these foods were forgotten please go back and write them in now.

Yes  ☐  No  ☐

☒  ☐ Was this day reasonably typical of your usual diet

If No, please explain ________________________________

Please turn over and fill in any known recipes of home-cooked meals or baking

If you have any queries, please contact Jane Easton or Fiona Leshy on (03) 639 3744

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Appendices

Appendix 1. Questionnaire Forms

24-Hour Dietary Recall (Cont.)
Appendices

Appendix 1. Questionnaire Forms.
24-Hour Dietary Recall (Cont.)

<table>
<thead>
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<th>Patient Identifiers</th>
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</tr>
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<td>0.1.5 Date of birth</td>
<td>Registration label here</td>
</tr>
<tr>
<td>0.2.5 Initials</td>
<td></td>
</tr>
<tr>
<td>0.4.5 Assessment type (pick one only)</td>
<td>Assessment date:</td>
</tr>
<tr>
<td>☐ Baseline</td>
<td>0.5.5</td>
</tr>
<tr>
<td>☐ 6 months</td>
<td>day</td>
</tr>
</tbody>
</table>

Recipes form

Instructions:
Please record the details of any home-made recipes in the table below. See page 9 of the booklet 'Guide to completing your 24-hour Dietary Recall' for more information.

<table>
<thead>
<tr>
<th>Name of recipe</th>
<th>Number of people it serves</th>
<th>Ingredients</th>
<th>Brand name (if known)</th>
<th>Preparation</th>
<th>Estimated amount</th>
</tr>
</thead>
</table>

If you have any queries, please contact Jane Easton or Fiona Leashy on (03) 639 3744
Appendix 1. Questionnaire Forms
Life in NZ – Physical Activity

Patient Identifiers

0.1.1 Date of birth __________ [day] __________ [month] __________ [year]

0.2.1 Initials __________ __________

0.4.1 Assessment type (pick one only)
☐ Baseline
☐ 6 months

Assessment date: __________ [day] __________ [month] __________ [year]
Appendices

Appendix 1. Questionnaire Forms. Life in NZ - Physical Activity (Cont.)

<table>
<thead>
<tr>
<th>Patient Identifiers</th>
<th>Registration number:</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1.2 Date of birth</td>
<td>0.2.2 Initials</td>
</tr>
<tr>
<td>day</td>
<td>month</td>
</tr>
<tr>
<td>0.4.2 Assessment type (tick one only)</td>
<td>Assessment date:</td>
</tr>
<tr>
<td>0.6 months</td>
<td>0.5.2</td>
</tr>
</tbody>
</table>

Recent Physical Activity (in the last 4 weeks)
- all housework, job, walking and spare time activities

1. How many hours have you spent recently doing any mild or moderate physical activity (time spent “on the move”)
   1.1 on a normal SATURDAY? ________ hours each Saturday
   1.2 on a normal SUNDAY? ________ hours each Sunday
   1.3 on a normal WEEKDAY? ________ hours each day

   Yes  No

2. Have you been doing any vigorous or strenuous physical activity recently (breathing hard or puffing a lot)?
   - If No, please go to question 3
   - If Yes, how many minutes do you breathe hard or puff a lot in a normal week (weekend and weekdays altogether)?
     ________ minutes per week

Please check questions 1 and 2 and then tick this box: ☐
Appendix 1. Questionnaire Forms. Life in NZ - Physical Activity (Cont.)

Recent Housework and Job Activity

3.  Have you done any housework at home recently (not gardening or repairs)?
   If No, please go to question 4
   If Yes, how many hours have you spent recently “on the move” doing this housework?
   DON’T count time spent just standing or sitting!
   3.1 housework on a normal SATURDAY: ________ hours “on the move”
   3.2 housework on a normal SUNDAY: ________ hours “on the move”
   3.3 housework on a normal WEEKDAY ________ hours “on the move”

4.  Have you done any full-time, part-time or voluntary work recently involving physical work or labour? (NOT your own housework!)
   If No, please go to question 5
   If Yes, answer these THREE questions:
   4.1 On how many days a week did this work involve physical work or labour?
      (Please circle one) 1 2 3 4 5 6 7
   4.2 On a normal day, how long did you spend doing this physical work or labour?
      (Please circle one) 15 30 1 2 3-5 >6
      min min hr hr hr hr
      When you did this physical work or labour, was it (tick one circle):
      mild or light  moderate  vigorous or heavy

Please check questions 3 and 4 and then tick this box: ☐
Appendix 1. Questionnaire Forms. Life in NZ - Physical Activity (Cont.)

![Patient Identifiers](image)

### Other Activities (in the last 4 weeks)

5. **Do you sometimes walk to or from work for 5 minutes or more?**
   - If No, please go to question 6
   - If Yes, answer these TWO questions:

5.1 **On how many days a week do you usually walk to or from work?**

   *Please circle one*

   1 2 3 4 5 6 7

5.2 **How many minutes of walking is this altogether each day?**

   [ ] minutes of walking each day

6. **How often have you done these activities recently?** *(circle one number on each line)*

<table>
<thead>
<tr>
<th>Activity</th>
<th>not at all</th>
<th>only once</th>
<th>less than once a week</th>
<th>once a week</th>
<th>a few times a week</th>
<th>once or more a day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lawnmowing</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>Gardening (NOT lawnmowing)</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>Chopping firewood</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>House renovating, painting, repairs etc</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>Cleaning car or bike</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>Going for a walk</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>Running or jogging</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>Cycling (not on an ergocycle)</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>Fitness classes (aerobics, jazzercise etc)</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>Fitness exercises at home</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
</tr>
</tbody>
</table>

*Please check questions 5 and 6 and then tick this box:  

If you have any queries, please contact Jane Easton or Fiona Leahy on (03) 639-2744
Appendix 1. Questionnaire Forms. Life in NZ - Physical Activity (Cont.)

<table>
<thead>
<tr>
<th>Patient Identifiers</th>
<th>Registration number:</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1.5 Date of birth</td>
<td>1 9</td>
</tr>
<tr>
<td>0.2.5 Initials</td>
<td></td>
</tr>
<tr>
<td>0.4.5 Assessment type (tick one only)</td>
<td>Assessment date:</td>
</tr>
<tr>
<td>☐ Baseline</td>
<td>0 5 5</td>
</tr>
<tr>
<td>☐ 6 months</td>
<td></td>
</tr>
</tbody>
</table>

7. ☐ Yes  ☐ No  Have you done any other physical activities or sports recently (such as, netball, golf, fishing, boating, Frisbee, skiing trips, play with kids etc)?
   ☐ If No, please go to question 8
   ☐ If Yes, please write them here and circle how often:

<table>
<thead>
<tr>
<th></th>
<th>not at all</th>
<th>only once</th>
<th>less than once a week</th>
<th>once a week</th>
<th>a few times a week</th>
<th>once or more a day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
</tr>
</tbody>
</table>

8. On an average workday, how long would you spend on your feet, either standing or moving about?
   ________ hours

9. Has the amount of exercise you do changed greatly in the last 5 years? (tick one circle):
   ☐ no change  ☐ increased  ☐ decreased

10. On a usual day how many hours do you spend sitting and watching television or videos?
    weekday ________ hours
    weekend ________ hours
Appendices

Appendix 1. Questionnaire Forms. Short Form -36 Question Quality of Life.

<table>
<thead>
<tr>
<th>Patient Identifiers</th>
<th>Registration number</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1.1 Date of birth</td>
<td>0.3.1</td>
</tr>
<tr>
<td>0.2.1 Initials</td>
<td></td>
</tr>
<tr>
<td>0.4.1 Assessment type (pick one only)</td>
<td>Assessment date</td>
</tr>
<tr>
<td>Baseline</td>
<td>0.5.1</td>
</tr>
<tr>
<td>6 months</td>
<td>2.0</td>
</tr>
</tbody>
</table>

Q4: SF-36 Health Survey

INSTRUCTIONS

I. This questionnaire asks about your health and well-being.

II. Answer the questions by circling the appropriate number (1 2 3)

III. Certain questions may look alike but each one is different. Some questions ask about problems you may not have. That’s great, but it’s important for us to know. Please answer each question.

IV. There are no right or wrong answers. If you are unsure how to answer a question, please answer as best as you can.

V. Please remember to bring this questionnaire with you for your next visit.
Appendix 1. Questionnaire Forms. Short Form -36 Question Quality of Life. (Cont.)

<table>
<thead>
<tr>
<th>Patient Identifiers</th>
<th>Registration number: 0.3.2</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1.2 Date of birth</td>
<td>1.9</td>
</tr>
<tr>
<td>0.2.2 Initials</td>
<td></td>
</tr>
<tr>
<td>0.4.2 Assessment type (tick one only)</td>
<td>Assessment date: 0.5.2</td>
</tr>
<tr>
<td>• Baseline</td>
<td>2.0</td>
</tr>
<tr>
<td>• 6 months</td>
<td></td>
</tr>
</tbody>
</table>

1. In general, would you say your health is:

(circle one)
- Excellent
- Very good
- Good
- Fair
- Poor

2. Compared to one year ago, how would you rate your health in general now?

(circle one)
- Much better now than one year ago
- Somewhat better now than one year ago
- About the same as one year ago
- Somewhat worse now than one year ago
- Much worse now than one year ago
Appendices

Appendix 1. Questionnaire Forms. Short Form -36 Question Quality of Life. (Cont.)

### Patient Identifiers

<table>
<thead>
<tr>
<th>Field</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Date of birth</td>
<td>1.9</td>
</tr>
<tr>
<td>Initials</td>
<td></td>
</tr>
<tr>
<td>Assessment type</td>
<td>Baseline</td>
</tr>
<tr>
<td>Assessment date</td>
<td>0.53</td>
</tr>
</tbody>
</table>

3. The following questions are about activities you might do during a typical day. Does your health now limit you in these activities? If so, how much?

<table>
<thead>
<tr>
<th>ACTIVITIES</th>
<th>Yes, Limited A Lot</th>
<th>Yes, Limited A Little</th>
<th>No, Not Limited At All</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. Vigorous activities, such as running, lifting heavy objects, participating in strenuous sports</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>b. Moderate activities, such as moving a table, pushing a vacuum cleaner, bowling, or playing golf</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>c. Lifting or carrying groceries</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>d. Climbing several flights of stairs</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>e. Climbing one flight of stairs</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>f. Bending, kneeling or stooping</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>g. Walking more than one kilometre</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>h. Walking half a kilometre</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>i. Walking 100 metres</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>j. Bathing or dressing yourself</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
</tbody>
</table>

4. During the past 4 weeks, have you had any of the following problems with your work or other regular daily activities as a result of your physical health?

<table>
<thead>
<tr>
<th>Problem</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. Cut down on the amount of time you spent on work or other activities</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>b. Accomplished less than you would like</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>c. Were limited in the kind of work or other activities</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>d. Had difficulty performing the work or other activities (for example, it took extra effort)</td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>

If you have any queries, please contact Jane Elston or Fiona Leahy on (08) 833 3744
Appendix 1. Questionnaire Forms. Short Form -36 Question Quality of Life. (Cont.)

5. During the past 4 weeks, have you had any of the following problems with your work or other regular daily activities as a result of any emotional problems (such as feeling depressed or anxious)? (circle one number on each line)

<table>
<thead>
<tr>
<th></th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. Cut down on the amount of time you spent on work or other activities</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>b. Accomplished less than you would like</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>c. Didn't do work or other activities as carefully as usual</td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>

6. During the past 4 weeks, to what extent has your physical health or emotional problems interfered with your normal social activities with family, friends, neighbours, or groups?

(circle one)

Not at all ............................................. 1
Slightly ............................................. 2
Moderately ........................................... 3
Quite a bit .......................................... 4
Extremely ........................................... 5

7. How much bodily pain have you had during the past 4 weeks?

(circle one)

No bodily pain ..................................... 1
Very mild .......................................... 2
Mild .................................................. 3
Moderate .......................................... 4
Severe ............................................. 5
Very severe ....................................... 6
Appendices

Appendix 1. Questionnaire Forms. Short Form -36 Question Quality of Life. (Cont.)

8. During the past 4 weeks, how much did pain interfere with your normal work (including both work outside the home and housework)?

(circle one)

- No bodily pain
- Not at all
- A little bit
- Moderately
- Quite a bit
- Extremely

9. These questions are about how you feel and how things have been with you during the past 4 weeks. For each question, please give the one answer that comes closest to the way you have been feeling. How much of the time during the past 4 weeks -

(circle one number on each line)

<table>
<thead>
<tr>
<th></th>
<th>All of the Time</th>
<th>Most of the Time</th>
<th>A Good Bit of the Time</th>
<th>Some of the Time</th>
<th>A Little of the Time</th>
<th>None of the Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. Did you feel full of life?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>b. Have you been a very nervous person?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>c. Have you felt so down in the dumps that nothing could cheer you up?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>d. Have you felt calm and peaceful?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>e. Did you have a lot of energy?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>f. Have you felt down?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>g. Did you feel worn out?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>h. Have you been a happy person?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>i. Did you feel tired?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
</tr>
</tbody>
</table>
Appendix 1. Questionnaire Forms. Short Form -36 Question Quality of Life, (Cont.)

10. During the past 4 weeks, how much of the time has your physical health or emotional problems interfered with your social activities (like visiting with friends, relatives, etc.)?

(circle one)

No bodily pain............................................1
All of the time...........................................1
Most of the time.........................................2
Some of the time.........................................3
A little of the time.....................................4
None of the time.......................................5

11. How TRUE or FALSE is each of the following statements for you?

(circle one number on each line)

<table>
<thead>
<tr>
<th></th>
<th>Definitely True</th>
<th>Mostly True</th>
<th>Don’t Know</th>
<th>Mostly False</th>
<th>Definitely False</th>
</tr>
</thead>
<tbody>
<tr>
<td>a.</td>
<td>I seem to get sick a little easier than other people</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>b.</td>
<td>I am as healthy as anybody I know</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>c.</td>
<td>I expect my health to get worse</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>d.</td>
<td>My health is excellent</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
</tbody>
</table>
Appendices

Appendix 1. Questionnaire Forms. Obesity Specific Quality of Life.

**Patient Identifiers**

<table>
<thead>
<tr>
<th>Date of birth:</th>
<th>Initials:</th>
<th>Registration number:</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1.1 day</td>
<td>19 month</td>
<td>0.2.1 year</td>
</tr>
<tr>
<td>0.4.1 Assessment type (tick one only)</td>
<td>Assessment date:</td>
<td>0.5.1 day</td>
</tr>
<tr>
<td>Baseline</td>
<td></td>
<td>0.5.1 month</td>
</tr>
<tr>
<td>6 months</td>
<td></td>
<td>2.0 year</td>
</tr>
</tbody>
</table>

**Q3: OSQOL Health Survey - Instructions**

1. This questionnaire asks about your health and well-being.
2. Answer the questions by circling the appropriate number.
3. Some questions may be about problems that you may not have. That's great, but it's important for us to know. Please answer each question.
4. There are no right or wrong answers. If you are unsure how to answer a question, please answer as best you can.
5. Please remember to bring this questionnaire with you to your next visit.

How TRUE or FALSE is each of the following statements for you (circle one number on each line).

<table>
<thead>
<tr>
<th>Statement</th>
<th>Definitely false</th>
<th>Mostly false</th>
<th>Don't know</th>
<th>Mostly true</th>
<th>Definitely true</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. I have trouble squatting</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>b. I cannot sit down in a very low armchair</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>c. I walk as little as possible</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>d. I have to stop to catch my breath after walking several hundred metres</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>e. I have trouble climbing stairs</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>f. People say that I am not very athletic</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>g. People often say that I am not agile</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>h. I often lack energy</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>i. I don't move around very much</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>j. I feel I'm being attacked when people talk about my weight</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>k. I feel very ill at ease</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
</tbody>
</table>

*If you have any queries, please contact Jane Easton or Fiona Leahy on (09) 630 3744.*

271
Appendix 1. Questionnaire Forms. Eating Attitudes Test – 12 Question

Patient Identifiers

<table>
<thead>
<tr>
<th>Date of birth:</th>
<th>Initials:</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1.1 day</td>
<td>1</td>
</tr>
<tr>
<td>0.1.9 month</td>
<td>1</td>
</tr>
<tr>
<td>0.2.1 year</td>
<td></td>
</tr>
</tbody>
</table>

Registration number: 0.3.1

Assessment type (tick one only)

- Baseline
- 6 months

Assessment date: 0.5.1 day

Q5: EAT-12 Health Survey: Instructions

I. Please circle the number on each line which best applies to each of the statements

II. All the results will be strictly confidential

III. Most of the questions directly relate to food or eating although other types of questions have been included.

IV. Please remember to bring this questionnaire with you to your next visit.

How TRUE or FALSE is each of the following statements for you (circle one number on each line)?

<table>
<thead>
<tr>
<th></th>
<th>Always</th>
<th>Usually</th>
<th>Often</th>
<th>Sometimes</th>
<th>Rarely</th>
<th>Never</th>
</tr>
</thead>
<tbody>
<tr>
<td>a.</td>
<td>I am preoccupied with a desire to be thinner</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>b.</td>
<td>I engage in dieting behaviour</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>c.</td>
<td>I feel uncomfortable eating sweets</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>d.</td>
<td>I think about burning up calories when I exercise</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>e.</td>
<td>I vomit after I have eaten</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>f.</td>
<td>I have gone on eating binges where I feel that I might not be able to stop</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>g.</td>
<td>I give too much thought to food</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>h.</td>
<td>I feel that food controls my life</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>i.</td>
<td>I cut my food into small pieces</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>j.</td>
<td>I take longer than others to eat meals</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>k.</td>
<td>Other people think I am too thin</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>l.</td>
<td>I feel that others pressure me to eat</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
</tbody>
</table>

If you have any queries, please contact Jane Easton or Fiona Leahy on (09) 630 3744.
Appendix 2. Adverse Events Form

Form Includes Serious Adverse Event Grading and Relationship to Study Treatment

Form X: Adverse Event

Date of birth: 01/01/1991
Registration number: 03.1

Please complete a new form for each new event. Please complete every section.

1. ___ __ 2:00 Date of notification of adverse event

2. ___ __ 2:00 Date of onset (If unknown, put an asterisk (*) for part or all of the date)

3. ___ __ 2:00 Date of resolution (If ongoing, leave blank and update when known. If resolved but date unknown, put an asterisk (*) for part or all of the date)

Adverse event

4. Description of event. Please report a diagnosis if possible. Enter ONE diagnosis per line (or symptom if diagnosis not available)

4.1

4.2

4.3

4.4

Office use

5. Yes No

Serious adverse event (SAE) (see categories below)

If No, go to question 6

If Yes, which of the following SAE categories does this event fulfil (answer all questions)

Yes No

5.1

Death

5.2

Life-threatening (an event in which the participant was at risk of death at the time of the event; does not refer to an event which hypothetically might have caused death if it were more severe)

5.3

Required inpatient hospitalisation or prolongation of existing hospitalisation

5.4

Persistent or significant disability/incapacity

5.5

Cancer

5.6

Congenital anomaly/birth defect

5.7

Other important medical event (a medical event not immediately life-threatening or resulting in death or hospitalisation but that may jeopardise the participant or may require medical/surgical intervention to prevent one of the above SAEs)

If Yes, please specify

Office use

6. Maximum intensity at its worst (tick ONE only)

Mild (awareness of event but easily tolerated)

Moderate (discomfort enough to cause some interference with usual activity)

Severe (inability to carry out usual activity)
Form X: Adverse Event

Date of birth:
0.1.2 | 1 | 9 | day month year
Initials: 0.2.2

Registration number:
0.3.2

Treatment

7. Yes | No
   | Study treatment adjustment
   | If No, go to question 8
   | If Yes,
   | Yes | No
   | Dosage modified (reduced)
   | If Yes, what is the new total number of capsules being consumed each day?
   | 7.1
   | Yes | No
   | Treatment discontinued
   | If Yes, date of discontinuation
day month year
   | 7.2
   | 7.2.1

Additional Information

8. Yes | No
   | Was this event anticipated in terms of volunteer history, physical findings and risk factors?

9. In the investigators opinion, was the event causally related to the study treatment (tick ONE only)
   | Yes
   | Probably
   | Possibly
   | Unlikely
   | No

Signature of Investigator

10. Signature  Date  20
    printed name  month  year

Once this form is complete, send the original to the Clinical Trials Research Unit.

Keep copies in the participant's Case Record Folder.
Appendix 3. Evaluable Numbers and Normal Range for Laboratory Tests

<table>
<thead>
<tr>
<th>Laboratory test</th>
<th>Evaluable Number N</th>
<th>Normal range</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Haematology</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hb, g/L</td>
<td>236</td>
<td>212-236</td>
</tr>
<tr>
<td>RBC, x10^{12}/L</td>
<td>236</td>
<td>115-154, 130-175</td>
</tr>
<tr>
<td>Hct, %</td>
<td>236</td>
<td>3.9-5.2, 4.3-6.0</td>
</tr>
<tr>
<td>MCV, fl</td>
<td>236</td>
<td>0.36-0.46, 0.40-0.51</td>
</tr>
<tr>
<td>MCH, pg</td>
<td>236</td>
<td>115-154, 130-175</td>
</tr>
<tr>
<td>ESR, mm/h</td>
<td>236</td>
<td>0-20, 0-10</td>
</tr>
<tr>
<td>Platelets, x10^{12}/L</td>
<td>236</td>
<td>150-400</td>
</tr>
<tr>
<td>Leukocytes, x10^{9}/L</td>
<td>236</td>
<td>4.1-11.7, 4.1-11.2</td>
</tr>
<tr>
<td>Neutrophils, x10^{9}/L</td>
<td>214</td>
<td>1.9-7.9</td>
</tr>
<tr>
<td>Eosinophils, x10^{9}/L</td>
<td>212</td>
<td>0-0.2</td>
</tr>
<tr>
<td>Basophils, x10^{9}/L</td>
<td>166</td>
<td>0-0.5</td>
</tr>
<tr>
<td>Monocytes, x10^{9}/L/L</td>
<td>212</td>
<td>0.3-1.0, 0-0.9</td>
</tr>
<tr>
<td>Lymphocytes, x10^{9}/L/L</td>
<td>212</td>
<td>1.0-4.0</td>
</tr>
<tr>
<td><strong>Biochemistry</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TC, mmol/L</td>
<td>243</td>
<td>&lt; 5</td>
</tr>
<tr>
<td>LDL-C, mmol/L</td>
<td>244</td>
<td>&lt; 3</td>
</tr>
<tr>
<td>HDL-C, mmol/L</td>
<td>243</td>
<td>&lt; 1.39, m &lt; 1.0</td>
</tr>
<tr>
<td>TC/HDL</td>
<td>244</td>
<td>&lt; 4.5</td>
</tr>
<tr>
<td>TG, mmol/L</td>
<td>244</td>
<td>&lt; 2</td>
</tr>
<tr>
<td>FPG, mmol/L</td>
<td>236</td>
<td>&lt; 5.4</td>
</tr>
<tr>
<td>HbA1c, %</td>
<td>217</td>
<td>4.0-6.0</td>
</tr>
<tr>
<td>Urate, mmol/L</td>
<td>236</td>
<td>0.14-0.36, 0.20-0.42</td>
</tr>
<tr>
<td>AlkPhos, U/L</td>
<td>236</td>
<td>25-130 U/L</td>
</tr>
<tr>
<td>ALT, U/L</td>
<td>230</td>
<td>&lt; 40, m &lt; 45</td>
</tr>
<tr>
<td>AST, U/L</td>
<td>233</td>
<td>&lt; 40, m &lt; 45</td>
</tr>
<tr>
<td>γGT, U/L</td>
<td>236</td>
<td>&lt; 50, m &lt; 60</td>
</tr>
<tr>
<td>Bilirubin, μmol/L</td>
<td>236</td>
<td>&lt; 20, m &lt; 30</td>
</tr>
<tr>
<td>Albumin, g/L</td>
<td>236</td>
<td>35-45</td>
</tr>
<tr>
<td>Globulin, g/L</td>
<td>236</td>
<td>18-34</td>
</tr>
<tr>
<td>CRP, mg/L</td>
<td>236</td>
<td>high sensitive range, 0-3</td>
</tr>
<tr>
<td>Ferritin, μg/L</td>
<td>235</td>
<td>&lt; 40, m &lt; 45</td>
</tr>
<tr>
<td>VitD, nmol/L</td>
<td>243</td>
<td>&lt; 20, m &lt; 30</td>
</tr>
<tr>
<td>βCaro, μmol/L</td>
<td>238</td>
<td>&lt; 20, m &lt; 30</td>
</tr>
<tr>
<td>sVitA, μmol/L</td>
<td>239</td>
<td>&lt; 20, m &lt; 30</td>
</tr>
<tr>
<td>VitE, μg/L</td>
<td>239</td>
<td>&lt; 20, m &lt; 30</td>
</tr>
<tr>
<td>Adpn, μg/ml</td>
<td>242</td>
<td>5-50, (unpub data, our lab, mean±sd), w 8.8(3.2), m 4.3(3.5),</td>
</tr>
</tbody>
</table>
## Appendix 4. Expanded Tables

### Table A. Baseline Adpn_{250} vs. Study Parameters Correlations: All, Women & Men

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Baseline Adpn</th>
<th>All, N=212-243</th>
<th>Women, n=176-199</th>
<th>Men, n=39-40</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Demographics</strong></td>
<td></td>
<td>Pearson r</td>
<td>p-value</td>
<td>Pearson r</td>
</tr>
<tr>
<td>Gender: Female</td>
<td></td>
<td>-0.17</td>
<td>0.009</td>
<td>0.28</td>
</tr>
<tr>
<td>Age, y</td>
<td></td>
<td>0.23</td>
<td>&lt;0.001</td>
<td>0.24</td>
</tr>
<tr>
<td>European</td>
<td></td>
<td>0.25</td>
<td>&lt;0.001</td>
<td>-0.17</td>
</tr>
<tr>
<td>Maori/Pacific</td>
<td></td>
<td>-0.18</td>
<td>0.005</td>
<td>0.20</td>
</tr>
</tbody>
</table>

### Anthropometry

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Baseline Adpn</th>
<th>All, N=212-243</th>
<th>Women, n=176-199</th>
<th>Men, n=39-40</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcohol &gt;2 U/d</td>
<td>-0.04</td>
<td>0.52</td>
<td>-0.04</td>
<td>0.62</td>
</tr>
<tr>
<td>Alcohol U/d</td>
<td></td>
<td>0.10</td>
<td>0.02</td>
<td>0.83</td>
</tr>
<tr>
<td>Reg. Alcohol Drinker</td>
<td>-0.12</td>
<td>0.06</td>
<td>-0.16</td>
<td>0.021</td>
</tr>
<tr>
<td>Ever Smoked</td>
<td></td>
<td>0.01</td>
<td>0.91</td>
<td>0.98</td>
</tr>
<tr>
<td>Reg. Cig. Smoker</td>
<td>0.23</td>
<td>&lt;0.001</td>
<td>0.23</td>
<td>0.001</td>
</tr>
<tr>
<td>Edu. Level &lt;Secondary</td>
<td>-0.06</td>
<td>0.37</td>
<td>-0.05</td>
<td>0.50</td>
</tr>
<tr>
<td>Number Cigs/d</td>
<td>-0.13</td>
<td>0.04</td>
<td>-0.14</td>
<td>0.05</td>
</tr>
</tbody>
</table>

### Haematology

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Baseline Adpn</th>
<th>All, N=212-243</th>
<th>Women, n=176-199</th>
<th>Men, n=39-40</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight, kg</td>
<td>-0.30</td>
<td>&lt;0.001</td>
<td>-0.29</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Height, m</td>
<td>-0.32</td>
<td>&lt;0.001</td>
<td>-0.28</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BMI, kg/m^2</td>
<td>-0.15</td>
<td>0.02</td>
<td>-0.19</td>
<td>0.007</td>
</tr>
<tr>
<td>Waist, cm</td>
<td>-0.20</td>
<td>0.002</td>
<td>-0.17</td>
<td>0.02</td>
</tr>
<tr>
<td>%Fat, %</td>
<td>0.07</td>
<td>0.32</td>
<td>-0.08</td>
<td>0.29</td>
</tr>
<tr>
<td>SBP, mmHg</td>
<td>0.00</td>
<td>0.10</td>
<td>0.02</td>
<td>0.82</td>
</tr>
<tr>
<td>DBP, mmHg</td>
<td>0.02</td>
<td>0.71</td>
<td>0.07</td>
<td>0.32</td>
</tr>
</tbody>
</table>

### Biochemistry

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Baseline Adpn</th>
<th>All, N=212-243</th>
<th>Women, n=176-199</th>
<th>Men, n=39-40</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC, x 1012</td>
<td>-0.13</td>
<td>0.05</td>
<td>-0.06</td>
<td>0.38</td>
</tr>
<tr>
<td>Hb, g/L</td>
<td>-0.05</td>
<td>0.45</td>
<td>0.03</td>
<td>0.64</td>
</tr>
<tr>
<td>Hct, %</td>
<td>0.18</td>
<td>0.006</td>
<td>0.17</td>
<td>0.02</td>
</tr>
<tr>
<td>MCV, fl</td>
<td>0.02</td>
<td>0.79</td>
<td>0.03</td>
<td>0.68</td>
</tr>
<tr>
<td>MCHC, g/L</td>
<td>0.08</td>
<td>0.20</td>
<td>0.10</td>
<td>0.16</td>
</tr>
<tr>
<td>RBCW %</td>
<td>-0.07</td>
<td>0.31</td>
<td>-0.04</td>
<td>0.54</td>
</tr>
<tr>
<td>Leukocytes, x10^9</td>
<td>-0.16</td>
<td>0.02</td>
<td>-0.13</td>
<td>0.06</td>
</tr>
<tr>
<td>Neutrophils, x10^9</td>
<td>-0.19</td>
<td>0.007</td>
<td>-0.15</td>
<td>0.05</td>
</tr>
<tr>
<td>Eosinophils, x10^9</td>
<td>0.08</td>
<td>0.24</td>
<td>0.06</td>
<td>0.41</td>
</tr>
<tr>
<td>Basophils, x10^9</td>
<td>0.04</td>
<td>0.61</td>
<td>0.07</td>
<td>0.41</td>
</tr>
<tr>
<td>Monocytes, x10^9</td>
<td>0.03</td>
<td>0.67</td>
<td>0.10</td>
<td>0.17</td>
</tr>
<tr>
<td>Lymphocytes, x10^9</td>
<td>-0.07</td>
<td>0.32</td>
<td>-0.07</td>
<td>0.35</td>
</tr>
<tr>
<td>Platelets, x10^12</td>
<td>-0.13</td>
<td>0.05</td>
<td>-0.14</td>
<td>0.06</td>
</tr>
<tr>
<td>ESR, mm/hr</td>
<td>-0.13</td>
<td>0.04</td>
<td>-0.16</td>
<td>0.03</td>
</tr>
</tbody>
</table>

### TC, mmol/L

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Baseline Adpn</th>
<th>All, N=212-243</th>
<th>Women, n=176-199</th>
<th>Men, n=39-40</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC, mmol/L</td>
<td>0.23</td>
<td>&lt;0.001</td>
<td>0.25</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HDL-C, mmol/L</td>
<td>0.40</td>
<td>&lt;0.001</td>
<td>0.38</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LDL-C, mmol/L</td>
<td>0.13</td>
<td>0.05</td>
<td>0.12</td>
<td>0.08</td>
</tr>
<tr>
<td>TG, mmol/L</td>
<td>-0.12</td>
<td>0.07</td>
<td>-0.06</td>
<td>0.37</td>
</tr>
<tr>
<td>TC/HDL</td>
<td>-0.26</td>
<td>&lt;0.001</td>
<td>-0.23</td>
<td>0.001</td>
</tr>
<tr>
<td>FPG, mmol/l</td>
<td>-0.12</td>
<td>0.08</td>
<td>-0.12</td>
<td>0.11</td>
</tr>
<tr>
<td>HbA1c, %</td>
<td>-0.11</td>
<td>0.10</td>
<td>-0.12</td>
<td>0.12</td>
</tr>
<tr>
<td>Urate, mmol/L</td>
<td>-0.26</td>
<td>&lt;0.001</td>
<td>-0.19</td>
<td>0.007</td>
</tr>
<tr>
<td>Ferritin, ug/L</td>
<td>-0.06</td>
<td>0.36</td>
<td>0.03</td>
<td>0.73</td>
</tr>
<tr>
<td>Parameters</td>
<td>Baseline Adpn</td>
<td>All, N=212-243</td>
<td>Women, n=176-199</td>
<td>Men, n=39-40</td>
</tr>
<tr>
<td>----------------------------------------</td>
<td>---------------</td>
<td>----------------</td>
<td>------------------</td>
<td>--------------</td>
</tr>
<tr>
<td></td>
<td>Pearson r</td>
<td>p-value</td>
<td>Pearson r</td>
<td>p-value</td>
</tr>
<tr>
<td>Bilirubin, umol/L</td>
<td>0.11</td>
<td>0.10</td>
<td>0.10</td>
<td>0.19</td>
</tr>
<tr>
<td>AlkPhos, U/L</td>
<td>0.06</td>
<td>0.40</td>
<td>0.03</td>
<td>0.69</td>
</tr>
<tr>
<td>ALT, U/L</td>
<td>-0.08</td>
<td>0.24</td>
<td>-0.09</td>
<td>0.25</td>
</tr>
<tr>
<td>AST, U/L</td>
<td>-0.07</td>
<td>0.27</td>
<td>-0.06</td>
<td>0.38</td>
</tr>
<tr>
<td>ALT./AST</td>
<td>-0.05</td>
<td>0.42</td>
<td>-0.07</td>
<td>0.35</td>
</tr>
<tr>
<td>GGT, U/L</td>
<td>-0.12</td>
<td>0.07</td>
<td>-0.08</td>
<td>0.28</td>
</tr>
<tr>
<td>Tot Protein, g/L</td>
<td>-0.20</td>
<td>0.00</td>
<td>-0.19</td>
<td>0.009</td>
</tr>
<tr>
<td>Albumin, g/L</td>
<td>-0.06</td>
<td>0.34</td>
<td>-0.03</td>
<td>0.68</td>
</tr>
<tr>
<td>Globulin, g/L</td>
<td>-0.17</td>
<td>0.01</td>
<td>-0.19</td>
<td>0.009</td>
</tr>
<tr>
<td>hsCRP, mg/L</td>
<td>-0.15</td>
<td>0.02</td>
<td>-0.14</td>
<td>0.06</td>
</tr>
<tr>
<td>INR(VitK)</td>
<td>-0.04</td>
<td>0.60</td>
<td>-0.12</td>
<td>0.11</td>
</tr>
<tr>
<td>Insulin, μU/L</td>
<td>-0.25</td>
<td>&lt;0.001</td>
<td>-0.26</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HOMA-IR mmol/μU/L</td>
<td>-0.20</td>
<td>0.002</td>
<td>-0.24</td>
<td>0.001</td>
</tr>
<tr>
<td>Adpn mg/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leptinµg</td>
<td>0.04</td>
<td>0.50</td>
<td>-0.02</td>
<td>0.82</td>
</tr>
<tr>
<td>sFStVitamin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sVitD, ng/L</td>
<td>0.22</td>
<td>0.001</td>
<td>0.25</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>sβCaro, μg/L</td>
<td>0.30</td>
<td>&lt;0.001</td>
<td>0.28</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>sVitA, μg/L</td>
<td>0.06</td>
<td>0.33</td>
<td>0.14</td>
<td>0.05</td>
</tr>
<tr>
<td>sVitE, μg/L</td>
<td>0.13</td>
<td>0.05</td>
<td>0.18</td>
<td>0.01</td>
</tr>
<tr>
<td>INR (VitK)</td>
<td>-0.04</td>
<td>0.60</td>
<td>-0.12</td>
<td>0.11</td>
</tr>
<tr>
<td>Diet</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water, mL</td>
<td>0.02</td>
<td>0.73</td>
<td>-0.03</td>
<td>0.68</td>
</tr>
<tr>
<td>Energy, J/d</td>
<td>-0.03</td>
<td>0.69</td>
<td>0</td>
<td>0.97</td>
</tr>
<tr>
<td>Alcohol mL/d</td>
<td>0.07</td>
<td>0.28</td>
<td>0.13</td>
<td>0.08</td>
</tr>
<tr>
<td>CHO g/d</td>
<td>-0.01</td>
<td>0.87</td>
<td>0</td>
<td>0.99</td>
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<tr>
<td>Fibre g/d</td>
<td>0.08</td>
<td>0.23</td>
<td>0.12</td>
<td>0.08</td>
</tr>
<tr>
<td>Starch, g/d</td>
<td>-0.10</td>
<td>0.12</td>
<td>-0.04</td>
<td>0.54</td>
</tr>
<tr>
<td>Tot Sugars, g/d</td>
<td>0.08</td>
<td>0.21</td>
<td>0.05</td>
<td>0.48</td>
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<tr>
<td>Tot Sucrose, g/d</td>
<td>0.05</td>
<td>0.46</td>
<td>0.02</td>
<td>0.82</td>
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<tr>
<td>Glucose, g/d</td>
<td>0.10</td>
<td>0.13</td>
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<tr>
<td>Fructose, g/d</td>
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<tr>
<td>Protein, g/d</td>
<td>-0.08</td>
<td>0.19</td>
<td>-0.05</td>
<td>0.50</td>
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<tr>
<td>Tot Fat, g/d</td>
<td>-0.03</td>
<td>0.60</td>
<td>-0.02</td>
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</tr>
<tr>
<td>Sat Fat, g/d</td>
<td>-0.06</td>
<td>0.35</td>
<td>-0.05</td>
<td>0.48</td>
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<tr>
<td>Poly Fat, g/d</td>
<td>0.02</td>
<td>0.78</td>
<td>0.03</td>
<td>0.63</td>
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<td>Cholesterol, mg/d</td>
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<tr>
<td>fVitD, μg/d</td>
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<td>0.1</td>
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<tr>
<td>fβCaro, mg/d</td>
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<td>0.34</td>
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<tr>
<td>fVitA, mg/d</td>
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<tr>
<td>fVitE, mg/d</td>
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<tr>
<td>Tot fVitA Equivs., mg/d</td>
<td>-0.04</td>
<td>0.51</td>
<td>-0.06</td>
<td>0.39</td>
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<tr>
<td>Thiamin, mg/d</td>
<td>0.01</td>
<td>0.93</td>
<td>0.02</td>
<td>0.79</td>
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<td>VitC, mg/d</td>
<td>0.02</td>
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<tr>
<td>Riboflav, mg/d</td>
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<td>0.96</td>
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<td>Niacin, mg/d</td>
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<td>0.94</td>
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<tr>
<td>Niacin/Tryptophan</td>
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<td>-0.03</td>
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<td>Niacin Equivs.</td>
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<td>0.45</td>
<td>-0.01</td>
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<td>VitB6, mg/d</td>
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<td>0.09</td>
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<td>VitB12, ug/d</td>
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<td>0.30</td>
<td>-0.06</td>
<td>0.37</td>
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### Baseline Adpn Parameters

<table>
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<th>All, N=212-243</th>
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<th>Men, n=39-40</th>
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<tr>
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<td>Sodium, mg/d</td>
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<td>Potassium, mg/d</td>
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<tr>
<td>Magnesium, mg/d</td>
<td>0.08</td>
<td>0.21</td>
<td>0.09</td>
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<td>Calcium g/d</td>
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<td>Phosphorous mg/d</td>
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<td>Iron, mg</td>
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<td>Zinc, mg</td>
<td>-0.08</td>
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<td>-0.03</td>
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<tr>
<td>Manganese, mg</td>
<td>0.02</td>
<td>0.78</td>
<td>0.04</td>
</tr>
<tr>
<td>Copper, mg</td>
<td>0.00</td>
<td>0.94</td>
<td>-0.01</td>
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<tr>
<td>Selenium, mg</td>
<td>0.06</td>
<td>0.37</td>
<td>0.09</td>
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</table>

**Metabolic Syndrome**

<table>
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<tr>
<th></th>
<th>IDF/JIS MetSMct</th>
<th>NCEP/ADA MetSMct</th>
<th>NCEP MetSMct</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>-0.25 &lt;0.001</td>
<td>-0.22 0.001</td>
<td>-0.23 0.001</td>
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</tbody>
</table>

y year; U unit; d day; reg regular; Cig cigarette; Edu education; weight body weight; BMI body mass index; waist waist circumference; %Fat body fat percentage; SBP systolic blood pressure; DBP diastolic blood pressure; RBC red blood cell; Hb haemoglobin; Hct haematocrit; MCV mean cell volume; MCHC mean cell haemoglobin concentration; RBCW red blood cell width; ESR erythrocytes sedimentation rate; TC total cholesterol; HDL-C high density lipoprotein cholesterol; LDL-C low density lipoprotein cholesterol; TG triglyceride; TC/HDL total cholesterol / high density lipoprotein; FPG fasting plasma glucose; HbA1c haemoglobin A1c; AlkPhos alkaline phosphatase; ALT alanine transferase; AST aspartate transferase; γGT Gamma glutamyl transferase; tot total; hsCRP high sensitive C-reactive protein; INR(VitK) international normalised ration (vitamin K); HOMA-IR homeostasis model analysis-insulin resistance; Adpn adiponectin; VitD vitamin D; βCaro beta carotene, VitA vitamin A; VitE vitamin E; CHO carbohydrate; sat saturated; poly polyunsaturated; VitC vitamin C; riboflavin riboflavin; VitB6 Vitamin B6; VitB12 vitamin B12; g gram; J joule; IDF International Diabetes Federation; JIS Joint interim statement; MetSMct metabolic syndrome marker count; NCEP National Cholesterol Education Panel; ADA American Diabetes Association.
Appendix 4. Table B. Change in HMW$_{30}$ vs. Study Parameters, Correlations: All, Women & Men

<table>
<thead>
<tr>
<th>Change in HMW</th>
<th>All, Change in HMW n=13-20</th>
<th>Women, Change in HMW n=9-14</th>
<th>Men, Change in HMW n=5-6</th>
</tr>
</thead>
<tbody>
<tr>
<td>δ Parameter</td>
<td>Pearson r   p value</td>
<td>Pearson r   p value</td>
<td>Pearson r   p value</td>
</tr>
</tbody>
</table>

**Anthropometry**
- δ Weight, kg
  -0.58 0.007
- δ BMI, kg/m$^2$
  -0.59 0.006
- δ Waist, cm
  -0.40 0.08
- δ %Fat, %
  -0.41 0.10
- δ SBP mmHg
  -0.29 0.21
- δ DBP mmHg
  -0.14 0.55

**Haematology**
- δ RBC, x 10$^{11}$
  -0.15 0.55
- δ Hb g/L
  -0.13 0.60
- δ Hct, %
  -0.32 0.18
- δ MCV, fl
  -0.41 0.09
- δ MCHC, L/L
  -0.03 0.89
- δ RBCW%
  -0.20 0.42
- δ Leukocytes, x10$^9$
  -0.24 0.32
- δ Neutrophil, x10$^9$
  -0.18 0.46
- δ Eosinophils, x10$^9$
  0.06 0.80
- δ Basophils, x108
  -0.35 0.26
- δ Monocytes, x108
  -0.45 0.06
- δ Lymphocytes, x108
  -0.39 0.11
- δ Platelets, x1011
  -0.19 0.43
- δ ESR, mm/hr
  0.37 0.12

**Biochemistry**
- δ TC, mmol/L
  -0.13 0.58
- δ HDL-C, mmol/L
  0.23 0.32
- δ LDL-C, mmol/L
  0.06 0.79
- δ TG, mmol/L
  -0.24 0.31
- δ TC/HDL
  -0.47 0.04
- δ FPG, mmol/l
  -0.23 0.34
- δ HbA$_{1c}$, %
  -0.58 0.03
- δ Urate, mmol/L
  -0.18 0.46
- δ Ferritin, µg/L
  -0.13 0.60
- δ Bilirubin, umol/L
  0.68 0.003
- δ AlkPhos U/L
  0.14 0.57
- δ ALT U/L
  0.11 0.66
- δ AST U/L
  -0.18 0.50
- δ ALAT/AST
  0.15 0.58
- δ γGT U/T
  -0.26 0.31
- δ Tot Protein g/L
  -0.14 0.57
- δ Albumin g/L
  -0.08 0.74
- δ Globulin, g/L
  -0.16 0.54
- δ hsCRP, mg/L
  -0.15 0.55

**Hormones/Cytokines**
- δ Insulin
  -0.02 0.94
<table>
<thead>
<tr>
<th>Parameter</th>
<th>All Change in HMW n=13-20</th>
<th>Women Change in HMW n=9-14</th>
<th>Men Change in HMW n=5-6</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pearson r</td>
<td>p value</td>
<td>Pearson r</td>
</tr>
<tr>
<td>HOMA-IR mmol/L·miU/L</td>
<td>-0.03</td>
<td>0.92</td>
<td>-0.09</td>
</tr>
<tr>
<td>δ Leptin, µg/ml</td>
<td>-0.47</td>
<td>0.04</td>
<td>-0.45</td>
</tr>
<tr>
<td>δ Adipin, mg/L</td>
<td>0.92</td>
<td>&lt; 0.001</td>
<td>0.91</td>
</tr>
<tr>
<td>δ HMW10, mg/L</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>δ MMW10, mg/L</td>
<td>0.25</td>
<td>0.30</td>
<td>0.23</td>
</tr>
<tr>
<td>δ LMW30, mg/L</td>
<td>0.72</td>
<td>&lt; 0.001</td>
<td>0.69</td>
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</table>

**Serum FSVitamins**

| δ VitD, ng/L               | 0.10      | 0.66    | 0.33       | 0.25     | -0.77      | 0.08     |                      |
| δ βCaro, µg/L              | -0.04     | 0.88    | 0.06       | 0.84     | -0.69      | 0.13     |                      |
| δ sVitA, µg/L              | 0.24      | 0.33    | 0.10       | 0.74     | 0.70       | 0.12     |                      |
| δ sVitE, µg/L              | -0.09     | 0.72    | -0.03      | 0.91     | -0.54      | 0.27     |                      |
| δ INR(VitK)                | -0.57     | 0.01    | -0.68      | 0.01     | 0.45       | 0.37     |                      |

**Metabolic Syndrome**

| δ NCEP MetS Mct           | -0.16     | 0.51    | -0.09      | 0.76     | -0.52      | 0.30     |                      |
| δ IDF/JIS MetS Mct         | -0.15     | 0.52    | -0.03      | 0.92     | -0.80      | 0.06     |                      |

**Dietary 24Hr Food Recall Nutrients**

| δ Energy J/day             | 0.35      | 0.14    | 0.30       | 0.33     | 0.63       | 0.18     |                      |
| δ Alcohol                  | 0.05      | 0.93    |            |          |            |          |                      |
| δ Protein g/day            | 0.34      | 0.16    | 0.25       | 0.42     | 0.63       | 0.18     |                      |
| δ Tot Fat, g/day           | 0.34      | 0.16    | 0.25       | 0.42     | 0.53       | 0.28     |                      |
| δ Sat Fat, g/day           | 0.11      | 0.66    | 0.04       | 0.91     | 0.48       | 0.34     |                      |
| δ Mono Fat, g/day          | 0.44      | 0.06    | 0.44       | 0.14     | 0.39       | 0.44     |                      |
| δ Poly Fat, g/day          | 0.27      | 0.27    | 0.27       | 0.37     | 0.11       | 0.84     |                      |
| δ Cholesterol, g/d         | 0.20      | 0.41    | -0.01      | 0.98     | 0.60       | 0.21     |                      |
| δ CHO g/day                | 0.11      | 0.66    | 0.17       | 0.59     | -0.20      | 0.71     |                      |
| δ Fibre g/day              | 0.20      | 0.41    | 0.30       | 0.33     | -0.28      | 0.59     |                      |
| δ Starch, g/day            | -0.11     | 0.65    | -0.09      | 0.76     | -0.52      | 0.30     |                      |
| δ Tot Sugars g/day         | 0.14      | 0.58    | 0.27       | 0.37     | -0.19      | 0.72     |                      |
| δ Tot Sucrose g/day        | 0.33      | 0.17    | 0.41       | 0.17     | 0.56       | 0.25     |                      |
| δ Glucose g/day            | -0.05     | 0.84    | 0.18       | 0.56     | -0.51      | 0.30     |                      |
| δ Fructose g/day           | -0.05     | 0.84    | 0.14       | 0.66     | -0.41      | 0.42     |                      |
| δ VitD, µg/d               | -0.17     | 0.50    | -0.24      | 0.42     | 0.04       | 0.95     |                      |
| δ βCaro, µg/d              | -0.03     | 0.92    | 0.00       | 0.99     | -0.16      | 0.80     |                      |
| δ Retinol Equivs., µg/d    | 0.09      | 0.71    | 0.10       | 0.75     | 0.34       | 0.51     |                      |
| δ VitE                     | -0.11     | 0.67    | -0.20      | 0.51     | 0.14       | 0.79     |                      |
| δ VitA, µg/d               | 0.00      | 0.10    | 0.02       | 0.94     | 0.05       | 0.93     |                      |
| δ Thiamine, µg/d           | -0.19     | 0.45    | -0.11      | 0.71     | -0.82      | 0.05     |                      |
| δ VitC                     | 0.19      | 0.42    | 0.23       | 0.45     | -0.24      | 0.65     |                      |
| δ Riboflavin, µg/d         | 0.29      | 0.23    | 0.31       | 0.31     | 0.08       | 0.89     |                      |
| δ Niacin, µg/d             | 0.25      | 0.29    | 0.54       | 0.06     | -0.75      | 0.09     |                      |
| δ Niacin Tryptophan, µg/d  | 0.18      | 0.47    | 0.08       | 0.80     | 0.40       | 0.44     |                      |
| δ Niacin Equivs., µg/d     | 0.42      | 0.08    | 0.52       | 0.07     | -0.49      | 0.33     |                      |
| δ VitB6, µg/d              | 0.23      | 0.34    | 0.38       | 0.20     | -0.07      | 0.90     |                      |
| δ VitB12, µg/d             | 0.10      | 0.68    | 0.06       | 0.85     | 0.14       | 0.79     |                      |
| δ Tot Folate mg/d          | 0.09      | 0.71    | 0.10       | 0.75     | 0.34       | 0.51     |                      |
| δ Sodium g/d               | 0.06      | 0.82    | 0.02       | 0.95     | -0.29      | 0.58     |                      |
| δ Potassium, g/d           | 0.22      | 0.36    | 0.29       | 0.34     | -0.08      | 0.88     |                      |
| δ Magnesium, mg/d          | 0.35      | 0.14    | 0.50       | 0.08     | -0.18      | 0.73     |                      |
| δ Calcium, mg/d            | 0.14      | 0.57    | 0.18       | 0.55     | -0.04      | 0.93     |                      |
| δ Phosphorus, mg/d         | 0.42      | 0.08    | 0.38       | 0.20     | 0.60       | 0.21     |                      |
## Appendices

<table>
<thead>
<tr>
<th>Change in HMW</th>
<th>All, Change in HMW n=13-20</th>
<th>Women, Change in HMW n=9-14</th>
<th>Men, Change in HMW n=5-6</th>
</tr>
</thead>
<tbody>
<tr>
<td>δ Parameter</td>
<td>Pearson r</td>
<td>p value</td>
<td>Pearson r</td>
</tr>
<tr>
<td>δ Iron, mg/dy</td>
<td>-0.01</td>
<td>0.98</td>
<td>0.32</td>
</tr>
<tr>
<td>δ Zinc, mg/dy</td>
<td>0.36</td>
<td>0.14</td>
<td>0.54</td>
</tr>
<tr>
<td>δ Manganese, mg/dy</td>
<td>0.23</td>
<td>0.35</td>
<td>0.48</td>
</tr>
<tr>
<td>δ Copper, mg/dy</td>
<td>0.33</td>
<td>0.16</td>
<td>0.27</td>
</tr>
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<td>δ Selenium, mg/dy</td>
<td>0.13</td>
<td>0.60</td>
<td>-0.08</td>
</tr>
</tbody>
</table>

weight body weight; BMI body mass index; waist waist circumference; %Fat body fat percentage; S/DBP systolic/diastolic blood pressure; DBP diastolic blood pressure; RBC red blood cell; MCV mean cell volume; Hb haemoglobin; Hct haematocrit; MCV mean cell volume; MCHC mean cell haemoglobin concentration; RBCW red blood cell width; ESR erythrocytes sedimentation rate; TC total cholesterol; HDL-C high density lipoprotein cholesterol; LDL-C low density lipoprotein cholesterol; TG triglyceride; TC/HDL total cholesterol / high density lipoprotein; FPG fasting plasma glucose; HbA1c haemoglobin A1c; AlkPhos alkaline phosphatase; ALT alanine transferase; AST aspartate transferase; γGT Gamma glutamyl transferase; Alp total; hsCRP high sensitive C-reactive protein; HOMA-IR homeostasis model analysis-insulin resistance; Adpn adiponectin; HMW high molecular weight adiponectin substudy; MMW medium molecular weight; LMW low molecular weight; VitD vitamin D; βCaro beta carotene; f food/dietary; s serum; VitA vitamin A; VitE vitamin E; INR(VitK) international normalised ration (vitamin K); NCEP National Cholesterol Education Panel; MetSMCt metabolic syndrome marker count; IDF International Diabetes Federation; JIS Joint interim statement; sat saturated; mono monounsaturated; poly polyunsaturated; CHO carbohydrate; equivs equivalence;
### Appendix 4. Table C. Baseline Fat Soluble Vitamin Correlations

<table>
<thead>
<tr>
<th>Parameters</th>
<th>sFS Vitamins</th>
<th>sVitD</th>
<th>p-value</th>
<th>sβCaro</th>
<th>p-value</th>
<th>sVitA</th>
<th>p-value</th>
<th>sVitE</th>
<th>p-value</th>
</tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight, kg</td>
<td>-0.22</td>
<td>0.001</td>
<td>-0.36</td>
<td>&lt; 0.001</td>
<td>-0.11</td>
<td>0.08</td>
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<tr>
<td>Height, m</td>
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<td>&lt; 0.001</td>
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<td>-0.18</td>
<td>0.006</td>
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</tr>
<tr>
<td>BMI, kg/m²</td>
<td>-0.20</td>
<td>0.00</td>
<td>-0.29</td>
<td>&lt; 0.001</td>
<td>-0.24</td>
<td>&lt; 0.001</td>
<td>-0.13</td>
<td>0.05</td>
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</tr>
<tr>
<td>Waist, cm</td>
<td>-0.14</td>
<td>0.03</td>
<td>-0.36</td>
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<td>0.62</td>
<td>0.08</td>
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<td></td>
</tr>
<tr>
<td>Fat %</td>
<td>-0.09</td>
<td>0.27</td>
<td>-0.22</td>
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<td>0.33</td>
<td>-0.08</td>
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</tr>
<tr>
<td>SBP, mmHg</td>
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<td>0.14</td>
<td>-0.09</td>
<td>0.16</td>
<td>0.22</td>
<td>0.001</td>
<td>0.30</td>
<td>&lt; 0.001</td>
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<tr>
<td>DBP, mmHg</td>
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<td>0.66</td>
<td>-0.08</td>
<td>0.22</td>
<td>0.19</td>
<td>0.003</td>
<td>0.25</td>
<td>&lt; 0.001</td>
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</tr>
<tr>
<td><strong>Haematology</strong></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RBC, x 10¹²</td>
<td>-0.07</td>
<td>0.31</td>
<td>-0.06</td>
<td>0.37</td>
<td>0.10</td>
<td>0.14</td>
<td>0.05</td>
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<td>0.58</td>
<td>-0.01</td>
<td>0.85</td>
<td>0.29</td>
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<td>0.18</td>
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<td>&lt; 0.001</td>
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<td>0.79</td>
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<td>MCHC, L/L</td>
<td>0.12</td>
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<td>0.27</td>
<td>0.27</td>
<td>&lt; 0.001</td>
<td>0.15</td>
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<td>RBCW %</td>
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<td>Basophils, x10⁹</td>
<td>(n=164-166)</td>
<td>0.20</td>
<td>0.009</td>
<td>-0.11</td>
<td>0.16</td>
<td>0.23</td>
<td>0.00</td>
<td>0.11</td>
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<tr>
<td>Monocytes, x10⁹</td>
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<td>-0.08</td>
<td>0.23</td>
<td>0.09</td>
<td>0.17</td>
<td>0.08</td>
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<tr>
<td>Lymphocytes, x10⁹</td>
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<td>Platelets, x10¹²</td>
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<td>0.49</td>
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<tr>
<td>ESR, mm/hr</td>
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<td>0.58</td>
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<td>0.96</td>
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<tr>
<td>TC, mmol/L</td>
<td>0.09</td>
<td>0.14</td>
<td>0.26</td>
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<td>&lt; 0.001</td>
<td>0.62</td>
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<td>HDL-C, mmol/L</td>
<td>0.08</td>
<td>0.21</td>
<td>0.28</td>
<td>&lt; 0.001</td>
<td>0.10</td>
<td>0.13</td>
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<tr>
<td>LDL-C, mmol/L</td>
<td>0.06</td>
<td>0.32</td>
<td>0.23</td>
<td>&lt; 0.001</td>
<td>0.14</td>
<td>0.04</td>
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<td>TG, mmol/L</td>
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<td>0.90</td>
<td>-0.07</td>
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<td>0.39</td>
<td>&lt; 0.001</td>
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<td>TC/HDL</td>
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<td>0.96</td>
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<td>0.11</td>
<td>0.10</td>
<td>0.30</td>
<td>&lt; 0.001</td>
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<td>FPG, mmol/l</td>
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<td>-0.18</td>
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<td>HbA₁c, %</td>
<td>-0.17</td>
<td>0.01</td>
<td>-0.19</td>
<td>0.006</td>
<td>0.01</td>
<td>0.88</td>
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<td>Urate, mmol/L</td>
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<td>0.79</td>
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<td>0.33</td>
<td>&lt; 0.001</td>
<td>0.19</td>
<td>0.004</td>
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<td>Ferritin, µg/L</td>
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<td>0.27</td>
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<td>0.84</td>
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<td>&lt; 0.001</td>
<td>0.29</td>
<td>&lt; 0.001</td>
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<td>0.05</td>
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<td>AlkPhos U/L</td>
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<td>-0.09</td>
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<td>ALT/AST</td>
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<td>-0.08</td>
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<td>0.59</td>
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<td>0.22</td>
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<tr>
<td>Tot Protein g/L</td>
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<td>0.63</td>
<td>-0.14</td>
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<td>sVitD</td>
<td>p-value</td>
<td>r</td>
<td>p-value</td>
<td>βCaro</td>
<td>VitA</td>
<td>sVitA</td>
<td>sVitE</td>
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<td>---------</td>
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<td>Albumin g/L</td>
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<td>0.18</td>
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<td>Globulin, g/L</td>
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<td>-0.15</td>
<td>0.02</td>
<td>-0.19</td>
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<td>hsCRP, mg/L</td>
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<td>0.30</td>
<td>-0.28</td>
<td>&lt; 0.001</td>
<td>-0.03</td>
<td>0.63</td>
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<tr>
<td>IDF/JIS MetSct</td>
<td>-0.10</td>
<td>0.11</td>
<td>-0.23</td>
<td>0.001</td>
<td>0.16</td>
<td>0.01</td>
<td>0.24</td>
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<tr>
<td>IDF/JIS MetS</td>
<td>-0.04</td>
<td>0.58</td>
<td>-0.16</td>
<td>0.01</td>
<td>0.19</td>
<td>0.004</td>
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<td>NCEP/ADA MetSct</td>
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<td>0.22</td>
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<td>0.25</td>
<td>&lt; 0.001</td>
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<tr>
<td>NCEP/ADA MetS</td>
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<td>0.36</td>
<td>-0.18</td>
<td>0.006</td>
<td>0.24</td>
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<td>0.21</td>
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<td>-0.22</td>
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<td>0.27</td>
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<td>0.25</td>
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<td>0.004</td>
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<tr>
<td>Fat Soluble Vitamins</td>
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<tr>
<td>VitD, ng/L</td>
<td>-</td>
<td>0.15</td>
<td>0.02</td>
<td>0.33</td>
<td>&lt; 0.001</td>
<td>0.06</td>
<td>0.36</td>
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<tr>
<td>β-Caro, µg/L</td>
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<td>0.02</td>
<td>-</td>
<td>0.12</td>
<td>0.08</td>
<td>0.16</td>
<td>0.02</td>
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<td>VitA, µg/L</td>
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<td>&lt; 0.001</td>
<td>0.12</td>
<td>0.08</td>
<td>-</td>
<td>0.46</td>
<td>&lt; 0.001</td>
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<tr>
<td>VitE, µg/L</td>
<td>0.06</td>
<td>0.36</td>
<td>0.16</td>
<td>0.02</td>
<td>0.46</td>
<td>&lt; 0.001</td>
<td>-</td>
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<tr>
<td>Hormones/Cytokines</td>
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<tr>
<td>Insulin, uU/L</td>
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<td>0.15</td>
<td>-0.22</td>
<td>0.001</td>
<td>0.03</td>
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<tr>
<td>HOMA mmol/uU/L</td>
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<tr>
<td>Adpn mg/L</td>
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<td>0.001</td>
<td>0.30</td>
<td>&lt; 0.001</td>
<td>0.06</td>
<td>0.33</td>
<td>0.13</td>
<td>0.05</td>
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<td>Adpn_mg/L</td>
<td>0.23</td>
<td>0.23</td>
<td>0.21</td>
<td>0.22</td>
<td>-0.25</td>
<td>0.19</td>
<td>0.17</td>
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<tr>
<td>HMW_mg/L</td>
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<td>0.35</td>
<td>0.18</td>
<td>0.29</td>
<td>-0.21</td>
<td>0.27</td>
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<tr>
<td>MMW_mg/L</td>
<td>0.27</td>
<td>0.14</td>
<td>0.20</td>
<td>0.25</td>
<td>-0.21</td>
<td>0.27</td>
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<td>0.99</td>
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<tr>
<td>LMW_mg/L</td>
<td>0.20</td>
<td>0.28</td>
<td>0.22</td>
<td>0.21</td>
<td>-0.34</td>
<td>0.07</td>
<td>0.16</td>
<td>0.41</td>
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<tr>
<td>%HMW_mg/L</td>
<td>-0.01</td>
<td>0.97</td>
<td>0.14</td>
<td>0.43</td>
<td>0.12</td>
<td>0.53</td>
<td>0.34</td>
<td>0.07</td>
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<tr>
<td>%MMW_mg/L</td>
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<td>0.86</td>
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<tr>
<td>%LMW_mg/L</td>
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<td>0.60</td>
<td>0.08</td>
<td>0.66</td>
<td>-0.30</td>
<td>0.12</td>
<td>-0.16</td>
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<td>0.31</td>
<td>-0.20</td>
<td>0.002</td>
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<td>24Hr Food Recall Nutrients</td>
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<tr>
<td>Food Weight, g</td>
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<td>0.33</td>
<td>0.09</td>
<td>0.18</td>
<td>0.04</td>
<td>0.55</td>
<td>0.11</td>
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<tr>
<td>Water, ml/d</td>
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<td>0.36</td>
<td>0.08</td>
<td>0.23</td>
<td>0.05</td>
<td>0.47</td>
<td>0.13</td>
<td>0.08</td>
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<tr>
<td>Energy J/d</td>
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<td>0.55</td>
<td>0.06</td>
<td>0.32</td>
<td>0.01</td>
<td>0.91</td>
<td>-0.02</td>
<td>0.74</td>
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</tr>
<tr>
<td>Alcohol ml/d</td>
<td>0.09</td>
<td>0.16</td>
<td>0.05</td>
<td>0.47</td>
<td>0.09</td>
<td>0.23</td>
<td>0.12</td>
<td>0.10</td>
<td></td>
</tr>
<tr>
<td>Protein g/d</td>
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<td>0.39</td>
<td>0.01</td>
<td>0.85</td>
<td>-0.05</td>
<td>0.48</td>
<td>-0.02</td>
<td>0.74</td>
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</tr>
<tr>
<td>Tot Fat, g/d</td>
<td>0.03</td>
<td>0.60</td>
<td>0.01</td>
<td>0.83</td>
<td>0.01</td>
<td>0.91</td>
<td>-0.01</td>
<td>0.92</td>
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<tr>
<td>Sat Fat, g/d</td>
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<td>0.51</td>
<td>0.01</td>
<td>0.88</td>
<td>0.03</td>
<td>0.68</td>
<td>0.00</td>
<td>0.96</td>
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<tr>
<td>Mono Fat g/d</td>
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<td>0.56</td>
<td>-0.01</td>
<td>0.87</td>
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<tr>
<td>Poly Fat, g/d</td>
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<td>0.50</td>
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</table>

Weight body weight; BMI body mass index; waist waist circumference; %Fat body fat percentage; SBP systolic blood pressure; DBP diastolic blood pressure; RBC red blood cell; Hb haemoglobin; Hct haematocrit; MCV mean cell volume; MCHC mean cell haemoglobin concentration; RBCW red blood cell width; ESR erythrocytes sedimentation rate; TC total cholesterol; HDL-C high density lipoprotein cholesterol; LDL-C low density lipoprotein cholesterol; TG triglyceride; TC/HDL total cholesterol / high density lipoprotein cholesterol; FPG fasting plasma glucose; HbA1c haemoglobin A1c; AlkPhos alkaline phosphatase; ALT alanine transferase; AST aspartate transferase; tot total; d day; g grams; s serum; VitD vitamin D; VitE vitamin E; βCaro beta carotene; VitA vitamin A; equivs equivalents; VitC vitamin C; tryptophan; VitB6 Vitamin B6; VitB12 vitamin B12.
### Study Parameters

<table>
<thead>
<tr>
<th>Change in sβCaro &amp; sVitE</th>
<th>Δ sβCaro</th>
<th>Δ sVitE</th>
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<td><strong>Parameter</strong></td>
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<td>Women, n=120-125</td>
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<td>p-value</td>
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<td>Δ Waist, cm</td>
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<td>Δ DBP mmHg</td>
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<td>Δ MCH, pg/L</td>
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<td>Δ Neutrophil, x10⁹</td>
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<td>Δ Eosinophils, x10⁹</td>
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<td>Δ Monocytes, x10⁹</td>
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<td>Δ Lymphocytes, x10⁹</td>
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<td>Δ Platelets, x10¹²</td>
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<td>Δ Albumin g/L</td>
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### Appendices

#### Change in sβCaro & sVitE

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<th>Parameter</th>
<th>δ sβCaro All, n=145-155</th>
<th>δ sβCaro Women, n=120-125</th>
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#### Metabolic Syndrome

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<th>δ NCEP MetS</th>
<th>δ IDF/JIS MetSMCt</th>
<th>δ IDF MetS</th>
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#### Serum Fat Soluble Vitamins

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<th>δ sβ-Carot, µg/L</th>
<th>δ sVitA, µg/L</th>
<th>δ sVitE, µg/L</th>
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#### Hormones/Cytokines

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<th>δ Adpn30, mg/L</th>
<th>δ HMW30, mg/L</th>
<th>δ MMW30, mg/L</th>
<th>δ LMW30, mg/L</th>
<th>δ %HMW30</th>
<th>δ %HMW30</th>
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#### 24Hr Food Recall Nutrients

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<tr>
<td>δ Selenium, mg/d</td>
<td>-0.08</td>
<td>0.35</td>
<td>-0.06</td>
<td>0.48</td>
<td>-0.05</td>
<td>0.58</td>
<td>0.18</td>
<td>0.35</td>
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δ change; weight body weight; BMI body mass index; waist waist circumference; %fat body fat percentage; SBP systolic blood pressure; DBP diastolic blood pressure; RBC red blood cell; Hb haemoglobin; Hct haematocrit; MCV mean cell volume; MCH mean cell haemoglobin; RBC W red blood cell width; ESR erythrocytes sedimentation rate; TC total cholesterol; HDL-C high density lipoprotein cholesterol; LDL low density lipoprotein-cholesterol; TG triglyceride; TC/HDL total cholesterol/high density lipoprotein; FPG fasting plasma glucose; HbA1c haemoglobin A1c; AlkPhos alkaline phosphatase; U unit; ALT alanine transferase; AST aspartate; ALT/AST alanine transferase/aspartate; γGT Gamma glutamyl transferase; tot total; hsCRP high sensitive C-reactive protein; NCEP National Cholesterol Education Programme; MetSMct metabolic syndrome marker count; Mets metabolic syndrome; IDF International Diabetes Federation; JIS Joint interim statement; s serum; VitD vitamin D; βCaro beta carotene; VitA vitamin A; VitE vitamin E; INR(VitK) internationalised normalised ratio vitamin K; Adpn adiponectin; HMW high molecular weight; MMW medium molecular weight; LMW low molecular weight; %HMW percentage high molecular weight; tot total; sat saturated; mono monounsaturated; poly polysaturated; C cholesterol; CHO carbohydrate; equivs equivalents; VitC vitamin C; trypto tryptophan; VitB6 vitamin B6; VitB12
REFERENCES


References


References


64. WHO/IASO/IOTF. The Asia-Pacific perspective: redefining obesity and its treatment. in Health Communications Australia (Melbourne, 2000).


References


References


217. Clifton, P.M., Keogh, J.B., Noakes, M., Clifton, P.M., Keogh, J.B. & Noakes, M. Trans fatty acids in adipose tissue and the food supply are associated with myocardial
References


References


255. Dalwoo. Chitosan Structure with Chitin and Cellulose (Figure). (http://dalwoo.com/chitosan/structure.htm, 2002).


283. European Food Safety Authority. Health claims related to various food(s)/food constituent(s) with antioxidant properties. *EFSA J* **8**, 1752 (2010).


References


References


References


520. Pla, G.R. Disordered eating as correlate in the development of obesity. in *Obesity: Epidemiology, Pathophysiology and Prevention* (eds. Debasis B & Harry G Preuss) 479 (Taylor and Francis, 2007).


References


References


Rabbani, N., Chittari, M.V., Bodmer, C.W., Zehnder, D., Ceriello, A. & Thornalley, P.J. Increased Glycation and Oxidative Damage to Apolipoprotein B100 of LDL Cholesterol in Patients With Type 2 Diabetes and Effect of Metformin. *Diabetes* 59, 1038-1045 (2010).


References


698. Kwon, J., Suzuki, T., Yoshida, H., Kim, H., Yoshida, Y. & Iwasa, H. Concomitant lower serum albumin and vitamin D levels are associated with decreased objective physical
References


732. Fargnoli, J.L., Fung, T.T., Olenczuk, D.M., Chamberland, J.P., Hu, F.B. & Mantzoros, C.S. Adherence to healthy eating patterns is associated with higher circulating total and...
References


References


References


References


References

335


References


