The effects of ruminant trans fatty acids and dairy food on cardiovascular disease and cardiometabolic risk.

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Contents

Abstract 4

Background: 4

Aim: 4

Methods: 4

Results 4

Conclusion: 5

Acknowledgments 6

List of tables 9

List of Figures 12

Research Aims 15

Specific aims 15

Publications 16

Oral Presentations at conferences 17

Posters 17

Grants related to this dissertation 17

Abbreviations 19

CHAPTER 1 22

Section 1: Trans-fatty acid and coronary artery disease. (10) 25

Section 2: Ruminant Trans fatty acids 33

Section 3: Dairy foods 62

Conclusion 82

Chapter 2 83

Section 1: Plasma fatty acid levels in New Zealanders with severe coronary artery disease. 84

Section 2: Trans-fatty acids in New Zealand patients with coronary artery disease. (66) 94

Section 3: Saturated and ruminant trans fatty acids do not predict mortality in patients with severe coronary artery disease. 106

Abstract 106

Chapter 3 116

Section 1: A randomised trial evaluating the effects of change in dairy food consumption on cardio-metabolic risk factors. (1) 117

Section 2: The effects of changing dairy food intake on trans and saturated fatty acid levels- results from a randomised controlled study. (284) 138

Conclusion 155

Chapter 4 156

Abstract 156

Introduction 157

Conclusion 168

Chapter 5 170

Plasma Fatty acid levels in vegans 170

Chapter 6 182

Introduction 182

Effects of high and low fat dairy food consumption on cardio-metabolic risk factors: A meta-analysis of randomised studies. (308) 183

Chapter 7 219

Summary 219

Conclusion 223

Appendix 1: Common fatty acids. 225

Appendix 2: Food frequency questionnaire 230

Appendix 3: Study protocol for the randomised study 236

Appendix 4: Study protocol for meta-analysis 250

Appendix 5: Methodology for analysis of levels of fatty acids in milk and plasma 253

References 258

Abstract

Background:

Dairy food is the richest source of animal fats in the human diet and comprises saturated, unsaturated and trans fatty acids. There is lack of clear evidence on the effects of dairy fat, especially trans fatty acids, and food on cardiovascular health. This has led to incongruent advice on how much dairy fat and food is part of a healthy eating plan.

Aim:

To evaluate the effects of ruminant trans fatty acids and whole fat dairy food on cardiometabolic risk factors and cardiovascular disease.

Methods:

An observational study evaluated associations between plasma ruminant trans fatty acid levels and cardiovascular disease. A randomized study in 180 healthy adults assessed effects of changing dairy food intake on cardiometabolic risk factors and plasma levels of fatty acids. Fatty acid levels in whole milk and in plasma from vegans were evaluated. A meta-analysis evaluated the effects of dairy food on cardiometabolic risk.

Results:

The observational study suggests that two thirds of plasma trans fatty acids in New Zealanders with significant coronary artery disease are those thought to originate from ruminant sources. These trans fatty acids are associated with increased risk of recent myocardial infarction and polyvascular disease, but not increased mortality. However, in the randomized study, advice to change dairy food intake had little effect

on fatty acids including saturated and trans fatty acids thought to be exclusively ruminant. Analyses of milk confirmed the presence of these trans fats, however; however, levels markedly varied between types of milk and between a year of normal rainfall and drought. Trans fatty acids, principally palmitelaidic acid were found in the plasma of vegans.

The randomized study and a meta-analysis suggest that increasing whole and low fat dairy food increases weight but has no significant effects on other cardio-metabolic risk factors.

Conclusion:

The main source of trans fatty acids in the New Zealand diet is probably from dairy food. These fatty acids are associated with increased cardiovascular risk, suggesting that all trans fatty acids regardless of source may be harmful. However, modifying the intake of dairy food alone has no effect on plasma levels, and trans fatty acids were found in vegans implying that they are also from other sources.

TFA levels in milk depend on feeding practices, suggesting that exposure to TFA in the diet could be reduced by changing farming practices. Increasing public awareness on all sources of TFA in the diet could be considered.

Dietary advice to increase intake of low fat, and to a lesser extent whole fat dairy food, is associated with weight gain but has little effect on plasma fatty acids or cardio metabolic risk factors. Whilst small effects on cardiometabolic risks accumulating over a long period cannot be excluded, evidence suggests that dairy food is neither harmful nor beneficial for cardiovascular health.

There is no evidence to support the traditional food pyramid approach to dietary advice where 3 or more servings of dairy food each day are recommended.

Acknowledgments

I wish to express my gratitude to Professor Ralph Stewart, Cardiologist; Department of Medicine who encouraged me to present this thesis. He was both supervisor and collaborator for much of the research that was undertaken. I also wish to thank both the nurses and doctors in the Cardiovascular Research Unit who supported me for these last few years. In particular, I would like to acknowledge the nurses who assisted with recruitment into the studies. Karishma Sidhu helped me enormously with the analysis of the research. Together we learnt how to perform a meta-analysis, and this has been invaluable for my ongoing work – thank you, Karishma!

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Finally, I would like to acknowledge my late father who passed away before I finished this thesis. He was so proud of my academic pursuits and was an inspiration to me.

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List of tables

TABLE	TITLE	PAGE NO
1	Common trans fatty acid isomers.	28
2	Common trans fatty acids in dairy food.	36
3	The effects of ruminant trans fatty acids on lipids and atherosclerosis- animal studies.	40
4	Effects of ruminant trans fatty acids on surrogate markers of cardiovascular disease in humans.	50
5	The association of intake of ruminant trans fatty acids and cardiovascular disease. Results are relative risk (95% confidence interval).	57
6	Possible effects of various fats from dairy food on cardio- metabolic risk.	65
7	Plasma fatty acids levels world-wide as percentage of total plasma fat.	68
8	Effects on dairy food on cardiometabolic risk factors. Results from observational studies.	70
9	Baseline characteristics of study population in the observational study. Results are number (percent) or mean (standard deviation as appropriate).	87
10	Plasma phospholipid levels in New Zealanders with coronary artery disease. The FA level is expressed as % total phospholipids and results are median and IQR.	88
11	Characteristics of study subjects in the lowest, middle and highest thirds of total TFA in the observational study.	99
12	Plasma levels of trans fatty acid isomers in patients with higher and lower plasma levels of C-reactive protein, with and without polyvascular disease and	102

	with and without recent myocardial infarction. The trans fatty acid level is expressed as the median percentage weight and inter-quartile range.	
13	Baseline characteristic of those who survived or died at 7.5 year follow up. Results are number (percent).	111
14	Predictors of total mortality in patients 7.5 years after angiogram. Results are median and interquartile range. When multivariate analysis was performed adjusting for age and creatinine, N-BNP remained significant ($p<0.001$), but CRP was no longer significant ($p=0.052$)	111
15	Saturated fatty acids and total mortality at 7.5 years. Results are median and interquartile range.	112
16	Baseline characteristics according to randomization. Results are mean and (standard deviation).	127
17	Dairy food intake and plasma fatty acid levels at baseline and 1 month from the food frequency questionnaire for each randomized group. Results are mean and (standard deviation).	127
18	The effects of changing dairy food consumption on blood pressure and heart rate. Results are mean and standard deviation, and 95% confidence interval of mean difference between baseline and one month measurements.	129
19	Effects of changing dairy food intake on lipids, C - reactive protein and insulin resistance as calculated using the Homeostasis Model Assessment. Results are mean and standard deviation, and 95% confidence interval of mean difference between baseline and one month measurements. C- reactive protein has been log transformed	130
20	The effects of changing dairy food intake on weight, waist and hip circumference. Results are mean and standard deviation, and 95% confidence interval of mean difference between baseline and one month measurements	132

21	Comparison between randomized groups expressed as the standardized mean difference (95%CI). Results are presented as higher versus lower dairy intake	133
22	Fatty acids in milk and in human plasma .Fatty acids are measured by gas chromatography-mass spectrometry.	142
23	Plasma levels of fatty acids across the three randomized groups at baseline and one month. The change in plasma levels across the three groups was assessed using ANCOVA. ANCOVA was used unless assumptions were not met. ANOVA was used for all others (Table 24) Fatty acids are standardized to d4- alanine and presented as median (interquartile range).	148
24	The difference across the three dairy food groups using ANOVA for those fats that did not meet the assumptions for ANCOVA.	150
25	Baseline Characteristics of vegans compared to aged matched controls from the dairy randomised study. Results are median and (interquartile range).	176
26	Fatty acid levels in vegans expressed as a percentage of total fats and standard deviation.	177
27	Baseline characteristics of studies included in the meta-analysis.	191
28	Studies stratified by study duration, study population normal weight or overweight and source of funding on cardio-metabolic risk factors.	195

List of Figures

FIGURE	TITLE	PAGE NO
1	Stearidonic acid.	24
2	Chemical structure of saturated, unsaturated and trans fatty acids.	26
3	Possible mechanism of development of atherosclerosis with TFA.	29
4	Bio hydrogenation of unsaturated fats in the rumen of a cow.	34
5	Correlation between plasma levels of vaccenic and palmitelaidic acids. The close correlation suggests that these TFA are derived from a similar or common dietary source	91
6	Correlation between plasma levels of palmitelaidic and elaidic acids. The weak correlation implies that these TFA are derived from different dietary sources	91
7	Proportion of study subjects in the lowest, middle and highest thirds of total plasma trans-fats who had polyvascular disease and recent myocardial infarction.	101
8	Study schematic of randomised study.	123
9	The effect size of dairy food on cardio metabolic risk with 95% confidence interval	134
10	Fatty acids found in New Zealand milk. Plasma fatty acids are measured by gas chromatography mass spectrometry and are [percentage of total fat.	144
11	Change in plasma levels of fatty acids with a high dairy compared to a low dairy food diet.	146
12	Correlation between fatty acid levels in milk and change in plasma fatty acid levels in those participants asked to increase dairy food intake.	151

	Results are mean levels and are normalised to d4 alanine	
13	Correlation between fatty acid levels in milk and change in fatty acid levels in those participants asked to decrease dairy food intake. Results are mean levels and are normalised to d4 alanine.	152
14	Trans fatty acid levels in milk in 2011 as a percentage of total fat. Milk is non-homogenised unless otherwise stated	161
15	Trans fatty acid levels in milk in 2013 as a percentage of total fat. Milk is non-homogenised unless otherwise stated.	162
16	Saturated fatty acid levels in milk in 2011 as a percentage of total fat. Milk is non-homogenised unless otherwise stated.	164
17	Saturated fatty acid levels in milk in 2013 as a percentage of total fat. Milk is non-homogenised unless otherwise stated.	165
18	Unsaturated fatty acid levels in milk 2011 as a percentage of total fat. Milk is non-homogenised unless otherwise stated.	166
19	Unsaturated fatty acid levels in milk in 2013 as a percentage of total fat. Milk is non-homogenised unless otherwise stated.	167
20	Plasma trans fatty acid levels in vegans expressed as mg/mL.	179
21	Study flow chart of the meta-analysis.	189
22	Effects of whole & low fat dairy food on weight.*	199
23	Effects of whole & low fat on dairy food waist circumference.*	200
24	Effects of whole fat dairy food on fasting plasma glucose.*	202
25	Effects of whole fat dairy food on insulin resistance assessed by Homeostasis Model Assessment.-Insulin Resistance.*	203
26	Effects of whole & low fat dairy food on LDL-cholesterol.*	205
27	Effects of whole & low fat dairy food on HDL-cholesterol.*	206
28	Effects of whole & low fat dairy food on C-reactive protein. *	208

29	Effects of whole & low fat dairy food on systolic blood pressure.*	209
30	Effects of whole & low fat dairy food on diastolic blood pressure.*	210
31	Funnel plots of effects of fat dairy food on HOMA-IR, glucose, LDL-c, HDL-c, C - reactive protein, systolic and diastolic blood pressure. These funnel plots indicate that the most common outliers is the study from Stancliffe et al.	212

Appendices

APPENDIX	**TITLE**	**PAGE NO**
Appendix 1	Lists of common fatty acids	225
Appendix 2	Food frequency questionnaire	230
Appendix 3	Study protocol for the randomised study	236
Appendix 4	Study protocol for the meta-analysis	250
Appendix 5	Methodology for analysis of levels of fatty acids in milk and plasma	253

Contribution:

Every experiment was conceived, designed and executed by the author, including developing protocols, gaining ethics approval and funding, recruitment of participants, data entry, statistical planning and analysis. The author is also the first author of all the published papers.

Research Aims

To evaluate the effects of ruminant trans fatty acids and dairy food on cardiovascular disease and cardiometabolic risk.

Specific aims

1. To evaluate plasma levels of trans fatty acids levels in New Zealanders.
2. To evaluate the relationship between plasma ruminant trans-fatty acids levels with clinical markers of vascular disease.
3. To evaluate the effects of changing dairy food intake on plasma trans fatty acid levels
4. To evaluate the effects of changing dairy food intake on cardiometabolic risk factors

Publications

Some of the original work presented or referred to in this thesis has been published:

1. **Benatar JR**. Trans fatty acids and coronary artery disease. Open access journal of clinical trials 2010:2 9–13. (Chapter 1)
2. **Benatar JR,** Gladding P, White HD, Zeng I and Stewart RAH. Trans-fatty acids in New Zealand patients with coronary artery disease. European journal of cardiovascular prevention and rehabilitation. 2011; 18: 615-20. (Chapter 2)
3. **Benatar JR.** There is no Relationship with Plasma Fatty Acid Levels in New Zealanders with Severe Coronary Artery and Mortality. Journal nutritional disorders and therapy 4:146. (2014). doi: 10.4172/2161-0509.1000146. (chapter 2)
4. **Benatar JR**, Jones E, White H, Stewart RA. A randomized trial evaluating the effects of change in dairy food consumption on cardio-metabolic risk factors. European journal of preventative cardiology. 2014; 21(11):1376-86. (Chapter 3)
5. **Benatar JR**; Stewart RAH. The effects of changing dairy intake on trans and saturated fatty acid levels- results from a randomized controlled study. Nutrition journal 2014, 13:32 doi:10.1186/1475-2891-13-32. (Chapter 3)
6. **Benatar JR,** Sidhu K, Stewart RAH (2013) Effects of High and Low Fat Dairy Food on Cardio-Metabolic Risk Factors: A Meta-Analysis of Randomized Studies. PLoS ONE 8(10): e76480. doi:10.1371/journal.pone.0076480. (Chapter 6)

Oral Presentations at conferences

1. Trans-fatty acids in New Zealand patients with coronary artery disease. Cardiac Society of New Zealand. 2010
2. A randomized trial evaluating the effects of change in dairy food consumption on cardio-metabolic risk factors. Cardiac Society of New Zealand. 8 June 2013.

Posters

1. Effects of increasing dairy food on cardio-metabolic risk factors: a meta-analysis of randomized studies. Cardiac Society of New Zealand, June 2013.

Awards and nominations

1. Winner Green Lane Scientific Sessions 2011 for presentation “The Fat of the Land”
2. Benatar JR, Jones E, White HD, Stewart RAH. A randomized trial evaluating the effects of change in dairy food consumption on cardio-metabolic risk factors. European Journal of Preventive Cardiology 2013- nominated as best from Department of Medicine 2013 for the doctoral showcase.

Grants related to this dissertation

Jocelyne Benatar, Ralph Stewart	The effect of dietary intervention on ruminant TFA and CRP levels in New Zealand	February 2011/ August 2012	GLREF/10/41/4081	$50,000
Jocelyne Benatar, Ralph Stewart	The effect of dietary intervention on ruminant TFA, CRP and insulin	February 2011/ August 2012	A+ trust- 4943- SPG-1104-001	$9,982

	resistance levels in New Zealand			

Abbreviations

(In alphabetical Order)

AMI	**Acute myocardial infarction**
ANCOVA	Analysis of covariance
ANOVA	Analysis if variance
ANZCTR	Australian New Zealand Clinical Trials Registry
ARR	Absolute risk reduction
BMI	Body mass index
BNP	Brain Natriuretic peptide
BP	Blood pressure
CHD	Coronary heart disease
CI	Confidence interval
CLA	Conjugated linoleic acid
cm	centimetres
CRP	c-reactive protein
CVD	Cardiovascular disease
DASH	Dietary Approaches to Systolic Hypertension
EA	Elaidic acid
ECG	Electrocardiogram
FDA	Food and Drug Administration
GC-MS	Gas Chromatography- Mass Spectrometry
HDL-c	High density lipoprotein cholesterol
HOMA	Homeostasis Model Assessment
HOMA-IR	Homeostasis Model Assessment for insulin resistance
HR	Hazard Ratio

HsCRP	High sensitivity c- reactive protein
ICAM	Intercellular Adhesion Molecule
IQR	Interquartile range
iTFA	Industrial trans fatty acids
kg	kilogram
L	litre
LA	Linoleic acid
LC	Long chain
LDL-c	Low density lipoprotein cholesterol
MC	Medium chain
MFGM	milk fat globule membrane
mg	milligram
MUFA	Monounsaturated fatty acids
ND	Not detected
OR	Odds ratio
P value	Probability value
PKE	Palm kernel expeller or extract
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
PUFA	Polyunsaturated fatty acids
PVD	Peripheral vascular disease
RA	Rumenic acid
RR	Relative risk
RRR	Relative risk reduction
rTFA	Ruminant trans fatty acids
SC	Short chain
SD	Standard deviation

SFA	Saturated fatty acids
TC	Total cholesterol
TFA	Trans fatty acids
TG	Triglycerides
VA	Vaccenic acid
VCAM	Vascular cell adhesion molecule
WC	Waist circumference
WHO	World Health Organisation

CHAPTER 1

Introduction

The topic of this dissertation is the result of my interest in the role of dietary foods in the primary and secondary prevention of cardiovascular disease and diabetes. The concept of 'good' and 'bad' fats is imbedded in dietary advice with little supporting evidence. (1) For example, consumers are told to avoid trans (2) and saturated (3) fatty acids which are considered harmful for health and are thought to increase the risk of cardiovascular disease (CVD). (For a description of fatty acids, see "A preamble on fatty acids" below).

In the human diet, the most abundant source of saturated fat is from dairy food, which also includes unsaturated and trans fatty acids (TFA). (4) However, the evidence that whole fat dairy food is harmful is not conclusive. This has led to inconsistent health advice to consumers, with some authorities recommending 3-4 servings per day (5) and labelling dairy a 'superfood'. (6) On the other hand, cardiovascular guidelines recommended avoidance of whole fat dairy food, (7, 8) with some recommending avoiding dairy food as much as possible. (9)

More data is clearly needed to better inform dietary guidelines. This dissertation will, therefore, attempt to shed light on this issue by focusing on the cardiovascular health effects of ruminant TFA and dairy food. It would be remiss to ignore the health effects of saturated fatty acids (SFA) so some sections will include analysis that includes these fatty acids.

This chapter provides an overview of the evidence to date. Section 1 is an article titled' Trans-fatty acid and coronary artery disease, published in the 'Open Access Journal of Clinical Trials'. (10) It is a general overview of the evidence linking TFA and cardiovascular disease (CVD). Section 2 reviews current evidence on ruminant TFA and potential effects on cardiometabolic risk and atherosclerosis. Section 3 reviews what is known about dairy food and possible effects on cardiometabolic risk and atherosclerosis.

A preamble on fatty acids

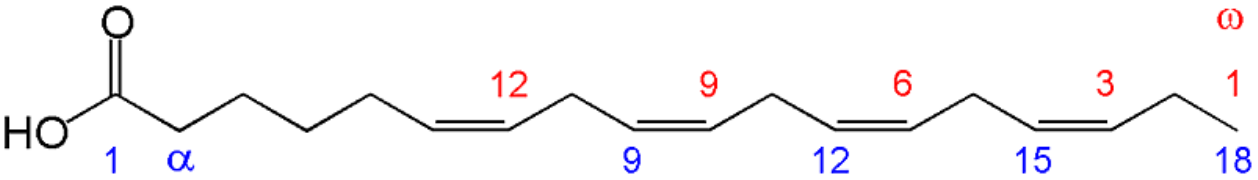
Fatty acids are molecules that are made up of a carboxylic acid () with a long aliphatic tail chain (Figure 1). The carboxylic acid end is called the alpha (α) end and the methyl end is called the omega (ω) end. Fatty acids are either saturated or unsaturated with hydrogen atoms. Saturated fats therefore only have a single bond between carbon atoms, and unsaturated fatty acids have double bonds between carbon atoms. The double bond can either be in the cis or trans position (Figure 2). (11)

Fatty acids are an important source of energy and are essential for many cellular functions and structures. They are either synthesised in the body or originate from the diet. Fatty acid synthesis in humans mainly occurs in the liver and in the lactating mammary gland but, is also synthesised in adipose tissue. Excess protein and carbohydrate may also be converted into fatty acids and transported and stored as triglycerides. As a general rule, humans are only able to synthesise long-chain fatty acids with even numbers of carbon atoms, but humans can convert certain saturated fats to unsaturated fats through a process called desaturation. Trans, omega (ω)-3 and ω-6 fatty acids cannot be synthesised by humans and are only available from the diet. (12) Some dietary fatty acids, for example linoleic and α-linolenic acid, are essential to human health and these are therefore called nutritionally essential fatty acids. Omega-6 fatty acids generally come from eggs, avocados, and corn, sunflower and safflower oils. Omega-3 fats come from fish, eggs, fruit, and flax and chia seeds. (13)

Appendix 1 contains a list of fatty acids together with their systematic names, lipid number and chemical structures and is a useful reference for anyone reading this thesis. I will attempt to use the common name, if available, and the lipid number throughout the text. I have chosen to describe the lipid number in this manner: carbon chain number; number of double bonds; position of the double bond (trans or cis) from the "omega" (ω) end. For example, 18:2 (n-7t, 9c), means the fatty acid contains 18 carbon atoms with two double bonds. The double bond in the ω-7 position is trans, and the ω-9 position is cis. I have chosen to convert all fatty acids to this nomenclature as it is practical and easy to understand.

The figure below can be used to illustrate the nomenclature. This is an omega 3 fatty acid as the first double bond in in the ω-3 position, has 18 carbon atoms and 4 double bonds. Unfortunately, it is impractical to show each double bond in the cis position, as the molecule is 3 dimensional and curves. The lipid number for this fat is 18:4 (n-3c, 6c, 9c, 12c) and its common name is stearidonic acid.

Figure 1: Stearidonic acid.



Moreover, the reviewer can swiftly decipher whether it is saturated with no double bonds (e.g. 15:**0**); an omega-3 unsaturated fat (e.g. 18:3 (n-**3c**, 6c, 9c); an omega-6 unsaturated fat (e.g.) 18:2 (n-**6c**, 9c) or a trans-fat 16:1 (n-7**t**).

SFAs are considered short-chain (2-5 carbon atoms), medium-chain (6-12 carbon atoms), long-chain (13-20 carbon atoms) and very long-chain (> 21 carbon atoms).

Section 1: Trans-fatty acid and coronary artery disease. (10)

Abstract

Over the last 40 years, there has been a significant increased consumption of TFA in the developed world as we have embraced processed and takeaway foods in our diet. These fatty acids are not essential for human nutrition and are hazardous to health. They increase the risk of cardiovascular disease more than any other macronutrient including saturated fat, through multiple mechanisms including adverse effects on lipids, endothelial function and inflammation. They are readily incorporated into cell structures such as cell membranes and the Golgi apparatus, resulting in unintended effects on multiple biological pathways. The majority of TFA in our diet are artificially manufactured by a process of partial hydrogenation of vegetable oil with little coming from natural sources. It should be possible to replace these harmful fats in the food chain at source with concerted efforts from food manufacturers and legislators.

Introduction

Approximately a century ago the discovery was made that liquid vegetable oil could be converted by a process of hydrogenation to a useful solid fat. The process for making hydrogenated and hardened fats from cheaper sources of vegetable oils was widely adopted after the Second World War and the consumption of these fats soared in the 1970's. (14)

The unintended effect of the process was the creation of a by-product called TFA. TFA are not essential for human nutrition and have subsequently been found to be hazardous to health with adverse effects on cardiovascular disease (CVD), prostate cancer and female infertility. This article reviews the evidence linking cardiovascular disease and TFA intake.

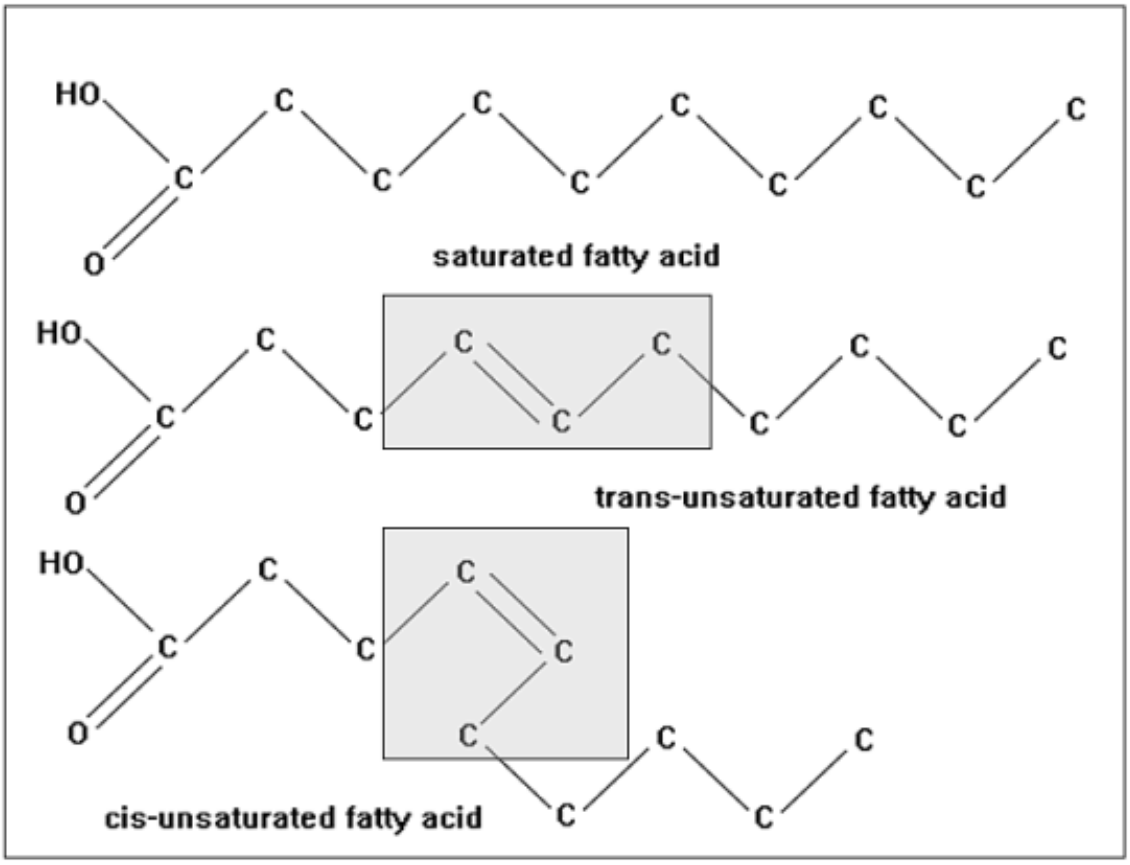
What are trans fatty acids?

Fatty acids are water–insoluble molecules with long carbon chains (Figure 1) that have a wide variety of functions within cells including maintenance of electrochemical gradients across membranes, first and second messenger signalling, energy storage, subcellular partitioning, protein trafficking and membrane anchoring. (12)

The term saturated, polyunsaturated and unsaturated, relates to the presence or absence of double bonds between the carbon atoms (Figure 2). Saturated fats have no double bonds between carbon atoms whereas unsaturated fats have double bonds between carbon atoms. Fatty acids with a single double bond are monounsaturated and those with many double bonds are polyunsaturated. (15)

The double bond mainly exists in the cis configuration, and when that double bond is 'flipped over', it exists in the trans configuration. The trans configuration inherently changes the properties of the fatty acid including the ability of the molecule to 'bend'. The type of fatty acid incorporated into cell structures has the potential to affect various cellular functions including cell membrane receptor activity.

Figure 2: Chemical structure of saturated, unsaturated and trans fatty acids.



Source of TFA

Humans do not produce TFA, and all comes from the diet. Two sources of TFA are found: in products derived from ruminant animals, such as milk and meat, and in foods that contain artificially manufactured

hydrogenated vegetable oil. Both are derived from hydrogenation of unsaturated fats but in the case of ruminant TFA (rTFA), the bacteria in the ruminant animal's gut hydrogenate the fatty acids.

The amount of TFA and the kind of TFA produced in milk and meat are determined by what the animal eats (16) with cows that graze producing more TFA than those that are grain fed. The predominant isomer of TFA found in milk is vaccenic acid (18:1 (n-7t)) .It makes up 55% of total trans18:1 isomers in milk fat from grazing cows, compared with 33% in milk from cows fed a grain diet. In most Western countries, only trace amounts of TFA ingested is from dairy sources and studies to date have not convincingly shown that rTFA are harmful. (17, 18)

In contrast, there is considerable data suggesting hydrogenated vegetable oils are harmful. (19-23) Hydrogenated oils are solid at room temperature, have a high smoke point and long shelf life. This allows for an unsaturated fat as an alternative spread to butter, an attractive option to consumers worried about saturated fat intake. It enables fast food outlets to fry foods at high temperatures, and manufacturers to produce goods such as biscuits and snacks, with a long shelf life. Industrially processed foods have been embraced in the Western world and account for the majority of TFA intake in Western countries. The most common isomer of TFA found in industrially processed food is elaidic acid (18:1 (n-9t)).

Table 1: Common trans fatty acid isomers.

Shorthand designation	Common name	Systematic name	Source
18 :1 (n-7t)	Vaccenic acid	trans -11-octadecenoic acid	Ruminant
18:1 (n-9t)	Elaidic acid	trans-9-octadecenoic acid	Industrial
18:2 (n-9t, 12t)	Linoelaidic acid	trans-9,trans-12-Linoleic acid	Industrial
16:1 (n-7t)	Palmitelaidic acid	9-trans-hexadecenoic acid	ruminant, palm oil derivative

Human intake of trans fatty acids

Humans ingest several isomers of TFA and the common ones are listed in table 1. In most countries, TFA derives predominantly from hydrogenated vegetable oils, but in some countries like Denmark and New Zealand, there is little intake of processed food and a high intake of dairy food. Dietary surveys show that there has been a large increase in industrial TFA (iTFA) consumption from the 1970's onwards and that the average consumption in North America nowadays is 2-3% of total calories consumed with less than 0.5% derived from ruminant sources. (14)

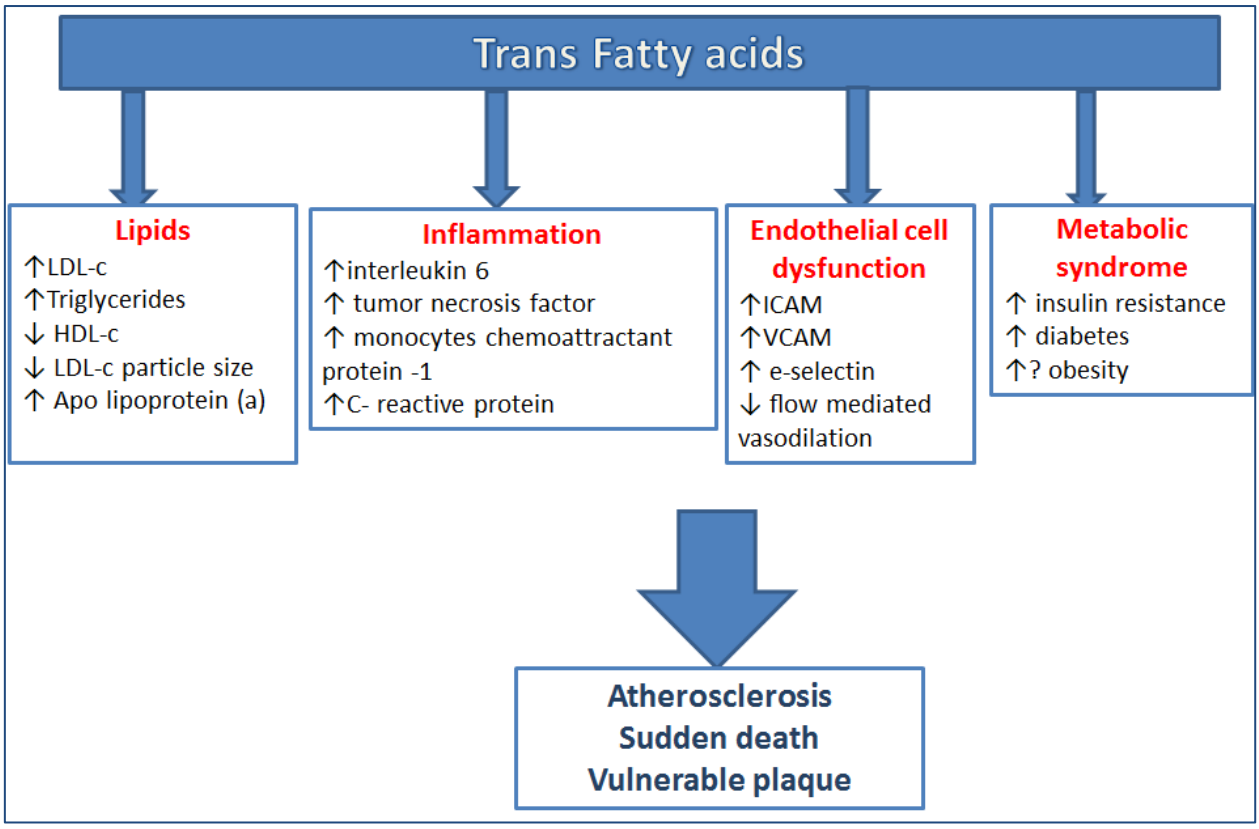
Biological effects of TFA

Studies have shown that TFA have adverse effects on lipids, (24-26) inflammation, (27-30) cell wall fluidity and endothelial function (Figure 3). (31, 32)

Metabolic and meta-analysis show a clear and consistent association between increased TFA intake and reduced high-density lipoprotein cholesterol (HDL-c), increased low-density lipoprotein cholesterol (LDL-c) and triglyceride levels. These studies have also shown that TFA increase lipoprotein (a) and reduce the

particle size of LDL-c, both of which contribute to an increased risk of CVD. Changes in lipids caused by TFA do not completely account for the increased risk of CVD seen in epidemiological studies. Residual risk is due to effects on inflammation and endothelial function.

Figure 3: Possible mechanism of development of atherosclerosis with TFA.



(ICAM-Intracellular adhesion molecule, VCAM-Vascular cell adhesion molecule)

Effects on inflammation are seen in both epidemiological and experimental studies. A study of over 700 nurses showed that those in the highest quartile of TFA consumption had blood levels of C-reactive protein (CRP) that were 73% higher than those in the lowest quartile. (32) Other studies have also linked significant intake of TFA with increased circulating concentrations of inflammatory molecules such as interleukin-6, tumour necrosis factor-alpha, CRP, and monocyte chemo-attractant protein-1. (33)

Several studies suggest that TFA cause endothelial dysfunction. After adjustment for other risk factors, greater intake of TFA was associated with increased levels of several markers of endothelial dysfunction, including soluble intercellular adhesion molecule 1 (ICAM-1), soluble vascular-cell adhesion molecule

1(VCAM-1), and E-selectin. (21, 34) In another trial, consumption of TFA impaired endothelial function, as reflected by a reduction in brachial artery flow-mediated vasodilatation by 29% as compared with intake of SFA. (28)

Cardiovascular disease and trans fat intake

Studies have shown a graded association between increased dietary intake of TFA, predominantly from hydrogenated vegetable oil and the risk of cardiovascular disease. In a meta-analysis of three prospective cohort studies which included ~140 000 subjects, an increase in 2% of energy from TFAs (or a teaspoonful a day) estimated from detailed food-frequency questionnaires was associated with a ~25% increase in the risk of CVD. (21)

The major evidence for the effect of TFA on CVD comes from the Nurses' Health Study (NHS); a cohort study that has been following 120,000 female nurses since its inception in 1976. In this study, data from 900 coronary events from the NHS population during 14 years of follow-up was analysed. CVD risk almost doubled for each 2% increase in TFA calories consumed. To put this in context, SFA needs to be 15% of total calories to achieve the same level of risk. (35, 36)

Studies testing TFA tissue levels in serum, adipose tissue and erythrocytes have shown a strong positive correlation between risk for CVD and tissue concentration of TFA. (17, 19, 23, 37-39) Two studies have linked the risk of sudden death from cardiac causes and levels of TFA, specifically the iTFA, linoelaidic acid. Studies are not consistent about whether specific TFA isomers have different effects on CVD risk. Elaidic acid has been associated with increased risk of sudden death, fatal ischemic heart disease and acute coronary syndrome. (17, 19, 23, 37-39) The data on effects of rTFA is less convincing than for iTFA. (18) The lack of biological effect of rTFA in these studies may in part be due to low levels of intake (typically less than 0.5% of total energy intake) in most Western countries.

Trans fatty acids, the metabolic syndrome and diabetes

There is no consistent data as to whether TFA increase the risk of the metabolic syndrome or diabetes. (40-46) The association between TFA intake and obesity disappears when corrected for caloric intake. The

Nurses' Health Study showed that the intake of TFA was significantly related to the risk of diabetes in 84 941 nurses. (43) This was, however, not validated in two other studies, viz. the Physician Health Study (47) and a study of women in Iowa. (48) Possible reasons for the discrepancy are that nurses had a significantly higher intake of TFA compared to their physician counterparts and the Iowa study was flawed in that diabetes was based on self-reported diagnosis, and diet was assessed only at baseline.

Reducing the exposure to trans fatty acids

The elimination of industrially manufactured non-essential TFA should be achievable. (34) This laudable goal needs to be reached without inadvertently replacing TFA with SFA. There is also growing concern that emphasizing the danger of TFA consumption may shift the focus away from the dangers of saturated fat consumption and add to the complexity consumers face when reading food labels.

The World Health Organisation (WHO) recommends that TFA intake be less than 1% of total caloric intake with most advice tailored towards reducing iTFA. (49) The Food and Drug Administration (FDA) has recommended that all TFA be on the nutrition label. Food labelling may give consumers choice, but requires extensive education programs. The pragmatic approach is to eliminate TFA at source by reducing levels in the food chain.

Healthier alternative oils with high smoke points such as rice bran oils are now available for deep fat fryers and changed formulations have allowed the elimination of TFA from margarines. Food manufacturers are investigating hydrogenation of fatty acids without producing the harmful TFA by- product. In countries with high rTFA intake, a number of methods can be considered to reduce TFA levels including manipulating the animal's diet and filtration of the milk. A number of countries, such as Denmark and Switzerland, have effectively legislated to eliminate iTFA. Others, such as the Unites States and Britain have implemented voluntary measures to reduce TFA in food.

Conclusion

Our consumer culture has produced many environmental hazards in the name of progress and convenience. Processed food consumption is linked with a number of health hazards, some attributable to the presence of TFA. TFA are non-essential fatty acids, with no benefit for human health, conversely; most studies have

shown that TFA consumption is hazardous to health specifically cardiovascular health. Whilst it may be impossible to eliminate TFA from the food chain, steps to minimize exposure to hydrogenated vegetable oils is achievable with concerted efforts from legislators, manufacturers and consumers. The experience from countries like Denmark indicates that this is an achievable goal.

Section 2: Ruminant Trans fatty acids

Introduction

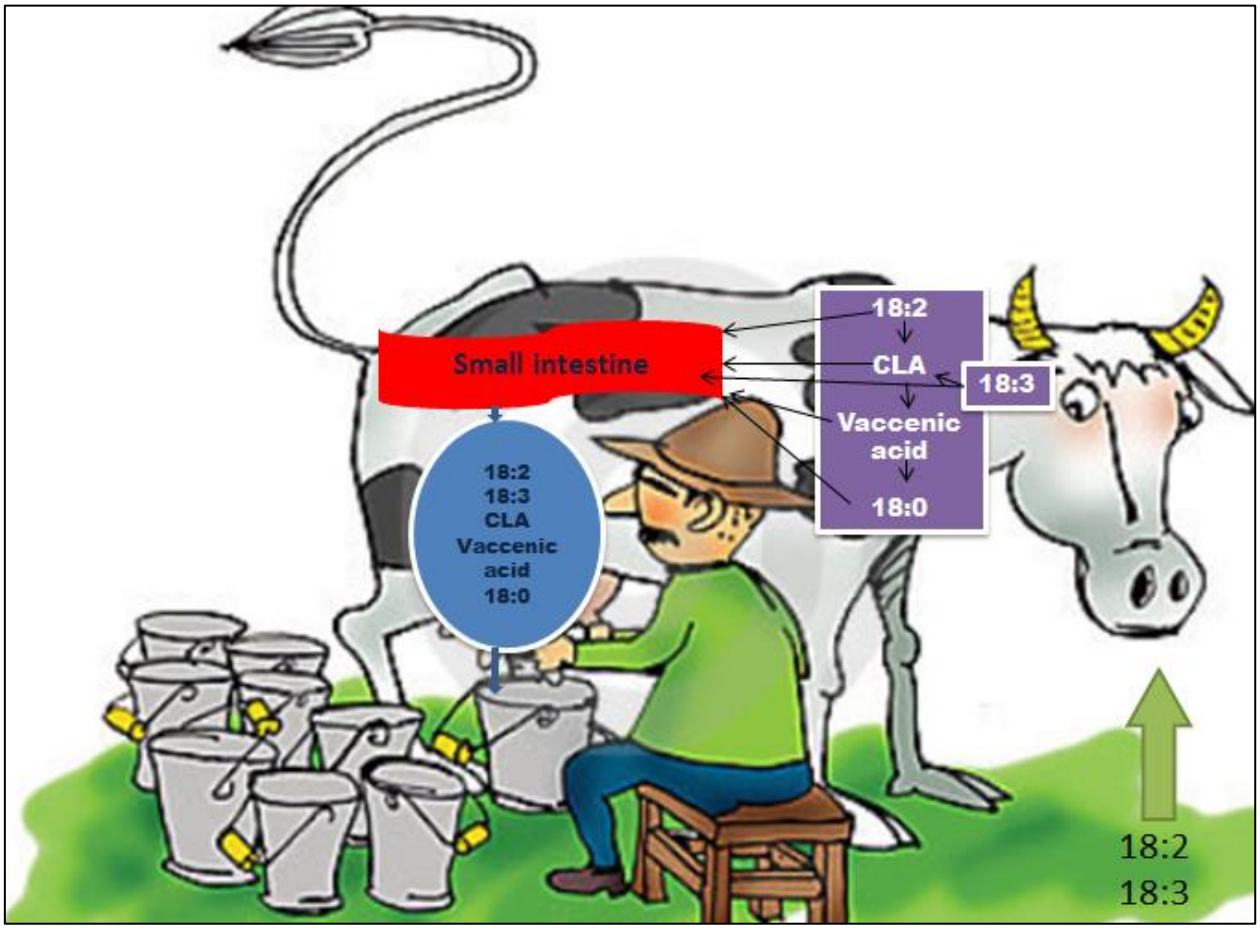
Ruminant TFA (rTFA) are unsaturated fatty acids present in by-products of ruminant animals such as meat and dairy. They arise through partial hydrogenation and/or isomerization of cis-unsaturated fatty acids to the trans configuration from the animal fodder by hydrogen produced during oxidation of substrates. (50, 51) Bacteria, like butyrivibrio fibrisolvens and clostridium protoclasticum (52) in the ruminant gut act as catalysts for this reaction. The principal rTFA are vaccenic acid (18:1 (n-7t)) and palmitelaidic acid (16:1 (n-7t)). In addition, ruminant fats contain small amounts of an unsaturated fat that contains both 1 cis and 1 trans bond called conjugated linoleic acid, (CLA) (18:2 (n-7t, 9c)). (53)

The double bond in the trans configuration for rTFA is mainly in the n-7 position whereas, for most common iTFA, this is in the n-9 position (Table 2).

Food composition and dietary intake of ruminant trans fatty acids

Dairy food is the most common source of animal fats with cheese consisting of up to 40% fat and butter 80% fat. In contrast, meat is less than 10% fat and is not considered the most important source of animal fats. rTFA are a small portion of the fat in dairy products (typically 2–5% of total fatty acids) and beef and lamb (3–9% of total fatty acids), (54, 55) with variations in fatty acid compositions due to feeding practices as well as geographical and seasonal change. (55, 56) Partially hydrogenated vegetable oil can consist of up to 60% of total fatty acids as TFA. (57, 58)

Figure 4: Bio hydrogenation of unsaturated fats in the rumen of a cow.



(CLA- conjugated linoleic acid).

Both the isomers and quantities of TFA in meat and milk are dependent on the feed of the animal. (54, 59, 60) For example, feeding cows in clover-rich pastures has been shown to increase TFA levels in milk due to the presence of the cis-vaccenic acid in plants. (61, 62) In New Zealand, cows are pasture fed in clover rich pastures, and this is thought to account for the higher levels of TFA (63) in their by-products than cows from Europe and the USA (10% vs. 2% respectively). (4, 54, 63, 64)

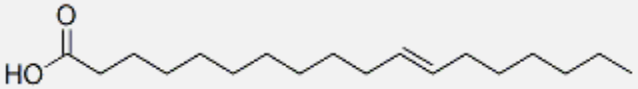
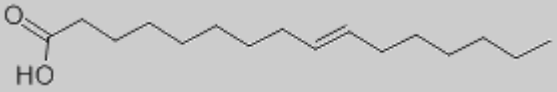
Generally rTFA consumption is thought to be too low to have biological effects, but in countries like New Zealand and Norway, this has not been found to be the case. (65, 66) In New Zealand (67-69) intake is higher than most Western countries (70) with low intake of processed foods. (69) The relative effects of dairy fats in this population, especially rTFA may be more apparent than in countries where rTFA consumption is low.

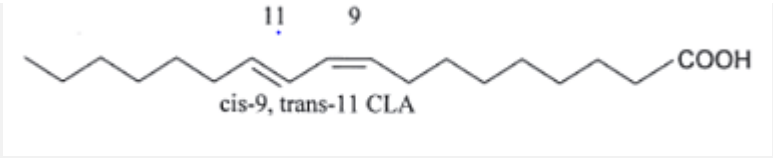
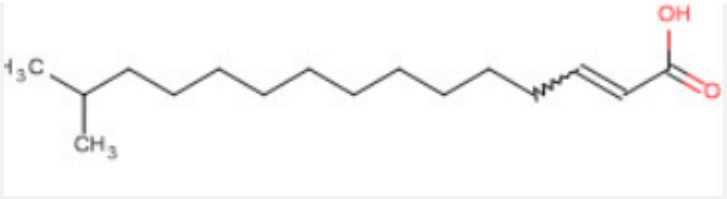
The major determinants of trans fatty acid levels in milk

The factors that affect FA levels in milk are nutrition, parity, stage of lactation and breed of the cow. (71, 72) Both the isomers and quantities of TFA in meat and milk are mainly dependent on the feed of the animal. (54, 59, 60) For example, feeding cows in clover rich pastures or adding sunflower oil to the feed has been shown to increase TFA levels in milk due to the presence of the cis-vaccenic acid in plants. (61, 62, 73) Milk from cows that graze grass has a higher proportion of unsaturated fat (including CLA) than milk from silage-fed cows. (74) Experimental studies show that SFA and unsaturated fats but not rTFA are affected by breed. (75) No studies have shown that manipulating ruminant bacteria affects rTFA levels.

Unsaturated fats in milk can also be isomerised by physical means. For example, heating milk at 63 ± 1.0°C for 30 min or microwaving it for 5 minutes significantly increases levels of vaccenic acid by 19 and 31%, respectively. (76) This suggests that vaccenic and palmitelaidic acids may be found in processed food depending on which unsaturated fats are used during the process of partial hydrogenation. (77)

Table 2: Common trans fatty acids in dairy food.

	Names	Molecular formula	Type of fatty acid	Sources	Structure
Vaccenic acid	Trans-vaccenic acid, trans-11-octadecenoic acid, (11E)-octadecenoic acid, (E)-11-octadecenoic acid, (11E)-octadec-11-enoic, 18:1 (n-7t), or t11-18:1	$C_{18}H_{34}O_2$	Trans	Ruminant by-products (predominant TFA). Lesser extent in industrial fat.	
Palmitelaidic acid	9-trans-hexadecenoic acid, $C_{16}H_{30}O_2$, (E)-9-Hexadecenoic acid; 9-trans-hexadecenoic acid; palmitelaidic acid; trans-9-hexadecenoic acid; trans-palmitoleic acid. 16:1(n-7t) or t9-16:1	$C_{16}H_{30}O_2$	Trans	Ruminant by-products. No other sources known at present.	

Conjugated Linoleic acid (CLA)	9Z,11E-octadecadienoic acid, Bovinic acid, Linoleic acid (10-trans, 12-cis);10(E) ,12(Z)-octadecadienoic acid 18:2 (n-9c, 7t) or C9, t11-18:2	$C_{18}H_{32}O_2$	Unsaturated (both trans and cis)	Ruminant by-products.	
Myristelaidic acid	C14:1T; 9-trans-tetradecenoic acid; delta 9 trans tetradecenoic acid; 14:1 (n-5t) or t10-14:1	$C_{14}H_{28}O_2$	Trans	Ruminant by-products (found in NZ milk samples- see chapter 4).	
10-trans-Pentadecenoic acid	C15_1n-5t), Methyl trans-10-pentadecenoic	$C_{15}H_{30}O_2$	Trans	Ruminant by-products (found in NZ milk samples- see chapter 4).	

Ruminant trans fatty acid isomers

Vaccenic acid (18:1 (n-7t))

This is the predominant TFA found in dairy food, and this can make up to 2/3 of the total TFA in milk, (4) although small quantities have been found in processed food. It is the precursor of conjugated linoleic acid (CLA) (78) which is thought to have health benefits. Animal studies to date have shown mixed effects (Table 3) with most effects seen when vaccenic acid is combined with CLA. Very few randomised studies have assessed the effect of vaccenic acid on cardiometabolic risk, mainly due to the limited availability of purified rTFA isomers. One randomised study found no effect on lipids, (79) another found no effect on inflammation. (80)

Conjugated Linoleic acid

CLA are a family of at least 28 isomers of linoleic acid with two conjugated unsaturated double bonds at various carbon atom positions in the fatty acid chain. Each double bond can be cis or trans, but those with one trans double bond are bioactive. (64)

CLA is formed as an intermediate during the bio-hydrogenation of linoleic acid, to stearic acid (Figure 4) (81) in the rumen by a bacterium called Butyrivibrio fibrisolvens (82-84) or from tissue synthesis of CLA by Δ 9-desaturase conversion of vaccenic acid. There is some evidence that mammals including humans may utilize the same pathway in the liver to produce some CLA (85-88) from ingested vaccenic acid in beef and dairy products.

The predominant CLA in dairy food is 18:2 (n-9c, 11t) CLA and for this reason, is also called rumenic acid (RA). CLA is widely sold as a supplement in the form of capsules that contain a mix of RA and 18:2 (n-10t, 12c) CLA. These CLA-preparations are promoted for weight loss, although studies in humans have been inconclusive. (78, 89)

Animal models suggest that dairy CLA may have beneficial effects on cardiometabolic risk (table 3). Dietary trials indicate that consumption of CLA has mixed effects on markers of inflammation and immune function.

(78, 80, 90-92) It has been shown to have beneficial effects on insulin resistance, lipoprotein metabolism (91, 93, 94) and weight. (92-98)

In the United States, trans linkages in a conjugated system are not counted as TFA for the purposes of nutritional regulations and labelling, (5), so CLA is not labelled as a TFA.

Palmitelaidic acid (16:1 (n-7t))

Palmitelaidic acid has recently been described in milk. Unlike vaccenic acid and CLA, it is a C16 fatty acid. One observational study has linked this TFA with reduced insulin resistance. (99)

Myristelaidic acid (14:1 (n-5t))

Myristoleic acid is an omega-5 fatty acid. It is biosynthesized from myristic acid by the enzyme delta-9 desaturase, but it is uncommon in nature. (100) No reports on the trans isomer were found in publications, nor were there any reports on the health effects of this rTFA.

Effects of ruminant trans fatty acids on cardiovascular disease

Animal Studies

Table 3 summarises findings from animal studies. There is a suggestion that at low dose, vaccenic acid and 18:2 (n-9c, 11t) (CLA) have a beneficial impact on blood lipid levels (mainly by lowering triglycerides), inflammatory markers and atherosclerosis. At higher doses, effects seem to mimic harmful effects seen with iTFA.

Table 3: The effects of ruminant trans fatty acids on lipids and atherosclerosis- animal studies.

Reference **(Year Published)** **Animal Model**	**Intervention**	**Length** **(weeks)**	**Result**	**Summary**
Arbones-Mainar et al. (101) 2006 Cholesterol-fed ApoE-/-mice	1% (w/w) 1. RA 2. 18:2 (n-6c, 8t) CLA 3. LA	12	1. RA decreased aortic lesions. 2. RA decreased plasma TC, FFA, glucose/insulin, and aortic lesions, increased Apo lipoprotein A-I. 3. 18:2 (n-6c, 8t)-CLA had a profound pro-atherogenic effect.	1. RA reduced atherosclerosis development 2. 18:2 (n-6c, 8t) CLA had a pro-atherogenic effect.
Bauchart et al. (102) 2007 Male New Zealand white rabbits	One of 3 butters: 1. 18:1 (n-8t) (11.8%) 2. VA (7%) +CLA (2.6%), 3. control diet low in all 3 fatty acids	12	VA + CLA butter resulted in lower VLDL-C, LDL-C, TG, plasma TC, phospholipids, and Apolipoprotein B.	VA/CLA butter improved plasma lipoprotein profile. Results suggest a neutral effect of CLA.

Cooper et al. (103) 2007 Cholesterol-fed Apo E-/-mice	0.5% (wt:wt) 1. LA 2. RA 3. 18:2 (n-6c, 8t) CLA 4. 1:1 CLA mixture	12	1. Compared to LA, CLA supplementation had no effect on lesion area in the aorta or aortic root cross-sections. 2. Plasma TG and cholesterol concentrations were higher in the 18:2 (n-6c, 8t) CLA group than all other treatment group.	1. CLA does not affect atherosclerosis 2. 18:2 (n-6c, 8t) CLA causes adverse changes in adipocyte function and plasma and liver lipid metabolism, which are partially ameliorated by the inclusion of RA
Dupasquier et al. (104) 2007 LDL receptor-deficient mice	1. regular fat 2. EA shortening 3. regular butter 4. VA butter 5. 2% cholesterol 6. 2% cholesterol + elaidic shortening 7. 2% cholesterol + regular	14	1. VA butter decreased serum cholesterol and TG. 2. VA butter was more effective when the diet contained higher cholesterol.	1. EA shortening diet was atherogenic. 2. VA butter diet was not atherogenic.

	butter 8. 2% cholesterol + VA butter			
Gavino et al. (105) 2000 Hamsters fed hydrogenated coconut oil and cholesterol	1. 1% CLA 2. 0.2% RA 3. 0.2% LA	6	1. CLA mixture led to lower plasma TC and TG. 2. No differences between RA and LA.	Effects of CLA mixture on CVD risk factors were greater than RA alone.
Jacome-Sosa et al. (106) 2010 JCR:LA-cp rats	1. VA+CLA 2. RA 3. control	16	VA + CLA and RA decreased TC, LDL-C, and TG.	VA + CLA had beneficial effects on lipids and lipoproteins.
Kritchevsky et al. (107) 2004	0.5% 1. 18:2 (n7-t, 9c) CLA 2. 18:2 (n-6c, 8t) CLA 3. RA + C18:2 (n-6c, 8t) CLA	12	1. Both 18:2 (n-7t, 9c) CLA and mixture decreased atherosclerotic lesions in arch and thoracic area of the aorta.	CLA improved atherosclerotic lesions; efficacy was higher in high-cholesterol diet.

Rabbits with atheromatous Lesions			2. 18:2 (n-6c, 8t) CLA only reduced lesions in the aortic arch.	
LeDoux et al. (108) 2007 Cholesterol-fed hamsters	One of 3 butters: 1. 20% standard butter 2. 5% standard butter+15% VA/CLA butter 3. 15% standard butter+5% iTFA	4	VA/CLA butter decreased plasma cholesterol, VLDL-C, and ratio of (VLDL-C+LDL-C): HDL-C.	VA/CLA butter improved plasma lipoprotein profile and reduced risk of atherosclerosis
Lock et al. (109) 2005 Cholesterol-fed hamsters	1. 20% standard butter 2. 5% standard butter+15% VA/CLA butter 3. 15% standard butter+5% iTFA	4	VA/CLA butter decreased plasma cholesterol, VLDL-C, and ratio of (VLDL-C + LDL-C): HDL-C.	VA/CLA butter improved plasma lipoprotein profile and reduced risk of atherosclerosis.
Meijer et al. (110)	1. Medium-chain fatty acids (8:0+10:0)	5	1. VA decreased LDL-C and TG compared to SFA.	1. Both EA and VA lowered CVD risk factors 2. EA is not more harmful than VA.

2001 Cholesterol-fed hamsters	2. SFA (16:0) 3. MUFA (18:1 (n-9c)) 4. EA 5. VA		2. No difference in LDL-C, VLDL-C, TG, or TC compared to MUFA, EA, or MCFA. 3. VA increased LDL-C:HDL-C ratio compared to EA.	
Mitchell et al. (111) 2005 High-fat, high-cholesterol–fed hamsters	1% wt:wt 1. RA 2. 18:2 (n-6c, 8t) CLA 3. LA	12	1. RA decreased non-HDL-C/HDL-C and aortic fatty streak lesion. 2. No effect of RA on plasma TG and LDL-C.	Individual CLA isomers beneficially affect lipoprotein profile and reduce atherosclerotic lesion development, but not different than LA.
Nestel et al. (112) 2006 ApoE-/-mice	1. RA 2. Normal diet	20	1. RA decreased TG and increased HDL-C. 2. No differences in aortic arch atherosclerosis between RA and control.	1. RA did not to reduce the severity of aortic atherosclerosis. 2. RA reduced TG and increased HDL-c.

Rice et al. (113) 2010 Cholesterol-fed guinea pigs	1. iTFA 2. rTFA 3. coconut oil, 4. soybean oil	8 or 12	3. Compared with soybean oil group, rTFA and iTFA groups had higher TC, LDL-C, and HDL-C. 4. No differences between rTFA and iTFA for plasma TC, LDL-C, HDL-C, or TG. 5. Total and small HDL particles were significantly higher in rTFA group vs. iTFA group.	At higher doses, iTFA and rTFA have similar effects on lipoprotein profile.
Roy et al. (114) 2007 Male New Zealand white rabbits	One of 3 butters: 1. 18:1 (n-8t) (11.8%) 2. VA (7%) +CLA (2.6%), 3. control diet low in all 3 fatty acids but with high-fat cholesterol	12	1. 18:1 (n-8t) raised VLDL-C more than the other butters. 2. 18:1 (n-8t) raised TC, LDL-C, non-HDL-C/HDL-C, and aortic lipid deposition vs. VA + CLA butter. 3. VA+CLA decreased HDL and increased liver TG vs. other butters.	VA had a neutral effect on plasma lipoprotein profile and tended to decrease atherosclerotic lesion development in rabbits.

Toomey et al. (115) 2003 Apo E-/-mice	1. RA 2. COX-1 inhibitor	16	RA decreased atherosclerotic lesion development and induced aortic lesion regression.	RA decreases atherosclerosis
Tyburczy et al. (116) 2009 Cholesterol-fed hamsters	1. Control Western diet 2. partially hydrogenated vegetable oil 3. VA 4. elaidic acid	4	Vaccenic and elaidic acid decreased plasma TC: HDL-C and non-HDL-C: HDL-C ratios, while partially hydrogenated vegetable oil increased these.	Adverse effect on lipoproteins of partially hydrogenated vegetable oil is independent of vaccenic and elaidic acid
Valeille et al. (117) 2004 Cholesterol-fed hamsters	1. Control diet 2. 0.6% RA 3. 1.2% RA 4. 1.2% RA +1.2% fish oil 5. control diet + 1.2% fish oil	8	RA increased LDL-receptor and scavenger receptor type B^{-1}.	Part of the beneficial effects was boosted by fish oil.

Valeille et al. (118) 2005 Cholesterol-fed hamsters	20% butter with 1. Nil added 2. 1% RA rich oil 3. 1% fish oil	12	RA 1. had lowest aortic lipid deposition 2. reduced plasma non-HDL/HDL. 3. improved anti-oxidized LDL paraoxonase activity. 4. down regulated expression of inflammatory related genes (TNF-a, IL-1b, COX-2.)	RA reduced atherogenic process in hyperlipidaemia hamsters.
Wang et al. (119) 2008 Lean and obese JCR:LA-cp rats	1. VA 2. control	3	1. VA decreased in TG in obese rats. 2. VA decreased IL-10 in lean and obese rats.	VA had significant hypo-triglyceridaemic effects in dyslipidaemia obese rats
Wang et al. (120) 2009	1. VA 2. control	16	1. VA decreased fasting plasma TC, LDL-C, TG, and hepatic fatty acid synthesis. 2. VA decreased postprandial TG and Apo lipoprotein. B.	Chronic VA supplementation significantly improved dyslipidaemia in both the fasted and postprandial state.

Obese JCR:LA-cp rats				
Wilson et al. (121) 2006 Cholesterol-fed hamsters	0.5% wt:wt 1. RA 2. 18:2 (n6c, 8t) CLA 3. LA	12	RA decreased plasma TC, HDL-C, non-HDL-C, TG, and cholesterol accumulation in aortic arch.	RA improved plasma lipoprotein profile and atherogenesis.

Rumenic acid (RA) is C18:2 (n-9t,11c) CLA; Vaccenic acid (VA); Conjugated linoleic acid (CLA); Elaidic acid (EA); Saturated fatty acid (SFA); Monounsaturated fatty acids (MUFA); Poly unsaturated fatty acids (PUFA); Linoleic acid (LA)

Human Studies

Effects of ruminant trans fatty acids on surrogate markers of cardiovascular disease

Studies have shown that all TFA, particularly iTFA, have adverse effects on lipids, (25, 26, 122, 123), inflammation (28-30) cell wall fluidity, and endothelial function. (28, 32, 124) Metabolic studies and meta-analysis show a clear and consistent association between increased overall TFA intake and reduced HDL-c, (25, 26, 122) increased LDL-c (25, 26, 122, 123, 125) and increased triglycerides. (24)These studies have also shown that TFA increase lipoprotein (a) (125, 126) and reduce the particle size of LDL-c, (125) both of which contribute to an increased risk of cardiovascular disease.

Data on the effects of rTFA on plasma lipoproteins in humans are limited with small trials assessing the possible differences between iTFA and rTFA (Table 4). (33, 79) One study found adverse effects of high intakes, but not of low intakes of rTFA. (127) Another study suggested that rTFA produce higher LDL and HDL cholesterol levels than iTFA in women, but not in men. (128) One review on the effects of rTFA on LDL and HDL-c has concluded that published data suggest that all TFA raise the ratio of plasma LDL to HDL-c. (53)

These experimental studies suggest that the dose of TFA matters. At low doses, rTFA has little observed effects, but at very large doses, may have negative effects on the lipid profile. This limits the ability to apply results of these feeding studies to the ‘real world’ for two reasons; the doses used in experimental models are much higher than can be consumed by the population and effects of rTFA may be ameliorated by other elements in dairy food.

Table 4: Effects of ruminant trans fatty acids on surrogate markers of cardiovascular disease in humans.

Study **Year Published** **Population**	**Design**	**Numbers**	**Intervention**	**Length of intervention (weeks)**	**Comments**	**Result**
Chardigny et al. (128) 2008 Healthy French men and women.	Randomised, double-blind, crossover	40	Food items containing (11–12 g/d, w 5% of daily energy). 1. rTFA 2. iTFA	3	1. Differences in MUFA and SFA between treatments may have confounded results. 2. Basal diets not controlled 3. High amount of rTFA feed.	rTFA diet significantly increased LDL-C and HDL-C vs. iTFA diet, in women only.
Kuhnt et al. (80) 2007 Healthy German	Randomised, parallel	24	1. 18:1 (n-7t) and 18:1 (n-6t) in equal amounts 2. Oil without TFA or CLA.	6	Basal diets were not controlled.	No effect of 18:1 supplementation on inflammatory markers (IL-6, IL-8, TNFα) or immune function.

men and women.						
Motard-Belanger et al. (127) 2008 Healthy Canadian men.	Randomised, double-blind, crossover	48	Four isocaloric diets: 1. high in rTFA (10.2 g) 2. moderate in rTFA (4.2 g) 3. high in iTFA (10.2 g) 4. control diet low in TFA from any source (2.2 g)	4	Controlled-feeding.	1. LDL-C significantly higher after high-rTFA diet vs. control or moderate-rTFA diet. 2. HDL-C significantly lower after high-rTFA vs. moderate-rTFA diet. 3. No significant differences between moderate rTFA diet and control
Naumann et al. (94) 2006 Dutch men and	Parallel	87	Dairy product with 3g: 1. 18:2 (n-9c, 11t) CLA. 2. 18:2 (n-6c, 8t) CLA. 3. no CLA.	13		No effects of either CLA on lipoproteins.

women with LDL phenotype B.						
Riserus et al. (90) 2004 Obese Swedish men.	Randomised, double-blind, placebo-controlled parallel	25	1. 18:2 (n-9c,11t) CLA (3g) 2. olive oil (control)	12	18:2 (n-9c, 11) CLA increased urinary 8-iso-PG F2a.	No effects on lipids and lipoproteins.
Sluijs et al. (97) 2010 Overweight and obese Dutch men.	Randomised, double-blind, placebo-controlled parallel	401	1. 4 g/d CLA (2.5 g/d 18:2 (n-9c, 11t) CLA+ 0.6 g/d 18:2 (n-6c, 8t) CLA) 2. placebo	26		No effects on lipids and lipoproteins.
Tardy et al. (129) 2009	Randomised, double-blind, parallel	63	1. low-TFA (0.54 g/d) 2. rTFA (4.86 g/d) 3. iTFA (5.58 g/d1).	4	Fatty acid composition of diets was not matched.	No effects of rTFA blend (enriched in VA) on lipids and lipoproteins.

Overweight French women.						
Tholstrup et al. (79) 2006 Healthy young Danish men	Randomised, double-blind, parallel	42	Butter 1. high in VA (3.6 g/d) 2. low in VA	5	1. Differences in MUFA and SFA between treatments may have confounded results 2. Basal diets were not controlled.	1. VA butter decreased plasma TC and HDL-C vs. control. 2. No differences in TC/HDL-C.
Tricon et al. (130) 2006 Healthy British men	Randomised double-blind, crossover	49	3 doses of highly enriched 1. 18:2 (n-9c,11t) CLA (0.59, 1.19, and 2.38 g/d) 2. 18:2 (n-6c, 8t) CLA (0.63, 1.26, and 2.52	8		1. 18:2 (n-9c, 11t) CLA significantly decreased TC: HDL-C ratio. 2. 18:2 (n-6c, 8t) CLA significantly increased TC: HDL-C ratio, LDL-C: HDL-

			g/d).			C ratio, TG.
Wanders et al. (131) 2010 Healthy Dutch men and women	Randomised, crossover	61	Isocaloric diets with 7% of energy (w20 g/d) from 1. Oleic acid 2. iTFA, 3. mixture of 80% 18:2 (n-9c, 11t) CLA+ 20% 18:2 (n6c, 8t) CLA	3	1. Controlled-feeding 2. High amount of rTFA was fed.	Compared to oleic acid diet, TC: HDL-C ratio was significantly higher after iTFA and CLA diets.

Observational studies

Observational studies evaluating the association with rTFA levels and CVD are presented in Table 5. Observational studies are limited because they are confounded by many factors. For example, dairy intake is associated with higher social and educational levels. (132, 133) It is also impossible to separate the effects of rTFA and those of other components in dairy food such as SFA, unsaturated fatty acids, protein and calcium.

Observational studies (20-22, 39, 134) that estimate TFA consumption from industrial and ruminant sources; have not found an adverse effect of rTFA in the low amounts usually consumed. This is thought to be partly accounted for by in vivo mammalian conversion of vaccenic acid to CLA which is thought to be beneficial to health. (33, 135, 136) Data from a Norwegian population study which followed more than 71,000 people for around 25 years linked higher intake of rTFA to greater risk of death from coronary heart disease and CVD (P=0.05 to P=0.002 for trend). (65) In this population, intake of iTFA is low with higher rTFA intake. A Danish study (137) showed no significant associations between TFA with abdominal fatness, inflammatory markers, blood lipids, blood pressure and insulin homeostasis. Norwegian and Danish populations are similar to New Zealand, (138) with a low intake of iTFA intake and a relatively high intake of rTFA.

No observational study has shown effects of rTFA on specific components of atherogenesis, such as endothelial dysfunction, inflammation or lipoproteins. Studies have assessed effects of total TFA intake on inflammation and lipids, but most TFA was from industrial sources. The American study of over 700 nurses (29) showed that those in the highest quartile of TFA consumption had blood levels of the inflammatory marker CRP that were 73% higher than those in the lowest quartile. Other studies have also linked significant intake of TFA with increased circulating concentrations of inflammatory molecules such as interleukin-6, tumour necrosis factor-alpha, CRP, and monocyte chemo-attractant protein-1. (27) Others show association with increased levels of several markers of endothelial dysfunction, including soluble intercellular adhesion molecule 1, soluble vascular-cell adhesion molecule 1, and E-selectin. (32) A recent comprehensive review (139) has not found a significant association of either rTFA or iTFA intake with risk of CVD (risk ratio (RR) =0.92 (0.76-1.11); P=0.36) and (RR=1.21 (0.97-1.50); P=0.09) respectively. This review

suggests that it is impossible to know whether the source of TFA is important and that the lack of evidence about effects of rTFA on health may be due to relatively low levels of intake.

Table 5: The association of intake of ruminant trans fatty acids and cardiovascular disease. Results are relative risk (95% confidence interval).

Study (Year) Population	**Design**	**Number**	**Assessment of intake**	**Endpoint**	**Result**	**Conclusion**
Ascherio et al. (140) 1994 American Men and women with first AMI.	Case-control	239 cases/282 controls	Absolute and energy adjusted intake of rTFA (FFQ)	Risk of first AMI	Absolute rTFA: RR = 1.23 (0.60 to 2.50, p = 0.09).	1. Trend for a positive association between absolute intake of rTFA and risk of AMI. 2. No association between energy adjusted intake of rTFA and risk of AMI.
Bolton-Smith et al. (141) 1996 Scottish	Cross-sectional	10 359	Energy-adjusted intake of rTFA and iTFA (FFQ)	Risk of CVD	Men: RR = 0.65 (0.41 to 1.04, p = 0.02). Women: RR = 0.96 (0.58 to 1.59; p 0.78).	1. Inverse association between rTFA and CHD in men. 2. No association between rTFA and CVD among women.

women and men						
Jakobsen et al. (134) 2008 Danish adults	Prospective	3 686	Absolute and energy adjusted dietary intake of rTFA(7-d weighed food records, dietary interviews)	Risk of CVD	Absolute rTFA: RR = 0.97 (0.91to 1.04). Energy-adjusted rTFA: RR = 1.05 (0.92 to1.19).	1. No significant association between rTFA and CVD. 2. Trend for an inverse association among women.
Oomen et al. (19) 2001 64–84 y Dutch men	Prospective	667	Energy-adjusted dietary intake of rTFA (dietary surveys)	Risk of CVD	RR 1.17 (0.69 to1.98).	1. No significant association between rTFA and CVD. 2. Trend for a direct association.
Pietinen et al.	Prospective	21 930	Energy-	Risk of	RR = 0.83 (0.62 to 1.11; p-= 0.035).	1. Significant positive association

(142) 1997 Finnish male smokers			adjusted dietary intake of rTFA (FFQ)	coronary death		between the intake of trans-fatty acids and the risk of coronary death. 2. No association between intakes of saturated or cis-monounsaturated fatty acids, linoleic or linolenic acid, or dietary cholesterol and the risk of coronary death.
Smit et al. (143) 2010 Costa Ricans with first AMI.	Case-control	1 813 in each arm	Concentration of 18:2 (n-7c,9t) CLA in adipose tissue	Risk of MI	RR = 0.51 (0.36 to 0.71; p= 0.0001).	Adipose 18:2 (n-7c,9t) CLA associated with lower risk of MI.
Sun et al. (22) 2007 American	Case-control	166 cases/327 controls	Concentration of VA in erythrocytes	Risk of CVD	1. Total 18:1(n-7t) associated with increased risk of CHD; P-trend, 0.01. 2. Association for individual 18:1t isomers were similar to total	1. Concentration of VA in erythrocytes was higher. 2. Direct association between VA and CVD.

women with CHD.					18:1t.	
Willett et al. (20) 1993 American women	Prospective	69 181	Energy-adjusted dietary intake of rTFA (FFQ)	Risk of CVD	RR = 0.59 (0.30 to 1.17; p=0.230).	1. No significant association between rTFA and CHD. 2. Trend for an inverse association.

AMI- acute myocardial infarction, FFQ- food frequency questionnaire, RR- relative risk.

Randomised studies

No randomised studies have been reported the effects of dairy food or rTFA alone on hard endpoints like major adverse cardiac events. The PREDIMED study (144) and the Lyon Heart study, (145) are the only randomised controlled studies that show a dietary intervention that is beneficial for cardiovascular endpoints. These assessed the Mediterranean diet that involved multiple interventions, including increased fruit, vegetables, nuts and olive oil intake. A small component of the diet was intake of low-fat dairy food. It is impossible to determine from this study whether dairy food has any effect on cardiovascular disease.

Conclusion

It is difficult to assess the specific effects of rTFA on CVD. This is partly the result of other fatty acids found in dairy such as SFA, mono-unsaturated fatty acids (MUFA) and poly-unsaturated fatty acids (PUFA) that are thought to have varied effects on CVD. Separating the effects of iTFA and rTFA on CVD is complicated by the fact that both contain similar isomers of TFA, and studies were done when the ability to discriminate between these isomers, especially at low concentrations, is likely to have been limited by available computer software at the time of testing.

These studies suggest that a significant intake of rTFA may be harmful. However, rTFA makes up only a small percentage of the fat in dairy food, and it is virtually impossible to consume enough from dairy food to replicate these experiments. Effects could possibly accumulate over time, especially in countries like New Zealand, where feeding practices increase rTFA in dairy food, and where the population consumes significant servings of dairy food.

Section 3: Dairy foods

Archaeological evidence suggests that dairy food has been a part of the human diet for a relatively short period with the development of the lactase enzyme in Northern Europe within the last 7 000 years. (146) This allowed for the inclusion of a rich source of protein and fat in the diet and may have conferred a survival or breeding advantage to this population. The long term health effects of dairy food on humans are unlikely to be answered in long term clinical studies. The best evidence at present comes from cohort studies.

Raw milk contains approximately 87% water, 4.6% lactose, 3.4% protein, 4.2% fat, 0.8% minerals and 0.1% vitamins. (4) It is one of the most complex foods with components that could theoretically, both increase and decrease cardiovascular risk. Milk fat includes a series of complex lipids such as phospholipids, gangliosides and cholesterol that are organized into a trilaminar structure known as milk fat globule membrane (MFGM). (4) MFGM compounds have been suggested as the main components in promoting beneficial effects on cardiovascular disease and diabetes. Calcium is thought to increase thermogenesis and may improve weight loss, (147, 148) however, calcium supplements may be associated with increase cardiovascular events. (149) SFA and TFA may increase CVD, (18, 56, 140) whilst unsaturated fats may reduce CVD. (150) The protein in dairy may increase satiety (151) and promote weight loss. (152)

The most common dietary SFA are palmitic (16:0) and stearic acids (18:0). These are mainly present in animal fat (~a half and a quarter of total SFA in beef, respectively), and to a lesser extent in plant fat. They are also the major SFA in human plasma and tissues. (153) Dairy fat is unique in that it contains a broad spectrum of short-chain (SC), medium-chain (MC) and long-chain (LC) SFA, as well as monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA). (154) The possible interaction between all of the fatty acids makes it virtually impossible to draw conclusions about the effects of one component such as TFA on cardiometabolic risk.

Hypothesised effects of each component of dairy fats are summarised in table 6. SFA is thought to have effects on CVD through multiple pathways including effects on insulin resistance, cellular function and circulating lipids.

Effects on insulin resistance and the pancreas

SFA particularly palmitic acid are thought to promote insulin resistance through a number of mechanisms:

1. Decreasing phosphorylation of the insulin receptor and insulin receptor substrate-1, thereby affecting cell signalling (in rats). (155)
2. Decreasing oxidation of fatty acids and glucose in muscle cells. (156)
3. Decreasing adiponectin production from visceral fat. (156)
4. Stimulating β-cells in a glucose-like fashion. (157)
5. Direct lipotoxic effects leading to inflammation and to β-cell apoptosis. (158, 159)
6. Decreased expression of the insulin gene. (160)
7. Direct lipotoxic effects on beta cell dysfunction. (161)

Cellular Effects

Dietary SFA can decrease membrane fluidity (162) in the cells that can affect an array of metabolic pathways and in doing so induce a pro-inflammatory gene expression pattern in adipose tissue (163) and apoptosis. (164)

Circulating Lipids

High SFA intake may increase levels of very low density cholesterol (VLDL-c) by stimulating peroxisome-proliferator-activated receptor-γ co-activator-1β and stimulating lipogenic gene expression. (165) In the human liver, Stearoyl-CoA desaturase-1 can contribute to the synthesis of cholesteryl oleate from dietary or endogenous SFA including 16:0 and 18:0 and this is thought to promote atherosclerosis via formation of foam cells. (166)

SFA have traditionally been considered the most harmful fats for heart heath. Comparison of plasma fatty acid levels (%) in different countries world-wide is presented in Table 7. SFA levels are similar for those with and without CVD, supporting the conclusion of a recent meta-analysis that suggests that trans but not saturated fats are a risk factor for heart disease. (167) However data from different studies need to be interpreted with caution; methods of testing for plasma fatty acids are not

consistent and many studies do not list all types of fats. An attempt to address this issue with testing is made by converting all fats in to a percentage of total fats. This table suggests that plasma SFA and TFA levels are marginally higher in the countries with high dairy intakes, such as the Netherlands.

Other components in dairy food that are thought to have some effects on cardiometabolic risk factors

1. Calcium has been shown to increase thermogenesis (168) and is thought to promote weight loss. (169) However, whilst calcium in the diet is thought to have no effect on cardiovascular events, (170) calcium supplementation has been shown to increase cardiovascular events. (149)
2. Magnesium is thought to improve blood pressure, (171) and reduce insulin resistance. (172)
3. Protein in dairy is thought to promote satiety and weight loss. (151)

Table 6: Possible effects of various fats from dairy food on cardio- metabolic risk.

	Common examples	**Adverse effect**	**Positive effect**
Short-chain saturated fats 2-5 carbon atoms	• Formic acid • Acetic acid • Propionic acid • Isobutyric acid (2-methylpropanoic acid) • Butyric acid • Isovaleric acid (3-methylbutanoic acid) • Valeric acid (pentanoic acid)		• Stimulate glucagon-like peptide-1 secretion- improve insulin sensitivity. • Reduce circulating levels free fatty acids.
Medium-chain saturated fats 6-12 carbon atoms	• Caproic acid • Enanthic acid • Caprylic acid • Pelargonic • Capric acid • Undecylic acid • Lauric acid	• Lauric acid increases LDL-c.	• Increase thermogenesis. • Reduce LDL-c. • Reduce triglyceride. • Lauric acid increases HDL-c.

Long-chain saturated fats 13-20carbon atoms	• Myristic acid (C14:0) • Pentadecanoic acid (C15:0) • Palmitic acid (C16:0) • Margaric acid (C17:0) • Stearic acid (C18:0) • Arachidic acid (C20:0)	• increase LDL –c and increase cardiovascular ris.* • increase fibrinogen. • promotes insulin resistance by decreasing phosphorylation of the insulin receptor and insulin receptor substrate-1.	• Stearic acid has neutral effects on LDL-c. • Little data on effects of pentadecanoic or margaric acid.
Very long-chain saturated fats > 21 carbon atoms	• Behenic acid • Lignoceric acid	• Benhenic acid increased TC and LDL-c.(173)	
Vaccenic acid		• At high doses may increase LDL-c.(136)	• Metabolized to CLA in humans which may be beneficial. (86)
Palmitelaidic acid			• Associated with reduced insulin resistance.* (99)
Trans-9-Heptadecenoic acid		• No data on effects.	• No data on effects.

Conjugated linoleic acid		• In overweight men may increase inflammation and insulin resistance. (174)	• Reduces insulin resistance. (136) • Reduces weight. (89) • Increase HDL-c and reduces LDL-c. (78)
Mono and polyunsaturated fats			• Reduce cardiovascular disease when saturated fats replaced by PUFA.

* Observational studies

Table 7: Plasma fatty acids levels world-wide as percentage of total plasma fat.

Study name	Country	Year	Number of patients	Mean age (SD)	CVD	SFA	MUFA	Omega 3 PUFA	Omega 6 PUFA	Omega 9 PUFA	PUFA	TFA
Rotterdam (175)	Netherlands	1991	157	51 (8)	Yes	46	11	ND	ND	ND	43	0.35
EPIC–Norfolk (CHD) (176)	United Kingdom	1993-1997	2 434	65 (8)	Yes	40	12	8	40	ND	50	0.10
Finland (177)	Finland	1985	20	42 (5)	No	43	18	ND	ND	ND	39	ND
Italy (177)	Italy	1985	21	43 (0.4)	No	44	20	ND	ND	ND	36	ND
Cardiovascular Health Study (177)	America	1985	20	44 (0.3)	No	41	18	ND	ND	ND	41	2.52
Pennsylvania (178)	America	2000	10	ND	No	41	12	6	38	ND	45	ND
EPIC-Norfolk (176)	United Kingdom	1993-1997	4930	60 (8)	No	40	12.3	8	ND	ND	48	0.10
JELIS Study (179)	Japan	2010	15534	61 (9)	No	32	25	10	ND	ND	43	ND
Zutphen Elderly Study (19)	Netherlands	1990	667	71(5)	No	45	ND	ND	ND	ND	38	4.31

ND- not described

Observational studies in humans

Effects on dairy food on cardiometabolic risk factors in observational studies are presented in Table 8. These studies suggest that dairy food intake is associated with less weight gain, reduced risk of diabetes and reduced insulin resistance. Some studies show an association with lower blood pressure, but this is not consistent. In total, studies suggest a reduction in the metabolic syndrome in the subjects with the highest milk consumption (RR 0.74; 95% CI 0.64 to 0.84).

These observational studies have influenced food guidelines; the American Diabetes Association labels dairy food a 'superfood' (6) because of its effects on insulin resistance and the American guidelines (5) recommend 3-4 servings of dairy a day. However other food guidelines (7, 180) advocate the intake of low-fat dairy food to avoid SFA and TFA. These guidelines are somewhat influenced by the observational studies suggesting the benefit of dairy food for diabetes and osteoporosis. The Harvard University School of Public Health advocates avoidance of all dairy food, because there is no evidence of benefit for either cardiovascular health or osteoporosis. (181)

Clearer evidence is needed to better inform dietary guidelines. A meta-analysis of randomised studies was therefore performed and is described in Chapter 6.

Table 8: Effects on dairy food on cardiometabolic risk factors. Results from observational studies.

Study **Year published** **Design**	**Population** **Sample size (% female)** **Age (mean ± SD**	**Length of follow up (months)** **Method of follow up**	**Effects on lipids**	**Effects on glucose/insulin/ diabetes or insulin resistance**	**Effects on blood pressure**	**Effects on weight**	**Effects on inflammatory markers**	**Conclusion**
Alonso (182) 2005 Prospective cohort	Healthy Spanish university graduates (SUN cohort) 5 880 (61.2) 37 ±10.7 years	27 Mailed questionnaires	NA	NA	Significantly reduced BP in highest quintile dairy intake even after adjustment for main risks HR: 0.44; 95% CI: 0.25, 0.77.	No change	NA	Women, physically active participants, and younger persons had a higher total dairy and low-fat dairy consumption. Low-fat dairy consumption was directly associated with fruit and

								vegetable consumption and with potassium and fibre intake and was inversely associated with alcohol and saturated fatty acid intake.
Azadbakht (183) 2005 Cross-sectional study	Iranian adults (Tehran Lipid and Glucose Study (TLGS) cohort) 827(57%) 38 ± 12 years	NA FFQ and clinic visits	Increase in HDL-c for highest quartile of compared to lowest quartile– p for trend < 0.03.	No change	Decrease for highest quartile compared to lowest quartile - p for trend <0.03.	Significant decrease in weight for highest quartile of compared to lowest quartile– p for trend <	NA	

						0.01.		
Beydoun (184) 2008 Prospective cross sectional surveys	American (NHANES cohort) 17 061 (64%) 25-74 years	NA FFQ and clinic visits	No change when accounting for calories etc.	No change when accounting for calories etc.	No change when accounting for calories etc.	No change when accounting for calories etc.	NA	Dairy consumption is significantly higher among subjects with more than a high school education, and significantly lower among women and minority groups. Large ethnic disparities exist for intakes of dairy and calcium, and for all metabolic outcomes.

Brooks (185) 2006 Cross sectional	American young adults (Bogalusa Heart Study cohort) 1306(61%) 29.7 ± 5.2 years	NA FFQ and clinic visit	NA	NA	NA	No difference	NA	
Choi (186) 2005 Prospective	Male American Doctors(Health Professionals Follow-up Study cohort) 41 254(0%) 53 ± 9 years	144 FFQ and self-reported measurement and events	NA	Relative risk for type 2 diabetes in men in the top quintile of dairy intake was 0.77 (95% confidence interval [CI], 0.62-0.95; P for trend, .003.	NA	NA	NA	

Elwood (187) 2007 Prospective	England (Caerphilly cohort) 2375 (0%) 45–59 years	240 FFQ and clinic visits (% yearly)	Odds of having the metabolic in the highest quartile relative to lowest quartile of dairy intake was 0.43 (0.20 to 0.95).					Did not report effects on individual components like BP, lipids and IR.
Engberink (188) 2008 Prospective cross-sectional	Healthy Dutch adults(Monitoring Project on Risk Factors for Chronic Diseases cohort 21 553 (55%) 42.3±11 years	NA FFQ and clinic visit	NA	NA	No relationship between intake of dairy and incident hypertension.	No difference.	NA	
Engberink (188)	Doetinchem subgroup from Monitoring Project	60 FFQ and clinic	NA	NA	No relationship between	No difference	NA	

2008 Prospective cohort	on Risk Factors for Chronic Diseases cohort 3 454 (55%) 49.5 ± 9.6 years	visit			intake of dairy and incident hypertension.			
Fumeron (189) 2011 Prospective	French adults (DESIR study cohort) 5 212(51%) 46 ± 5 years	108 FFQ and clinic visits	Trend to decreased diastolic BP with increased dairy	No difference when corrected for BMI	No difference.	Increased dairy food associated with less weight gain.	NA	Reduced metabolic syndrome with increased dairy food 0.86 (0.78–0.95); P = 0.002.
Liu (190) 2006	American woman (The Women's	120 FFQ and clinic	NA	Higher intake of dairy food associated with	NA	NA	NA	Women with higher dairy food intake had healthier

Randomised, double-blind, placebo-controlled trial-low-dose aspirin and vitamin E in the primary prevention of cardiovascular disease and cancer	Health Study cohort) 37 183 (100%) 55 ± 7 years	visits		reduced incidence of diabetes – adjusted (RR 0.79 [0.67– 0.94]; P for trend 0.007).				lifestyle patterns especially those consuming low fat dairy food.
Lutsey (191) 2008 Prospective	Healthy American adults (Atherosclerosis Risk in Communities (ARIC) cohort)	108 FFQ and clinic visits	Odds of having the metabolic in the highest quartile relative to the lowest quartile of dairy food intake was 0.87 (0.77–0.98).					Did not report effects on individual components like BP lipids and is IR.

	9514(56%)							
Margolis (192) 2011 Prospective cohort study	American Woman (Woman's Health Initiative cohort) 82 076(100%) 60 ± 10years	64 FFQ	NA	NA	NA	NA	Low-fat dairy food consumption inversely associated with the risk of type 2 diabetes. RR 0.5–0.6 in the upper quintiles compared with the lowest quintile.	
Mennen (193) 2000	French adults (DESIR study cohort)	NA FFQ and clinic visit	Metabolic syndrome reduced for those consuming >4 servings/day RR 0.63 (0.40, 0.99).					No assessment of foods like fruit and vegetables.

Cross sectional	5 212(51%) 46 ± 5 years							
Newby (194) 2004 Cross sectional	American adults (Baltimore Longitudinal Study of Aging cohort) 459 (49%) 58 ± 29 years	NA FFQ and clinic visit	NA	NA	NA	No difference.	NA	Low-fat dairy food intake associated with healthier eating patterns.
O'Neil (195) 2009 Cross-sectional	American Woman (Mothers with children in Head Start cohort) 609 (100%)	NA FFQ	NA	NA	NA	No difference when adjusted for income and diet.	NA	Those who consumed a higher amount of low-fat dairy products also had better overall diet quality.

	29.5 ± 0.24years							
Pereira (196) 2002 Prospective	American (CARDIA cohort) 3 157 (60%) 25.2 ± 0.5years	120 FFQ and clinic visits	Some decrease (p for trend <0.01).	Decreased by 72 (42-86) % for highest quintile compared to lowest.	Significant decrease for highest quintile compared to lowest quintile - p for trend. <0.01.	Significant decrease in obesity and overweight for highest quintile compared to lowest quintile– p for trend < 0.001).		Those with a higher dairy food intake were more likely to be female and not black population. No significant associations in those with BMI < 25 kgm2.
Poddar (197) 2009 Prospective	American adults 76(86%) 19.2 ± 0.2 years	8 FFQ and clinic visits	NA	NA	NA	Less weight gain in those who consumed more dairy	NA	Those who consumed a higher amount of low-fat dairy products had better diet quality.

						food.		
Snijder (198) 2008 Prospective cohort	Dutch adults (HOORN cohort) 1 124 (55%) 60.1 ± 6.6 years	76 FFQ and clinic visit	No significant change in LDL-c, HDL-c, TG.	No significant change in fasting and 2 hour glucose.	No change.	No change.	No change.	Relatively high dairy food intake compared to other studies (mean 5 servings per day).
Tsai (199) 2007 Cross sectional;	Elderly Taiwanese (Survey of Health and Living Status of the Elderly in Taiwan cohort) 4440(61%)	NA In home interviews	NA	NA	NA	Reduced BMI in regular dairy consumers with trend <0.01.	NA	Likely due to reverse bias.

	Age not described> 53 years							
Vergnaud (200) 2008 Prospective	Supplémentation en Vitamines et Minéraux Antioxydants (SU.VI.MAX) Study cohort 2267(45%) 51.2 ±4.3 years.	72 FFQ and clinic visits	NA	NA	NA	NA	NA	6-y changes in weight and WC only in men who were initially overweight.

Conclusion

In most countries, rTFA intake in humans is thought to be too low to have biological effects, and justifiably the focus is on reducing intake of iTFA. There is little evidence to suggest differential effects of rTFA and iTFA. Most of the evidence is based on animal experiments or human feeding studies. Animal studies of atherosclerosis are flawed; animals do not develop atherosclerotic lesions like humans. The fatty streaks that develop in the lines of mammals used for atherosclerosis models do not develop into the calcified plaque seen in humans. (201) Feeding studies do not replicate the 'real world'; doses used are not physiological, and time frames are usually too short to see long-term effects. They are also not able to account for interactions with other elements in the whole food.

Dairy food is highly complex with elements that could be both beneficial and harmful for cardiometabolic health. For example, experimental models suggest that certain components of CLA may be beneficial, but the interaction of CLA with saturated fats may negate effects. Observational studies suggest that dairy food intake is associated with reduced insulin resistance, lower blood pressure and less weight gain.

Assessing the effect of whole dairy foods on health is a more pragmatic approach; it ensures that all possible synergistic effects (202) are taken into account and may be more relevant to nutritional guidelines that recommend intake of food groups rather than specific nutrients

Chapter 2

Introduction

This chapter describes an observational study in New Zealand patients with significant CVD. The primary objective of the study was to find novel biomarkers (for example fatty acids) associated with clinical markers of CVD in New Zealand patients. Section 1 describes plasma fatty acid levels in this population and is titled 'Plasma fatty acids in New Zealanders with severe coronary artery disease'. Section 2 evaluates the association between rTFA and clinical markers of cardiovascular disease. This paper titled 'Trans-fatty acids in New Zealand patients with coronary artery disease' is published in the ***European Journal of Cardiovascular Prevention & Rehabilitation (****now the European J*ournal of Preventive Cardiology). (66) I believed it would be good to get follow up data on this cohort so have added Section 3 which briefly describes the association between fatty acids and mortality after 7.5 years of follow up. The paper titled 'There is no relationship with plasma fatty acid levels and mortality in New Zealanders with severe coronary artery disease' is published in the ***Journal of Nutritional Disorders & Therapy***. (203)

The study received funding from the Health Research Council of New Zealand and was approved by the Northern X Ethics Committee. All participants provided written informed consent.

Ethics approval: AKX/03/12/337

Section 1: Plasma fatty acid levels in New Zealanders with severe coronary artery disease.

Abstract

Background: This study describes plasma fatty acid levels in New Zealanders who have significant coronary artery disease.

Methods: Fasting plasma samples were taken in in 420 consecutive patients with angiographic diagnosis of severe coronary disease requiring coronary artery bypass surgery. Plasma levels of fatty acids were measured by gas chromatography-mass spectrometry.

Results: The mean age of participants was 68 (standard deviation 10) years and 83% were male. Saturated fats were 46.52 (standard deviation 1.17) %, unsaturated fats were 51.8 (standard deviation 1.31) %, trans fatty acids 1.07 (standard deviation 0.69) % of total fats.

The most common saturated fatty acids were palmitic acid 28.40 (standard deviation 1.60) % and stearic acid 14.42 (standard deviation 1.31) %. The most common unsaturated fat was linoleic acid 17.16 (standard deviation 2.71) %. The most common trans fatty acid was vaccenic acid 0.63 (standard deviation 0.50) %, and this correlated strongly with palmitelaidic acid ($r=0.94$, $p<0.0001$). Together these two trans fatty acids made up 79% of total plasma trans fatty acids.

Conclusion: Saturated and trans fatty acid levels are higher than expected from dietary modeling. More than three quarters of trans fatty acids is from dairy food and meat. Dietary modeling does not accurately reflect exposure to fatty acids in this population.

Introduction

Diet surveys suggest that the typical New Zealand diet comprises around 35% of total energy as fat (15% SFA; 12% MUFA; 7% PUFA and <1% as TFA). (204) New Zealand is relatively unique in the Western world, in that little processed food is consumed, but dairy food intake is high. (138) For this reason, the New Zealand Government has not prioritized legislation of TFA in food, (205) and the National Heart Foundation has not addressed TFA for fear it would detract from its message to reduce SFA from the diet. (205)

Meal diaries and food frequency questionnaires have been found to be unreliable in assessing dietary intake (206, 207) and it is now suggested that objective measurements of dietary intake be more widely used. One such measurement is plasma levels of fatty acids, which reflect dietary intake for the last 2-3 weeks. (177) In this study, plasma level of all fatty acids including TFA isomers was measured in New Zealand patients with severe symptomatic coronary artery disease.

Methods

Study population and clinical data

Four hundred and twenty two sequential patients with symptomatic severe coronary artery disease diagnosed by angiography were invited to participate in this observational cross sectional study from August 2004 to September 2006. Ethics approval was obtained from the Northern X Ethics Committee. All participants provided written informed consent after the diagnostic coronary angiogram.

Measurement of FA

Fasting blood samples were taken in ethylene-diamine-tetra acetic acid tubes. Plasma was separated and stored at -70^{o} Celsius until analysis. Plasma phospholipids analysis was performed at the Nutrition and Functional Food Science Laboratory, the University of Adelaide, Australia. Total lipids were extracted with AR Methanol and fatty acid methyl esters formed by transmethylation. (208) Serum phospholipid fatty acid composition was assessed by gas chromatography (Hewlett Packard 6890 Gas Chromatograph with an SGE BPX70 column and a Flame Ionisation Detector). Vaccenic

acid, elaidic acid, linoelaidic acid), palmitelaidic acid and total TFA were measured. Levels are expressed as the percentage of the g/100g of the plasma phospholipids acids.

Statistical Analysis

Results are presented as mean and standard deviation (SD) for population characteristics and median and interquartile range (IQR) for FA levels. The relationship between the isomers of TFA was assessed by Spearman correlation coefficients.

Results

Baseline characteristics for the 422 patients included in the study are presented in Table 9. The mean age was 68 (standard deviation (SD) 10) years and 83% were male. The majority of participants were of European descent (55%), with a smaller proportion Maori (14%), Pacific Islander (6%), South Asian 27 (7%) or other ethnicity 92 (23%). Cardiovascular risk factors included a history of present or past smoking (73%), hypertension (53%) and diabetes treated with pharmacotherapy (27%). The average body mass index was 28 (SD 4.7) kg/m2 and waist circumference 101 (SD 12.5) cm.

Table 9: Baseline characteristics of study population in the observational study. Results are number (%) or mean (standard deviation as appropriate).

No. of Subjects	422
Age	68 (±10)
Male	350 (83%)
Smoker	312 (74%)
Body mass index	28.0 (±4.7)
Waist circumference (cm)	100.5 (±12.5)
Diabetes	114 (27%)
Hypertension	287(53%)
Statin	248 (59%)
Beta blocker	241 (57%)
LDL cholesterol (mmol/l)	2.5 (±1.0)
HDL cholesterol (mmol/l)	1.3 (±0.4)

The median fat levels expressed as % total plasma phospholipid are displayed in Table 10. SFA were 46.58 (interquartile range (IQR) 45.83 to 47.25) %, unsaturated fats were 51.82 (IQR 50.54 to 53.012) %, TFA 0.84 (IQR 0.67 to 1.20) %.

The median plasma levels of the four trans fatty acids measured were as follows: palmitelaidic acid 0.081 (IQR 0.04 to 0.22) %, vaccenic acid 0.45 (IQR 0.35 to 0.72) %, elaidic acid 0.19 (IQR 0.13 to 0.25) % and linoelaidic acid 0.09 (IQR 0.07 to 0.11) %.

The most common SFA were palmitic acid 28.45 (IQR 27.33 to 29.36) % and stearic acid 14.37(IQR 13.53 to 15.28) %. The most common unsaturated fat was linoleic acid 16.93 (IQR 15.273 to 18.98) %.

Table 10: Plasma phospholipid levels in New Zealanders with coronary artery disease. The FA level is expressed as % total phospholipids and results are median and IQR.

	Median	**Interquartile range**
Total saturated fats	**46.58**	**45.83 to 47.25**
Caprylic acid (8:0)	0.00	0.00 to 0.00
Pelargonic acid (9.0)	0.00	0.00 to 0.00
Capric acid (10:0)	0.00	0.00 to 0.00
Undecylic acid (11:0)	0.00	0.00 to 0.00
Lauric acid (12:0)	0.00	0.00 to 0.01
Tridecylic acid (13:0)	0.00	0.00 to 0.00
Myristic acid (14:0)	0.21	0.18 to 0.26
Pentadecanoic acid (15:0)	0.19	0.16 to 0.22
Palmitic acid (16:0)	28.45	27.39 to 29.36
Margaric acid (16:0)	0.48	0.42 to 0.53
Stearic acid (18:0)	14.37	13.53 to 15.28
Arachidic acid (20:0)	0.50	0.43 to 0.59
Behenic acid (22:0)	0.49	0.41 to 0.56
Lignoceric acid (24:0)	0.59	0.51 to 0.67
Total trans fats	**0.84**	**0.67 to 1.20**
Palmitelaidic acid (16:1 (n-7t))	0.081	0.04 to 0.22
Elaidic acid (18:1 (n-9t))	0.19	0.13 to 0.25
Vaccenic acid (18:1 (n-7t))	0.45	0.35 to 0.72

Linoelaidic acid (18:2 (n-6t, 9t))	0.09	0.07 to 0.11
Conjugated Linoleic acid	0.02	0.01 to 0.03
Rumenic acid (18:2 (n-9c,11t))	0.026	0.018 to 0.028
Conjugated Linoleic acid (18:2 (n-10t, 12c))	0.00	0.00 to 0.00
Total monounsaturated fats	**12.87**	**11.95 to 13.86**
Undecenoic acid (11:1 (n-1c))	0.00	0.00 to 0.00
10Z-dodecenoic acid (12:1 (n-2c))	0.00	0.00 to 0.00
8-tridecenoic acid (13:1 (n-5c))	0.00	0.00 to 0.00
Myristoleic acid (14:1 (n-5c))	0.00	0.00 to 0.00
Pentadecenoic acid (15:1)	0.05	0.00 to 0.07
cis-10-Pentadecenoic acid (16:1 (n-6c))	0.15	0.13 to 0.181
Palmitoleic acid (16:1 (n-7c))	0.49	0.39 to 0.61
Heptadecenoic acid (17:1 (n-7c))	0.07	0.00 to 0.09
Oleic acid (18:1 (n-9c))	9.20	8.44 to 10.03
Methyl octadecenoate (18:1 (n-11c))	1.53	1.36 to 1.72
Methyl Petroselinate (C18:1 (n-6c))	0.00	0.00 to 0.00
10-nonadecenoic acid (19:1 (n-9c))	0.05	0.05 to 0.06
9-eicosenoic acid (20:1 (n-11c))	0.03	0.02 to 0.28
11-Docosenoic acid (22:1 (n-11c))	0.16	0.12 to 0.22
Erucic acid (22:1 (n-9c))	0.02	0.02 to 0.03
Nervonic acid (24:1 (n-9c))	0.74	0.62 to 0.91
Total Omega 3 fatty acids	**6.741**	**6.01 to 8.10**
Hexadecatrienoic acid (16:3 (n-3c,6c,12c))	0.04	0.03 to 0.26
Alpha-linolenic acid (18:3 (n-3c,6c,9c))	0.12	0.09 to 0.15

Stearidonic acid (18:4 (n-3c,6c,9c,12c))	0.00	0.00 to 0.00
Eicosatrienoic acid (20:3 (n-3c,6c,9c))	0.09	0.082 to 0.099
Timnodonic acid (20:5 (n-3c,6c,9c,12c,15c))	1.03	0.80 to 1.36
Clupanodonic acid (22:5 (n- 3c,6c,9c,12c,15c))	1.12	0.97 to 1.29
Cervonic acid (22:6 (n- 3c,6c,9c,12c,15c,18c))	4.29	3.62 to 5.17
Total omega 6 fats	**32.01**	**30.75 to 33.52**
Linoleic acid (18:2 (n-6c,9c))	16.93	15.27 to 18.97
Gamma-linolenic acid (18:3 (n-6c,9c,12c))	0.13	0.10 to 0.38
Eicosadienoic acid (20:2 (n-6c,9c))	0.30	0.26 to 0.34
Dihomo-gamma-linolenic acid (20:3 (n-6c,9c,12c))	3.36	2.86 to 3.98
Arachidonic acid (20:4 (n-6c,9c,12c,15c))	10.21	8.57 to 11.88
Docosadienoic acid (22:2 (n-6c,9c))	0.20	0.16 to 0.23
Adrenic acid (22:4 (n-6c,9c,12c,15c))	0.31	0.25 to 0.36
Osbond acid(22:5 (n-6c,9c,12c,15c,18c))	0.01	0.00 to 0.03
Total omega 7 fats	**2.03**	**1.81 to 2.28**
Total omega 9 fatty acids	**10.62**	**9.85 to 11.54**
Oleic acid (18:1 (n-9c))	0.05	0.04 to 0.06
Gondoic acid (20:1 (n-9c))	0.07	0.05 to 0.09
Mead acid (20:3 (n-9c,12c,15c))	0.20	0.17 to 0.26

There was a strong correlation between plasma levels of vaccenic acid and palmitelaidic acid (r=0.94, p<0.0001) (Figure 5), and between elaidic acid and linoelaidic acid (r=0.51, p<0.0001). In contrast, there was no correlation between plasma levels of palmitelaidic acid and elaidic acid (r=0.11, p=0.03) (Figure 6). Vaccenic acid and palmitelaidic acid made up 79% of total plasma TFA.

Figure 5: Correlation between plasma levels of vaccenic and palmitelaidic acids. The close correlation suggests that these TFA are derived from a similar or common dietary source

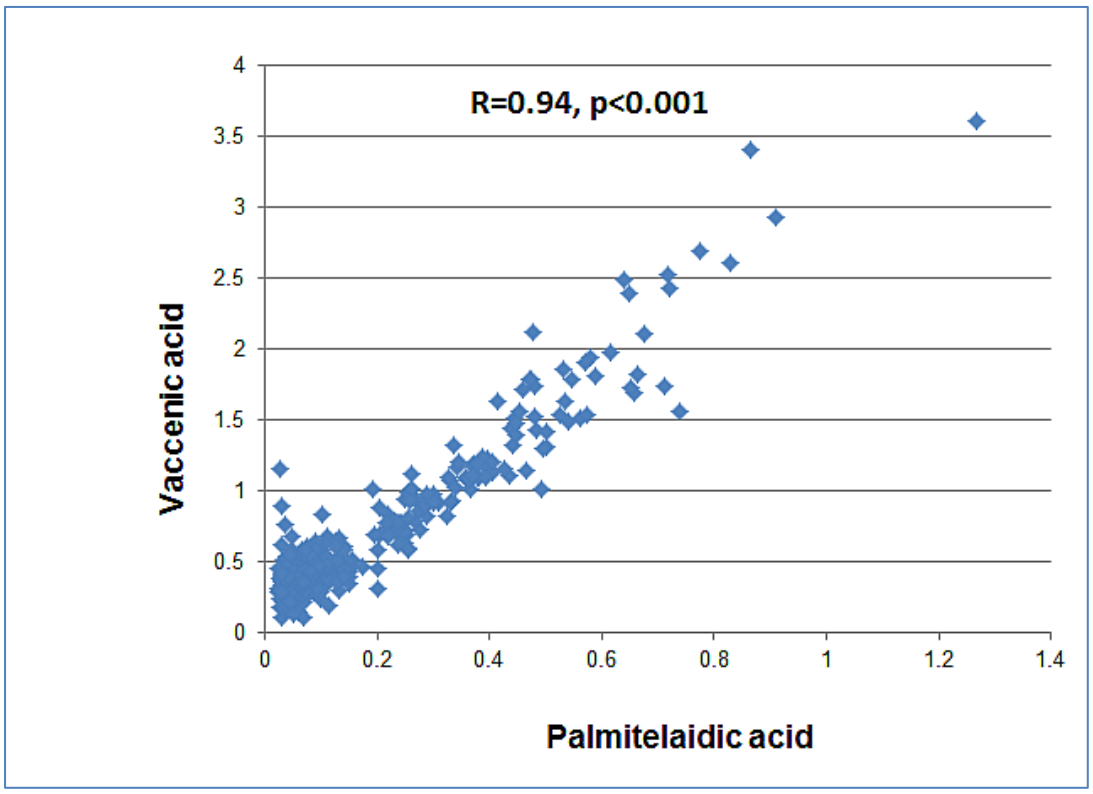
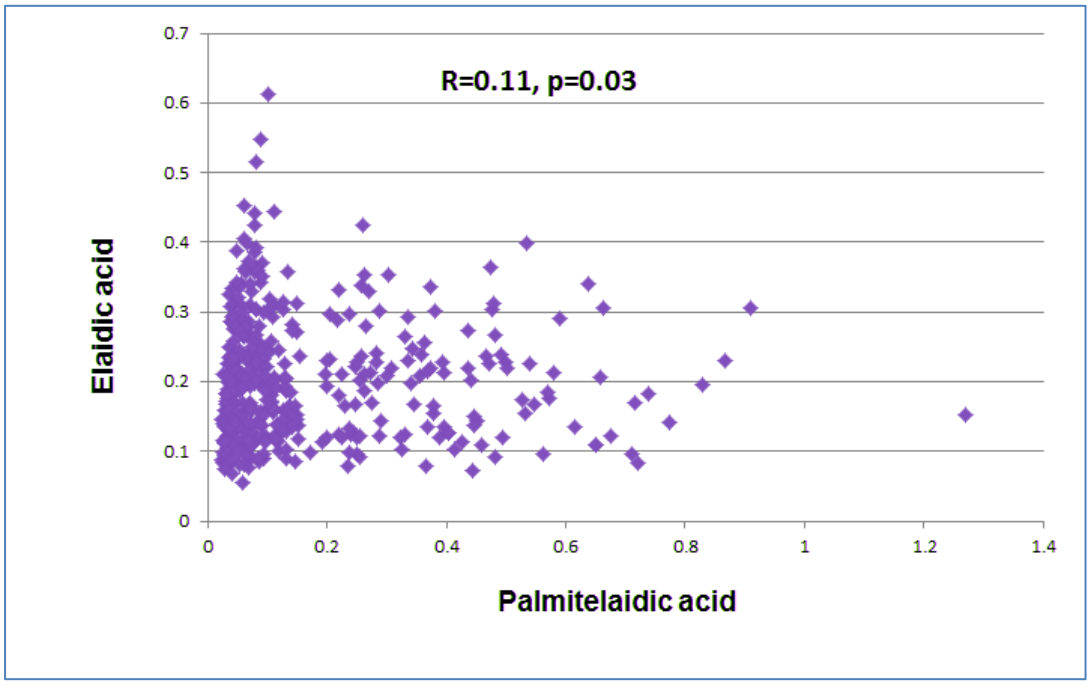


Figure 6: Correlation between plasma levels of palmitelaidic and elaidic acids. The weak correlation implies that these TFA are derived from different dietary sources



There was a good correlation between margaric and pentadecanoic acids (r= 0.56, p<0.001), but no correlation between pentadecanoic and vaccenic acids (r=0.011, p=0.03), pentadecanoic and palmitelaidic acids (r= -0.033, p<0.001), margaric and vaccenic acids (r= -0.0054) or margaric and palmitelaidic acids (r= -0.085, p<0.001).

Discussion

SFA and TFA levels in this population are higher that than expected from diet surveys. (69) The most common fats in this population with significant cardiovascular disease are those fats considered 'harmful' to heart health, palmitic and stearic acid. This population have not yet been exposed to 'heart healthy' dietary advice or cardiac rehabilitation; therefore, plasma levels reflect the intake of fats at time of diagnosis.

The relatively high levels of SFA and TFA may reflect the high intake of dairy food in the New Zealand population. Dairy food is the most significant source of animal fat and New Zealanders have a relatively high intake of dairy food. (69) As one of the world's most significant producers of dairy food, (68) this has been strongly promoted as part of healthy diet. The relatively high TFA levels may also be partly explained by the practice of grass feeding cows in New Zealand that increases TFA content in milk. (209)

Levels of total TFA in this study population are lower than those seen in other Western countries and comprise different isomers than typically seen. (19, 176-179, 210, 211) TFA levels in Europe and America are 2-4% of total fatty acids with more than 70% being iTFA (elaidic and linoelaidic acid). (19, 21-23, 47, 212). In this study mean TFA levels are 1.07% with vaccenic acid the most plentiful. The strong correlation between plasma levels of vaccenic acid and palmitelaidic acid suggest that palmitelaidic acid also originates from ruminant food. These two make up ¾ of the total TFA level in this population, and the absolute level is approximately twice as high as levels seen in the European and American studies. This is in contrast with duet surveys that suggest that 40% of TFA in New Zealand are from ruminant animals. (138)

The lack of correlation between these two TFA and the SFA pentadecanoic and margaric acids is harder to explain. These two SFA are considered to be markers of dairy food intake, (213) and are important constituents of butterfat and milk. It is possible that these SFA are from other sources.

SFA levels in this population are most similar to the Dutch population (Table 7). (175) Dietary surveys (214, 215) suggest that this population, like the New Zealand population, has a high intake of ruminant by-products. In contrast, the Japanese population, (179) that do consume little dairy food, (216) have a significantly lower plasma level of SFA (32%).

This observational study describes fatty acid levels in people with significant cardiovascular disease. Further studies are needed to determine SFA and TFA levels in the normal population. Further studies are also needed to evaluate the effects of specific dietary interventions on tissue levels of these fatty acids, and markers of cardiovascular risk. Strategies to reduce TFA levels in meat and dairy products, such as modifying the animal feed (62) could be considered, especially in countries with relatively high dairy food intake such as New Zealand and Norway.

The limitations of this study are that information on diet was not collected and only one blood test was collected, therefore, the relation between plasma levels of different fatty acids and diet could not be directly assessed. Also, a single blood test excludes evaluation of variation of levels over time. Dietary studies suggest that the majority of the population have a consistent eating pattern, so it is expected that there is a small variation of FA levels within individuals. (194) The study has been undertaken in patients with severe coronary artery disease so it is possible that the FA levels seen in this study do not reflect FA levels in the general population.

In conclusion, this study suggests that in New Zealand patients with significant coronary artery disease, total plasma TFA levels are low and are mainly derived from meat and dairy food. SFA and TFA levels are higher than expected from dietary modelling.

Section 2: Trans-fatty acids in New Zealand patients with coronary artery disease. (66)

Abstract

Background: Dietary surveys indicate that New Zealanders have a low intake of trans fatty acids with little derived from industrial sources. This observational cross sectional study in New Zealand patients with surgical coronary artery disease assesses the plasma levels of trans fatty acids, and the association of trans fatty acids levels with clinical markers of vascular disease.

Methods: 390 patients with severe coronary artery disease had fasting blood tests taken. Plasma levels of four trans fatty acids derived from hydrogenated vegetable oils and ruminant animal products were measured by gas chromatography. Relationships between plasma trans fatty acids levels and the recent occurrence of myocardial infarction, the presence of polyvascular disease, and serum levels of C-reactive protein were assessed.

Results: The median trans fatty acids level was 0.85% by weight of total fatty acids (interquartile range 0.59, 1.79%), with a skewed distribution to the right. For the lowest (< 0.74%), middle (0.74%-1.07%) and highest (>1.07%) thirds of total trans fatty acids, the proportion of patients with polyvascular disease was 10%, 16%, and 27%, (p=0.0004) respectively. Plasma C-reactive protein also increased by tertile of trans fatty acids (median 2.0, 2.9, 3.2 mg/ L, p=0.007).The association between polyvascular diseases and C-reactive protein remained significant after adjustment for risk factors. Significant associations were present between plasma trans fatty acids from both ruminant and hydrogenated vegetable oil sources and these markers of cardiovascular risk.

Conclusions: There is an association between relatively low plasma levels of total trans fatty acids, mostly derived from ruminant sources, and an increased risk of polyvascular disease and increased C- reactive protein in patients with severe coronary artery disease. These high-risk patients may benefit from a targeted approach to minimise all sources of trans fatty acids in the diet.

Introduction

TFA are unsaturated fats which have a double bond in the trans-configuration and are derived from the diet only. The major source of TFA is processed foods which contain partially hydrogenated vegetable oil, predominantly elaidic acid (18:1 (n-9t). (11) A second dietary source of TFA is milk products and meat from ruminant animals (22, 134), with the predominant isomer being vaccenic acid (trans 18:1 (n-7t). (134)

In epidemiological studies, there is a dose dependent association between increased dietary intake of TFA, especially industrial TFA, and the risk of CVD. (17, 19-21, 23, 47) The New Zealand population has a low intake of total TFA with most deriving from 'natural' ruminant sources. (138) It is possible but not known whether this source of TFA is associated with cardiovascular risk in populations with higher consumption of meat and dairy food products, even though consumption of hydrogenated vegetable oils from processed and fast foods is low.

Most studies which evaluated the adverse effects of TFA have been undertaken in healthy populations. However, TFA could be particularly hazardous in patients with established CVD. The aim of the current study is to determine whether plasma levels of TFA from different dietary sources are associated with the presence of clinical markers of increased vascular disease in patients with known severe coronary artery disease. Markers of vascular disease assessed are the recent occurrence of myocardial infarction, (217) the presence of polyvascular disease. (47) Markers of vascular risk assessed are lipids and the plasma level of C-reactive protein (CRP). (218)

Method

Study population and clinical data

This is an observational cross sectional study. Sequential patients with symptomatic severe coronary artery disease thought by the investigating cardiologist to need coronary artery bypass surgery at angiogram were invited to participate in this study from August 2004 to September 2006. Written informed consent was obtained after the diagnostic coronary angiogram and a fasting blood sample was taken. A study nurse then completed the case report form which included data on cardiovascular risk factors, prior cardiovascular history and cardiac investigations. Recent myocardial infarction was defined as, presenting within the last two weeks with a rise and/or fall of the 4th generation troponin T above the cut point of 0.03µg/L with at least one of the following: ischemic symptoms; development of pathological Q waves on the electrocardiogram (ECG) or ECG changes indicative of ischemia (>1mm ST segment elevation or 1mm depression). (217)

Polyvascular disease was defined as clinical disease in another arterial territory, either peripheral artery disease or cerebrovascular disease. (47) Peripheral artery disease was defined of any of the following: claudication (at rest or exertion), amputation due to arterial insufficiency, vascular reconstruction, bypass surgery or percutaneous intervention to the extremities, documented aortic aneurysm or a positive non-invasive test (e.g. ankle brachial index < 0.9). Cerebrovascular disease was defined as a history of prior stroke. Of the 447 patients approached plasma TFA were measured in 390 patients who form the current study population.

Measurement of trans fatty acids and C-reactive protein

Blood samples were taken in ethylene-diamine-tetra acetic acid tubes. Plasma was separated and stored at -70^{o} Celsius until analysis. Plasma phospholipids analysis was performed at the Nutrition and Functional Food Science Laboratory, University of Adelaide, Australia. Total lipids were extracted with AR Methanol and fatty acid methyl esters formed by transmethylation. Serum phospholipid fatty acid composition was assessed by gas chromatography (Hewlett Packard 6890 Gas Chromatograph with a SGE BPX70 column and a Flame Ionisation Detector). Vaccenic acid, elaidic acid, linoelaidic acid, palmitelaidic acid and total TFA were measured. Levels are expressed as the percentage of the total weight (%) of the fatty acids. CRP was measured using a high sensitivity assay using the Roche CRPLX immunoturbidimetric method on a Roche Modular analyser.

Statistical Analysis

Patients are divided into lowest, middle and highest thirds by the levels of total TFA. Analysis of variance was used for continuous variables and the Cochran-Armitage Trend test for binary outcomes across TFA tertile groups. CRP and LDL-c levels were natural log transformed in the analysis. Mann-Whitney U tests are used to compare TFAs levels according to the presence or absence of cardiovascular risk markers. Results are presented as mean and standard deviation (SD) or median and inter-quartile range (IQR). Multiple generalized logistic regressions were used to assess the associations between TFA tertile groups and MI adjusted by polyvascular disease and the cardiac risk factors, LDL-c, CRP and age.

Results

The study population and plasma levels of fatty acids are described in Section 1 of this Chapter.

Clinical data are presented in Table 11 for the lowest, middle and highest thirds of total TFA for the study population. There was no significant association between TFA and history of smoking, hypertension, diabetes, obesity or plasma lipids.

However, there was a graded increase in the plasma level of CRP from the lowest to the highest (2.0, 2.9, 3.2 mg/L) tertile of TFA respectively (p=0.007). The association between CRP and TFA remained statistically significant (p=0.01) after adjustment for LDL-c, recent myocardial infarction and polyvascular disease. Patients with higher plasma levels of TFA, were more likely to have polyvascular disease (p=0.0004) (Figure 7), and this association remained statistically significant (p=0.0007) after multivariate adjustment for age, recent myocardial infarction, LDL-c and CRP. Patients in the lowest tertile of TFA were less likely to have a recent myocardial infarction. (p=0.006), but this association was not statistically significant (p=0.28) after adjustment for age, polyvascular disease, LDL-c and CRP.

Associations between individual TFA and CRP, presence of polyvascular disease and occurrence of recent myocardial infarction are presented in Table 12. Subjects with polyvascular disease had higher plasma levels of vaccenic acid and palmitelaidic acid, while those with recent myocardial infarction had

higher levels of elaidic acid and linoelaidic acid. Subjects with a CRP ≥3mg/dl had higher levels of palmitelaidic acid and elaidic acid.

Table 11: Characteristics of study subjects in the lowest, middle and highest thirds of total TFA in the observational study.

Trans fat level (tertiles)	Lowest	Middle	Highest	p value[a]
Range (weight %)	<0.74	0.74 to 1.07	>1.07	
No. of Subjects	130	130	130	
Age [b]	67 (±11)	68 (±10)	68 (±10)	0.61
Male	108 (83%)	106 (82%)	110 (85%)	0.74
Smoker	96 (74%)	96 (74%)	95 (73%)	0.89
Body mass index [b]	28.0 (±4.8)	28.0 (±4.7)	28.6 (±4.8)	0.46
Waist circumference (cm) [b]	100.5 (±11.8)	100.6 (±13.0)	103.1 (±12.6)	0.18
Diabetes	38 (29%)	23 (18%)	42 (32%)	0.60
Hypertension	68 (52%)	61 (48%)	72 (56%)	0.53
Statin	77 (59%)	77 (59%)	78 (60%)	0.45

Beta blocker	68 (57%)	58 (44%)	61 (44%)	0.08
LDL cholesterol (mmol/l	2.5 (±1.0)	2.8 (±1.3)	2.6 (±1.0)	0.23
HDL cholesterol (mmol/l)	1.3 (±0.4)	1.3 (±0.8)	1.2 (±0.3)	0.25
C-reactive protein (mg/dl)[c]	2.0 (0.5, 5.7)	2.9 (1.1, 6.7)	3.3 (1.5, 11.4)	0.007

Values are n (%), mean (SD) or median (IQR).

[a] Analysis of variance F test.

[b] Two-sided Cochran–Armitage Trend test.

[c] medium (IQR

Figure 7: Proportion of study subjects in the lowest, middle and highest thirds of total plasma trans-fats who had polyvascular disease and recent myocardial infarction.

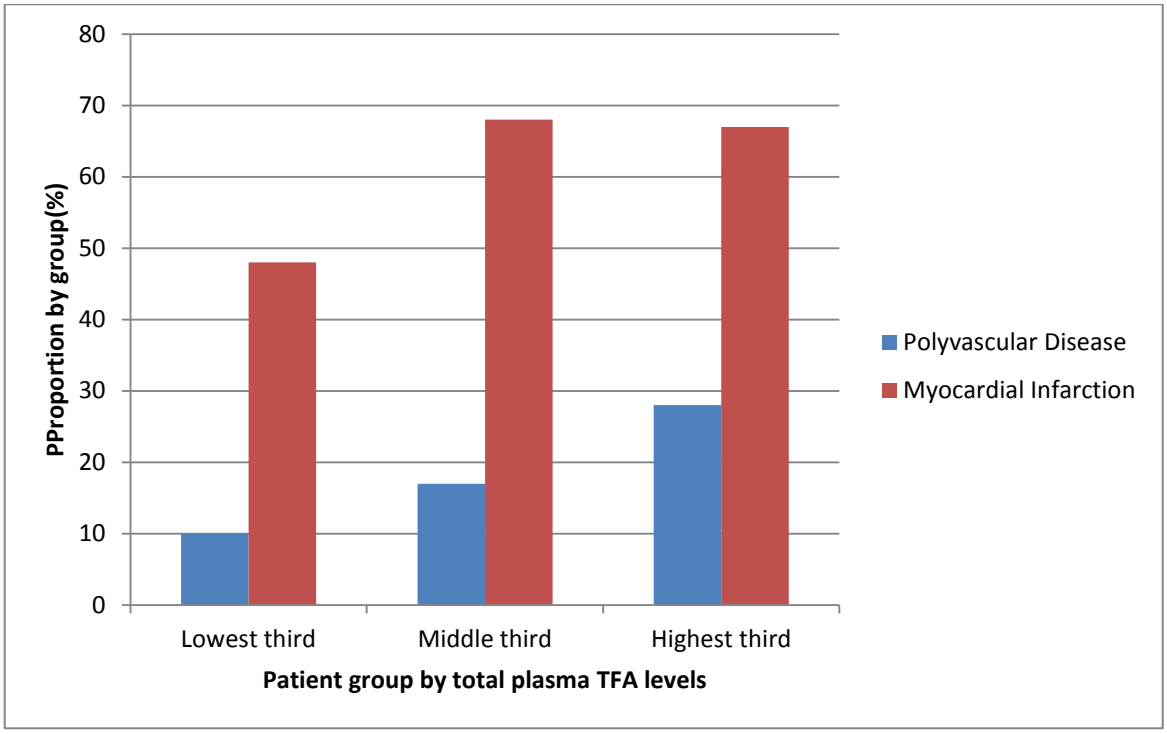


Table 12: Plasma levels of trans fatty acid isomers in patients with higher and lower plasma levels of C-reactive protein, with and without polyvascular disease and with and without recent myocardial infarction. The trans fatty acid level is expressed as the median percentage weight and inter-quartile range.

	CRP >=3mg/dl	**CRP <3mg/dl**	**P***
Number of subjects	**179**	**206**	
Total TFA	0.87 (0.72, 1.47)	0.83 (0.66,1.16)	**0.04**
Palmitelaidic acid	0.10 (0.05, 0.27)	0.08 (0.04, 0.22)	**0.02**
Vaccenic acid	0.46 (0.37, 0.90)	0.44 (0.35, 0.71)	**0.18**
Elaidic Acid	0.19 (0.14, 0.27)	0.17 (0.12,0.24)	**0.02**
Linoelaidic acid	0.09 (0.07, 0.10)	0.09 (0.07, 0.10)	**0.64**
	Polyvascular disease	**No polyvascular disease**	
Number of subjects	**68**	**315**	
Total TFA	1.16 (0.77, 1.79)	0.84 (0.66,1.17)	**0.001**
Palmitelaidic acid	0.14 (0.07, 0.36)	0.08 (0.04, 0.20)	**0.0003**
Vaccenic acid	0.61 (0.41,1.09)	0.44 (0.35,0.72)	**0.002**

Elaidic Acid	0.21 (0.13, 0.27)	0.17 (0.12, 0.24)	**0.06**
Linoelaidic acid	0.09 (0.07,0.10)	0.09 (0.07, 0.10)	**0.96**
	Recent myocardial infarction	**No recent myocardial infarction**	
Number of subjects	**240**	**149**	
Total TFA	0.87 (0.73, 1.40)	0.82 (0.59, 1.16)	**0.009**
Palmitelaidic acid	0.09 (0.05, 0.26)	0.08 (0.04, 0.23)	**0.06**
Vaccenic acid	0.45 (0.37, 0.82)	0.46 (0.32, 0.77)	**0.22**
Elaidic Acid	0.21 (0.15, 0.27)	0.14 (0.11, 0.21)	**<0.0001**
Linoelaidic acid	**0.09 (0.07, 0.11)**	**0.08 (0.07, 0.10)**	**0.01**

Discussion

This study examines a population with severe coronary heart disease who have relatively low intake of TFA mainly derived from dairy food and meat products. Most previous studies have investigated the adverse effect of TFA in healthy populations with higher intake of total TFA predominantly derived from partially hydrogenated vegetable oils. (2, 19, 21-23, 29, 39, 47, 134, 219, 220) Those studies have found associations between total TFA and increased cardiovascular risk. (19-23) However, the adverse effects of TFA on cardiovascular risk may be much greater in patients with known coronary artery disease, because they have a much higher absolute risk of cardiovascular events.

In this study, patients who had higher plasma levels of TFA were more likely to have polyvascular disease. This association has not been reported previously. Patients with polyvascular disease have poorer outcomes, with a greater burden of atherosclerosis and a higher risk of acute cardio-vascular events and death. (47) Patients who had higher TFA plasma levels were also more likely to have a recent history of myocardial infarction although this association was not statistically significant after adjusting for other factors.

TFA, predominantly from industrial sources, can increase cardiovascular risk through multiple mechanisms, including adverse effects on plasma lipids, (32) inflammatory markers and endothelial function. (20, 134, 221) In this study, patients with higher plasma levels of TFA were more likely to have an elevated plasma level of C-reactive protein. However, there was no clear association between TFA and plasma lipids. The high use of statins would decrease the ability to detect modest association between lipid levels and TFA.

In this study palmitelaidic acid and vaccenic acid were derived from meat and dairy food and were most closely associated with peripheral vascular disease. Palmitelaidic acid has been associated with cardiac death in two studies, (134, 212) but has not been associated with poly vascular disease. Few studies have distinguished between the two major trans 18:1 isomers (vaccenic acid and elaidic acid) which make up to 75% of TFA intake, so it is difficult to assess their individual contribution to adverse effects. (21, 23, 39, 212)

Linoelaidic acid (18:2 (n-6t, 9t)) has been most consistently associated with cardiovascular risk in previous studies. The absence of a clear association between linoelaidic acid and markers of vascular disease in the current study may be related to the low plasma levels in this population. (18)

The relatively low intakes of ruminant TFA in most Western countries may explain the lack of clear evidence that rTFA may be harmful. (212) TFA from dairy products and meat may be relatively more important risk factors for CVD in countries where TFA consumption derived from hydrogenated vegetable oils is low or reduced by legislation, and where intake from ruminant sources is relatively high. (18)

Study limitations

Some limitations of this study are described previously in this chapter. Because this was a cross sectional study it is possible that observed associations were not causal. However TFA are derived from diet alone and plasma levels are unlikely to be higher because of more severe cardiovascular disease. A clinical outcome trial with coronary artery disease patients randomised to either standard dietary advice or specific advice to eliminate TFA would be needed for conclusive evidence on the importance of eliminating TFA in high risk patients.

Conclusion

Relatively low plasma levels of TFA are associated with an increased prevalence of polyvascular disease and higher plasma CRP in patients with severe coronary heart disease. Clinical trials that assess the effects of eliminating both ruminant and industrial TFA in patients with vascular disease are required.

Section 3: Saturated and ruminant trans fatty acids do not predict mortality in patients with severe coronary artery disease.

The previous section describes an association with rTFA and increased prevalence of polyvascular disease and higher plasma CRP in patients with symptomatic severe coronary artery disease. The cohort of this prospective observational study had higher rTFA levels than in other populations, thereby offering a unique opportunity to assess the relationship of rTFA with mortality. In this section 7½ year follow up of the original cohort is described. This section has been submitted in the Journal of Nutritional Disorders and Therapy. The Journal article amalgamates section 1 and 3, in that it describes fatty acid levels and the relationship of these with total and cardiovascular mortality.

Abstract

Background: Objective measures of fatty acid intakes such as tissue levels of fatty acids more accurately reflect dietary intake compared to food frequency questionnaires. This study describes plasma fatty acid levels in New Zealanders with significant coronary artery disease and the relationship with mortality at 7.5 years.

Methods: This is prospective observational study. Fasting plasma samples were taken in in 420 consecutive patients with angiographic diagnosis of severe coronary disease requiring coronary artery bypass surgery. Plasma levels of fatty acids were measured by gas-chromatography mass-spectrometry. Mortality data was obtained by accessing details of the most recent contact with health professionals, review of clinical notes and the National Health Index database and death certificates*.*

Results: The mean age of participants was 68 (± 10) years and 83% were male. Saturated fats were 46.5 (±1.2) %, unsaturated fats were 51.8 (±1.3%) %, trans fatty acids 1.1 (±0.69) % of total fats. Ruminant trans fatty acids made up 67% of total plasma trans fatty acids. Saturated fats and ruminant trans fatty acids levels were not associated with increased total mortality (hazard ratio 0.93 (0.75 to 1.16) p=0.53

and.14 (0.85 to 1.53) p=0.39 respectively or cardiovascular mortality (hazard ratios 0.93 (0.75 to 1.16) p=0.53 and 0.91 (0.61 to 1.37, p=0.66).

Conclusion: Saturated and trans fatty acid levels in this population are higher than expected from food frequency questionnaires. More than two thirds of trans fatty acids is from dairy food and meat. Neither saturated fats nor trans fatty acids are associated with increased cardiovascular and total mortality.

Introduction

Evidence suggests that there may be a relationship between certain fatty acids and cardiovascular disease. For example, observational studies suggest that trans fatty acids (TFA) are associated with increased total and cardiovascular mortality. (33, 139) However, most TFA in those populations is derived from partially hydrogenated vegetable oil, with little coming from ruminant sources. (2, 19-23, 27, 39, 134, 220, 222) The effects of TFA derived from animal byproducts is less clear with one author suggesting that all TFA regardless of source is associated with increased mortality, (65) but others suggesting that ruminant TFA(rTFA) have no effect. (139)

A Cochrane review contends a small, but potentially important, reduction in cardiovascular risk with reduced SFA intake.(223) The postulated effects of SFA on heart heath are thought to depend on the type of SFA. For example, long chain fatty acids are thought to be harmful (3) but medium chain fatty acids are thought to have neutral or beneficial effects. However, the recent meta-analysis by Rajiv Chowdhury (167) and review by Siri-Tano (224) suggests that there is little distinction between the effects of different SFA isomers.

Meal diaries and food frequency questionnaires have been found to be unreliable to assess dietary intake (206, 207) and it is now suggested that objective measurements of dietary intake be more widely used. One such measurement is plasma levels fatty acids which reflect dietary intake for the last 2-3 weeks. (177) In this study, plasma level of all fatty acids including TFA isomers was measured in New Zealand subjects with severe symptomatic coronary artery disease. The relationship between total and cardiovascular mortality at7 ½ years and fatty acid levels was described.

Methods

This is a prospective observational study. The primary study endpoint of this study is all-cause mortality by the 28 February 2014. The secondary study endpoint is early and late all cause and cardiovascular mortality.

Attainment of mortality status

This was obtained initially by reviewing the National Health index database. Patients were deemed alive as of 01February 2014 if no date of death was listed in the National Health Database and evidence of patient contact was found. Contact was defined as evidence of a face to face visit to the general practitioner, hospital, laboratory or community pharmacy in the month of February 2014. Contact was determined by checking hospital notes, laboratory results and contacting the general practitioner.

For those who had died, hospital notes, laboratory and radiology tests and death certificates were reviewed, and when not available, information was obtained from the general practitioner.

Clinical definitions

Cardiovascular death was defined as death resulting from an acute myocardial infarction, sudden cardiac death, death due to heart failure, death due to stroke, and death due to other cardiovascular causes. (225) Early mortality was defined as death in the first year from informed consent; late mortality was defined as mortality from 1 year after informed consent to 1st February 2014.

Measurement of FA and biomarkers

Measurement of fatty acids has been described in Section 1. CRP was measured using a high sensitivity assay using the Roche CRPLX immunoturbidimetric method on a Roche Modular analyzer and levels are expressed as (mg/dl). BNP was measured using the Advia Centaur BNP assay (Bayer Diagnostics and levels are expressed as pmol/L. Creatinine was measured using Roche Modular P unit (Roche Diagnostics). Levels are expressed as mmol/l.

Statistical analysis

Categorical data is reported as frequencies and percentages and continuous data as medians with IQR. The Mann Whitney U test was conducted to determine differences in SFA and TFA levels between those who died and those still alive and to compare age, C-reactive protein, creatinine, white cell count and brain natriuretic peptide (BNP) between the two groups.

The distribution of ethnicity and gender between the groups was compared using the two-sample Z test. Univariate Cox proportional hazard regression was used to assess the relationships between SFA and TFA with one year mortality, late mortality, cardiovascular mortality and non-cardiovascular mortality. Statistical analyses were performed using the statistical package SAS version 9.3 (SAS Institute, Cary, NC). All p-values resulted from two sided tests and a p-value of <0.05 was considered statistically significant.

Results

Average follow up was for 7.5 (SD 0.8) years and mortality status and cause of death were available for 421 of the 422 of the cohort. One participant had left New Zealand and no follow-up information was retrievable. All but 13 of the original cohort had surgery and in total 95 (22.5%) were dead as of 28th February 2014. Of these 34 were dead within 1 year (early mortality) and 61 dead after one year (late mortality).

Gender and race did not predict mortality (table 13); however, the inverse was true for increased age, creatinine, inflammatory markers (C - reactive protein) and Brain- natriuretic peptide (BNP) (Table 14). When multivariate analysis was performed, CRP was no longer significant (p= 0.06), but age, creatinine and BNP remained significant (p<0.001 for all three).

Table 15 depicts the association of SFA and TFA with total mortality. Total SFA did not predict early mortality (hazard ratio (HR), 0.99 (0.67 to 1.47), p=0.97), late mortality (HR 0.98 (0.64 to 1.44), p=0.64, or cardiovascular mortality (HR 0.93 (0.75 to 1.16) p=0.53). Also, no saturated fat isomer was associated with increased mortality.

Similarly, total TFA did not predict early mortality (HR 0.78 (0.36 to 1.71), p=0.54), late mortality (HR 1.14 (0.85 to 1.53) p=0.39) or cardiovascular mortality (HR 0.91 (0.61 to 1.37, p=0.66). Whilst linoelaidic acid was associated with increased total mortality (p=0.023), levels were very low with markedly skewed distribution to the left.

Table 13: Baseline characteristic of those who survived or died at 7.5 year follow up. Results are number (%).

	Alive	**Died**	**P**
Female	52 (78%)	20 (22%)	0.43
Male	273 (79%)	75 (21%)	0.43
Asian	31 (9%)	6 (6%)	0.33
European	225 (68%)	66 (68%)	0.93
Maori	37 (11%)	14 (14%)	0.37
Other	22 (7%)	5 (5%)	0.60
Pacific Islander	18 (5%)	6 (6%)	0.77

Table 14: Predictors of total mortality in patients 7.5 years after angiogram. Results are median and interquartile range. When multivariate analysis was performed adjusting for age and creatinine, N-BNP remained significant (p<0.001), but CRP was no longer significant (p=0.052).

	Alive	**Dead**	
	Median (IQR)	**Median (IQR)**	**p**
N	327	95	
Age (years)	67.3 (59.6 to 74. 6)	73.9 (65.1 to 78.6)	<0.0001
CRP (mg/dl)	2.4 (1.1 to 6.2)	3.8 (1.4 to 13.7)	0.0045
Creatinine (mmol/l)	107 (92 to 125)	121 (100 to 150)	<0.0001
WBC (E+9/l	12.6 (10.3 to 15.2)	11.7 (8.9 to15.9)	0.18
BNP (pmol/L)	33 (15 to 88)	112 (40 to 309)	<0.0001

Table 15: Saturated fatty acids and total mortality at 7.5 years. Results are median and interquartile range**.**

	Alive	**Dead**	
Fatty acid (lipid Number)	**Median (IQR)**	**Median (IQR)**	**p**
N	327	95	
Caprylic acid 8:0	0.00 (0.00 to 0.00)	0.00 (0.00 to 0.00)	0.72
Pelargonic acid 9.0	0.00 (0.00 to 0.00)	0.00 (0.00 to 0.00)	0.72
Capric acid 10:0	0.00 (0.00 to 0.00)	0.00 (0.00 to 0.00)	0.10
Undecylic acid 11:0	0.00 (0.00 -0.00)	0.00	0.62
Lauric acid 12:0	0.00 (0.00 to 0.01)	0.00 (0.00 to 0.01)	0.30
Tridecylic acid 13:0	0.00 (0.00 to 0.00)	0.00 (0.00 to 0.00)	0.18
Myristic acid 14:0	0.21 (0.18 to 0.26)	0.21 (0.17 to 0.26)	0.83
Pentadecanoic acid 15:0	0.19 (0.16 to 0.22)	0.20 (0.18 to 0.23)	0.067
Palmitic acid 16:0	0.70 (0.62 to 0.80)	0.72 (0.63 to 0.80)	0.93
Margaric acid 17:0	0.48 (0.42 to 0.53)	0.50 (0.43 to 0.54)	0.16
Stearic acid	0.51	0.46	0.18

18:0	(0.33 to 0.66)	(0.00 to 0.67)	
Arachidic acid 20:0	0.50 (0.43 to 0.58)	0.51 (0.43 to 0.59)	0.32
Behenic acid 22:0	0.49 (0.41 to 0.56)	0.47 (0.40 to 0.56)	0.30
Lignoceric acid 24:0	0.59 (0.52 to 0.67)	0.57 (0.49 to 0.66)	0.12
Total saturated fats	**46.52** **(45.81 to 47.23)**	**46.52** **(45.66 to 47.29)**	**0.64**
Palmitelaidic acid 16:1 (n-7t)	0.08 (0.04 to 0.23)	0.09 (0.05 to 0.17)	0.62
Elaidic acid 18:1 (n-9t)	0.19 (0.13 to 0.25)	0.19 (0.12 to 0.27)	0.77
Vaccenic acid 18:1 (n-7t)	0.44 (0.35 to 0.77)	0.46 (0.37 to 0.67)	0.37
Linoelaidic acid 18:2 (n-6t, 9t)	0.09 (0.07 to 0.10)	0.10 (0.08 to 0. 11)	0.0023
Total trans fatty acids	**0.84** **(0.66 to 1.29)**	**0.89** **(0.71 to 1.20)**	**0.44**

Discussion

TFA is consistently associated with increased mortality in cohorts with higher TFA levels that are mainly derived from partially hydrogenated vegetable fat. (2,3,17) However, TFA levels in this study were relatively low and were mainly rTFA, consistent with food frequency questionnaires.(226) Little is known about effects of rTFA on mortality. One author suggests that all TFA regardless of source is associated with increased mortality, (65) but others suggest that rTFA have no effect. (139) Some observational

studies show an association with increased cardiovascular disease in a dose dependent manner (65, 66) and others show little effect. (134, 220). Experimental studies suggest that rTFA, has little effect on lipids and inflammatory markers at low doses, (33, 79) but has adverse effects on cholesterol homeostasis at higher doses.(127) In this study, total TFA and rTFA did not predict total or cardiovascular mortality. Whilst there is a suggestion that linoelaidic acid was associated with increased mortality, the results need to be interpreted with extreme caution as levels were very low and there was a markedly skewed distribution to the left.

Most of the SFA in this population were the long chain fatty acids and these were not associated with increased mortality. Levels of short chain and medium chain fatty acids were too low to reliably draw conclusions. This study contrast with others that suggest that long chain fatty acids and total SFA are associated with increased cardiovascular mortality,(37, 216) but is consistent with the most recent meta-analysis by Chowdhury. (167) Emerging evidence also suggests that a low fat diet may not be effective at reducing cardiovascular disease, (144) compared to a diet where SFA is substituted with PUFAs. (144, 227) In countries that have embraced a low fat diet, there are increased rates of diabetes and obesity, thought to be due to a compensatory increase in the consumption of carbohydrates. (228, 229)

In this study, creatinine, age and BNP measured at time of diagnosis are associated with increased risk of death after coronary artery bypass surgery. These finding concur with other studies [24-26] that have found that these risk factors are associated with increased mortality in patients with cardiovascular disease. However, the findings that pre-operative BNP predict mortality e after patients have had complete revascularization despite adjustments for the most significant risk factors for post-operative mortality, (230, 231) is novel and will need further study.

The strength of this study is that survival information on all but 1 participant was available, and the cause of death was able to be ascertained on all participants.

The limitations of this study are that information on diet was not collected and only one blood test was collected. Relation between plasma levels of different FA and diet could therefore not be directly

assessed. The single blood test excludes evaluation of variation of levels over time. Dietary studies suggest that the majority of the population have a consistent eating pattern, so it is expected that there is a small variation of fatty acid levels within individuals. (194) The study has been undertaken in patients with severe coronary artery disease; it is possible that the fatty acid levels seen in this study do not reflect levels in the general population.

Conclusion

This study suggests that in New Zealand patients with significant coronary artery disease, total plasma, SFA and TFA levels are higher than expected from dietary modeling. These fatty acids are not associated with increased risk of total and cardiovascular mortality.

Chapter 3

The observational study generated the hypotheses that most TFA in New Zealanders arise from ruminant sources and that they are associated with markers of increased risk. This Chapter describes a randomised study designed to evaluate the effects of changing dairy food intake on cardio-metabolic risk factors, (1) and to assess which fatty acids in human plasma originate from dairy food. Studies have suggested that pentadecanoic and margaric acids (213) are markers of dairy food intake. Observational studies, rather than interventional studies, also suggest that vaccenic and palmitelaidic acids are TFA derived exclusively from dairy food. (66, 99) These theories evolved from correlating vaccenic acid, mainly thought to be ruminant in origin, with palmitelaidic acid.

Observational studies show associations between high dairy food intake and lower rates of obesity, (184, 185) reduced blood pressure (188) and reduced insulin resistance. (187) Whilst these studies are helpful, they do not allow assessment of causality. In observational studies, the biggest predictor of the incidence of cardiovascular disease, (232) cardiometabolic risk factors, (233) obesity, (234, 235) diabetes (236, 237) and life expectancy (238) is socio economic status. For example, the average life expectancy in countries with high levels of socio economic deprivation (like Swaziland), is 31.9 years whereas it is > 80 years in countries with high socioeconomic status (like New Zealand). (239) Even within countries like New Zealand, men in the least deprived areas are expected to live 8.8 years longer compared with those who live in the most deprived areas. (238) The ability to afford certain food is the strongest determinant of dietary intake. Dairy food is expensive and not surprisingly, the biggest predictor of dairy food intake is socioeconomic status .(133) It is thus impossible to separate the effects of dairy food intake on health and the effects of socio economic status in observational studies.

Randomised studies eliminate the effects of these confounders and are therefore considered the ‘gold standard’ to assess the effects of interventions on health. However, the limited time-frames do not allow for the assessment of effects that can accumulate over long periods of time. In the Dietary Approaches to Systolic Hypertension (DASH diet), effects on blood pressure were seen within 2 weeks. In a post hoc

analysis, dairy food was thought to account for a 2.2mmHg drop in blood pressure. (240) This was congruent with observational studies, so dietary guidelines recommend 2-3 serves of dairy food per day. However, this diverges with advice to avoid saturated fats of animal origin, when the most common source of such fat in the diet is dairy food.

Ethics approval was obtained for this study and it was funded by grants from the Auckland DHB Charitable trust as well as the Green Lane Research and Educational Fund. Section1 describes the effects of changing dairy food consumption on cardio-metabolic risk factors and is published in the *European Journal of Preventive Cardiology*. (1) Section 2 describes the effects of changing dairy food intake on the fatty acids and is published in *Nutrition*. (241)

Trial registration number: ACTRN12612000574842

Ethics approval number: NTX/10/11/115

Section 1: A randomised trial evaluating the effects of change in dairy food consumption on cardio-metabolic risk factors. (1)

Abstract

Background: It is currently not known whether dairy food consumption influences the risk of cardiovascular disease or diabetes. This study evaluates the effects of changing dairy food intake on cardiometabolic risk factors.

Methods: 180 healthy volunteers were randomised to increase, reduce or not change their dairy food intake for one month in response to dietary advice. Body weight, waist circumference, blood pressure, fasting plasma lipids, insulin resistance, and C-reactive protein were measured at baseline and after one month and compared by dietary group.

Results: 176 (98%) subjects completed the study. The average change in self-reported dairy fat intake for the increased dairy food group was +09 standard deviation 1.1g/day (+71%), the group with no change to their intake was -2.1 standard deviation 0.4g/day (-15%) and the decreased dairy food group was -10.8 standard deviation 1.2g/day (-77%) respectively. There was no statistically significant change in LDL or HDL cholesterol, triglycerides, systolic or diastolic blood pressure, C-reactive protein, glucose or insulin, with 95% confidence interval standard mean differences < 0.2 for all and CRP <0.3. There was a small increase in weight (+0.4 kg, standard deviation 3.1) in those asked to increase dairy food consumption.

Conclusions: In healthy volunteers dietary advice to change dairy food intake for one month did not have a clinically significant effect on cardio-metabolic risk factors. These observations suggest that dairy food can be included as part of a normal healthy diet without increasing cardio-metabolic risk.

Background

The influence of dairy food on the risk of CVD and diabetes is currently uncertain. For many years reduction in high fat dairy food was recommended in order to decrease saturated fat intake (5, 8) but some more recent guidelines place less emphasis on the importance of a low fat diet. (7, 9) Clearer guidance is important, particularly given the high and increasing prevalence of CVD and diabetes in most countries, and the increasing global consumption of dairy food. (239) Food pyramids recommend consumption of 3-4 servings of dairy food per day, (5, 242) but other major guidelines (7, 180, 243, 244) recommend substituting dairy food with non-animal sources of protein.

Most evidence on the relationship between dairy food and cardio-metabolic risk comes from observational studies. (187, 188, 196, 245, 246) A major concern has been that dairy food is a source of both SFA and TFA which are thought to increase cardiovascular risk. (66, 224, 247) However, dairy food has not been shown to have adverse effects on lipids (248, 249) and a number of studies suggest that it could have beneficial effects on blood pressure, (188, 191, 196, 246, 250) weight (148, 187, 191, 196) and insulin resistance. (148, 187, 191, 196, 198) Dairy products are complex foods and an important source of protein, vitamin D, potassium, phosphorus, magnesium and calcium, (240) and could potentially improve cardiometabolic risk through a number of mechanisms. Small experimental studies suggest that dietary

calcium induces thermogenesis and weight loss, (168, 169) magnesium reduces blood pressure (240) and vitamin D improves endothelial function. (251) There is also evidence that the negative effects of SFA could be offset by the health benefits of CLA. (78, 91)

Data from randomised clinical trials is currently limited to small (n <100) narrowly focused studies, (129, 249, 252-254) or larger studies which are confounded by multiple interventions. (240) More evidence on the effects of changing dairy food intake on overall cardio-metabolic risk from randomised trials in real world settings is needed to guide recommendations on dairy food consumption as part of a healthy diet. Evidence from previous studies show that effects on blood pressure (BP), (240) lipids (25, 255) and weight (249) are apparent in the first month following consumption, and that compliance to dietary changes over long periods is difficult. We therefore undertook a randomised clinical trial comparing the effects of increasing, decreasing or not changing daily dairy food intake for one month on cardiometabolic risk factors in 180 healthy volunteers.

Methods

Study population

Healthy volunteers living in Auckland, New Zealand regularly consuming dairy food and who were willing to modify dairy intake for one month were recruited by advertisement from February 2011 to September 2011. Exclusion criteria included an inability to tolerate dairy food, known diabetes, cardiovascular disease, inflammatory conditions, currently taking any lipid or glucose modifying medication and age ≤18 years. Ethics approval was obtained from the Northern X Ethics Committee and all participants provided written informed consent. 180 participants were randomised into the study.

Study procedures

After written consent was obtained, height, weight and waist circumference were measured, a 3 day food frequency questionnaire was completed and a fasting blood sample collected. Participants were then randomised by a computer generated randomisation algorithm to one of three possible arms; increased dairy food consumption, reduced dairy food consumption or no change in dairy food consumption for 1 month. Participants then met with a study coordinator with experience in dietary advice for 15 minutes. Dietary sheets with advice on how to increase or decrease dairy were given to participants. Participants

randomised to increase dairy were asked to consume an extra 2-3 servings per day, and to change to high fat milk and dairy solids which are high in fat. Participants randomised to decreased dairy were asked to eliminate all possible sources of dairy food. Alternatives such as rice milk or soya were suggested. A follow-up assessment was arranged for one month at which time the above assessments were repeated. Subjects were phoned after two weeks to encourage dietary compliance.

Sitting BP was measured in the right arm after sitting for 5 minutes. Waist and hip circumference were measured according to the International Standards for Anthropometric Assessment, with the subject in a relaxed standing position and with arms folded across the chest. Waist circumference was measured at the halfway mark between the iliac crest and the bottom rib; hip circumference was measured at the level of the greater trochanter. Weight was measured on a calibrated digital scale.

Fasting blood tests were taken from all participants at baseline and after one month. For blood glucose and lipids, blood was taken in a heparin tube and analysed within 60 minutes using standard Roche Modular analyser. The following methods were used: glucose: glucose oxidase; TC: cholesterol oxidase; HDL-c: PEG-modified cholesterol esterase and oxidase, with dextran sulphate; TC: lipoprotein lipase. Low density lipoprotein cholesterol (LDL-c) was calculated using the Friedwald equation. To measure insulin, blood was taken in a plain tube. Serum was separated in a 4^{o} centrifuge and stored at -70^{o} Celsius until analysis. Serum insulin was measured by chemiluminescence immunoassay using an Abbott Architect analyser. The homeostatic model assessment (HOMA) was calculated from fasting glucose and insulin levels using a standard formula. (256)

To confirm changes in dairy food intake on different diets, plasma levels of the phospholipids 15:0 pentadecanoic acid and 17: 0 Heptadecanoic acid or margaric acid, which are known to be derived from dairy food, (219) were measured from blood samples taken in ethylene-diamine-tetra acetic acid tubes. Phospholipids were extracted with AR Methanol and fatty acid methyl esters formed by trans methylation. (208) Plasma phospholipid fatty acid composition was assessed by GC-MS (Hewlett Packard 6890 Gas

Chromatograph with a SGE BPX70 column and a Flame Ionisation Detector, with levels expressed as the percentage of the total weight of the fatty acids (Appendix 5 as a more detailed description of methodology).

The National Cancer Institute Diet History Questionnaire, (257) a validated food frequency questionnaire (FFQ) was used to assess all dairy food and red meat intake during the preceding 3 days at baseline and at 1 month. Questions on dairy food and meat intakes were unchanged but those related to alcohol, fruit, vegetables, grains and sweeteners were not included. This shortened FFQ was not separately validated. Serving sizes were defined using the United States Department of Agriculture criteria. (258) For example one serving size was equivalent to 250ml 3% milk, 250ml yogurt, 500ml 1.5% milk or 1/3 cup cheddar cheese. The total dairy fat content ingested in g/day was calculated based on the reported intakes of each food, and the fat content from manufacturers' labels. (259)

Statistical analysis

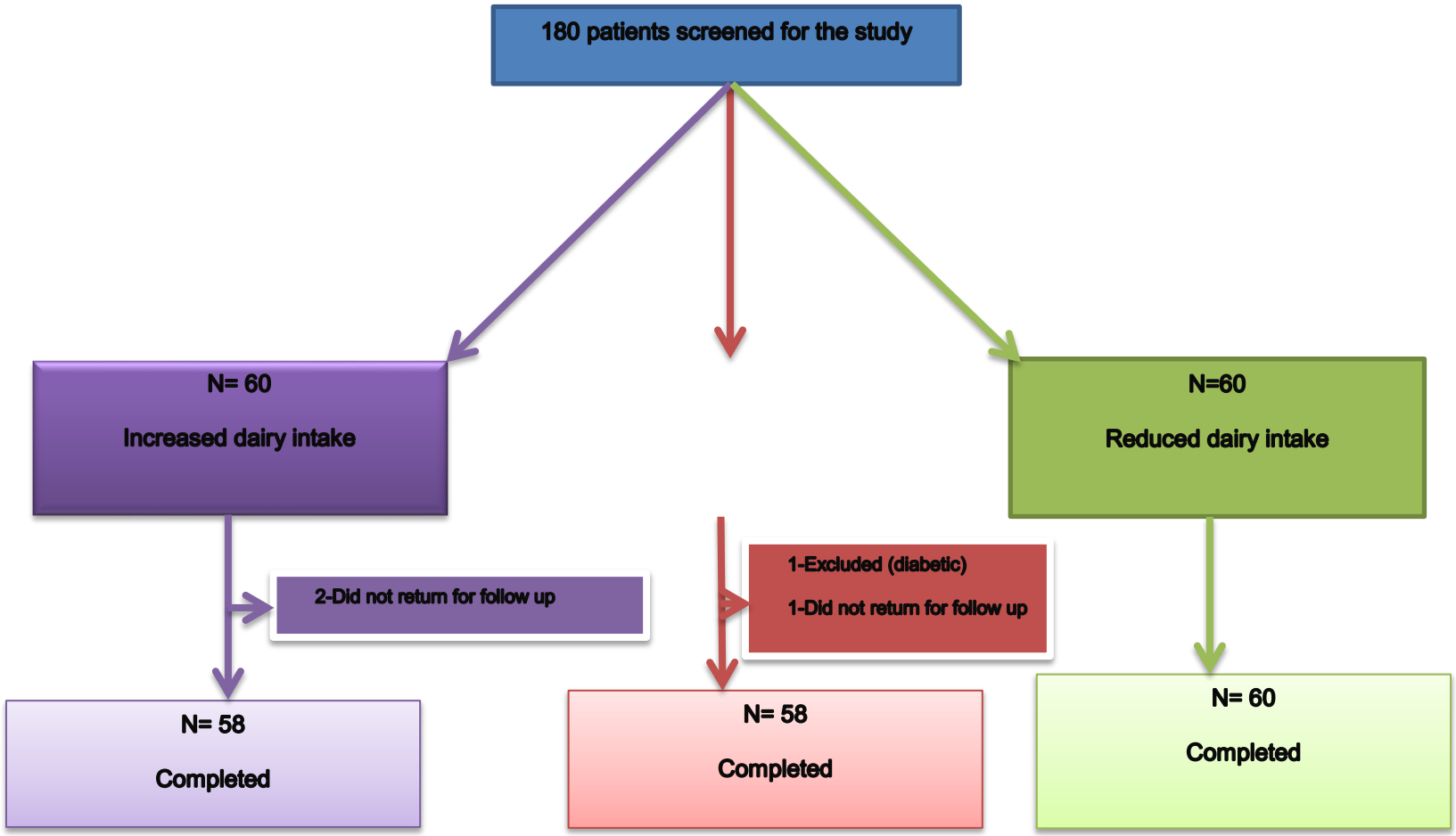
Demographic data, baseline and one month visit measurements and change in these measurements between baseline and one month were summarized as mean and standard deviation or frequency and percentage as appropriate. CRP was log transformed. Sixty participants in each group was estimated to give 80% power to detect a treatment difference with a two-sided 0.05 significance level, if the true difference in BP between treatments is 3.5mmHg with a SD of 7.

Baseline characteristics and scores were compared across the three arms using analysis of variance (ANOVA). Differences in measurements between the arms at one month were also assessed using analysis of covariance (ANCOVA) adjusting for the baseline scores. All p-values resulted from two sided tests. We adjusted for change in waist circumference and weight to assess effects on HOMA, BP, TC, HDL-c, LDL-c, HDL/LDL Ratio and TG. The effect of each intervention for all variables was summarized as standardized mean difference (SMD). Statistical analyses were performed with SAS software version 9.3 (SAS Institute, Cary, NC).

Results

176 of 180 randomised participants completed the study (Figure 9). The mean age of the population was 47 (IQR: 38-55) years and 70% of the participants were female. Participants had normal weight with an average body mass index of 24.5 (SD 4.0), and were normotensive with an average BP 110/70 (SD10/8) mmHg. Nine participants met the criteria for the metabolic syndrome according to the American Heart Association criteria. (260) There were no significant differences in baseline characteristics by dietary group (Table 16).

Figure 8: Study schematic of randomised study.



Change in dairy fat intake for the increased dairy food diet was +09 SD 1.1g/day (71%), no change was -2.1, SD 0.4g/day (15%) and decreased dairy food diet was -10.8, SD1.2g/day (77%) respectively (Table 16). There was no significant change in intake of red meat by randomised group. Change in levels of pentadecanoic acid (15:0) and Heptadecanoic acid (17:0) were consistent with the reported change in dairy food consumption in the diet for each group (Table 17).

Table 16: Baseline characteristics according to randomization. Results are mean and (standard deviation).

	Reduced dairy food intake	**Same dairy food intake**	**Increased dairy food intake**
N	60	60	60
Female (%)	62	66	63
Age (years)	48.6 (12.0)	45.3 (12.4)	46.3 (10.5)
Heart rate (Beats per minute)	63.7 (8.2)	63.0 (9.1)	64.1 (8.6)
Systolic blood pressure	116.6 (12)	114.2 (10)	113.9 (11)
Diastolic blood pressure	70.3 (9)	70.0 (8)	70.2 (8)
Waist circumference (cm)	84.2 (12.3)	83.2 (12.6)	84.3 (9.8)
Hip circumference(cm)	100.1 (8.8)	100.8 (10.1)	101.7 (7.1)
BMI	24.2 (4.0)	24.8 (4.0)	24.6 (4.1)
TC (mmol/L)	5.31 (0.97)	5.13 (0.98)	5.15 (0.93)
HDL (mmol/L)	1.70 (0.42)	1.68 (0.47)	1.68 (0.48)
LDL (mmol/L)	3.12 (0.88)	2.97 (0.86)	2.98 (0.78)
TG (mmol/L)	1.12 (0.81)	1.12 (0.72)	1.04 (0.64)
LDL:HDL ratio	3.21 (0.94)	3.10 (1.10)	3.31 (1.24)
CRP (mg/L)	2.61 (6.05)	2.41 (6.09)	2.93 (2.0)
Glucose (mmol/L)	5.33 (0.53)	5.21 (0.45)	5.41 (0.41)
Insulin (mU/L)	5.56 (2.97)	6.01 (4.22)	6.07 (4.4)
Insulin resistance (HOMA)	1.38 (0.82)	1.43 (1.08)	1.47 (1.1)

p >0.3 for all.

Table 17: Dairy food intake and plasma fatty acid levels at baseline and 1 month from the food frequency questionnaire for each randomised group. Results are mean and (standard deviation).

	Reduced dairy food intake	**Same dairy food intake**	**Increased dairy food intake**	**P value ***
Low fat milk (ml/day)				
Baseline	159 (23)	165 (28)	165 (26)	
1 month	82 (10)	113 (12)	276 (15)	
Change	-77 (8)	-52 (12)	+111 (11)	<0.001
High fat milk (ml/day)				
Baseline	154 (20)	143 (19)	131(20)	
1 month	41 (6)	131 (12)	471 (12)	
Change	-113 (10)	-18 (7)	+140 (11)	<0.001
High fat solids (g /day)				
Baseline	95 (8)	97 (8)	92 (7)	
1 month	21 (3)	80 (5)	183 (5)	
Change	-74 (4)	-17 (5)	+81 (6)	<0.001
Servings / day				
Baseline	3.5 (1.3)	3.5 (1.2)	3.5 (1.4)	
1 month	1.0 (0.9)	2.9 (01.2)	6.5 (1.8)	
Change	-2.5 (1.2)	-0.6 (0.2)	+3.0 (1.2)	<0.001
Dairy fat intake (g/day)				
Baseline	14.4 (11.1)	14.4 (9.2)	12.8 (8.3)	
1 month	4.12 (5.5)	10.9 (7.6)	25.3 (19.1)	
Change	-10.4 (10.1)	-3.4 (7.9)	+12.5 (15.7)	<0.001
Red meat intake(g/day)				

Baseline	26.5 (21.2)	26.8 (22.0)	26.3 (22.3)	
1 month	28.5 (22.4)	20.9 (20.3)	31.2 (21.9)	
Change	+2.0 (3.1)	-5.9 (6.0)	4.9 (3.9)	0.64
Pentadecanoic acid (%weight)				
Baseline	0.084 (0.034)	0.084 (0.026)	0.085 (0.030)	
1 month	0.079 (0.025)	0.085 (0.026)	0.098 (0.037)	
Change	-0.006 (0.028)	-0.00 (0.037)	+0.014 (0.046)	<0.001
Heptadecanoic (Margaric) acid (%Weight)				
Baseline	0.166 (0.084)	0.159 (0.074)	0.158 (0.080)	
1 month	0.142 (0.072)	0.158 (0.070)	0.168 (0.052)	
Change	-0.014 (0.083)	-0.001 (0.089)	+0.011 (0.090)	<0.001

*Comparisons are across the three groups.

There was no clinically or statistically significant change in systolic BP, diastolic BP, heart rate (Table 18), low or high density lipoprotein, triglycerides, CRP, glucose, insulin and insulin resistance (HOMA) (Table 19) at 1 month between the 3 randomised groups. For all comparisons, the 95% confidence interval excluded effect sizes of 20% (261) except for HOMA and CRP where the 95% CI were 30% (Figure 10).

Increased dairy food intake was associated with a small increase in waist circumference (Table20) compared to reduced dairy food intake (0.4, CI -0.4 to 1.2 cm vs. -0.7, CI -1.3 to -0.01 cm, p=0.03). There was a non-significant increase in weight in those asked to increase dairy food consumption compared to those asked to reduce dairy food consumption (0.4, CI 0.1 to 0.7 kg vs. -0.4 CI -0.5 to 0.2 kg, p= 0.07).

Changes in each cardio-metabolic risk factor between the 3 diets evaluated by ANCOVA are displayed in Table 21. No consistent trends were observed when taking a higher compared to lower dairy food intakes across all three comparisons. There were nominally statistically significant associations (p=0.04) for increased dairy compared to the usual diet for weight gain and higher LDL-c.

Table 18: The effects of changing dairy food consumption on blood pressure and heart rate. Results are mean and standard deviation, and 95% confidence interval of mean difference between baseline and one month measurements.

*Comparisons are across the three groups.

	Reduced dairy food intake	**Same dairy food intake**	**Increased dairy food intake**	**P value***
Systolic BP (mmHg)				
Baseline	116.7 (10.7)	114.2 (10.7)	113.9 (10.6)	
1 month	115.5 (10.0)	115.1 (11.2)	114 (10.2)	
Change	-1.2 (8.5) (CI-3.6, 1.25)	-0.9 (7.4) (CI-1.9,3.6)	-0.36 (8.4) (CI-2.5,1.8)	0.72
Diastolic BP (mmHg)				
Baseline	70.3 (7.8)	70.0 (8.4)	70.2 (7.8)	
1 month	68.4 (8.3)	67.5 (8.0)	69.7 (8.1)	
Change	-1.9 (7.0) (CI 3.9,-0.07)	-2.5 (6.4) (CI-4.9,-0.8)	-0.5 (7.1) (CI-3.7,0.67)	0.38
HR (beats per minute)				
Baseline	63.7 (8.1)	63.0 (8.1)	64.1 (9.1)	
1 month	64.5 (7.2)	63.5 (6.9)	64.3 (6.9)	
Change	0.8 (5.4) (CI-4.6,2.7)	0.5 (4.2) (CI-0.8,2.4)	0.2 (4.0) (CI-1.4,1.1)	0.15

Table 19: Effects of changing dairy food intake on lipids, C - reactive protein and insulin resistance as calculated using the Homeostasis Model Assessment. Results are mean and standard deviation, and 95% confidence interval of mean difference between baseline and one month measurements. C- reactive protein has been log transformed.

	Reduced dairy food intake	**Same dairy food intake**	**Increased dairy food intake**	**P value***
LDL- cholesterol (mmol/L)				
Baseline	3.12 (0.88)	2.97 (0.86)	2.98 (0.79)	
1 month	3.01 (0.82)	2.88 (0.82)	3.05 (0.81)	
Change	-0.11 (0.38)	-0.09 (0.40)	0.06 (0.39)	0.09
	(CI -0.21,-0.1)	(CI -0.19,0.02)	(CI -0.05,0.08)	
HDL- cholesterol (mmol/L)				
Baseline (SD)	1.70 (0.42)	1.68 (0.47)	1.68 (0.48)	
1 month (SD)	1.69 (0.37)	1.63 (0.43)	1.69 (0.48)	
Change	-0.01 (0.18)	-0.05 (0.18)	0.01 (0.15)	0.33
	(CI -0.06,0.03)	(CI-0.01,0.00)	(CI -0.03,0.05)	
Triglyceride (mmol/L)				
Baseline	1.12 (0.81)	1.12 (0.72)	1.04 (0.68)	
1 month	1.14 (0.73)	1.13 (0.75)	1.04 (0.75)	
Change	0.03 (0.76)	0.01 (0.40)	0.0 (0.41)	0.95
	(CI -0.18,0.23)	(CI-0.10,0.11)	(CI -0.11,0.11)	
Log C- reactive protein				
Baseline	0.36 (0.82)	0.35 (0.73)	0.41 (0.65)	
1 month	0.28 (0.67)	0.39 (0.58)	0.44 (0.75)	

Change	-0.09 (0.60) (CI -0.25,0.07)	-0.05 (0.65) (CI -1.2,0.22)	0.04 (0.52) (CI -11,1.8)	0.17
Glucose (mmol/L)				
Baseline	5.33 (0.53)	5.21 (0.45)	5.41 (0.41)	
1 month	5.30 (0.54)	5.17 (0.47)	5.41 (0.62)	
Change	-0.04 (0.44) (CI -0.16,0.09)	-0.02 (0.47) (CI -0.13,0.08)	0.00 (0.44) (CI -0.10,0.13)	0.68
Insulin (mU/L)				
Baseline	5.56 (2.97)	6.01 (4.22)	6.07 (4.41)	
1 month	6.12 (3.13)	6.85 (5.45)	6.04 (3.32)	
Change	0.47 (2.60) (CI -0.26,1.19)	0.84 (4.80) (CI -0.44, 1.13)	0.2 (3.58) (CI -0.8, 1.19)	0.73
Homeostasis Model assessment (IR) (units)				
Baseline	1.38 (0.82)	1.43 (1.08)	1.47 (1.1)	
1 month	1.47 (0.87)	1.64 (0.96)	1.47 (0.95)	
Change	0.09 (0.69) (CI-0.11,0.28)	0.24 (1.52) (CI -0.17,0.65)	0.08 (0.99) (CI -0.20,0.35)	0.8

*Comparisons are across the three groups

Table 20: The effects of changing dairy food intake on weight, waist and hip circumference. Results are mean and standard deviation, and 95% confidence interval of mean difference between baseline and one month measurements.

	Reduced dairy food intake	**Same dairy food intake**	**Increased dairy food intake**	**P value***
Waist circumference(cm)				
Baseline	84.2 (12.5)	83.1 (12.6)	84.3 (11.8)	
1 month	83.5 (12.5)	82.7 (12.5)	84.7 (11.9)	
Change	-0.7 (2.4) (CI -1.3,-0.01)	-0.4 (2.3) (CI -1.0,0.2)	0.4 (3.1) (CI -0.4,1.2)	0.03
Hip circumference (cm)				
Baseline	100.1 (8.7)	100.8 (10.1)	101.8 (8.9)	
1 month	99.9 (9.0)	100.6 (9.0)	102.0 (9.3)	
Change	-0.3 (2.0) (CI -0.9,2.6)	-0.2 (1.8) (CI-0.5,2.1)	0.2 (1.5) (CI -0.4,0.8)	0.22
Weight (kg)				
Baseline	72.6 (14.4)	72.8 (13.9)	72.9 (14.1)	
1 month	72.4 (14.4)	72.6 (14.1)	73.3 (13.9)	
Change	-0.2 (1.3) (CI-0.5,0.2)	-0.2 (1.2) (CI-5,0.9)	0.4 (1.3) (CI 0.1,0.7)	0.07

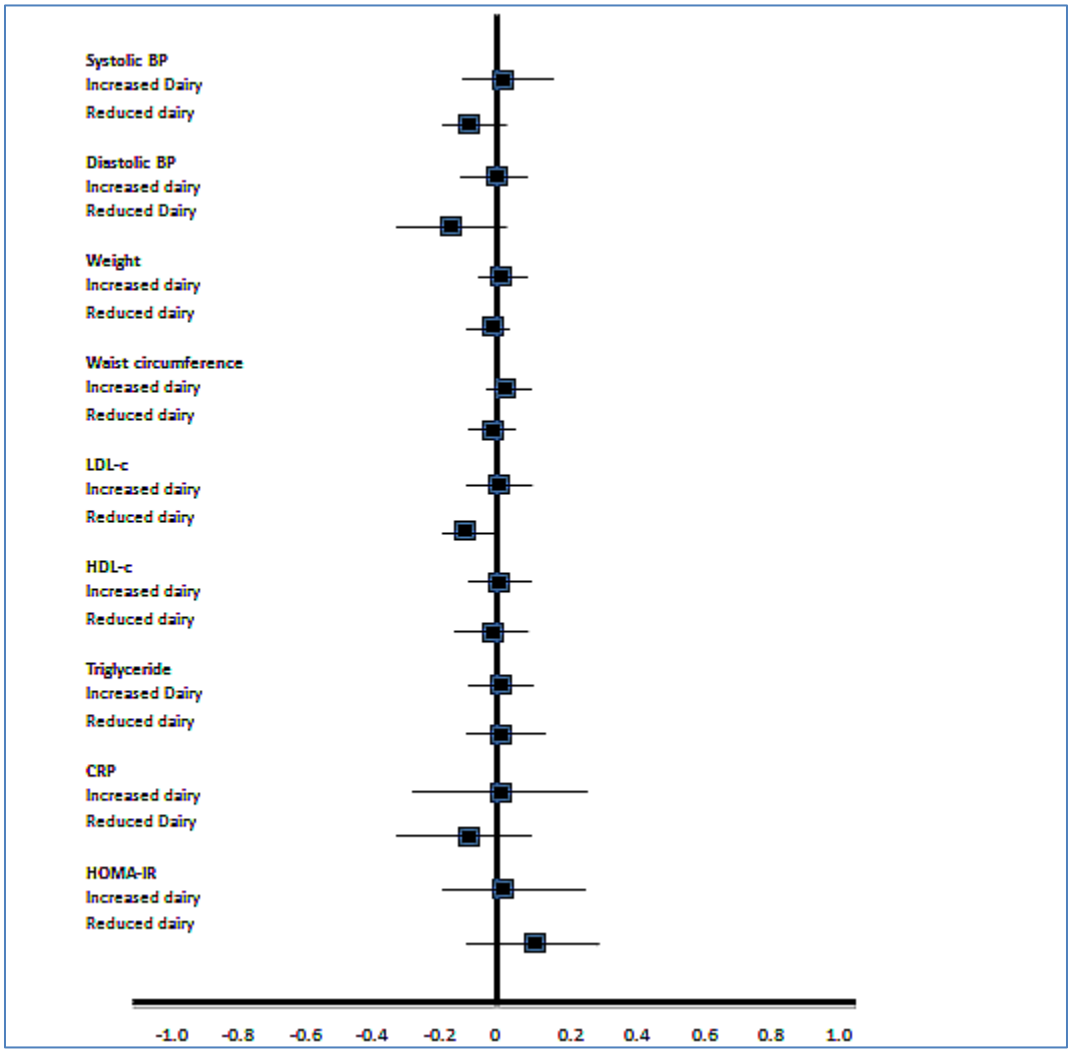
*Comparisons are across the three groups.

Table 21: Comparison between randomized groups expressed as the standardized mean difference (95%CI). Results are presented as higher versus lower dairy intake.

	Difference between no change and reduced dairy food intake	**Difference between increased and no change dairy food intake**	**Difference between increased and reduced dairy food intake**
Systolic Blood pressure (mmHg)	1.4 (-1.8 to 4.7)	-1.4(-4.6 to 1.9)	0.1 (-3.3 to 3.7)
Diastolic blood pressure (mmHg)	-0.7 (-3.3 to 1.9)	1.5 (-1.1 to 4.1)	0.8 (-1.8 to 3.5)
Heart rate (beats per minute)	-0.4 (-2.3 to 1.4)	-0.8 (-2.6 to 1.1)	-1.2 (-3.0 to 0.6)
LDL- cholesterol (mmo/L)	-0.024 (-0.19 to 0.15)	0.19 (0.019 to 0.37)*	0.16 (-0.01 to 0.34)
HDL- cholesterol (mmol/Ll)	-0.04 (-0.010 to 0.02)	0.06 (0.00 to 0.12)	0.02 (-0.04 to 0.08)
C- reactive protein (mg/L)	0.14 (-0.05 to 0.34)	-0.00 (-0.20 to 0.19)	0.14 (-0.06 to0.34)
Fasting Glucose (mmol/L)	-0.02 (-0.19 to 0.15)	0.10 (-0.07 to 0.27)	0.08 (-0.01 to 0.25)
Insulin (mU/L)	0.59 (-0.73 to 1.91)	-0.75 (-2.07 to 0.57)	-0.16 (-1.51 to 1.19)
Homeostatic model assessment (units)	0.23 (-0.18 to 0.65)	-0.23 (-0.64 to 0.18)	0.00 (-0.41 to 0.42)
Weight (Kg)	-2.26 (-4.87 to 0.35)	2.76 (0.16 to 5.37)	0.51 (-2.13 to 3.13)
Waist Circumference (cm)	0.20 (-1.03 to 1.43)	0.85(-0.38 to 2.07)	1.06 (-0.206 to 2.30)

Comparison is made using the analysis of covariance (ANCOVA). * p=0.04, for all other measurements p>0.09

Figure 9: The effect size of dairy food on cardio metabolic risk with 95% confidence interval.



Discussion

In the INTERHEART (262) and INTERSTROKE (263) studies, the major cardiovascular risk factors hypertension, central obesity, lipids and diabetes mellitus, accounted for about 70% of risk. In this study, change in dairy food intake for one month had no significant effect on these major cardiovascular risk factors. Another risk factor predictive of acute cardiovascular events is high sensitivity C-reactive protein (HsCRP). (264) In observational studies, dairy food intake is associated with lower levels of HsCRP (265) but in this study, HsCRP is similar on higher and lower dairy food intakes. The 95% CI for effect size is 20% (261) which suggest that advice to modify dairy food intake alone is likely to have little or no effect on the cardiovascular risk factors measured.

Much of the previous evidence on the associations between dairy food and cardio-metabolic risk factors is from large population based studies. In these studies higher dairy food intake has been associated with lower levels of inflammatory markers, (265, 266) lower blood pressure, (196, 246, 267) less insulin resistance or diabetes (151, 196, 198, 245) and lower body weight. (147, 151, 187, 191, 245, 268, 269) However, observational studies do not allow reliable evaluation of causality because of the potential for bias from confounders. For example higher dairy food intake is associated with better social economic status and higher educational attainment, (132, 133, 270) factors which are also associated with less obesity and lower cardio-metabolic risk. (234, 235)

Previous evidence on the effects of dairy food consumption on cardio-metabolic risk from randomised clinical trials is limited. Most studies have been small and assessed only one cardio-vascular risk factor. (147, 182, 253, 268). In the DASH (240) study, dairy food consumption was estimated to lead to a small reduction in blood pressure. However the DASH study included multiple interventions with increased fruits, vegetables, and low-fat dairy foods and had reduced amounts of SFA, total fat, and cholesterol, so the impact of dairy food consumption compared to other interventions such as reduced total fat consumption could not be reliably assessed.

In this study decreasing dairy food intake was associated with a small decrease in waist circumference and weight. This does not support the suggestion that increasing dairy food intake aids weight loss. Change in dairy food also had no effect on fasting glucose, insulin or HOMA. Observational studies suggest that increased dairy consumption could produce a favourable effect in persons with insulin resistance, (148, 187, 191, 196, 245) but limited data from randomised studies also found no association between dairy food intake and insulin resistance. (129, 253, 255, 271, 272) While cardiovascular prevention guidelines have suggested reducing high fat dairy food in order to lower SFA consumption, (5, 7, 8) evidence that change in dairy food influences plasma lipid levels is also limited. (252, 255, 271, 272) In the current study LDL and HDL cholesterol levels were similar after both increasing and decreasing dairy food intake, but the 95% confidence interval included an approximately 0.37mml/L increase in LDL cholesterol on the higher dairy food intake.

This study was designed to evaluate the real-world effect of dietary advice usually given to patients. (5, 7, 180) This contrasts with studies which have controlled intake with prepared meals, (182, 240) supplementary dairy products, (255, 273-275) or focus on specific types of dairy such as fermented vs. unfermented dairy, (276, 277) cheeses vs. whole milk. (278-280) These studies are often predicated on the assumption that specific dairy foods contain different concentrations of fatty acid isomers which may affect health. However, most diets contain dairy food from a range of sources and results of these focused studies are hard to apply to the general population.

At present several authoritative guidelines focus on specific food groups and place dairy food as an integral part of a healthy diet, (5) recommending at least 3 servings per day. This study suggests that the shift in focus of recent guidelines (7, 180, 243) and researchers (281, 282) from specific food groups like dairy products to broader dietary patterns is appropriate.

Limitations and strengths

The study evaluated the impact of simple advice, similar to that given as part of usual health care, and consistent with most current dietary guidelines which focus on food groups and dietary patterns. (7, 243) The study was not designed to evaluate the effects of changing different types of dairy food, such as high or low fat dairy. However, no clear association was observed between the change in dairy fat and change in any cardio-metabolic risk factor. Detailed food frequency questionnaires assessed the types of dairy food ingested and the self-reported changes in dairy intake were large and clinically relevant. Compliance to randomised groups was objectively assessed by measuring plasma fatty acid levels of 15:0 and 17:0.

Evaluating effects of both increasing and reducing dairy food intake allowed evaluation of the dose response if present. It is possible the duration of dietary change was too short to influence the cardio-metabolic risk factors. However, in experimental studies the effects of dietary change on blood pressure, weight, insulin resistance and cholesterol have been observed within one month, (240) and adherence to longer term dietary change in a clinical trial is difficult. (283) The study evaluated healthy volunteers in part to reduce confounding from effects of disease and treatments which influence cardio-metabolic risk.

Further study is needed to evaluate long term effects in patients with the metabolic syndrome and cardiovascular disease, but it is likely these will be similar to healthy volunteers.

Conclusion

Dietary advice to change dairy food intake for one month had no significant effects on major cardiovascular risk factors. These observations suggests that dairy food can be included as part of a normal healthy diet without adverse effects on cardio-metabolic risk.

Section 2: The effects of changing dairy food intake on trans and saturated fatty acid levels- results from a randomised controlled study. (284)

Abstract:

*Background***:** Dairy food is an important natural source of saturated and trans fatty acids in the human diet. This study evaluates the effect of dietary advice to change dairy food intake on plasma fatty acid levels known to be present in milk in healthy volunteers.

Methods: Twenty one samples of whole fat dairy milk were analysed for fatty acids levels. Changes in levels of plasma phospholipid levels were evaluated in 180 healthy volunteers randomised to increase, maintain or reduce dairy food intake for one month. Fatty acids were measured using gas chromatography–mass spectrometry and levels were normalised to d-4 alanine.

Results: The long chain fatty acids, palmitic (13.4%), stearic (16.7%) and myristic (18.9%) acid, were the most common saturated fats in milk. Four trans fatty acids constituted 3.7% of the total milk fat content. Increased dairy food intake by 3.0 (standard deviation 1.2) serves/ day for 1 month was associated with small increases in plasma levels of myristic (+0.05, 95% confidence level-0.08 to 0.13, p=0.07), pentadecanoic (+0.014, 95% confidence level -0.016 to 0.048, p=0.02) and margaric acid (+0.02, 95% confidence level -0.03 to 0.05, p=0.03). There was no significant change in plasma levels of 4 saturated, 4 trans and 10 unsaturated fatty acids. Decreasing dairy food intake by 2.5 (standard deviation 1.2) serves per day for one month was not associated with change in levels of any plasma fatty acid levels.

Conclusion: Dietary advice to change dairy food has a minor effect on plasma fatty acid levels.

Background

A healthy diet is important to reduce the risk of CVD (285) and diabetes. (286) In observational studies, high intake of SFA (150) and TFA (21) and low intake of PUFAs (175) is associated with increased cardiovascular risk. Studies also suggest that increased plasma levels of TFA (23) and SFA (176) are associated with an increased risk of CVD. Plasma levels of trans, odd-numbered saturated and polyunsaturated (n-3 and n-6) fatty acids reflect dietary intake as they are not endogenously synthesized. (287) The aim of dietary advice to reduce intake of foods with SFA and TFA is to reduce plasma levels of 'harmful' fats and increase levels of 'beneficial' PUFAs.

Dairy food is a highly complex food composed of constituents thought to be both harmful and beneficial for cardiovascular health. It is the most abundant source of animal fat in the diet including SFA and 'naturally occurring' rTFA. (4) It is rich in in long-chain SFAs such as myristic and palmitic acids; thought to be harmful for cardiovascular health. (49) However, dairy food also contains unsaturated, short and medium-chain SFAs thought to be beneficial for heart health. (59, 70) Whilst iTFA are considered harmful, the effects of TFA from dairy food on cardiovascular health are less clear. (33) Some studies suggest rTFA may be beneficial to health (136) but others suggest a neutral effect. (134, 139) This inconsistent data on the health effects of dairy food (183, 213, 265, 288) has led to confusing messages for consumers. Cardiovascular guidelines recommend avoidance of dairy fat, (289, 290) but others recommend at least 3-4 servings of dairy food per day. (5)

No randomised studies have assessed the effects of changing dairy food intake in the real world on rTFA, saturated and polyunsaturated fatty acid levels. In this study, we analysed the composition of fatty acids in dairy food in New Zealand. We then evaluated the effects of dietary advice to increase, decrease or not change daily dairy food intake for one month on plasma fatty acid levels in a randomised clinical trial with180 healthy volunteers.

Methods

Randomised study

A more detailed description of the randomised study and the effects of dietary intervention on cardio-metabolic risk factors have previously been reported. (1) A full description of the study and dietary intervention is described in Section 1 of Chapter 3.

For fatty acid analysis, participants were instructed to fast for 10 hours prior to clinic visits. Blood samples were collected in ethylene-diamine-tetra acetic acid tubes from all participants at baseline and after one month. Plasma was separated in a 4^0 Celsius centrifuge within 20 minutes of the blood draw and stored at -80^0 Celsius in nunc tubes until analysis.

Milk samples

Seven brands of whole (3.3% fat) cow's milk were purchased in April 2011. These included homogenised, non-homogenised, organic and non-organic brands. Three samples of each brand were collected (total n=21), decanted into nunc tubes and stored at -80^0 Celsius until analysis.

Analysis of milk and plasma

Fatty acid analysis of milk and plasma was performed at the School of Biological Science, Auckland University. A detailed description of the methodology is available at the beginning of Chapter 3.

Definitions

The United States Department of Agriculture criteria (258) were used to define serving size. For example one serving size was the equivalent of 250ml 3% milk, 250ml yogurt, 500ml 1.5% milk or 1/3 cup cheddar cheese. The total dairy fat content ingested in g/day was calculated based on the reported intakes of each food, and the fat content from manufacturers' labels. (259)

Statistical analysis

Baseline and one month visit levels of fatty acids and changes in these measurements between baseline and one month were summarized as median and interquartile range. A 30% difference in vaccenic acid levels was expected to be seen with the intervention. Sixty participants in each group were estimated to

give 80% power to detect a treatment difference with a two-sided 0.05 significance level, if the true difference between the treatments was 1.5.

Analysis was undertaken only on those participants who completed the study. Baseline characteristics and scores were compared across the three arms using ANOVA. ANCOVA was used to determine if there were significant differences in fatty acid values between the three dairy food groups. (291) ANCOVA was conducted on the follow up values adjusting for the baselines values. For the few variables that did not meet the ANCOVA assumptions, (292) ANOVA was used to determine significant differences in follow-up values between the three dairy food groups. Secondary analysis was performed to determine differences within randomised groups using ANOVA. Statistical analyses were performed with SAS software version 9.3 (SAS Institute, Cary, NC). The Bonferroni method was used to adjust for multiplicity testing. (293)

Results

Fatty acids in milk

Whole fat (3.3%) milk contained 81.95% SFA, 3.98% TFA and 14.06% unsaturated fats (Table 22). The most common SFA were the medium chain lauric acid 13.7 (SD 4.7) %, and long chain palmitic acid 15.9(SD 5.8) % and myristic acid 18.0 (SD 3.5) %. Vaccenic acid was the most plentiful rTFA (3.0% of milk fat), and oleic acid is the most common unsaturated fat (Figure 10).

Table 22: Fatty acids in milk and in human plasma .Fatty acids are measured by gas chromatography-mass spectrometry.

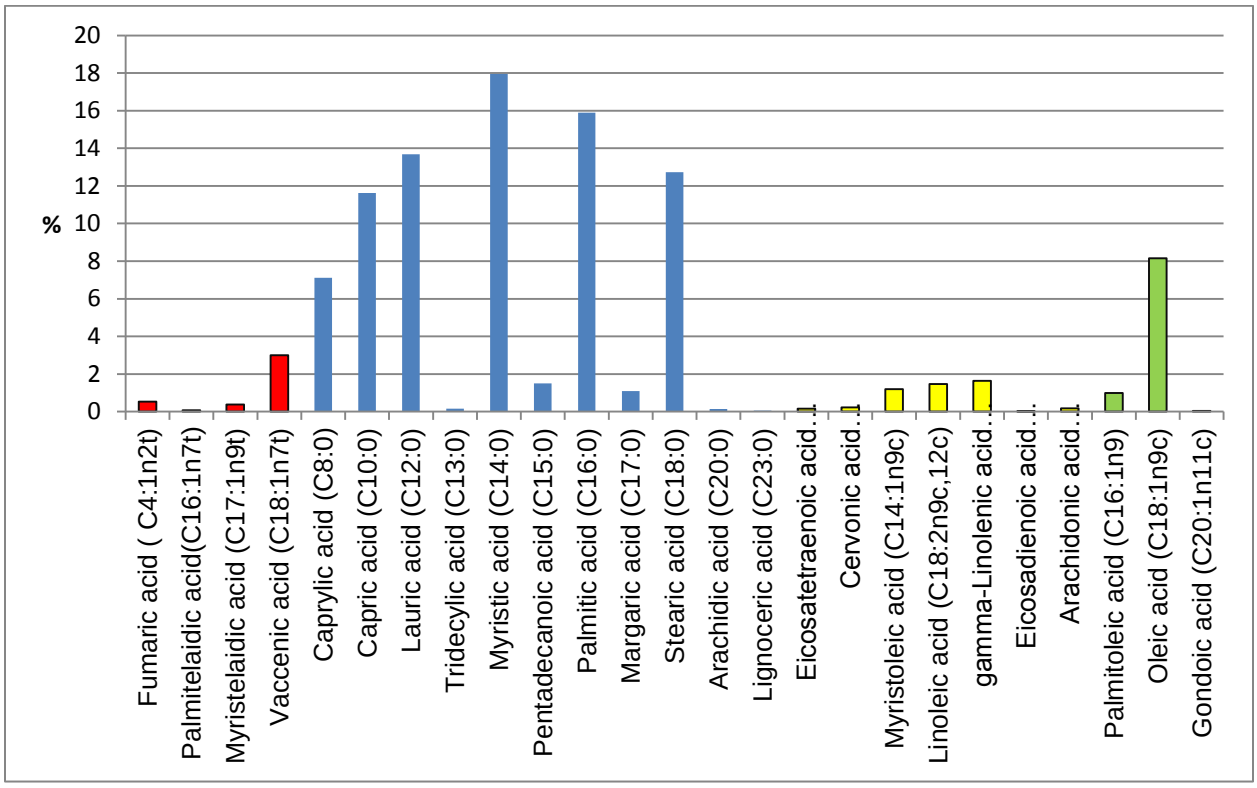
	Levels in whole fat milk N=21	**Coefficient of variation (%) milk**	**Plasma levels in humans at baseline**	**Coefficient of variation (%) plasma**
Trans fatty acids				
Fumaric acid	0.059 (0.004)	0.071	ND	ND
Palmitelaidic acid	0.007 (0.009)	1.292	0.022 (0.031)	1.593
Vaccenic acid	0.334 (0.183)	0.551	0.458 (0.210)	0.465
Myristelaidic acid	0.041 (0.006)	0.153	0.073 (0.313)	4.012
Saturated fats				
Caprylic acid	0.791 (0.213)	0.265	0.005 (0.003)	0.583
Capric acid	1.293 (0.347)	0.248	0.015 (0.019)	1.44
Lauric acid	1.520 (0.474)	0.322	0.034 (0.054)	1.633
Tridecylic acid	0.018 (0.003)	0.136	ND	ND
Myristic acid	1.996 (0.345)	0.179	0.262 (0.131)	0.476
Pentadecanoic acid	0.167 (0.088)	0.532	0.084 (0.032)	0.374
Palmitic acid	1.767 (0.265)	0.153	2.221 (0.635)	0.301
Margaric acid	0.122 (0.014)	0.824	0.160 (0.077)	0.534
Stearic acid	1.415 (0.137)	0.099	1.966 (0.548)	0.336
Arachidic acid	0.016 (0.002)	0.133	0.013 (0.004)	3.587
Lignoceric acid	0.006 (0.001)	0.224	ND	ND
Polyunsaturated fats				
Eicosatetraenoic acid	0.018 (0.003)	0.142	0.231 (0.868)	0.73
Linoleic acid	0.160 (0.012)	0.615	0.789 (0.373)	0.564
gamma-Linolenic acid	0.176 (0.038)	0.222	0.076 (0.097)	1.230

Eicosadienoic acid	0.002 (0.002)	0.412	0.041 (0.017)	0.489
Arachidonic acid	0.020 (0.006)	0.285	0.556 (0.146)	0.283
Cervonic acid	0.024 (0.004)	0.283	0.381(0.132)	0.374
Monounsaturated fats				
Myristoleic acid	0.12 (0.04)	0.33	ND	ND
Palmitoleic acid	0.106 (0.026)	0.18	0.081(0.055)	0.53
Oleic acid	0.898 (0.085)	0.10	0.69(0.21)	0.32
Gondoic acid	0.004 (0.004)	0.78	ND	ND

ND=not detected

* Monounsaturated fatty acids

Figure 10: Fatty acids found in New Zealand milk. Plasma fatty acids are measured by gas chromatography mass spectrometry and are [percentage of total fat.



Trans fatty acids

Saturated fatty acids

Poly- unsaturated fatty acids,

Mono -unsaturated fatty acids

Randomised study

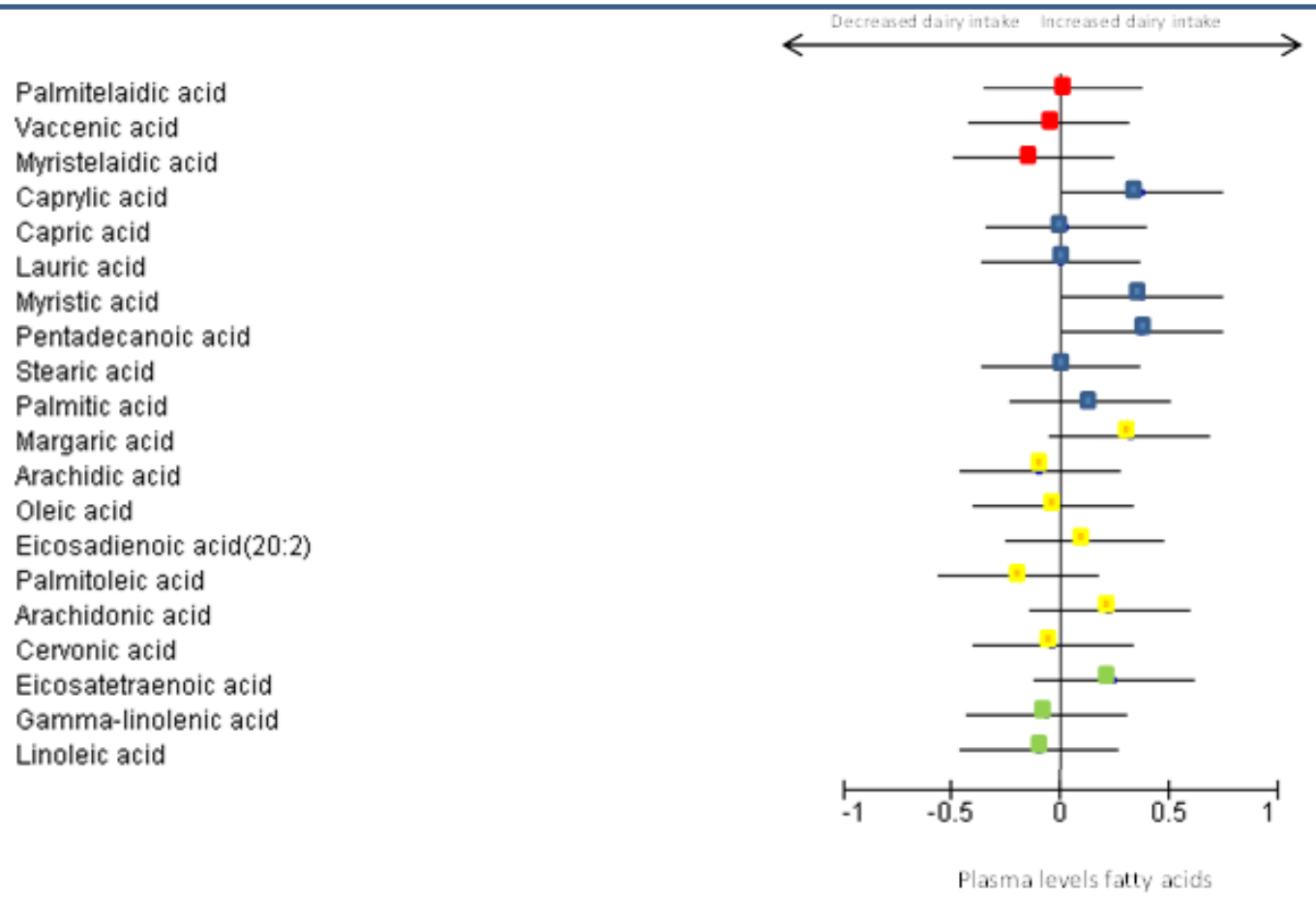
176 of 180 randomised participants completed the study. Baseline characteristic are presented in Table 16 and are described in section 1 of Chapter 3. There were no significant differences in baseline characteristics and fatty acid levels by dietary group.

Change in dairy food intake assessed by the FFQ for the increased dairy food diet was +3.0 (1.2) serves/day, p<0.001, no change in dairy food consumption was -0.6 (0.2) serves/day, p=0.78 and for the decreased dairy food diet -2.5 (1.2) serves/day, p<0.001. The total difference between those asked to

increase and reduced their dairy food intake was 5.5 (SD1.4) serves per day, p<0.001. Change in dairy fat for the reduced dairy food diet was -10.4 (10.1) g/day, no change in dairy food consumption was -3.4 (7.9) g/day, and for the increased dairy food diet was +12.5 (15.7) g/day. There was no significant change in intake of food from ruminant sources, such as meat or goat's milk and cheese, by randomised group.

The effect of changing dairy food intake on plasma fatty levels is displayed in Tables 23 and 24. There was no significant change in TFA between randomised groups. In those randomised to decrease dairy food intake, there was no significant change in fatty acid levels compared to the control group. In those randomised to increase dairy food intake, plasma levels of pentadecanoic (p=0.02) and margaric acids (p=0.03) increased compared to the control group. When comparison was made between increased and decreased dairy food intake (Figure 12), there was an increase in caprylic, myristic, pentadecanoic and margaric acids. Other long-chain and medium-chain saturated fats did not change significantly. After adjustment for multiple testing, changes in pentadecanoic acid levels between increased and reduced dairy food intake remained significant (p=0.02). However, change in other fatty acid levels was no longer significant.

Figure 11 Change in plasma levels of fatty acids with a high dairy compared to a low dairy food diet.



Results are expressed as a standard mean difference .This graph shows that increasing dairy food intake compared to the control group (no change) relatively increases plasma levels of caprylic, myristic, pentadecanoic and margaric acid, but has no effect on other fatty acids. However when correcting for multiplicity testing; only the change in pentadecanoic acid remained significantly different between high and low dairy diets.

There was no significant change in total SFAs for the increased dairy food diet +13.1 (95% confidence interval (CI) -1.2 to + 25.3) %, unchanged dairy food diet +2.4 (95% CI -9.2 to +11.3) % or the decreased

dairy food diet +3.5 (95%CI -5.5 to +11.6) %. The FFQ indicated that participants randomised to reduce dairy food intake switched to rice, almond and soy milk products.

Change within randomised groups was then analysed. There was no significant change in total TFA for the increased dairy food diet -5.5% (95%CI -15.4 to +10.2) %, unchanged dairy food diet -9.1(95CI % -20 to +1.2)%, and the decreased dairy food diet +10.1 (95%CI -2.4 to +22.5) %. Levels of the individual TFA isomers, vaccenic acid, palmitelaidic acid and myristelaidic acid also did not change. Pentadecanoic (15:0), myristic (14:0) and margaric (heptadecanoic (17:0)) acids increased with increased dairy intake (p=0.02 and 0.03 respectively); however, there was no significant change for reduced dairy intake.

Figure 13 shows associations between levels of milk fatty acids and effects of increasing dairy food intake on plasma levels of fatty acids. Figure 14 shows associations between levels of milk fatty acids and effects of reducing dairy food intake on plasma levels of fatty acids. Change in the plasma levels of fatty acid isomers did not correlate with the amount of that fatty acid in dairy food. For example, myristic acid and palmitic acid were the most plentiful SFAs in milk, but changing dairy food intake did not affect plasma levels.

Table 23: Plasma levels of fatty acids across the three randomised groups at baseline and one month. The change in plasma levels across the three groups was assessed using ANCOVA. ANCOVA was used unless assumptions were not met. ANOVA was used for all others (Table 24) Fatty acids are standardized to d4- alanine and presented as median (interquartile range).

Trans Fatty Acid	Reduced dairy food intake	Same dairy food intake	Increased dairy food intake	Across all three groups P	Reduced vs. same P	Same vs. increased P
Vaccenic acid						
Baseline	0.50 (0.32 to 0.63)	0.51 (0.36 to 0.61)	0.51 (0.37 to 0.61)			
1 month	0.54 (0.40 to 0.64)	0.49 (0.36 to 0.60)	0.50 (0.36 to 0.68)			
Change	0.05 (-0.14 to 0.16)	-0.03 (-0.23 to 0.26)	-0.04 (-0.22 to 0.30)	0.74	0.24	0.31
Myristelaidic acid						
Baseline	0.018 (0.015 to 0.024)	0.018 (0.013 to 0.023)	0.018 (0.015 to 0.023)			
1 month	0.019 (0.015 to 0.024)	0.018 (0.015 to 0.023)	0.018 (0.015 to 0.023)			
Change	0.001 (-0.003 to 0.005)	0.001 (-0.004 to 0.005)	0.001 (-0.004 to 0.005)	0.79	0.91	0.26
Saturated Fatty Acid						
Myristic acid						
Baseline	0.22 (0.17 to 0.30)	0.24 (0.18 to 0.32)	0.25 (0.19 to 0.33)			
1 month	0.25 (0.19 to 0.33)	0.24 (0.203 to 0.32)	0.29 (0.22 to 0.36)			
Change	0.01 (-0.05 to 0.1)	0.01 (-0.08 to 0.09)	0.05 (-0.08 to 0.13)	0.39	1.00	0.07
Palmitic Acid						
Baseline	2.33 (1.88 to 2.75)	2.32 (1.97 to 2.70)	2.20 (1.96 to 2.64)			
1 month	2.43	2.34	2.51			

	(2.09 to 2.89)	(1.97 to 2.91)	(2.06 to 2.93)			
Change	-0.52 (-0.51 to 0.90)	0.42 (-0.47 to 0.62)	1.37 (-0.41 to 9.37)	0.79	0.47	0.42
Stearic acid						
Baseline	1.98 (1.77 to 2.21)	1.98 (1.84 to 2.15)	1.97 (1.76 to 2.24)			
1 month	2.12 (1.88 to 2.35)	2.15 (1.91to 2.39)	2.12 (1.83 to 2.4)			
Change	0.05 (-0.27 to 0.48)	0.17 (-0.10 to 0.52)	0.13 (-0.32 to 0.56)	0.84	0.56	0.41
Pentadecanoic acid						
Baseline	0.084 (0.072 to 0.101)	0.084 (0.076 to 0.120)	0.085 (0.070 to 0.101)			
1 month	0.079 (0.054 to 0.088)	0.085 (0.068 to 0.104)	0.098 (0.078 to 0.120)			
Change	-0.006 (-0.015 to 0.00)	-0.00 (-0.015 to 0.017)	+0.014 (-0.016 to 0.048)	0.11	0.33	0.02
Margaric acid						
Baseline	0.17 (0.12 to 0.22)	0.17 (0.12 to 0.21)	0.16 (0.09 to 0.21)			
1 month	0.15 (0.08 to 0.19)	0.17 (0.08 to 0.18)	0.18 (0.14 to 0.24)			
Change	-0.02 (-0.09 to 0.03)	-0.001 (-0.08 to 0.02)	0.02 (-0.03 to 0.05)	0.95	0.54	0.03

Table 24: The difference across the three dairy food groups using ANOVA for those fats that did not meet the assumptions for ANCOVA.

Trans Fatty Acid	**Reduced dairy food intake**	**Same dairy food intake**	**Increased dairy food intake**	**P value**
Palmitelaidic acid				
Baseline	0.033 (0.00 to 0.45)	0.04 (0.00 to 0.43)	0.02 (0.00 to 0.32)	
1 month	0.049 (0.00 to 0.50)	0.039 (0.00 to 0.49)	0.034 (0.01 to 0.39)	
Change	0.00 (-0.09 to 0.09)	0.00 (-0.27 to 0.22)	0.00 (-0.07 to 0.14)	0.96
Linoelaidic acid				
Baseline	0.000 (0.000 to 0.0296)	0.000 (0.000 to 0.026)	0.000 (0.001 to 0.018)	
1 month	0.000 (0.000 to 0.0233)	0.000 (0.000 to 0.0274)	0.000 (0.000 to 0.033)	
Change	0.000 (-0.010 to 0.000)	0.000 (-0.014 to 0.016)	0.000 (0.000 to 0.018)	0.53
Saturated fats				
Lauric acid				
Baseline	0.025 (0.000 to 0.039)	0.026 (0.000 to 0.043)	0.025 (0.000 to 0.042)	
1 month	0.030 (0.000 to 0.056)	0.026 (0.000 to 0.041)	0.036 (0.025 to 0.052)	
Change	0.00 (-0.011 to 0.027)	0.000 (-0.022 to 0.024)	0.012 (-0.005 to 0.024)	0.12

Figure 12: Correlation between fatty acid levels in milk and change in plasma fatty acid levels in those participants asked to increase dairy food intake. Results are mean levels and are normalised to d4 alanine

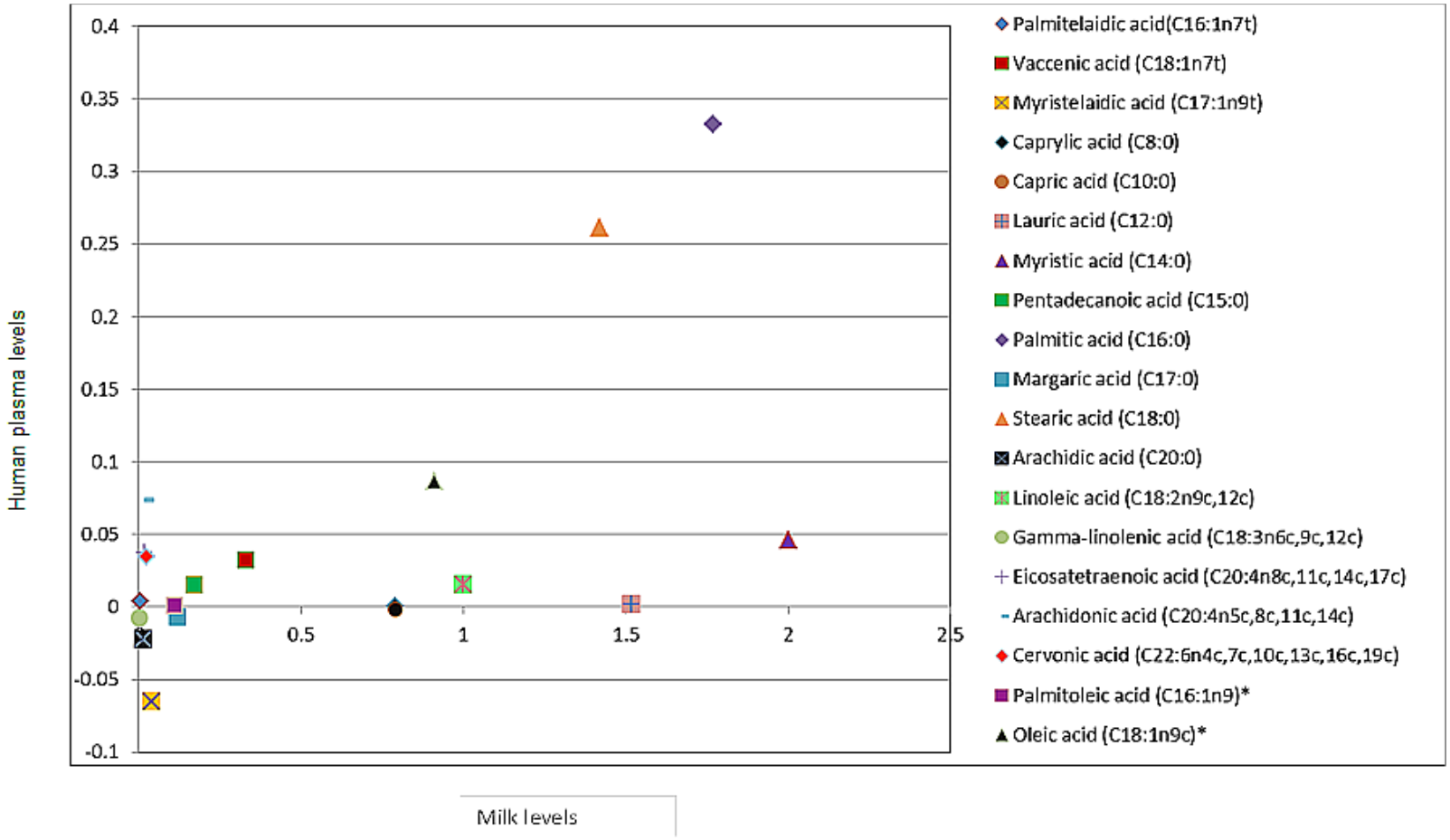
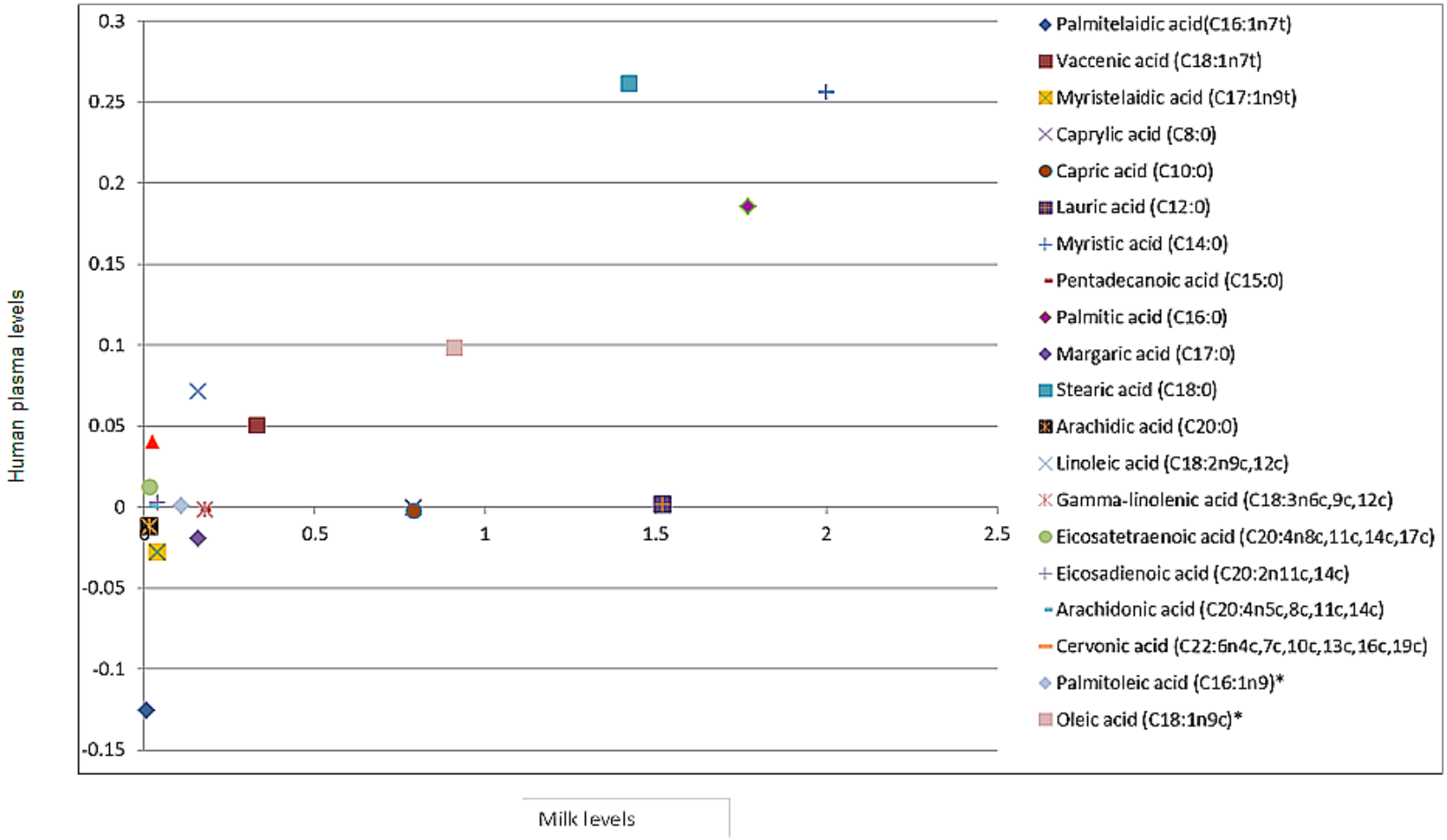


Figure 13: Correlation between fatty acid levels in milk and change in fatty acid levels in those participants asked to decrease dairy food intake.

Results are mean levels and are normalised to d4 alanine.



Discussion

Dairy food is the richest natural source of 'harmful' fats like long-chain SFA and TFA in the diet. (294) Changing dietary intake should significantly affect the plasma levels of fats, especially long-chain SFA and rTFA, thought to be almost exclusively sourced from dairy food. In this study milk was analysed to assess fatty acids present in dairy food. Fatty acid composition in milk is mainly dependent on the feed and season (75) and varies between countries. It was therefore essential to confirm fatty acids levels concurrent with the randomised study to allow for interpretation of study findings. Milk was considered the most useful dairy food to analyse as participants increased dairy food intake mainly by increasing their consumption of whole fat milk, yogurt and ice cream rather than cheese.

Intake of dairy food significantly changed for all randomised groups, with those that increased dairy food consuming on average 5.5 serves per day more than those that decreased dairy food intake. However, whilst there was a small increase in plasma levels of myristic, pentadecanoic acid and margaric acid with increased dairy food consumption, plasma levels of most fatty acids present in milk did not change with diet. After multiplicity testing, only pentadecanoic acid levels changed significantly.

Dietary guidelines recommend consumption of low fat dairy food to reduce the intake of long-chain fatty acids like myristic and palmitic acid that are associated with increased cardiovascular risk, (37) and stearic acid that is associated with reduced HDL cholesterol. (36) However the effects of the only fatty acid that changed with significant change in the diet, pentadecanoic acid, are not known. Some studies have suggested that margaric acid and pentadecanoic acid are markers of dairy food intake, (213) nevertheless, this study suggests that in the real world, large changes in dairy food intake does not lead to significant changes in these fatty acids. Moreover, reducing dairy food intake had no effect on plasma levels of these fats suggesting that these may also be present in other food sources. Ultimately, separating the health effects of these fatty acids is difficult to justify when giving dietary advice, as they are highly correlated because they are found in the same food (e.g. beef and dairy products).

Similarly, rTFA did not change with randomised groups. Dietary advice to reduce dairy food intake in patients with cardiovascular disease is not aimed at reducing plasma rTFA levels. Consumption of rTFA is

thought to be too low to have biological effects (135) and some studies suggests that vaccenic acid may not be harmful for human health. (136) Previous studies have identified palmitelaidic acid as exclusively from dairy food; (66, 99) however, this study suggests that these assumptions may be incorrect.

It is possible that, in persons who decreased dairy food consumption, rTFA from other sources increased. Oils containing precursors to vaccenic and palmitelaidic acid are increasingly used for processing and cooking food. Increased heat or pressure during cooking can raise rTFA levels in these oils. (76) Vaccenic acid is found in dairy-free processed foods such as takeaway potato chips in the United Kingdom, (77) and dairy components are also added to products like margarines, often assumed to be dairy- free. When these components are heated, TFA levels can be significantly increased. (76) A less likely possibility is that humans are able to catalyse a reaction to convert small amounts of cis fatty acids to TFA. Whilst humans are able to convert vaccenic acid to conjugated linoleic acid by delta-9 desaturase in the liver, (78, 86) bioconversion of cis to trans fatty acids has not been demonstrated.

Changing dairy food intake for one month could be too short to affect plasma fatty acid levels. However, in feeding studies changes in plasma TFA occur within 2-3 weeks, (128, 131) and other dairy fatty acids like pentadecanoic and margaric acid (213), changed with a change in dairy food intake within 3 weeks. There was a large difference in dairy food intake within and between groups. In contrast to some observational studies, (66, 99) no correlation was observed between vaccenic acid and palmitelaidic acid either at baseline or 1 month (r=0.053 and 0.089 respectively) suggesting they do not emanate exclusively from the same source.

Limitations

It is also possible that participants did not adhere to their randomised diet. However, food frequency questionnaires suggested good adherence and that consumption of other sources of TFA, such as processed food, and goat’s milk or meat, were not increased. Nevertheless, food frequency questionnaires might not accurately reflect dietary intake. (207, 295) Studies comparing food frequency measures with repeated dietary recalls generally show correlations of the order of 0.4–0.7. (296)

Conclusion

Dietary advice to change the intake of dairy food does not significantly change plasma fatty acid levels including ruminant trans fatty acids. Such advice may need to focus on total food patterns rather than individual food groups to affect plasma fatty acid levels.

Conclusion

The conclusion reached from Chapter 3 is that fatty acids including TFA, considered exclusively ruminant, do not alter following a significant change in dairy food intake. Changing dairy food intake also did not significantly affect cardio- metabolic risk factors. This contradicts the guidelines that assert that dairy food is the most significant source of certain fats (pentadecanoic acid, margaric acid, vaccenic acid and palmitelaidic acid) and that it has beneficial effects on insulin resistance and blood pressure.

Chapter 4

Observational studies, (66, 99) indicate that both vaccenic and palmitelaidic acids are derived exclusively from dairy food. However, results of the randomised study described in Chapter 3 suggest that these assumptions may be incorrect. Neither vaccenic acid nor palmitelaidic acids correlated with changes in dairy food intake, nor did they correlate with pentadecanoic or heptadecanoic acids. A number of possible explanations could account for this:

1. They are not found in dairy food in New Zealand.
2. These are no longer derived exclusively from ruminant sources in New Zealand and may now be found in other food (especially processed food).
3. Observational studies are inaccurate- the graph (Figure 5) demonstrates what one would expect to see when two variables correlate strongly. However this assumes that the data follows a normal distribution.
4. Humans are able to catalyse reactions that convert MUFA and PUFA to TFA in amounts that are too small to be previously detectable. TFAs in low concentration have until recently been unable to be measured due to the inability of computer software to distinguish between cis and trans configuration on mass spectrometry, i.e. false negative results

In this Chapter, the first possibility is explored through the analysis of seven types of whole fat milk in New Zealand. The first set of milk was analysed in 2011 when there was normal rainfall; the second, in 2013 after a drought. Unfortunately the samples in 2011 were not retrievable in 2014 when a better methodology was used to analyse the second set of milk samples, so direct comparison of TFA was not possible.

Abstract

Background: Observational studies suggest that vaccenic and palmitelaidic acids are from dairy food and that pentadecanoic and margaric acids are markers of dairy intake. However, changing dairy food intake

in a randomised study had little effect on levels of these fatty acids. In this study, whole fat milk is analysed to assess the presence of these fatty acids...

Methods: Seven brands of whole fat milk (3.3%), organic and non- organic with identical expiry dates were collected during of normal rainfall (2011) and during a drought (2013). Fatty acids were measured using gas chromatography, mass spectrometry and expressed as a percentage of total fat.

Results: Pentadecanoic, margaric, vaccenic and palmitelaidic acids were present in all milk. On average, milk fat was comprised of 82% saturated, 14% unsaturated and 4% trans fatty acids during normal rainfall and 55% saturated, 36% unsaturated and 12% trans fatty acids during drought. Total trans fatty acids concentrations were lower in organic non-homogenised milk compared to non-organic, non-homogenised milk (1.4% vs. 4.4%) in 2011. The difference was made up by increased levels of vaccenic acid (0.2% vs. 4.0%) in non-organic milk. In 2013 compared to 2011, vaccenic acid made up a higher percentage of total fat (4.9% vs. 2.2%) as did palmitelaidic acid (3.4% vs. 0.04%).

Conclusion: Whole fat milk contains TFA and saturated fats thought to be markers of dairy intake but proportions of these fats differ between organic and non-organic milk, and from year to year. Feeding practices and drought affect the proportion of fats in milk.

Introduction

New Zealand cows are generally fed on clover-rich pastures, a practice thought to increases rTFA levels. (72) Farmers have traditionally used grass or maize silage as supplementary feed when grass is less plentiful, but this has recently been supplanted by palm kernel expeller (PKE). (297, 298) PKE is a by-product of the palm oil industry in South East Asia. It is derived from the nut of the palm fruit after the oil is either mechanically extracted e.g. most PKE imported into NZ is the by-product from such extraction) or solvent extracted (with resulting lower nutritive value). Its use has now increased and, New Zealand now imports over 1.3 million tonnes of PKE per year. The effect of supplementation with PKE on fatty acid composition of dairy food has not been described.

Dairy food has also undergone other significant changes since the 1950's; milk is now homogenised. This process is used to prevent a cream layer from separating out of the milk. Milk is pumped at high pressures through very narrow tubes, to break up and emulsify the fat globules. This process facilitates the ability to reduce overall milk fat in milk, enabling the production, for example, of 2% milk. It is also useful to deal with the thick layer of white cells and bacteria that collect on the bottom of the milk during pasteurization. Homogenisation ensures that this bottom layer gets mixed through the milk. The process of making homogenised milk has resulted in longer lasting milk, and the ability to ship milk greater distances without spoilage. Homogenisation also facilitates the creation of 'designer milks' used to market the product to specific demographics, for example, 'Mega milk' that is marketed to children and 'Calci trim' that is marketed to woman to prevent osteoporosis.

Other dairy foods such as butter and cheese are made from non-homogenised milk, so the fatty acid concentrations in these products reflect effects of feeding practices. Milk fat contains approximately 400 different fatty acids, which make it the most complex of all natural fats with a variety of short, medium and long-chain SFA, TFA, PUFAs and MUFAS. SFA make up about 70% of the proportion of the milk fat. rTFA comprise a small proportion of the fat in dairy products (typically 2–5% of total fatty acids) and beef and lamb (3–9% of total fatty acids), (54, 55) with variations in fatty acid compositions due to feeding practices as well as geographical and seasonal change. (55, 56) In contrast, partially hydrogenated vegetable oil can consist of up to 60% of total fatty acids as TFA. (57, 58)

New Zealand is one of the world's most significant primary producers of dairy food in the world and accounts for about a third of the world's trade in dairy. (297) Exports began in 1846, six years after the Treaty of Waitangi between Tangata whenua and the British Crown was signed. Refrigerated shipments in 1882 opened new markets and laid the foundations for New Zealand's dominance as a dairy products exporter.

In 2013, New Zealand was affected by a drought that necessitated the increased use of PKE in both the North and South Island. Organic farmers cannot use PKE which is fumigated (298) to ensure pests are not imported into New Zealand.

This study evaluates the fatty acid content of whole fat organic and non-organic milk during 2011 when there was normal rainfall and 2013, during the drought.

Methods

Materials

Three sets of seven varieties of whole fat (3.3%) milk (total n=21) with identical expiry dates were purchased in April 2011 and in April 2013. The varieties of milk included 2 organic whole milks (1 homogenised, 1 not homogenised), and 5 non organic milk (2 not homogenised and 3 homogenised).

Analysis of samples

Milk samples were decanted into nunc tubes and stored at -80 degrees until analysis was completed. Samples were labelled by a third party who kept a master list to maintain blindness until all results were collated. Plasma Phospholipids analysis was performed at the School of Biological Science, Auckland University. Total lipids were extracted with AR Methanol and fatty acid methyl esters formed by trans methylation.

Milk phospholipid fatty and amino acid composition was assessed by GC-MS (Agilent 7890A gas chromatograph coupled to a 5975C mass spectrometer with a split/splitless inlet. The column used was a fused silica Rtx-2330 100 m long, 0.25 mm internal diameter, 0.2 µm highly polar stationary phase (90% biscyanopropyl 10% cyanopropylphenyl polysiloxane, Shimadzu) (Appendix 5).

Analysis of results

Three samples of each brand of milk were analysed. The mean fatty acid (standard deviation) and the coefficient of variation were calculated for each fatty acid. Milk fatty acid levels are described as a mean percentage of total milk fat. Comparison between organic and non-organic milk and between milk in 2011 and 2013 was made.

Results

Trans Fatty acids

On average, milk fat was comprised of 81.95% SFA, 14.06% unsaturated fat and 3.98% TFA in 2011. Figure 15 shows levels of the 4 TFA found in organic c vs. non organic milk in 2011. Total rTFA levels are lower in organic non-homogenised milk compared to non-organic, non-homogenised milk (1.3% vs. 5.0%). The difference is composed of increased levels of vaccenic acid (0.25% vs. 3.96%) and palmitelaidic acid (0.026% vs. 0.046%).

Figure 16 shows levels shows TFA in milk in 2013. Direct comparison with milk in 2011 is not possible as the new column used for GC-MS in 2013 was able to identify more TFA isomers. Total TFA is similar across all types of milk in 2013. However, organic milk had nominally more TFA than other milk on 2013, due to a relatively higher level of vaccenic acid.

Vaccenic acid made up a higher percentage of total fat in 2013 compared to 2011 (4.9% vs. 2.2%) as did palmitelaidic acid (3.4% vs. 0.04%).

Figure 14: Trans fatty acid levels in milk in 2011 as a percentage of total fat. Milk is non-homogenised unless otherwise stated.

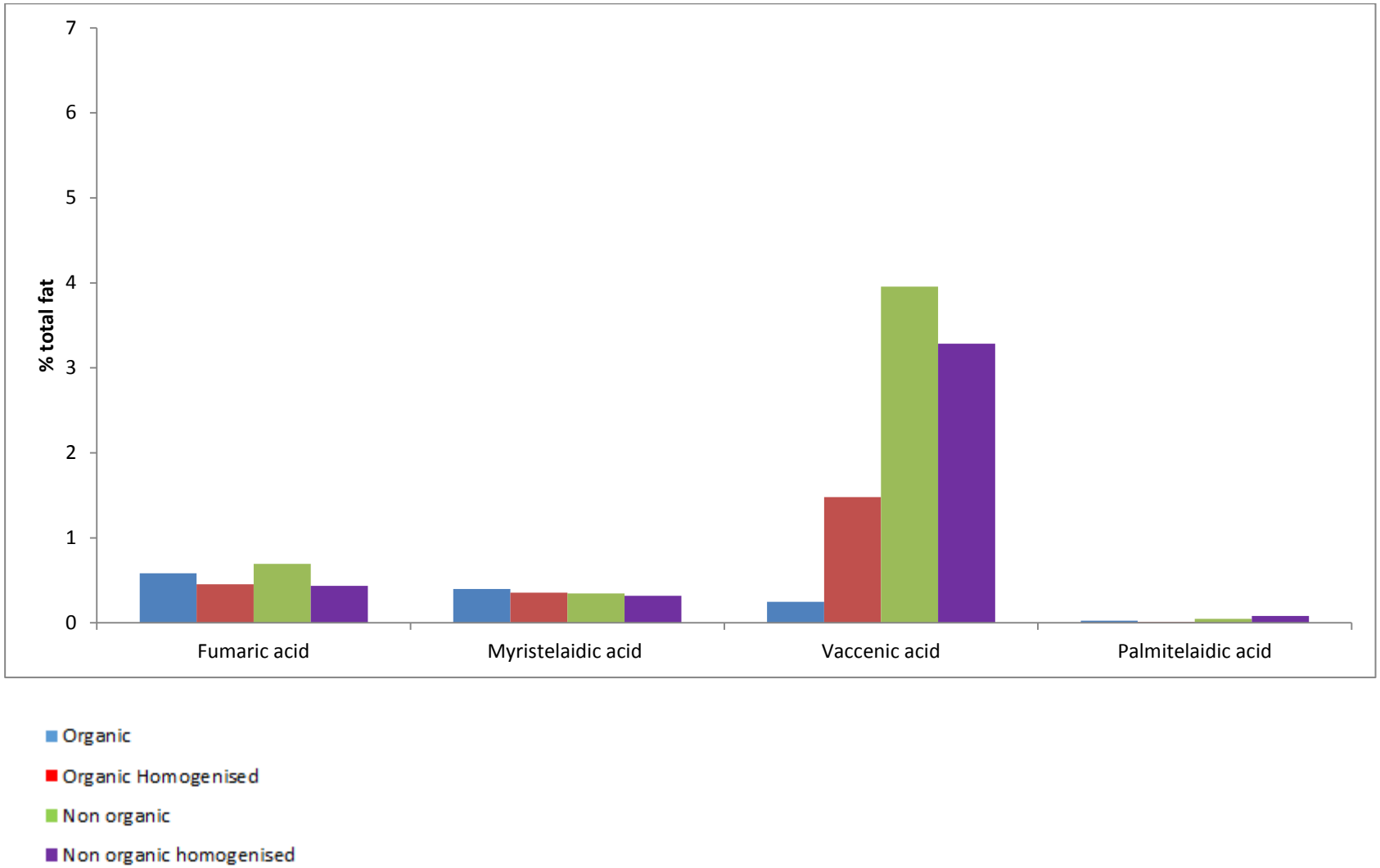
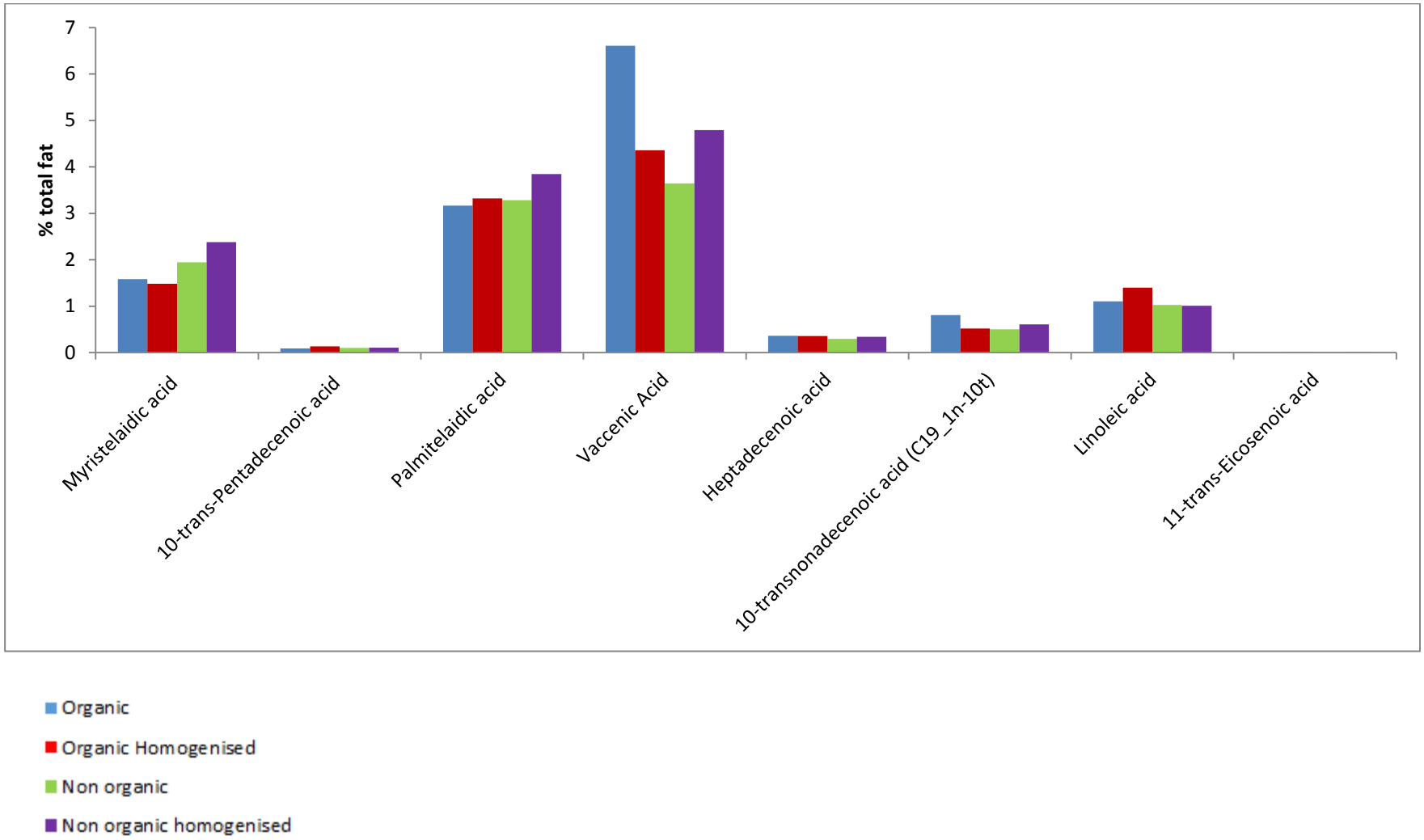


Figure 15: Trans fatty acid levels in milk in 2013 as a percentage of total fat. Milk is non-homogenised unless otherwise stated.



Saturated fatty acids

Levels of saturated fats in 2011 and 2013 are presented in Figures 16 and 17 respectively. In 2011, homogenised organic milk had the most saturated fatty acids compared to all other milks .For the non-homogenised milk, there was more saturated fat in the organic milk compared to the non-organic milk (79.2 %vs. 74.4%). However the relative proportion of saturated fatty acid isomers was similar. The most common SFA in milk were palmitic, myristic and stearic acids. Pentadecanoic and margaric acids were present in all milks but made up a low percentage of SFA, (1.04, SD 0.77) % and (1.0, SD 0.09) % respectively).

Compared to 2011, the saturated fat in all milk in 2013 had significantly higher concentrations of palmitic acid. Margaric and pentadecanoic acids were approximately 1-2% of total fats in all milk across both years; however, there was a reduced concentration of myristic and stearic acids in 2013 compared to 2011.

Unsaturated fatty acids

Levels of unsaturated fatty acids in milk are presented in Figures 18 and 19. There was a higher concentration of unsaturated fatty acids in 2013 compared to 2011 (36.2% vs.13.4%), mainly due to an increased concentration of oleic acid (30.9% vs. 7.9%).

Figure 16: Saturated fatty acid levels in milk in 2011 as a percentage of total fat. Milk is non-homogenised unless otherwise stated.

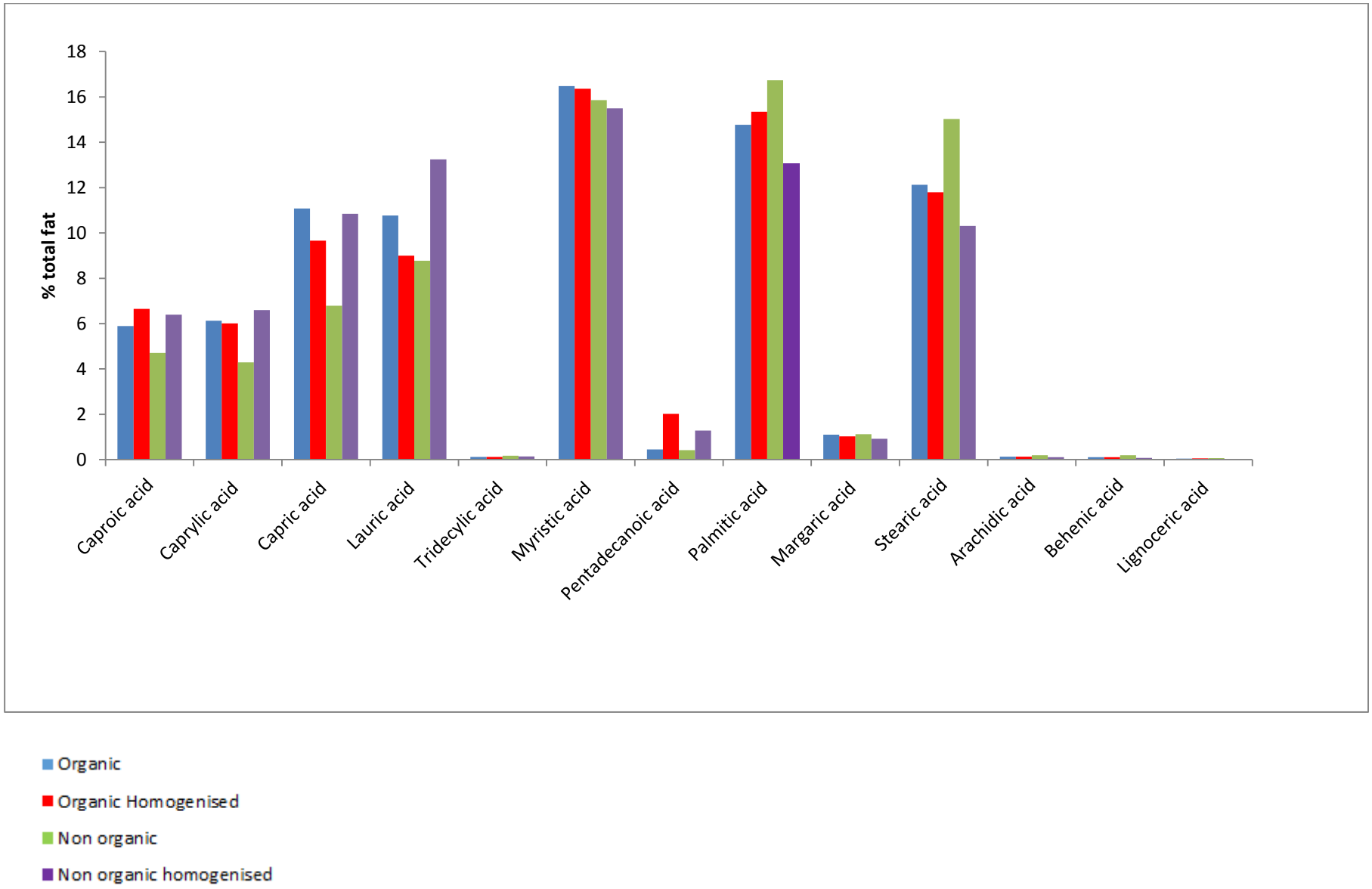


Figure 17: Saturated fatty acid levels in milk in 2013 as a percentage of total fat. Milk is non-homogenised unless otherwise stated.

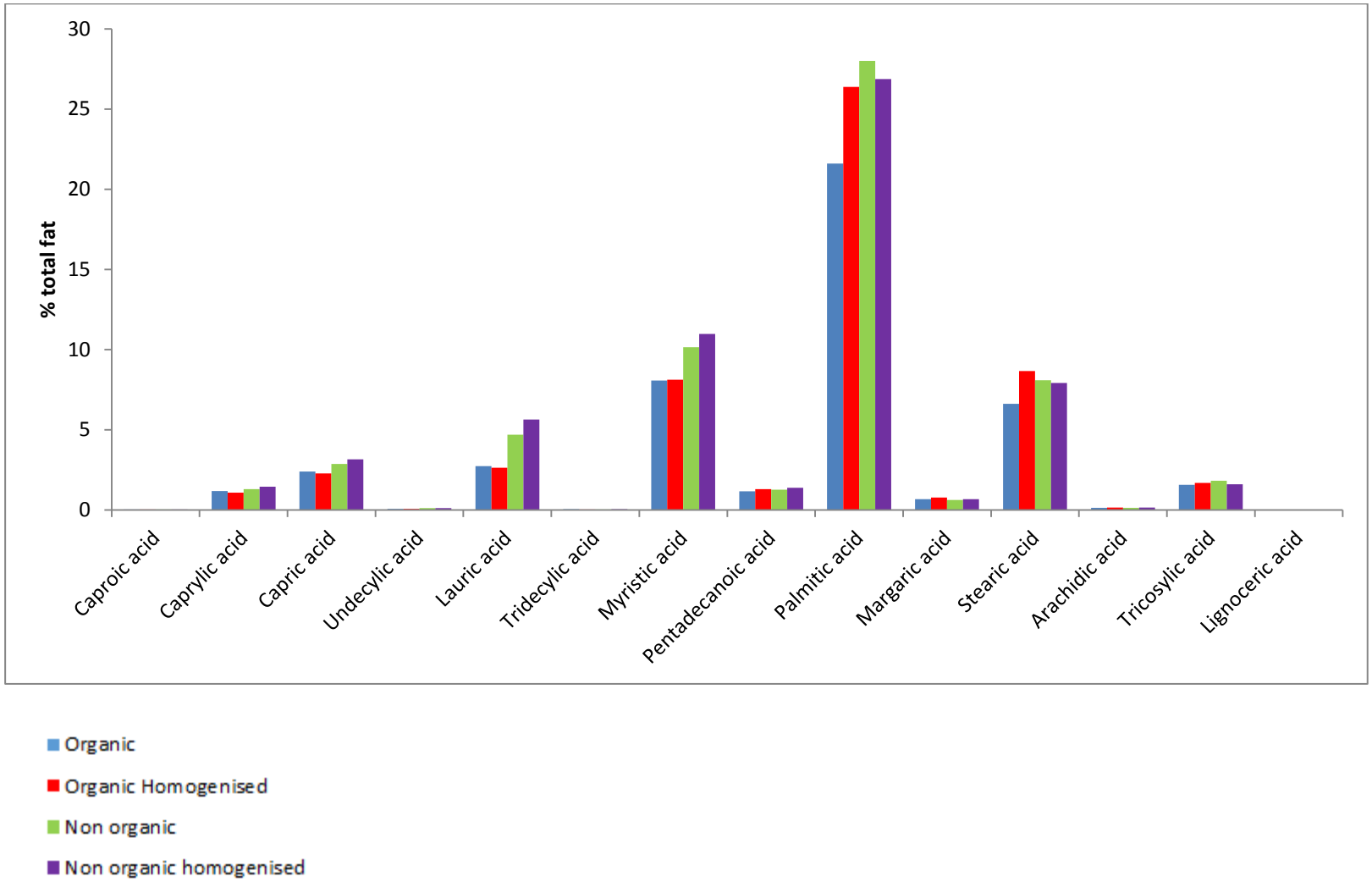


Figure 18: Unsaturated fatty acid levels in milk 2011 as a percentage of total fat. Milk is non-homogenised unless otherwise stated.

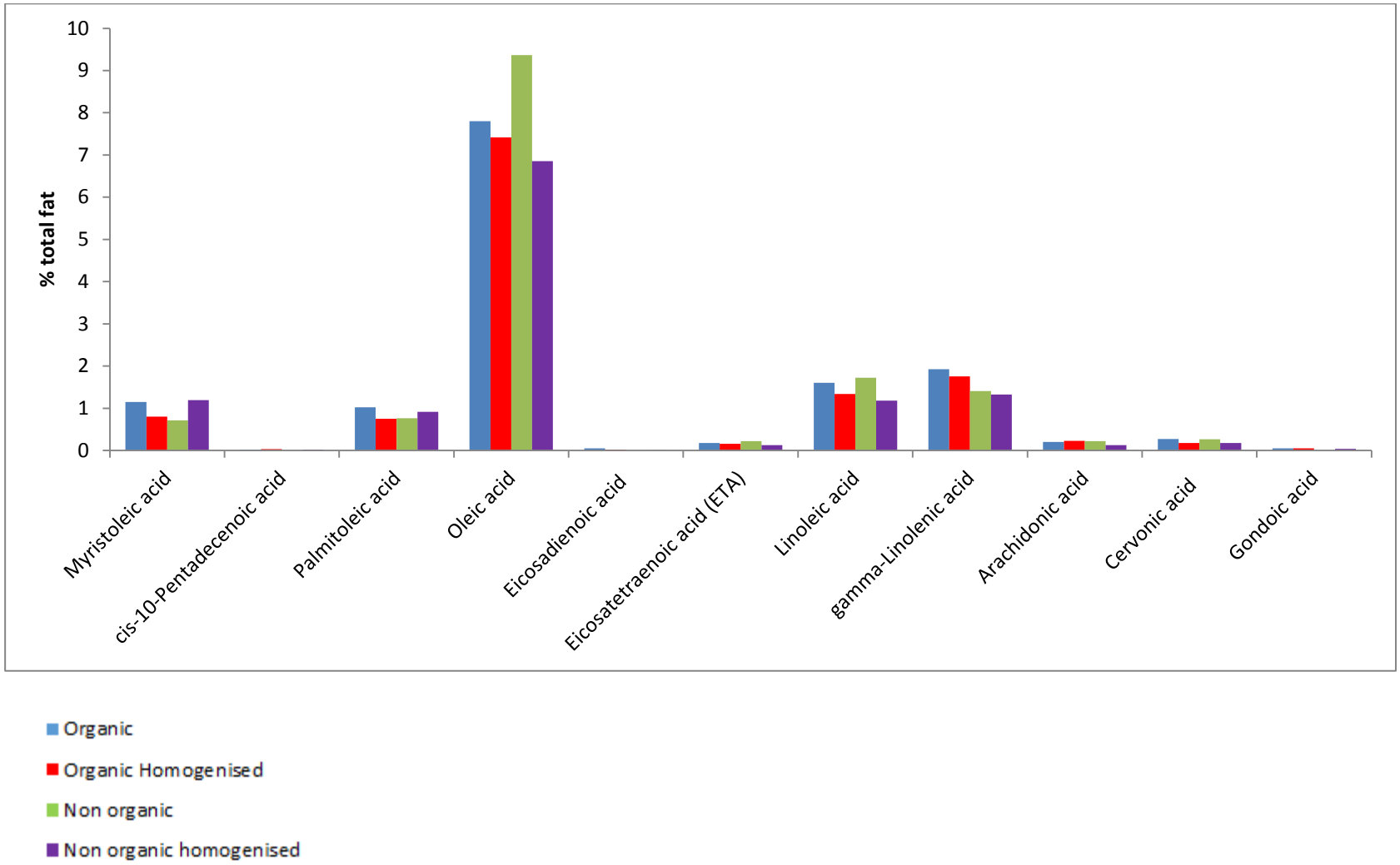
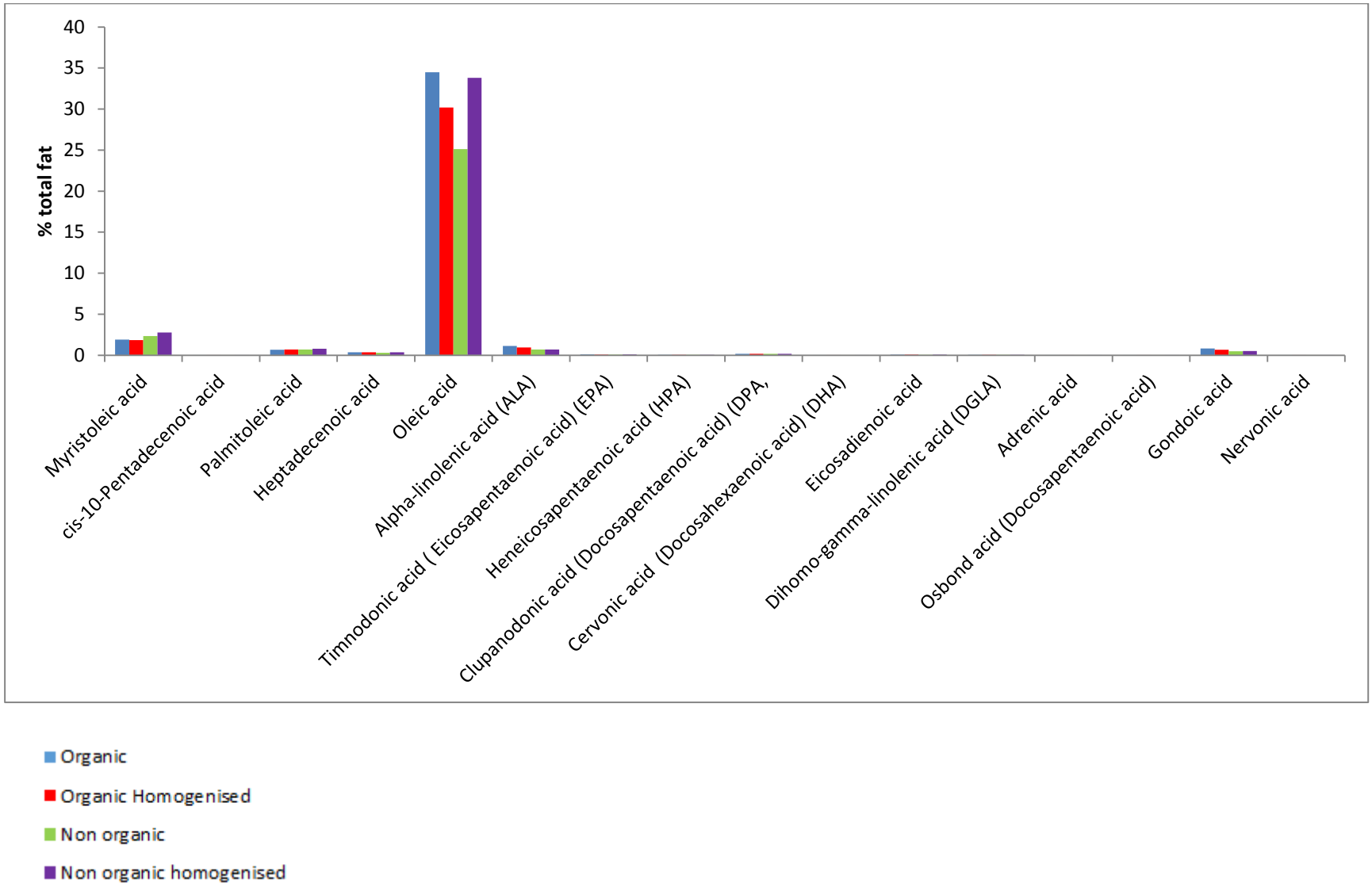


Figure 19: Unsaturated fatty acid levels in milk in 2013 as a percentage of total fat. Milk is non-homogenised unless otherwise stated.



Conclusion

This study assessed the composition of milk fatty acids of organic and non-organic milks, in years of good rainfall (2011) and drought (2013). This is primarily because these factors affect the animal feed, a strong determinant of fatty acid composition of milk. (72) Underpinning the study was the need to confirm the presence pentadecanoic, margaric, vaccenic and palmitelaidic acids in milk.

Organic dairy farmers are committed to not relying on pesticides, chemical fertilisers or drugs for the animals. Underlying the whole procedure is the aim of getting the soil active and balanced and creating an interactive ecosystem. This necessitates the use of a wider range of plants including herbs, instead of the typical dual species of ryegrass and clover, and the inclusion of deeper rooting species that enable a cycling of nutrients from a greater depth in the soil, a reduction in soil compaction from the soil structuring effect of roots and an increase in drought tolerance.(299) It is therefore expected organic milk will have different concentrations of fatty acids compared to non-organic milk.

The drought in 2013 necessitated the use of supplementary feeding. Organic farmers used stored hay, silage, maize, fodder and herbs to maintain milk supply. Non organic farmers used PKE and subsequently importation of PKE increased substantially in 2013. (300) This change in animal feed was also expected to have a major effect on milk fatty acid composition.

Trans fatty acids

This study suggests that vaccenic acid and palmitelaidic acid were both present in milk, however the concentration of these TFA in organic milk was almost four times lower than in non-organic milk in 2011 with vaccenic acid as the predominant TFA. However, this difference was no longer apparent in 2013, when all milk had significantly higher total concentration of TFA, mainly because of increased concentration of both vaccenic and palmitelaidic acids. Previous studies have shown that TFA in milk is only affected by the animal feed and not the breed of cow. (62) A possible explanation for the difference seen in 2011 is the composition of the pasture for organic and non-organic farms in times of good rain

differs enough to significant impact on TFA concentrations. However, in times of drought all supplemental feeds increased concentration of TFA. This suggests that strategies at production levels could be implemented to reduce the amount of TFA the New Zealand population is exposed to.

Saturated and unsaturated fatty acids

Margaric and pentadecanoic acids were found in low concentrations in all milk. (213) Concentrations of these fatty acids were remarkably similar in all milk tested suggesting that animal feed may not affect concentration of these fatty acids.

In 2011, palmitic, stearic and myristic acids were the most plentiful SFA in milk. Organic milk had slightly higher concentrations of saturated fatty acids than non-organic milk. However in 2013, concentration of all SFA was lower, mainly because of reduced concentration of myristic and stearic acids.

Oleic acid was the most predominant unsaturated fatty acid in milk, and concentrations of this fatty acid rose strikingly in 2013, suggesting that supplemental feed had a positive effect on this fatty acid.

Conclusion

This study suggests that pentadecanoic, margaric; vaccenic and palmitelaidic acids are present in all milk. However, feeding practices have an important effect on concentrations of various fatty acids and modifying the feed may be a possible way to reduce the exposure of the population to trans fatty acids.

Chapter 5

Plasma Fatty acid levels in vegans

This Chapter describes plasma fatty acid levels in avowed vegans. This study was designed to evaluate the possibility that humans are able to catalyse a reaction that isomerizes cis- unsaturated fatty acids to trans-unsaturated fatty acids. Current scientific thinking is that bacteria are the only biological organism able to do this. However, this theory was postulated when software was unable to discriminate fatty acids at very low concentrations. It is possible, though unlikely, that mammals may be able to catalyse this reaction. Vegans do not consume any form of animal product and almost all do not eat any processed vegetable food. They do not eat takeaway food to avoid the possibility of contamination from animal products. Trans fatty acids should therefore not be detected in their plasma.

Ethics approval number: 13/CEN/57

UTN: U1111-1141-2843

Trial Registration ACTRN: ACTRN12613000371796

Abstract

Background: Vegans do not consume animal products and eat almost no processed foods so no trans fatty acids derived from animal by-products or processed food should be detectable in their plasma. Previous studies also suggest that vegans have favourable cardiometabolic risk profiles compared to vegetarians and omnivores. This study evaluates fatty acids and the cardiometabolic risk profiles in vegans.

*Methods***:** Cardiometabolic risk factors and fasting plasma fatty acids were analysed in 25 avowed vegans. Cardiometabolic risk factors were compared with aged matched omnivores.

Results: Participants were vegan for a mean of 36 (interquartile range 24 to 72) months and 83% ate no processed food. Compared to omnivores, there was no difference in systolic BP 108 (interquartile range

104 to 118) mmHg vs. 110 (interquartile range 100 to 115) mmHg; p=0.66, diastolic BP 71 (interquartile range 62 to 80) mmHg vs. 70 (interquartile range 60 to 70) mmHg; p=0.25, weight 67.7 (interquartile range 62.0 to 74.0) kg vs. 69.3 (interquartile range 57.8 to 70.0) kg; p=0.61 or waist circumference 75.8 (interquartile range 72.8 to 82.0) cm, vs. 79.0 (IQR 71.0 to 87.0) cm; p=0.27. Compared to non-vegans, vegans had significantly lower total cholesterol (3.6 vs. 4.5mmol/l, p<0.0001) and LDL-c (1.7 vs. 2.5 mmol/l, p<0.0001).

Vegans had relatively lower percentage of saturated fatty acids in the plasma compared to non-vegans (33.5% vs. 63.6%). Trans fatty acids made up 1.5% of total fatty acids with palmitelaidic acid as the most predominant trans fatty acid at 1.4%.

Conclusions: Vegans have a more favourable cardio-metabolic risk profile and a low proportion of plasma saturated fatty acids compared to omnivores. Trans fatty acids are found in vegans, the most plentiful one being palmitelaidic acid.

Introduction

A recently completed randomised controlled study evaluated the effect of changing dairy food intake on rTFA. Plasma fatty acids exhibited no correlation with change in dairy food intake. More puzzling, there was no correlation between the ruminant TFA themselves (r =0.08, p=0.257). A number of possible explanations could account for this:

- Ruminant TFAs analysed are not present in dairy products in New Zealand - analysis of milk undertaken by us has discounted this possibility (Chapter 4).
- Ruminant TFA are not uniquely found in dairy food but are also found in other food sources- this possibility has not been discounted, but testing all foods is impractical.
- Humans are able to metabolise TFA- this possibility is the least likely hypothesis but some rodent studies hint at this possibility. (87)

The purpose of this study is to determine if rTFA as measured by plasma phospholipid levels in humans are only derived from animal products. Veganism is a form of vegetarianism which eliminates all animal products from the diet. Testing levels in avowed vegans would eliminate other (non-animal) sources of TFA. For example, if vaccenic acid is found in vegans, the inference is that it enters the food chain from another source and will explain previous study findings. Whilst vegans can eat certain processed foods, the philosophy that underpins the choice to become vegan often extends to ecological awareness and a rejection of processed food. Participants in this study revealed that all but two, eschewed any vegan processed foods. A number have made the transition to raw foods. This will serendipitously also allow for assessment of TFA in a population with almost no intake of processed food. It is highly unlikely that bio-hydrogenation of unsaturated fatty acids occurs in humans, and this unique population will allow the evaluation of this possibility.

Objectives

Primary Objective:

To evaluate plasma levels of TFA in vegans.

Secondary Objective

To assess the cardiometabolic risk profile in vegans.

Hypothesis

No TFA derived from ruminant by products are present in vegans.

Methods

Vegan participants

An advertisement was placed on the vegan society website in May and June 2013. Healthy vegan volunteers willing to participate in this study were asked to contact the author. Exclusion criteria included

ingestion of any animal by-product in the last 3 months, known diabetes, cardiovascular disease, and inflammatory conditions, currently taking any lipid or glucose modifying medication or age ≤18 years.

Control Samples

Participants in the randomised study (Chapter 3) who were of similarly age to the vegan participants constituted the age matched control group. Samples taken at baseline of the randomised study that were stored at -70^{o}C were retrieved for analysis. The original consent for the randomised study included permission to use stored samples for such future tests.

Study procedures

Ethics approval was obtained from the Multiregional Ethics Committee and all participants provided written informed consent. After written consent was obtained, height, weight and waist circumference were measured, a 3 day food frequency questionnaire was completed and a fasting blood sample collected.

Sitting BP was measured in the right arm after sitting for 5 minutes. Waist circumference was measured at the halfway mark between the iliac crest and the bottom rib; hip circumference was measured at the level of the greater trochanter. Weight was measured on a calibrated digital scale.

Fasting blood tests were taken from all participants. For lipids, blood was taken in a heparin tube and analysed within 60 minutes using standard Roche Modular analyser. The following methods were used: TC: cholesterol oxidase; HDL-c: PEG-modified cholesterol esterase and oxidase, with dextran sulphate; TC: lipoprotein lipase. LDL-c was calculated using the Friedwald equation.

Fasting blood samples were taken in ethylene-diamine-tetra acetic acid tubes, and plasma was stored at -80^{0} Celsius until analysis. Plasma phospholipid fatty acid composition was assessed by GC-MS (Agilent 7890A gas chromatograph coupled to a 5975C mass spectrometer with a split/splitless inlet). The column used was a fused silica Rtx-2330 100 m long, 0.25 mm internal diameter, 0.2 µm highly polar stationary

phase (90% biscyanopropyl 10% cyanopropylphenyl polysiloxane, Shimadzu) (Appendix5) . Results are expressed as percentage of total fat.

The National Cancer Institute Diet History Questionnaire, a validated food frequency questionnaire was used to assess all dairy product and red meat intake during the preceding 3 days at baseline and 1 month. Serving sizes were defined using the United States Department of Agriculture criteria. (270) For example one serving size was equivalent to 250ml 3% milk, 250ml yogurt, 500ml 1.5% milk or 1/3 cup cheddar cheese. The total dairy fat content ingested in g/day was calculated based on the reported intakes of each food, and the fat content from manufacturers' labels.

Statistical tests

Baseline characteristics are described as median (IQR). Differences in baseline characteristics between vegans and aged matched controls from the dairy study are compared with a two-tailed Student t test. The percentage of total fat is described for saturated fats, unsaturated fats and TFA.

Results

Twenty five vegans completed the study. The average age was 32.2 (SD 12.7) years and 75% were female (Table 25).

All participants were vegan for longer than 12 months, except for 1 who had been vegan for 5 months. Most (83%) did not consume any form of vegan processed food following a diet that consisted mainly of whole food. One quarter of the vegans was raw-food vegans; which meant that they only ate uncooked and unprocessed fruit, vegetables and nuts.

For vegans compared to non-vegans, there was no difference in systolic BP 108 (IQR 104 to 118) mmHg vs. 110 (IQR 100 to 115) mmHg; p=0.66, diastolic BP 71 (IQR 62 to 80) mmHg vs. 70 (IQR 60 to 70) mmHg; p=0.25, weight 67.7 (IQR 62.0 to 74.0) kg vs. 69.3 (IQR 57.8 to 70.0) kg; p=0.61 or waist circumference 75.8 (IQR 72.8 to 82.0) cm, vs. 79.0 (IQR 71.0 to 87.0) cm; p=0.27. Compared to non-

vegans, vegans had significantly lower total cholesterol (3.6 vs. 4.5mmol/l, $p<0.0001$) and LDL-c (1.7 vs. 2.5 mmol/l, $p<0.0001$).

Fatty acid concentrations are displayed in Table 26 Fatty acids in vegans was comprised of 64% (SD 17%) unsaturated fatty acids, 34% (SD 9%) SFA and 1.5% (SD 1.6%) TFA. TFA were present in every sample tested (Figure 15) and this was almost exclusively made up by palmitelaidic acid (1.4%). All other TFA were present at very low concentrations.

Table 25: Baseline Characteristics of vegans compared to aged matched controls from the dairy randomised study. Results are median and (interquartile range).

	Vegans	**Age matched controls**	**P value**
Number	25	40	
Age (Years)	28 (23 to 45)	29 (25 to 32)	0.24
Length of time exclusively vegan (months)	36 (24 to 72)	Not applicable	
Systolic blood pressure (mmHg)	108 (104 to 118)	110 (100 to 115)	0.66
Diastolic blood pressure (mmHg)	71 (62 to 80)	70 (60 to 70)	0.25
Weight (kg)	67.7 (62.0 to 74.0)	69.3 (57.8 to 70.0)	0.61
Waist circumference (cm)	75.8 (72.8 to 82.0)	79.0 (71.0 to 87.0)	0.27
Total Cholesterol (mmol/L)	3.6 (3.3 to 4.08)	4.50 (4.05 to 5.5	<0.0001
High Density Lipoprotein Cholesterol (mmol/L)	1.65 (1.6 to 1.7)	1.60 (1.9 to 1.9)	0.24
Low Density Lipoprotein Cholesterol (mmol/L)	1.7 (1.3 to 1.9)	2.5 (2.2 to 3.2)	<0.0001
Triglyceride (mmol/L)	0.6 (0.5 to 0.8)	0.8 (0.6 to 0.13)	0.015

Table 26: Plasma fatty acid levels in vegans expressed as percentage of total fat and standard deviation\

	Percentage total fat	Standard deviation
Myristelaidic acid	0.02	0.02
10-trans-Pentadecenoic acid	0.01	0.00
Palmitelaidic acid	1.36	0.63
Vaccenic acid	0.02	0.01
10-transnonadecenoic acid	0.05	0.01
11-trans-Eicosenoic acid	0.01	0.01
Elaidic acid	0.03	0.01
Total Trans fatty acids	**1.51**	**0.68**
Caproic acid	0.00	0.00
Caprylic acid	0.01	0.00
Capric acid	0.02	0.01
Undecylic acid	0.00	0.00
Lauric acid	0.17	0.20
Tridecylic acid	0.03	0.01
Myristic acid	0.00	0.00
Pentadecanoic acid	0.08	0.03
Palmitic acid	19.37	5.24
Margaric acid	0.15	0.05
Stearic acid	5.94	1.98
Arachidic acid	0.23	0.06
Heneicosylic acid	0.04	0.01
Behenic acid	0.90	0.21
Tricosylic acid	7.32	0.89
Lignoceric acid	0.02	0.01
Total Saturated Fatty acids	**34.28**	**8.7**
Myristoleic acid	0.08	0.04

cis-10-Pentadecenoic acid	0.01	0.00
Palmitoleic acid	0.15	0.05
Heptadecenoic acid	0.08	0.03
Oleic acid	23.28	6.30
cis- vaccenic acid	1.87	1.47
Linoleic acid	25.51	4.14
Gondoic acid	0.52	0.34
Alpha-linolenic acid	0.74	0.48
Eicosadienoic acid	0.22	0.09
Dihomo-gamma-linolenic acid	1.18	0.55
Arachidonic acid	6.30	2.30
Timnodonic acid	0.45	0.36
Nervonic acid	1.04	0.30
Adrenic acid	0.14	0.05
Osbond acid	0.12	0.05
Clupanodonic acid	0.48	0.25
Cervonic acid	1.40	0.58
Total Unsaturated fatty acids	**63.57**	**17.38**

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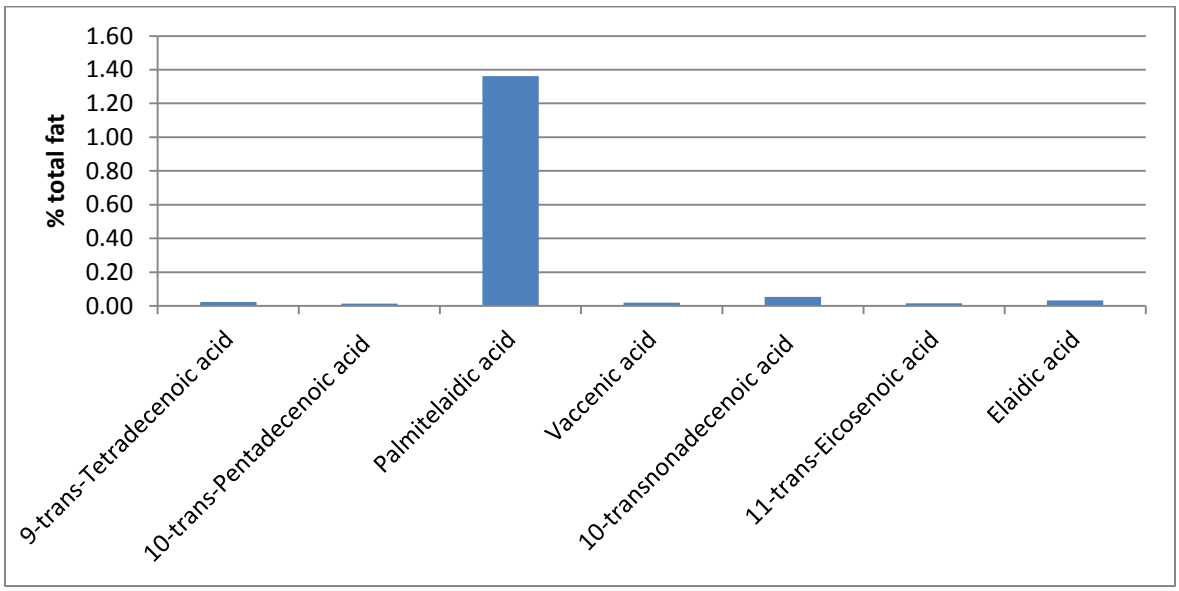
Figure 20: Plasma trans fatty acid levels in vegans expressed as percentage of total fat.



Discussion

Many studies have assessed effects of vegetarian diets on cardiometabolic risk, but few have looked at the effects of complete abstention of all animal products from the diet on cardio-metabolic risk. Vegetarians eschew meat but can have a high intake of animal fat as they consume dairy food. Veganism requires a high level of commitment in that almost all food products contain some form of animal by product, so vegans are generally limited to whole foods. Vegan studies are difficult to undertake as it is challenging to identify a large number of true vegans in a given population and randomised studies would almost certainly have large dropout rates. (283)

Vegans had similar body weights; waist circumferences and systolic blood pressure compared to non-vegans, but had lower total cholesterol and LDL-c. A recent meta-analysis (301) suggests that vegetarians have lower systolic and diastolic blood pressures than omnivores and that vegans have slightly lower blood pressures than vegetarians. In this study, there was little difference in blood pressure for age matched controls. However, compared to all participants in the dairy food diet who were older, (n=180, median age 47 (IQR 38-55) years, there was a modest difference in systolic BP (108mmHg vs. 114mmHg, p=0.03) and no difference in diastolic BP.

The cholesterol profile of vegans was more favourable with significantly lower triglycerides and LDL-c than non-vegans. This is in keeping with a recent randomised study (302) that found that those randomised to a vegetarian diet high in plant sterols had lower LDL-c compared to those randomised to a vegetarian diet with low-fat dairy products and whole grains. An observational study (303) suggests that vegans have lower LDL-c than vegetarians or omnivores. Traditionally, it is believed that most LDL-c is genetically and epigenetically predetermined with only 15% influenced by the diet. (304) This study suggests that complete abstention of all animal by-products is associated with a 32% lower LDL-c, one of the most significant risk factors for CVD. (305) An observational study (306) has shown a significant reduction in CVD in vegan men compared to omnivores (odds ratio 0.58, (IQR 0.38 to 0.89)). However, the observational studies cannot completely account for certain confounders that are associated with lower cardiovascular risk. Vegans generally have healthier health behaviours (307) with higher rates of exercise, lower rates of smoking and are generally from higher socio economic groups.

Vegans did have a lower percentage of saturated fats compared to omnivores suggesting that complete abstention of any animal product does lead to a more desirable fatty acid profile. However, TFA were present in plasma of these committed vegans, predominantly palmitelaidic acid. It has previously been suggested that all TFA in humans are from the diet because humans do not isomerise cis fatty acids to TFA. (2) This study suggests that this assumption may not be correct. It may explain why TFA levels did not change with a change in dairy food consumption that was seen in the randomised study described in Chapter 3.

It is difficult to explain why TFA was found in vegans. One possible reason is that this is a false positive result due to a systematic error in testing TFA levels. This is not likely as the test was repeated with the appropriate GC column and all results were manually cross checked. However, cis and trans fatty acids occur very close together and have exactly the same mass spectrum. If the sample has a very high concentration of one or many cis fatty acids, for example, oleic acid, it can obscure the adjacent ones (like vaccenic acid) that are present at lower concentrations. Palmitelaidic acid is not affected by this methodology, however, levels of vaccenic acid need to be interpreted with caution. Further studies in

vegans using a targeted method, like silver ion high-performance liquid chromatography, is needed to further assess vaccenic acid levels.

Another possibility is that this is a true positive result. It is possible, but highly unlikely that TFA are present in unprocessed plant based foods. It is also possible that some vegans may have consumed processed foods with partially hydrogenated vegetable oils. However, TFA from this source (elaidic and linoelaidic acids) were negligible and the predominant TFA, palmitelaidic acid is considered an rTFA. Whilst it is possible that some vegans may have eaten animal by-product, it is not probable as this TFA was present in every single sample at similar levels. The possibility that humans can catalyse a reaction to create small amounts of TFA cannot be discounted, and further studies are needed to evaluate this.

Limitations of this study are that one blood sample was tested and variation over time could not be assessed. Claims that no animal by product had been consumed for long periods preceding the blood test could not be independently verified.

Conclusion

Vegans had favourable cardiometabolic risk profiles and had a higher proportion of unsaturated fatty acids in their plasma compared to aged matched controls. TFA was present in plasma of every vegan suggesting that humans may possibly catalyse a reaction to create TFA. More studies are needed to confirm this finding.

Chapter 6

Introduction

This Chapter describes a meta-analysis published in PLOS One. (308) It evaluates the effects of increased dairy food (whole and low fat) consumption on cardiometabolic risk factors such as weight, insulin resistance, blood pressure, inflammatory markers and lipids in healthy adults. As such it consists of 10 separate meta-analyses in one paper

As part of the process of completing these meta-analyses, a number of new research skills were developed by the author. A list of key variables to be analysed and the protocol were drafted (Appendix 3) plus a literature search was performed by the author. For many papers, key variables, for example weight, were not the primary objective, (273, 309, 310) and this made the searches difficult. These articles were found after repeat searches using multiple databases, to ensure that all possible data was acquired. When data was not available, corresponding authors were contacted to request this information. When key variables like HOMA–IR were incorrectly reported, corresponding authors were contacted and informed. Repeat calculations ensured that the correct data was used in the meta-analysis.

Every paper was analysed using the PICO format and Jadad score. Baseline characteristics described in Table 27 are all collated and analysed by the author. The RevMan software version 5•2 (The Nordic Cochrane Centre, The Cochrane Collaboration, Copenhagen) was downloaded and the draft analysis was performed by a statistician and the author. This initial analysis erroneously used mean change and standard deviation of the population. The required the author repeat to the analysis on her own using standard mean change and standard deviation of the change. The author then stratified the analysis for whole-fat and low-fat dairy food. The final statistical analysis in the published paper was completed by the author, including, the description of included papers, baseline characteristics, the meta-analysis itself plus heterogeneity and sensitivity analysis. The paper includes 30 separate analyses that are combined to evaluate the effects of increased whole-fat, low-fat and total dairy food intake on 10 cardio-metabolic risk factors.

Trial Registration ACTRN: ACTRN12613000401752

Ethics approval number: NTX/10/11/115

Effects of high and low fat dairy food consumption on cardio-metabolic risk factors: A meta-analysis of randomised studies. (308)

Abstract

Importance: Clear guidelines on the health effects of dairy food are important given the high prevalence of obesity, cardiovascular disease and diabetes, and increasing global consumption of dairy food.

*Objective***:** To evaluate the effects of increased dairy food in a healthy population on cardiometabolic risk factors.

Data Sources: Searches were performed until April 2013 using Medline, Science Direct, Embase, Google, the Cochrane Central Register of Controlled Trials, reference lists of articles, and proceedings of major meetings.

Study Selection: Healthy adults randomised to increased dairy food for more than one month without additional interventions.

*Data Extraction and Synthesis***:** A standard list was used to extract descriptive, methodological and key variables from all eligible studies. If data was not included in the published report corresponding authors were contacted.

*Results***:** 20 studies with 1677 participants with a median duration of dietary change of 26 (interquartile range 10-39) weeks and mean increase in dairy food intake of 3.6 (standard deviation 0.92) serves/day were included.

Increased dairy food intake was associated with a modest weight gain (+0.60, 95% confidence interval 0.30 to 0.90kg, p<0.0001) but no significant change in waist circumference (-0.07 , -1.24 to 1.10 cm) ; insulin resistance (HOMA –IR -0.94 , -1.93 to 0.04 units); fasting glucose (+1.32 , 0.19 to 2.45 mg/dl) ; LDL-cholesterol (1.85 ,-2.89 to 6.60 mg/dl); HDL-cholesterol (-0.19 , -2.10 to 1.71 mg/dl); systolic (-0.4, -1.6 to 0.8 mmHg) and diastolic blood pressure (-0.4 , -1.7 to 0.8 mmHg) or C-reactive protein (-1.07 , -2.54 to 0.39 mg/L). Results were similar for studies with low-fat and whole-fat dairy interventions.

Limitations: Most clinical trials were small and of modest quality.

Conclusion: Increasing whole-fat and low-fat dairy food consumption increases weight but has minor effects on other cardio-metabolic risk factors. These findings suggest that dairy food can be included as part of a healthy diet.

Introduction

Clear guidelines on the health effects of dairy food are important given the high and increasing prevalence of obesity, (311) cardiovascular disease (312) and diabetes (313) in most countries, and the increasing global consumption of dairy food. (239) Many current dietary guidelines promote low- fat dairy products as a healthy food. (5, 6) This advice is supported by observational studies which report that increased dairy food consumption is associated with lower blood pressure, (188, 191, 196, 246, 250) weight reduction, (268) improved insulin sensitivity, (148, 191, 196, 198) less inflammation (265, 266) and a lower ratio of total to HDL cholesterol. (24) A modest inverse association between dairy consumption and cardiovascular disease has also been reported. (37, 48, 314)

In contrast, whole fat dairy food is not recommended in most food guidelines (7, 8, 180) because of the concern that saturated fat in dairy food may have an adverse effect on serum lipids which could increase the risk of cardiovascular disease. Despite these guidelines the effects of high fat dairy food on the risk of obesity, diabetes and cardiovascular disease are uncertain. A recent meta-analysis found no association between dietary saturated fat intake and the risk of cardiovascular disease. (224) Whole fat dairy foods contain many fatty acids, which may have favourable as well as unfavourable effects on lipids and other cardio-metabolic risk factors. (315) Also, the effects of reducing saturated fat from one food are determined by other dietary changes, including carbohydrates, and mono-unsaturated and poly-unsaturated fatty acids. (24)

The effects of a high dairy food diet on diabetes and cardiovascular disease have not been evaluated in randomised clinical outcome trials. The large long term randomised dietary intervention studies which evaluated the ‘Dietary Approaches to Stop Hypertension’ (DASH) (240) and ‘Mediterranean’ (144) diets on clinical outcomes, while including increased low fat dairy food in the intervention, do not allow an for evaluation of the independent effects of changes in dairy food intake. Health effects of whole and low fat dairy food would be more reliably evaluated in clinical trials than in observational studies, and by assessing a number, rather than just one cardio-metabolic risk factor. We therefore undertook a meta-analysis of randomised clinical studies that evaluated effects of changing whole and low fat dairy food

intake in healthy adults on a broad range of cardio-metabolic risk factors including weight, insulin resistance, lipids, blood pressure and CRP.

Methods

We followed the PRISMA (http://www.prisma-statement.Org) guidelines throughout the design, implementation, analysis, and reporting of this meta-analysis. A protocol for the study was designed (Appendix 4) and the study was registered with the Australian New Zealand Clinical Trials Registry.

Search Strategy

We searched for all trials that randomised adults to increased dairy for at least one month without additional interventions (e.g. caloric restriction, multiple dietary interventions), had an appropriate control group, and sufficient data to calculate estimates of effect with standard deviations on at least one of the following: weight, waist circumference, blood pressure, HDL and LDL cholesterol, fasting glucose, insulin resistance and CRP. Studies were excluded if they were observational or otherwise non-randomised; were commentaries, reviews, or duplicate publications from the same study. We restricted inclusion to studies of healthy adults who did not have diabetes, hypertension or vascular disease. Both feeding and dietary advice trials and studies with a crossover or parallel group study design were included.

Searches were performed of literature published through March 2013 using Medline, Science Direct, Embase, Google, the Cochrane Central Register of Controlled Trials, reference lists of articles, and proceedings of major meetings for relevant literature. The search terms were ‘dairy’ and each of the following; ‘cardiometabolic risk’, ‘weight’, ‘waist circumference’, ‘glucose’, ‘insulin’, ‘insulin resistance’, ‘inflammation’, ‘inflammatory markers’, ‘blood pressure’, ‘cholesterol’ and ‘lipids’.

Assessment of study eligibility and data extraction

One reviewer screened all abstracts and titles and, upon retrieval of candidate studies, two team members (JB, KS) reviewed the full text to determine eligibility. If the study was eligible, data were abstracted by JB. Through an iterative process, a standard list was used to extract descriptive, methodological and key variables from all eligible studies. Data extracted included years the study was

performed and reported, the primary aim of the study, population characteristics, funding source, control and intervention diets, duration of follow-up, estimates of effect and standard deviations. If data was not included in the published report corresponding authors were contacted. (252, 271) The quality of each study was rated using the Jadad score. (316) Questions arising during data abstraction were resolved by discussion with all team members.

Definitions

Dairy food with less than1% fat, such as trim or low fat milk was categorized as a low- fat dairy food. Dairy food that included full-fat milk (3-4% fat), cheese, butter, cream and ice cream, was categorized as whole fat dairy food.

The method used to quantify insulin resistance was the homeostatic model assessment- Insulin resistance (HOMA-IR). (256) This estimates steady state insulin sensitivity as units. The equation that is used is HOMA-IR = glucose (mmol/L) x insulin (munits/L) ÷22.5.

Statistical analysis

Each cardio-metabolic risk factor when on a higher and lower dairy food diet was compared between cases and controls from the same study. For those studies with 3 treatment groups, comparison was made between the control and the high dairy food group. Effects were measured at least 4 weeks after randomization, with the final results used for studies with more than one measurement during follow up. A negative effect size means that dairy food has a favourable effect on the cardiometabolic risk factor. Because an increase in HDL-cholesterol is considered beneficial, positive and negative exponents were switched to maintain consistency in presentation. In one study the standard deviation was not reported (182) but calculated from the 95% confidence intervals.

For each cardio-metabolic risk factor, the weighted mean change from baseline to follow up was calculated across all included studies within each randomised group. The inverse-variance method, whereby study differences are weighted according to the reciprocal of their variance, was used to pool all

standardized mean differences to yield an overall effect size with corresponding 95% confidence intervals.

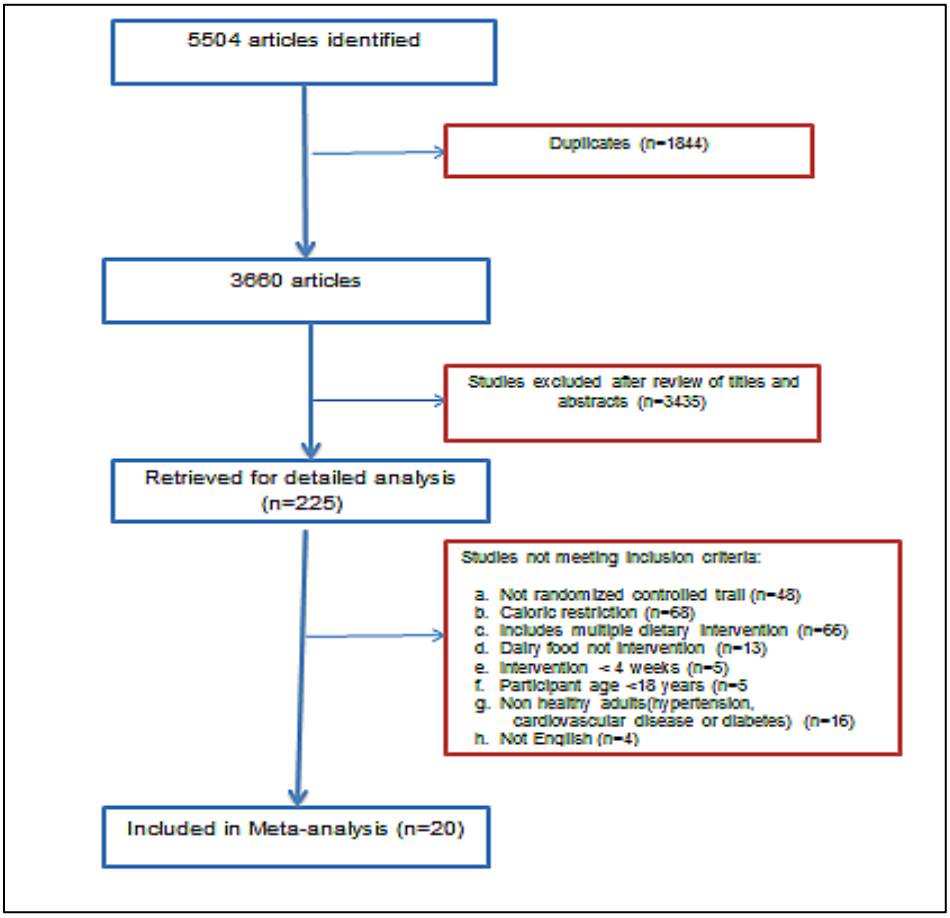
Each meta-analysis was assessed for heterogeneity by a Chi square test and I^2 statistic. A fixed effects model was used when heterogeneity was not present (I^2=0) and a random effects model was used when statistical heterogeneity (I2≥1%) was present. A p-value of <0.05 was considered statistically significant. Studies are presented in Forrest plots in order of statistical power. Stratified analyses was studied by low-fat and whole- fat dairy, duration of dietary intervention (less than or greater than 6 months), body weight of study participants, and industry or public source of funding. Studies where the intervention was skim, trim or <1% dairy food are low fat dairy food studies. Sensitivity analyses were also conducted to evaluate the impact of selected studies on overall pooled estimates and heterogeneity. The Statistical analyses were performed using RevMan software version 5·2 (The Nordic Cochrane Centre, The Cochrane Collaboration, Copenhagen).

Results

Search Results

The literature search yielded 5504 citations (2495 on Pub Med and 2849 on Science Direct, 160 on Google), which included 1844 duplicates. After title and abstract screening, the full text of 225 articles were evaluated, with 205 excluded either because the intervention included caloric restriction or had multiple dietary changes, or the study was in a population with disease (Figure 19). Twenty studies were included in the meta-analysis.

Figure 21: Study flow chart of the meta-analysis.



Characteristics of studies

Characteristics of the 20 included trials which included 1677 participants are summarized in Table 27. The average age was 51 (SD 16) years and 78% of participants were female. The median duration of follow up was 26 (IQR 10-39) weeks. The average difference in dairy food intake between groups was 3.12 (SD 0.62) standard serving sizes /day. Within studies there was no imbalance between randomised groups. One crossover study (252) had a higher dropout rate for subjects initially randomised to low compared to high dairy food intake (49% vs. 22%).

Sixteen studies had a parallel group design and 4 were cross-over studies. In 10 studies increased dairy food included whole fat dairy food, while in another 10 studies, only low -fat dairy was advised. Fifteen of the studies were at least partly funded by the dairy or food industry.

Change in risk factors on a high and a lower dairy food diet in all studies combined are displayed in figures 20-28. Results stratified by duration of intervention, participant body weight and by funding source are displayed in Table 28.

Table 27: Baseline characteristics of studies included in the meta-analysis

Trial Country Year published	Population Mean BMI (kg/M^2)	Funding source	Number of subjects[1] (% female)	Mean Age years (SD)	Design	Length of intervention (Weeks)	Primary outcome	Low dairy Food	High Dairy Food (+ serves /day)[1]	Jadad Score
Alonso (182) Spain 2009	Normotensive college students 23.6	Public	45 (60%)	19·9 (1·5)	Crossover	8	Blood pressure and weight	1·5 serves/day	3·5 whole-fat	2
Baran (317) America 1990	Healthy volunteers 22.8	Industry	37 (100%)	36.5 (3.4)	Parallel	156	Bone density	Usual diet	2.5 whole-fat or low-fat dairy	2
Barr (271) North America 2000	Healthy volunteers 25.8	Industry	198 (64%)	65·2 (6·7)	Parallel	12	Weight, blood pressure, lipids	Usual diet	3 low-fat dairy	3
Benatar (1) New Zealand 2013	Healthy volunteers 24.6	Public	120 (75%)	46·3 (12·0)	Parallel	4	Weight, blood pressure	Usual diet	3·5 whole-fat or low-fat dairy	3

Chee (273) Malaysia 2003	Postmenopausal Chinese woman 23.8	Industry	173 (100)	59·0 (3·2)	Parallel	104	Bone loss	Usual diet	2 low-fat dairy	3
Crichton (283) Australia 2012	Overweight and obese volunteers 31.5	Public	36 (83)	47·3 (15·1)	Crossover	26	Weight	≤1 serves/day	4 low-fat dairy	2
Eagan (318) North America 2006	Normal weight young women 22.5	Industry	37 (100)	20·0 (2·0)	Parallel	26	Fat mass	Usual diet	3 whole-fat or low-fat dairy	2
Gardner (255) North America 2007	Healthy volunteers 26.0	Industry	28 (79)	52(9)	Crossover	4	Lipids	Usual diet	2·5 low-fat dairy	3
Ghadirian (310) Canada 1995	Postmenopausal nuns 23.0	Industry	158(100%)	79 (9.5)	Parallel	4	Uric acid	0	3.6 whole-fat or low-fat dairy	2
Gunther (254) North America 2005	Healthy woman 22.2	Industry	99 (100)	20·0 (2·1)	Parallel	52	Weight	Usual diet	3 low-fat dairy	2

Kukuljan (319) Australia 2009	Older men (>50years) 27.6	Public	89(0%)	60.9 (7.5)	Parallel	52	Bone Density	Usual diet	1.7 low-fat dairy	2
Lau (309) China 2001	Postmenopausal women 22.2	Industry	185 (100)	57·0 (1·8)	Parallel	104	Bone loss	Usual diet	2 low-fat dairy	3
Manios (320) Greece 2009	Postmenopausal women 30.4	Industry	62(100)	61.2 (4.9)	Parallel	52	Weight	Usual diet	3 low-fat dairy	2
Palacios (249) Puerto Rica 2011	Obese adults 38.5	Public	16 (81)	37·0 (2·2)	Parallel	21	Weight, lipids	Usual diet	4 whole-fat or low-fat dairy	1
Stancliffe (253) North America 2011	Overweight and obese with metabolic syndrome 30.7	Industry	40 (53)	37·0 (9·9)	Parallel	12	Inflammatory markers	0·5 serves /day	3·5 whole-fat or low-fat dairy	2
Tardy (129) France 2009	Healthy women with abdominal obesity 32.6	Public	39 (100)	36·4 (7·7)	Parallel	4	HOMA	Usual diet + vegetable oils	3 whole-fat	2

Van Meilj (321) Holland 2010	Overweight adults 32.0	Industry	35 (71)	49·5(13·2)	Crossover	8	Blood pressure, Lipids, inflammatory marker, glucose	Usual diet	3 low-fat dairy	2
Wennesberg (272) Scandinavia 2009	Metabolic syndrome 30.0	Industry and Public	105 (67)	51·2 (8·0)	Parallel	26	Waist circumference	Usual diet	3-5 whole-fat or low-fat dairy	2
Zemell (322) North America 2010	Obese and overweight adults 30.0	Industry	20 (30)	31·0 (10·3)	Crossover	4	Inflammatory markers	0	3 low-fat dairy	3
Zemmel (phase 1) (323) North America 2005	Obese adults 34.9	Industry	34 (59)	41·7 (2·8)	Parallel	26	Weight	Usual diet	3 whole-fat or low-fat dairy	1

Table 28: Studies stratified by study duration, study population normal weight or overweight and source of funding on cardio-metabolic risk factors.

Metabolic risk factors	Diet Change <6 months duration	Diet change ≥ 6 months duration	Normal weight (BMI <25 kg/m^2)	Overweight or obese (BMI > 25kg/m2)	Industry funded studies	Public funded studies
Total number of studies	10	10	8	12	14	6
Total number of subjects	738	982	854	823	1246	431
Weight (kg)	+0.51* (0.14 to 0.88)	+0.74* (0.24 to 1.20)	+0.59* (0.29 to 0.89)	+0.61* (0.03 to1.24)	+0.44* (0.12 to 0.76)	+0.82* (0.32 to 1.33)
N	738	891	895	534	1198	431
Waist circumference (cm)	+0.24 (-1.22 to 0.70)	+0.22 (-1.91 to 2.35)	+0.80 ([-0.20 to 1.80) n=115	-0.29 (-1.67 to 1.10)	-0.73 (-3.11 to 1.65)	+0.59 (-0.12 to 1.29)
N	194	246	115	325	214	226

HOMA- IR (units)	-0.85 (-1.88 to 0.19)	0.00 (-0.25 to 0.25)	-0.16 (-0.56 to 0.24)	-0.79 (-1.94 to 0.37)	-1.59 (-4.77 to 1.59)	-0.40* (-1.01 to 0.21)
N	195	75	116	154	115	155
Fasting Glucose (mg/dl)	+0.36 (-0.90 to 1.80)	+0.72 (-4.32 to 5.76)	+0.36 (-2.70 to 3.42)	+0.36 (-1.08 to 1.80)	+0.54 (-1.08 to 2.16)	+0.18 (-2.16 to 2.52)
N	601	72	196	477	477	226
LDL-cholesterol (mg/dl)	+0.87 (-5.03 to 4.25)	+4.25 (-4.25 to 12.37)	+5.8* (0.39 to 11.2)	0.00 (-4.25 to 4.25)	-0.00 (-6.57 to 6.57)	+2.70 (-1.54 to 6.96)
N	480	222	115	587	460	242
HDL-cholesterol (mg/dl)	-1.16 (-4.25 to 1.55)	+0.00 (-2.32 to 2.70)	-3.09 (-7.73 to 1.93)	-0.00 (-1.93 to 1.93)	+ 1.16 (-6.12 to 8.50)	-6.57 (-16.24 to 3.48)
N	443	221	155	509	419	245

C- reactive protein (mg/L)	-1.97 (-4.61 to 0.67)	+0.13 (-0.72 to 0.98	+1.18 ([-0.68 to 3.04)	-1.56 (-3.22 to 0.10)	-1.89 (-3.84 to 0.07)	0.60 (-0.88 to 2.08)
N	268	183	116	335	265	186
Systolic blood pressure (mmHg)	-0.57 (-1.91 to 0.76)	+0.43 (-2.59 to 3.45)	+0.57 (-1.37 to 2.51)	-1.05 (-2.62 to 0.52)	-1.44 (-3.15 to 0.28)	+0.64 (-1.10 to 2.37)
N	526	185	216	495	423	288
Diastolic blood pressure (mmHg)	-0.21 (-1.78 to 1.36)	-1.31 (-3.28 to 0.66)	+1.03 (-0.73 to 2.79)	-1.08 (-2.41 to 0.26	-1.37 (-3.05 to 0.30)	+0.74 (-0.72 to 2.20)
N	526	185	216	495	423	423

*P <0.05

n = number of participants

Effects on body weight

Eighteen (129, 182, 249, 250, 252-254, 271-273, 309, 310, 317-320, 323, 324) studies reported effects on weight in 1629 individuals (Figure 20). The mean body mass index (BMI) at baseline was 25.6 (SD 6.2) kg/m^2 and weight 77.7 (SD 16.2) kg. Increased dairy food intake was associated with a modest weight gain (+0.60, 95% confidence interval 0.30 to 0.90kg, p<0.0001). In six studies (129, 252, 253, 272, 320, 324) with 440 individuals, waist circumference did not change significantly (-0.07, -1.24 to 0. 1.10cm) (Figure 21).

Weight gain was observed both in studies which increased low fat (+0.82, 0.35 to 1.28 kg, p<0.001) and whole fat dairy food (+0.41, 0.04 to 0.79kg, p=0.03). Modest weight gain was also observed in 10 studies (n= 692) which included overweight and obese subjects (+0.60, 0.01 to 1.19kg, p=0.03) and in 8 studies n= 937) of normal weight participants (+0.59, 0.31 to 0.87kg, p=0.02).

Figure 22: Effects of whole & low fat dairy food on weight.*

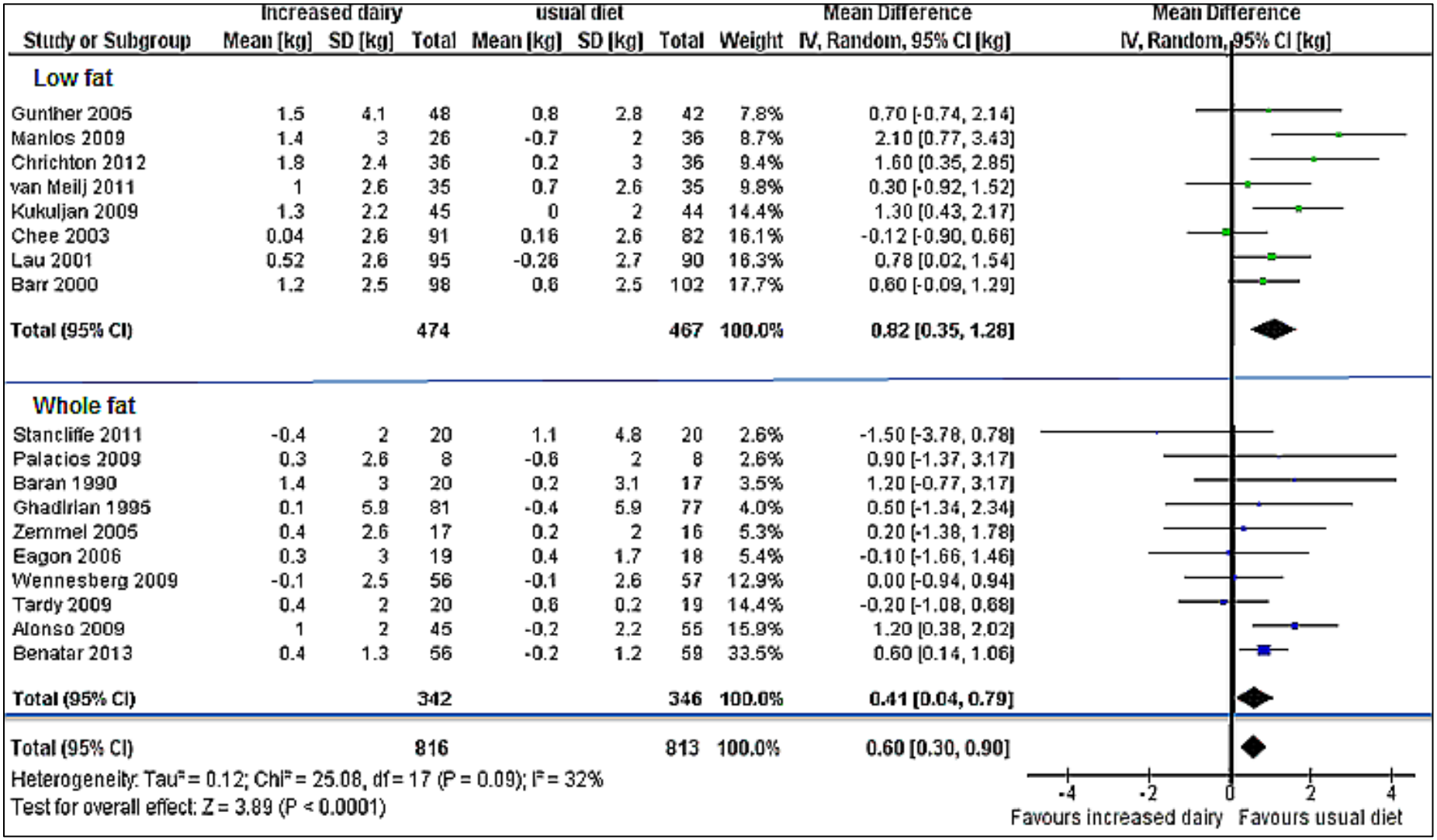
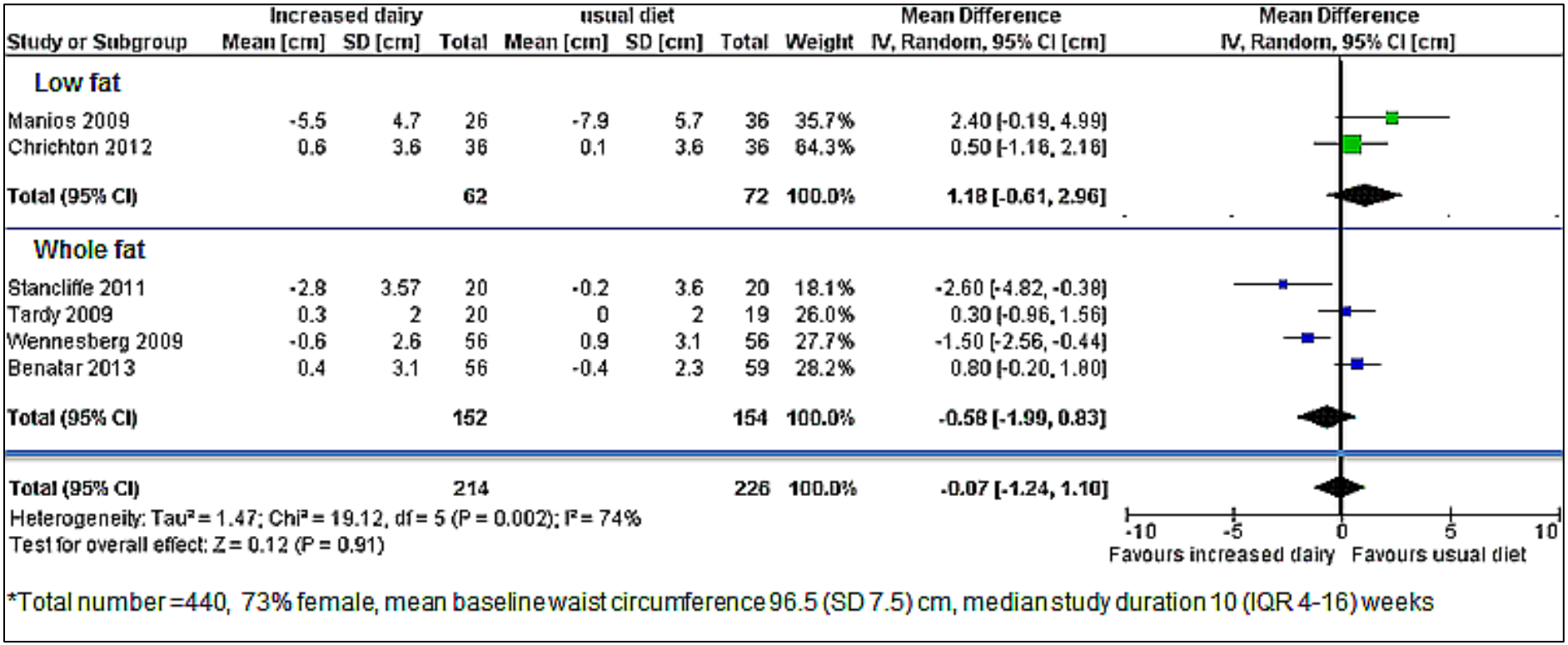


Figure 23: Effects of whole & low fat on dairy food waist circumference.*

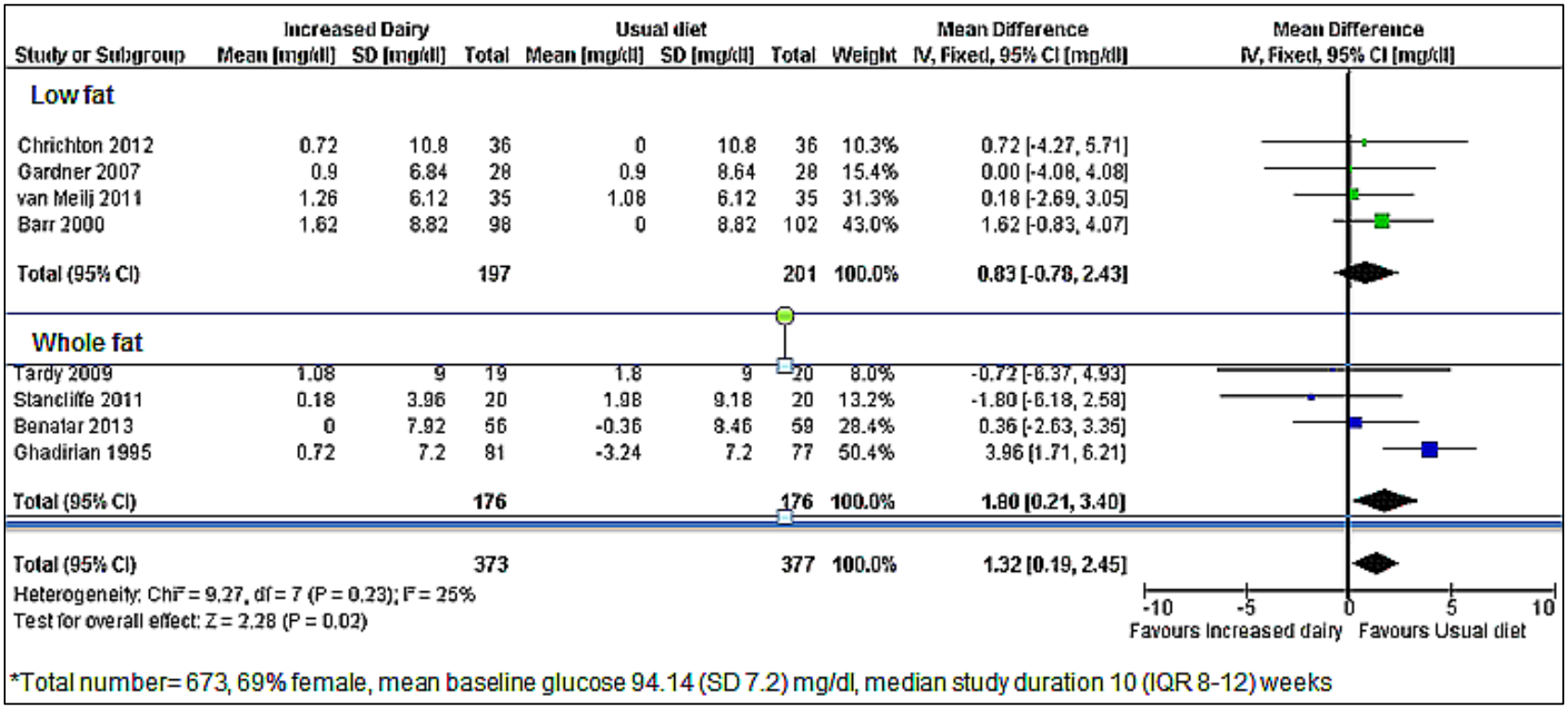


*Total number =440, 73% female, mean baseline waist circumference 96.5 (SD 7.5) cm, median study duration 10 (IQR 4-16) weeks

Effects on insulin resistance

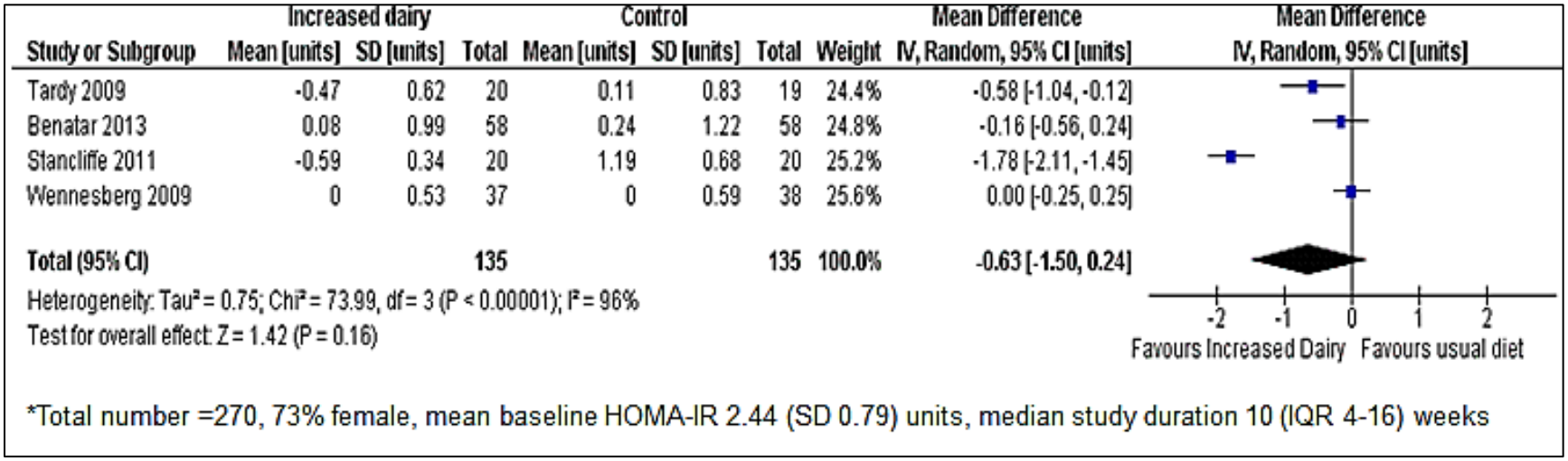
In 8 studies, (129, 250, 252, 253, 255, 271, 310, 324) there was no significant change in fasting glucose on a higher compared to a lower dairy food diet (Figure 22). Four studies, (129, 253, 272, 324) assessed effects on HOMA-IR in 270 subjects (Figure 23). One (129) did not report standard deviation so a weighted mean standard deviation from the other studies was used. HOMA-IR was recalculated in one study which used incorrect units. (272) For all studies combined HOMA-IR was slightly improved on the high dairy food diet (-0.94, -1.93 to 0.04 units, p=0.06). However there was heterogeneity between studies (I^2 =92%), accounted for by the two smallest studies (129, 253) which reported reduced insulin resistance on the high dairy food diet (-1.37, -1.64 to -1.10 units). HOMA-IR was similar on high and low dairy food diets in the two larger studies (272, 324) (-0.05, -0.26 to 0.17 units). HOMA-IR did not change significantly for studies stratified by body weight, duration of intervention or high versus low fat dairy food (Table 2).

Figure 24: Effects of whole fat dairy food on fasting plasma glucose.*



*Total number= 673, 69% female, mean baseline glucose 94.14 (SD 7.2) mg/dl, median study duration 10 (IQR 8-12) weeks

Figure 25: Effects of whole fat dairy food on insulin resistance assessed by Homeostasis Model Assessment.-Insulin Resistance. *

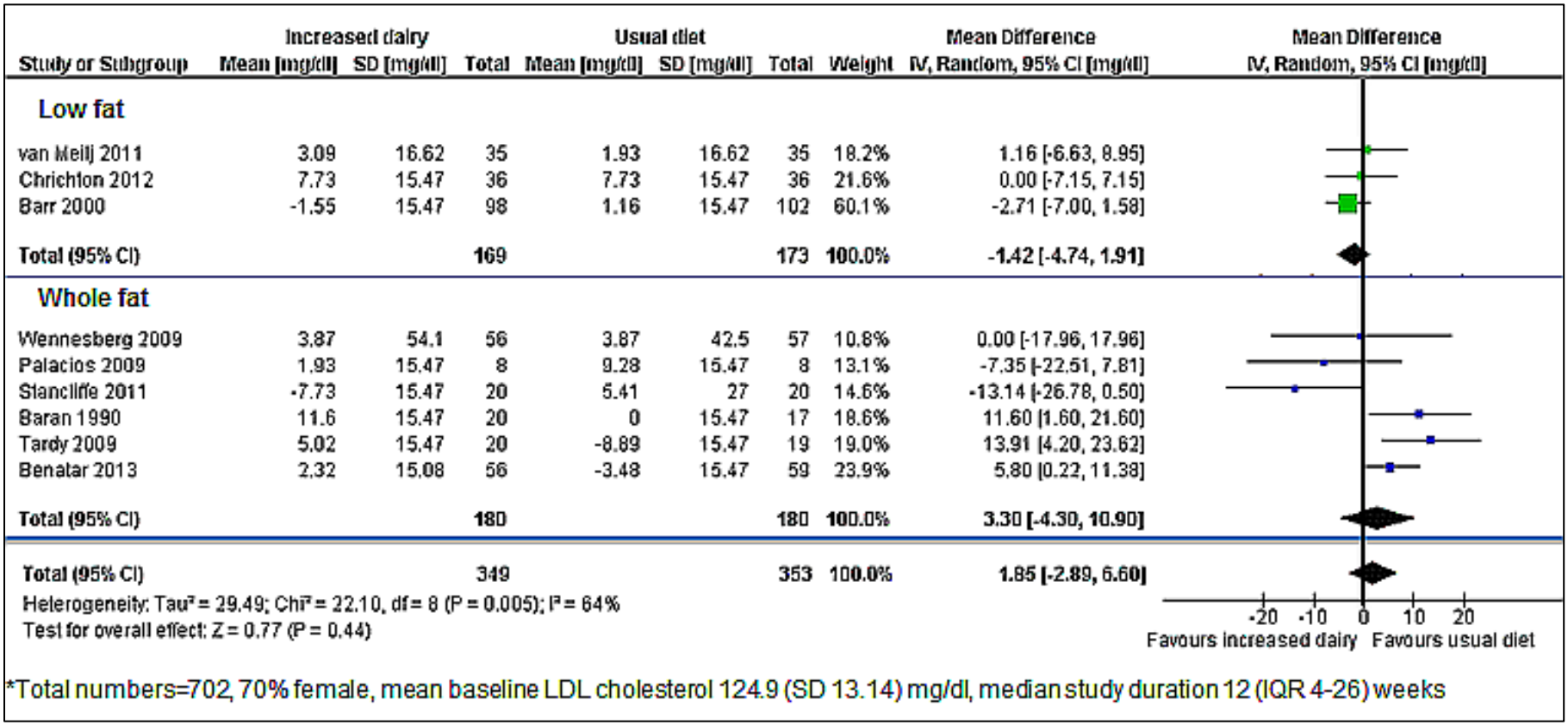


*Total number =270, 73% female, mean baseline HOMA-IR 2.44 (SD 0.79) units, median study duration 10 (IQR 4-16) weeks

Effects on LDL and HDL cholesterol

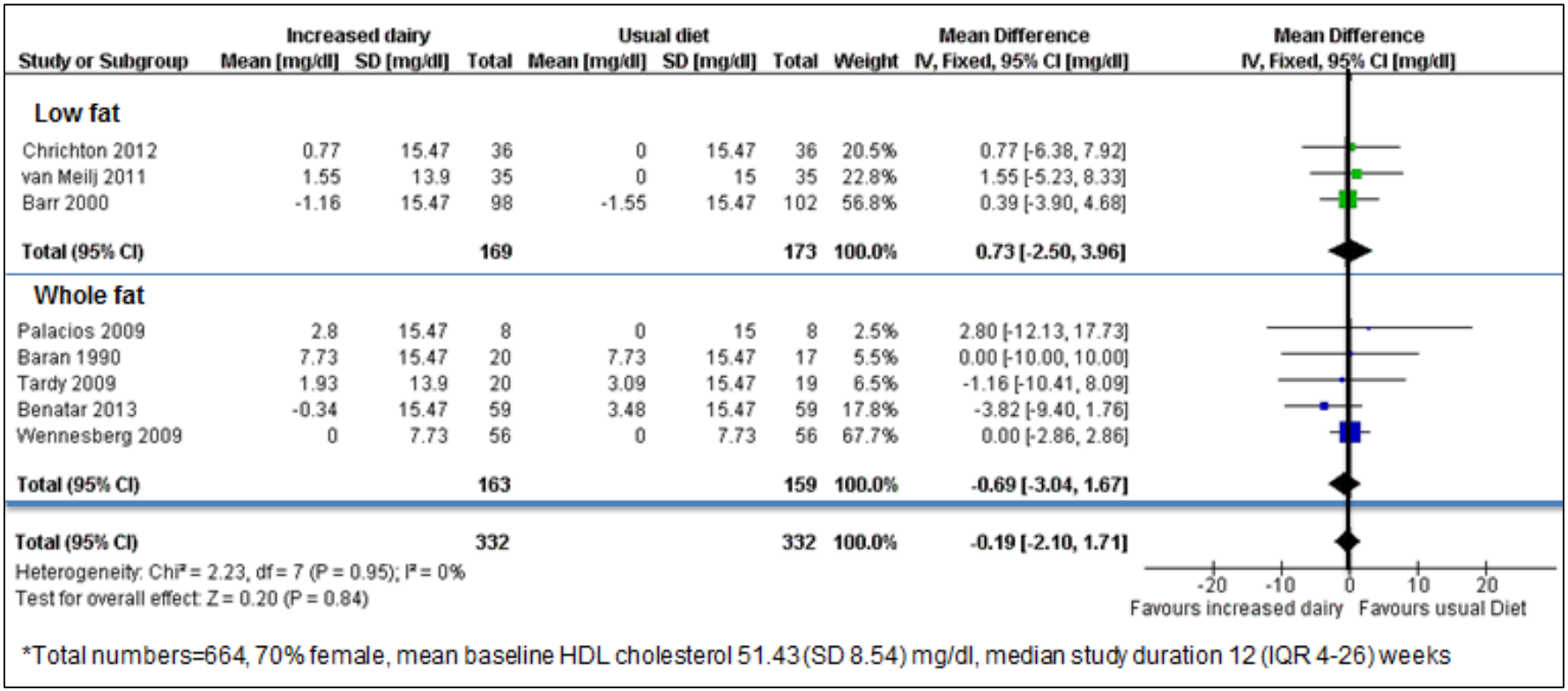
Nine studies (129, 249, 250, 252, 253, 271, 272, 317, 324) assessed effects on LDL- and HDL-cholesterol in 702 individuals. For all studies combined there was no significant change in either LDL or HDL-cholesterol after increasing dairy food (Figures 24 and 25). Effects of HDL-c were consistent (I^2=0%) across studies, but there was heterogeneity for LDL-c (I^2=49%). There was no change in LDL cholesterol when whole fat dairy food (+3.30, -4.30 to 10.90mg/dl]) or low fat dairy (-1.42,-4.74, to 1.91mg/dl) food was increased. Results were similar for shorter and longer periods of dietary intervention and for studies which included normal and overweight or obese participants.

Figure 26: Effects of whole & low fat dairy food on LDL-cholesterol.*



*Total numbers=702, 70% female, mean baseline LDL cholesterol 124.9 (SD 13.14) mg/dl, median study duration 12 (IQR 4-26) weeks

Figure 27: Effects of whole & low fat dairy food on HDL-cholesterol.*



*Total numbers=664, 70% female, mean baseline HDL cholesterol 51.43 (SD 8.54) mg/dl, median study duration 12 (IQR 4-26) weeks

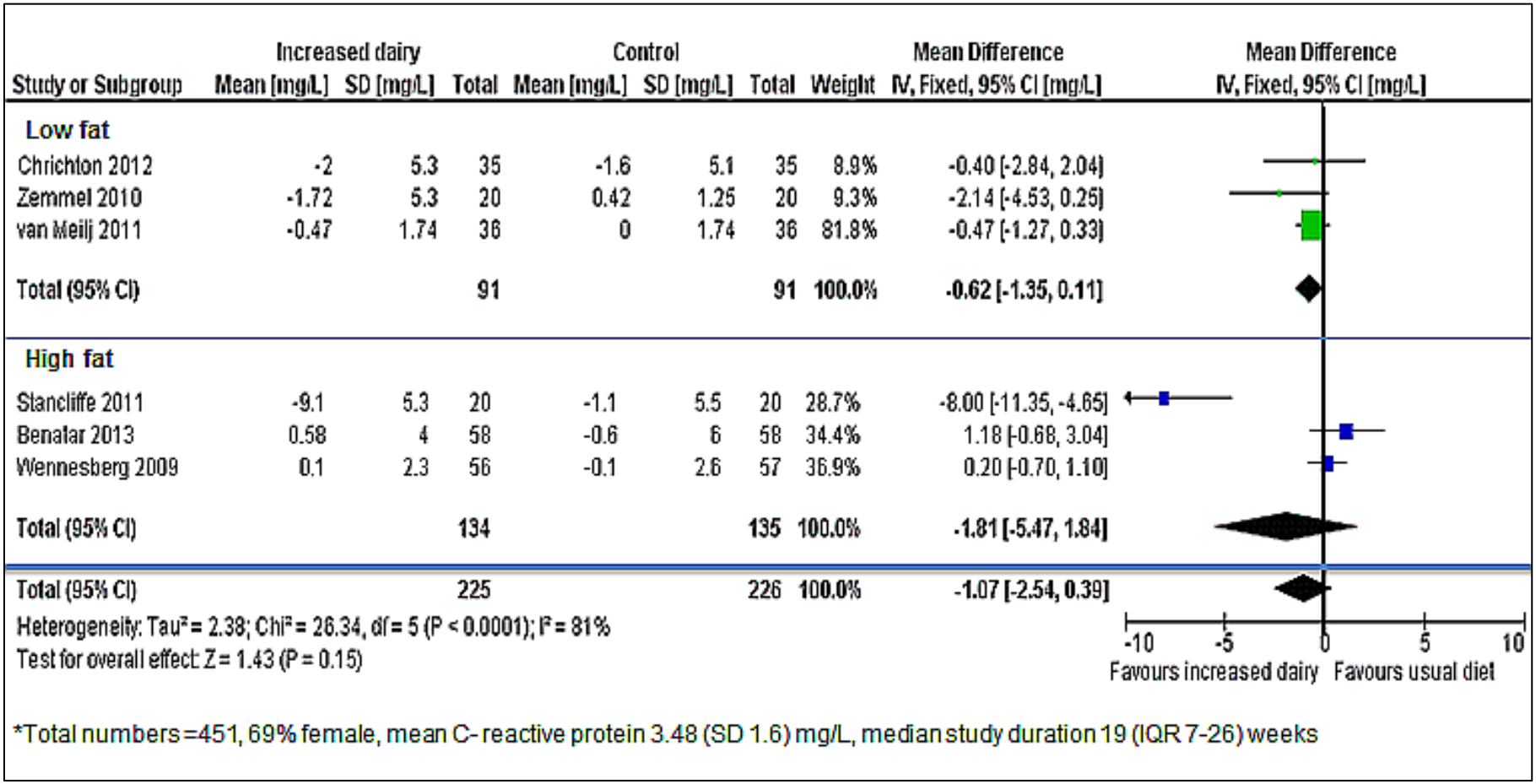
Effects on C-reactive protein

Six studies (252, 253, 272, 321, 322, 324) assessed effects on CRP in 400 individuals (Figure 26). For all studies combined there was no significant change in C-RP on a high dairy food diet. However, two smaller studies (253, 322) reported significant reductions in CRP with increased dairy food intake (-1.10, -2.27 to 0.06mg/L). There was no evidence for effects on CRP when studies were stratified by duration of dietary intervention, high and low fat dairy food, or normal or overweight subjects (Table 2).

Effects on blood pressure

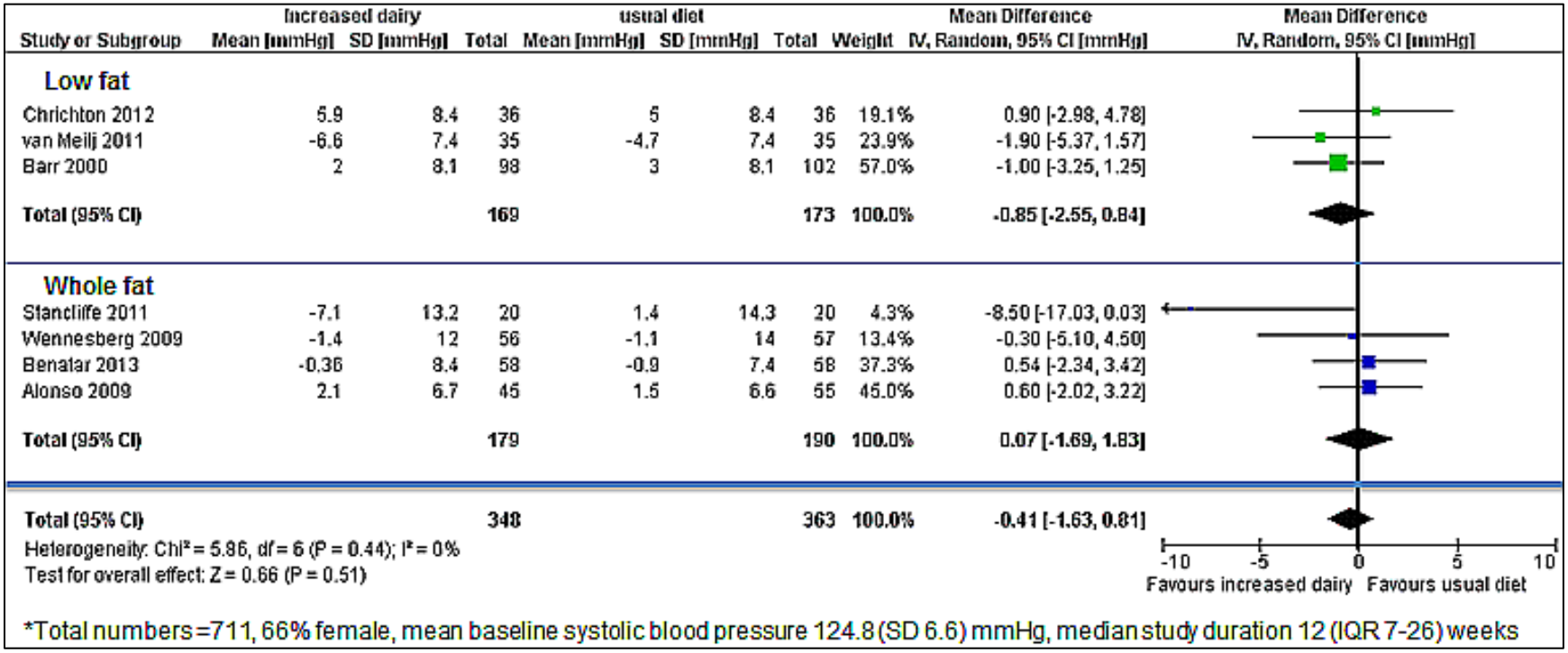
Seven studies (182, 250, 252, 253, 271, 272, 321, 324) assessed effects on blood pressure in 711 participants (Figures 27 and 28). For all studies there was no significant change in either systolic blood pressure or diastolic blood pressure. There was also no evidence for effects on blood pressure when studies were stratified by duration of dietary intervention, high and low fat dairy food or for normal or overweight subjects.

Figure 28: Effects of whole & low fat dairy food on C-reactive protein. *



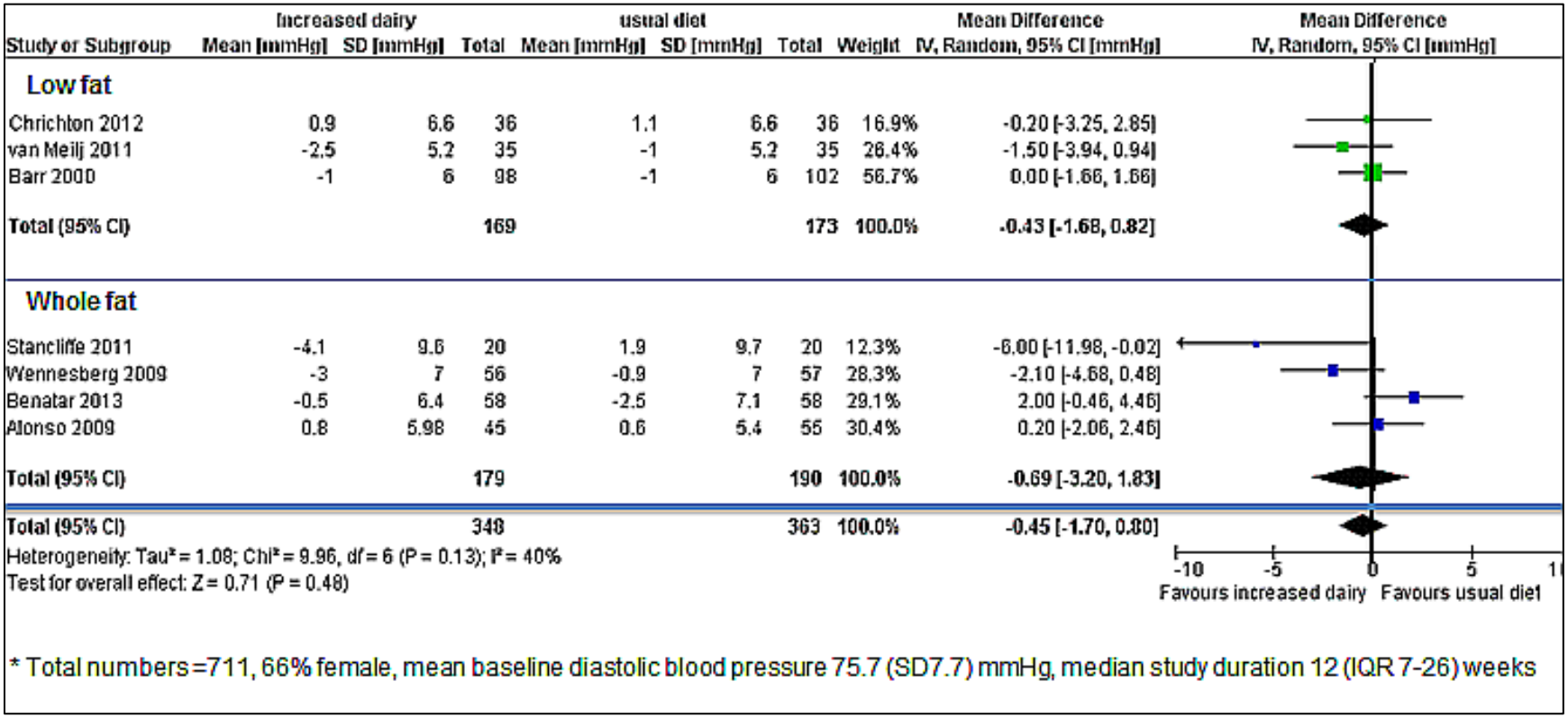
*Total numbers =451, 69% female, mean C- reactive protein 3.48 (SD 1.6) mg/L, median study duration 19 (IQR 7-26) weeks

Figure 29: Effects of whole & low fat dairy food on systolic blood pressure.*



*Total numbers =711, 66% female, mean baseline systolic blood pressure 124.8 (SD 6.6) mmHg, median study duration 12 (IQR 7-26) weeks

Figure 30: Effects of whole & low fat dairy food on diastolic blood pressure.*



* Total numbers =711, 66% female, mean baseline diastolic blood pressure 75.7 (SD7.7) mmHg, median study duration 12 (IQR 7-26) weeks

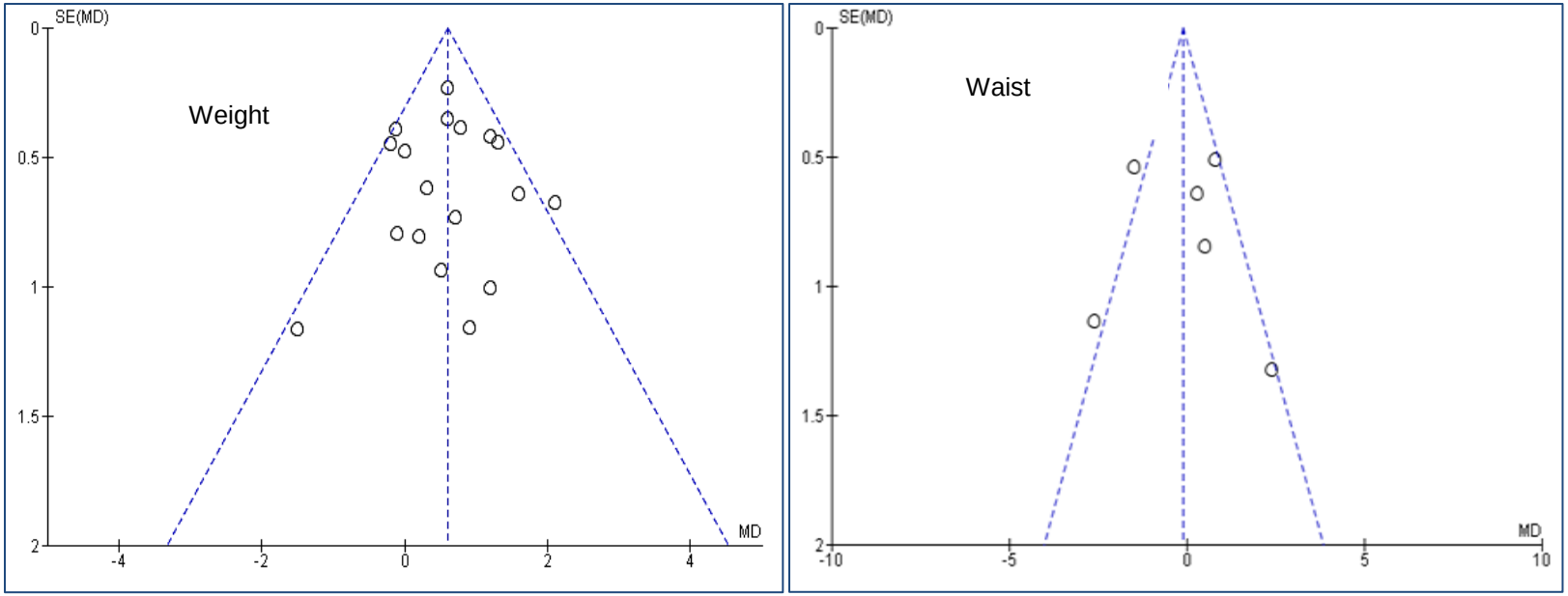
Evaluation of heterogeneity and sensitivity analysis

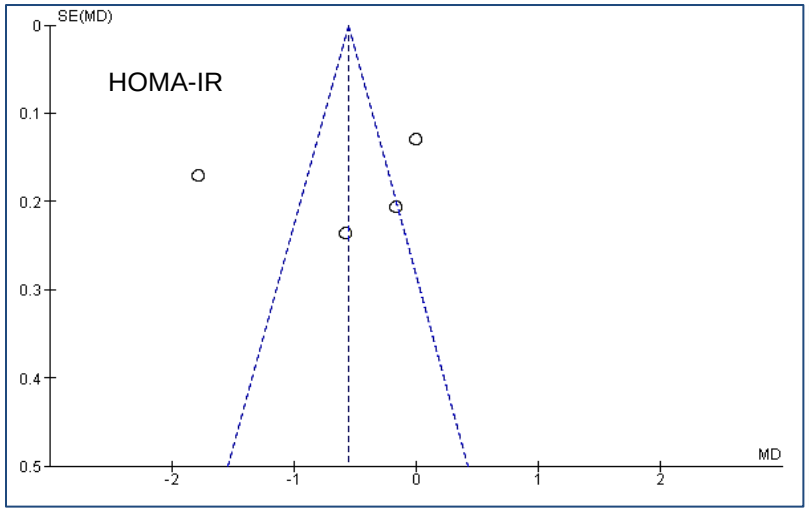
Industry sponsored studies were more likely to report favourable effects on risk factors than non-industry sponsored studies (Table 28). Funnel plots identified that the study by Stancliffe (253) reported decreases in LDL cholesterol, HOMA-IR, CRP and waist circumference on the increased dairy diet food beyond the 95% confidence range for all studies combined (Figure 30). This study also reported the greatest decrease in blood pressure and weight of all studies. Excluding this study in a sensitivity analysis substantially decreased heterogeneity, but overall effects were similar, and there was no other consistent difference between smaller and larger studies. The study by Manios (320) was the only study that fell outside the 95% confidence interval for weight, though excluding the study had no overall effect on results. This study had an imbalance in randomization with fewer people randomised to high dairy food (n=30) compared to the control group (n=40). The study by Ghadirian (310) was outside the 95% confidence interval for fasting plasma glucose. In this study, the control group had a statistically significant reduction in fasting plasma glucose, with little change for the high dairy food group'. Repeated sensitivity analysis excluding this study showed no overall effect (+0.02,-0.05 to 0.10mmol/L, p=0.53) and a reduction in heterogeneity (25→0%). Results were also similar when analysis was repeated excluding the 4 cross-over studies.

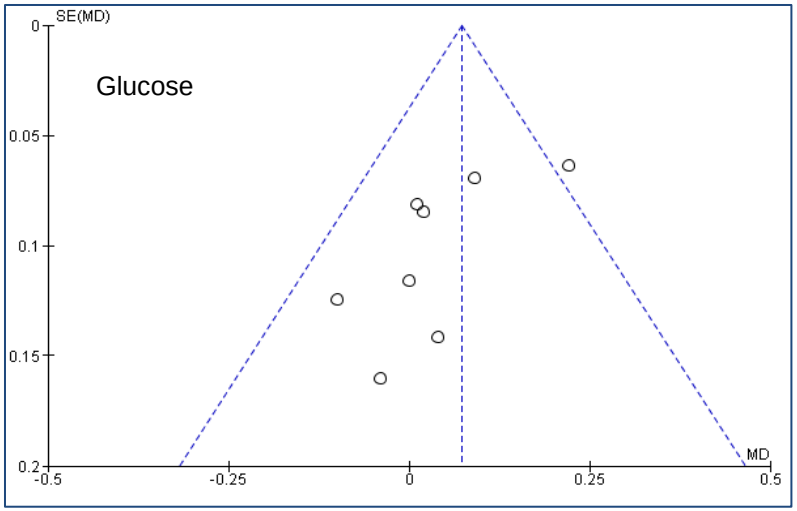
On the basis of a funnel plot and Begg's test, no significant publication bias was shown in the meta-analysis of body weight, waist circumference, insulin resistance, blood pressure, lipids and CRP.

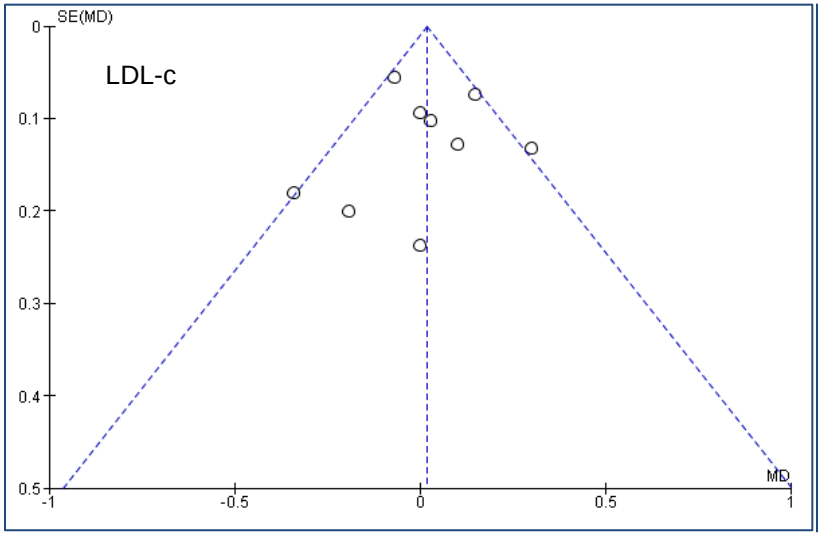
Figure 31: Funnel plots of effects of fat dairy food on HOMA-IR, glucose, LDL-c, HDL-c, C - reactive protein, systolic and diastolic blood pressure.

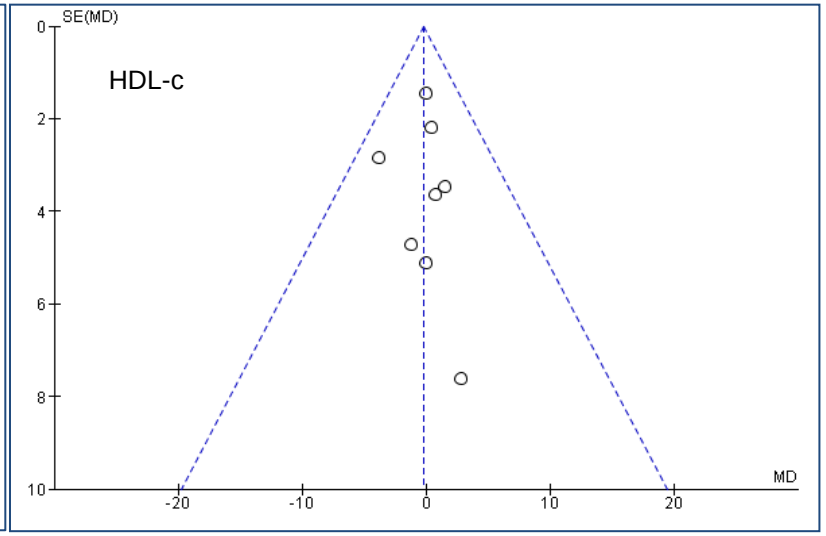
These funnel plots indicate that the most common outliers is the study from Stancliffe et al.

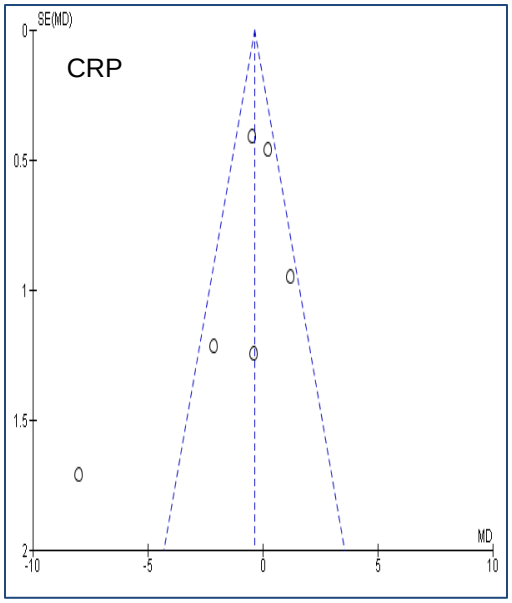


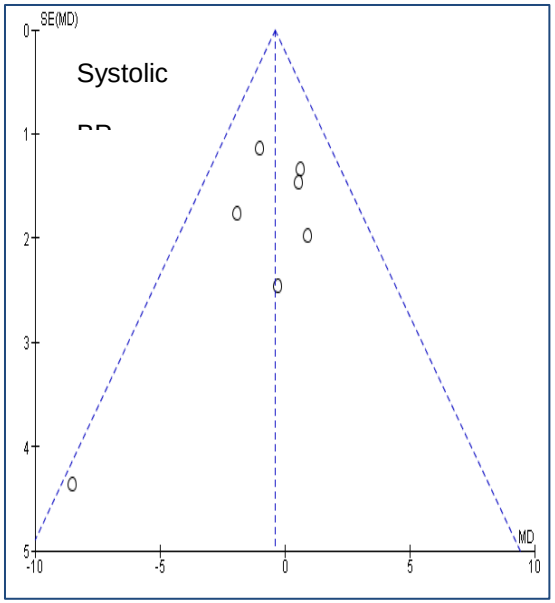

HOMA-IR
SE(MD)
0
0.1
0.2
0.3
0.4
0.5
-2
-1
0
1
2
MD

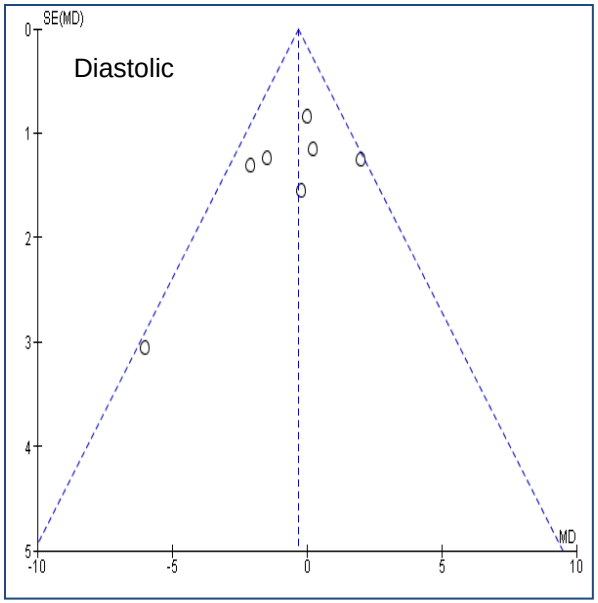

Glucose
SE(MD)
0
0.05
0.1
0.15
0.2
-0.5
-0.25
0
0.25
0.5
MD


LDL-c
SE(MD)
0
0.1
0.2
0.3
0.4
0.5
-1
-0.5
0
0.5
1
MD


HDL-c
SE(MD)
0
2
4
6
8
10
-20
-10
0
10
20
MD


SE(MD)
CRP
MD


SE(MD)
Systolic
MD


SE(MD)
Diastolic
MD

Discussion

This systematic analysis of randomised dietary intervention trials suggests that a moderate increase in dairy food consumption has no or small effect on the major cardiovascular and metabolic risk factors. (262) This conclusion contrasts with results from several large epidemiological studies, which concluded that dairy food may have favourable effects on insulin resistance and decrease the risk of type 2 diabetes. (187, 196) However, in these observational studies (182, 184, 195, 197) dairy food intake was associated with an overall healthier eating pattern, healthier lifestyle, higher socio economic status and educational attainment, which are each associated with more favourable cardiometabolic profiles. (325) Evaluating the effects of dairy food in randomised intervention trials, is likely to be more reliable than from observational studies, where associations may not be causal.

Several observational studies have suggested that dairy food may facilitate weight loss, particularly in obese and overweight individuals. (268) Also in randomised trials where the intervention included both increased dairy food and caloric restriction, weight loss has been reported. (326) However, in the current meta-analysis, which included studies which gave no advice on calorie restriction, increasing dairy food consumption resulted in a modest weight gain. Results were similar in studies which included overweight and obese participants. Whilst no direct comparison is possible, mean weight gain on low fat dairy food is double that of whole fat dairy food. This is counterintuitive but is in keeping with a recent viewpoint expressed in JAMA pediatrics (290) which suggest that trim milk is associated with increased weight in children. It is likely that the weight gain was the result of increased total calories, in studies which encouraged greater dairy food intake without making other changes in diet. It is uncertain whether weight gain also occurs when dairy food is taken as part of, rather than in addition to a balanced diet.

Several diabetes guidelines (6, 327, 328) recommend regular intake of low-fat dairy food because of its' low glycaemic index. (329) In observational studies (187, 288) persons in the highest quartile of dairy products consumption have less insulin resistance, and this association is strongest in those who are overweight or obese. In this analysis insulin sensitivity improved in two small studies (129, 253) with no demonstrated effect in the larger trials. (272, 324) In stratified analyses there was no effect in overweight

and obese participants, or with whole or low fat dairy food interventions. Based on these observations, it is uncertain whether increasing dairy food improves insulin sensitivity, and further well designed studies are needed to resolve this question. A recent review by the American Diabetes Association (330) concluded that 'none of the components of dairy appear to have an effect on glycaemic control or cardiovascular disease risk reduction', which is consistent with this analysis.

Many food guidelines encourage consumption of low- fat dairy food, but advise avoiding whole- fat dairy products. (180, 244) In the current analysis LDL-c did not change significantly when whole-fat dairy food consumption was increased. Whilst the risk of cardiovascular disease is reduced when saturated fats are replaced by unsaturated fats, (227) the reasons for this may be multifactorial. This study suggests that effects on LDL-cholesterol may not the primary reason.

Dairy food intake was associated with lower blood pressure in observational studies, (148, 246) and in the large randomised DASH study. (240) In the DASH study the intervention included increased low fat dairy food, reduced total and saturated fat, and increased fruit and vegetables. In a secondary analysis it was estimated that low-fat dairy food could account for about half of the observed 5.5mmHg decrease in systolic blood pressure. However it is not possible to reliably estimate the effects of each dietary component when the intervention includes multiple dietary changes. For this reason the DASH study and studies of the Mediterranean diet (144) were not included in this meta-analysis. In this meta-analysis, the confidence intervals exclude significant effects (>1.6mmHg) of increasing dairy food on systolic and diastolic blood pressure.

Limitations of meta-analysis

The majority of subjects included in the meta-analysis were women, but there is currently no evidence for different effects of diet by gender. The diverse population and age range of subjects included makes the results relevant to improving lifestyle risk factors for diabetes and cardiovascular disease in the general population. Studying healthy populations also avoids possible treatment and disease effects on the

outcomes of interest. Further research is needed to confirm similar neutral effects of dairy food in patients with established diabetes and cardiovascular disease.

It is possible that the duration of the dietary intervention was not long enough to identify effects on risk factors, but stratified analyses suggest similar results for longer and shorter periods of dietary intervention. The increase of 3.6 servings each day is a substantial dietary change, and previous studies suggest dietary interventions influence risk factors within one month. (240, 331)

Most studies included were relatively small. It is difficult to blind diet studies and the level of compliance with the dietary interventions was often uncertain. Several studies reported significant adverse or favourable effects of increasing dairy food on one or more risk factors, but sensitivity analyses suggested these studies had only a small effect on overall estimates. It is also possible that smaller studies which found no effects have not been published. Three quarters of the studies were funded by the dairy or food industry, and results were more favourable for industry compared to non-industry funded studies

.

This meta-analysis stratified studies by ‘low fat’ and ‘whole fat’ interventions, but a direct comparison of these studies may not be reliable, and no trials, which directly compare ‘low’ with ‘whole’ fat dairy food diets, have been reported. Because studies were small, and some may be unreliable, the analysis cannot exclude a small increase in LDL-c, with increase in whole-fat dairy food consumption. Studies are also needed to evaluate the effects of other components of dairy food. To provide a better estimate of health effects the meta-analysis evaluated associations with multiple rather than just one or two risk factors. However dairy food could influence the risk of cardiovascular disease or diabetes by pathways other than the risk factors measured. (149) The influence of dairy food on clinical outcomes rather than risk factors is most important for dietary guidelines. However, currently, no completed randomised trials allow independent assessment of the effects of changing dairy food on diabetic complications or cardiovascular events.

Conclusion

Increase in both whole and low fat dairy food, without other dietary interventions, is associated with a modest weight gain, with no or minor effects on other cardio-metabolic risk factors. These observations suggest that for most healthy individuals it is reasonable to include both low and whole fat dairy food as part of a healthy diet.

Chapter 7

Summary

Food patterns are emerging as an important factor in the prevention and management of obesity, diabetes and cardiovascular disease. Recent randomised studies suggest that the Mediterranean and DASH diets may be beneficial for cardiovascular health, but these studies have not addressed the effects of dairy food. Dairy food intake is increasing worldwide and is generally promoted as a healthy food, especially in emerging economies. It is considered an important source of protein and minerals such as calcium; and is the most abundant source of animal fats such as saturated and ruminant trans fatty acids in the human diet. Dairy food consists of components that could both increase and reduce cardiometabolic risk. For example, calcium and protein are thought to help reduce weight gain and improve insulin resistance, but saturated fats are thought to increase cardiovascular disease. Reviews suggest that replacing animal saturated fats with unsaturated fats may be beneficial, but little is known about the health effects of rTFA.

Exploring the effects of dairy food and rTFA in the New Zealand population is ideal because this population is unique in the developed world as there has been little exposure to processed food, with a high intake of dairy food. This thesis explores the cardiovascular health effects of rTFA and dairy food in the New Zealand population.

Ruminant trans fatty acids

Whilst 'man made' iTFA are known to be harmful to cardiovascular health, current literature suggests that there is little or no adverse effect of rTFA. Two reasons are thought to account for this, firstly these 'natural' TFAs might not be harmful, and secondly, it is thought that humans do not ingest these at a significant enough dose to cause noticeable clinical effects. However, as legislation reduces iTFA intake in the Western world, the relative effects of rTFA may become more important. New Zealand is one of the

few countries where exposure to processed food is low. This gives researchers a unique opportunity to assess the effects of dairy food and rTFA on cardiovascular health.

An observational study in New Zealanders with significant coronary artery disease suggested that a clear delineation between industrial and ruminant TFA exists. Two TFA, palmitelaidic and vaccenic acids were identified as ruminant. These two TFA comprise two thirds of all TFA found in the plasma and were associated with an increased risk of polyvascular disease and increased CRP. Polyvascular disease is a clinical maker of more significant cardiovascular disease. This study suggested that high risk patients may benefit from a targeted approach to minimize all sources of TFA in the diet. However, the long term follow up of the cohort suggests that rTFA levels are not associated with increased mortality.

To confirm that the plasma TFA were from dairy food, a randomised study in 180 healthy participants was undertaken. Participants were asked to modify dairy food intake and effects on plasma fatty acids and cardio- metabolic risk factors were measured. In this study, changing dairy food intake had little effect on fatty acids including plasma TFA levels. After multiplicity testing only 1 fatty acid, pentadecanoic acid, changed with dairy food intake. No correlation was found between the two TFA labelled ‘ruminant’, vaccenic and palmitelaidic acid, either at baseline or at one month. Moreover, these TFA did not correlate with pentadecanoic acid or heptadecanoic acid at baseline or one month. This study suggested that these TFA may no longer be exclusively from dairy food.

However, the observational study was completed a few years earlier than the randomised study. A number of explanations could account for this disparity. The most likely explanation is that food has changed in last few years with a rapid transition to processed foods that may contain a host of TFA. Likewise, dairy food itself has changed with different animal feeding and processing practices. Another possibility is that improvements in computer software has allowed for the improved discrimination of fatty acid isomers with GC-MS.

Two further studies explored these possibilities; the first measured fatty acids in milk and the second measured fatty acids in avowed vegans who also eschew processed foods.

The fatty acid content of milk and dynamic change over 2 years were assessed by analysis of milk during normal rainfall and then again during a drought. The first analysis was completed during the randomised study to evaluate TFA in milk. Vaccenic acid was the most abundant TFA and a novel TFA, myristelaidic acid, was identified which was more abundant than palmitelaidic acid. Organic milk had significantly lower TFA levels than non-organic milk. The breed of cows does not affect TFA levels, so the most probable explanation for this finding is that differences in farming practices affect dairy fat. Drought conditions in 2013 led to a significant uptake of supplementary PKE feed by non-organic farmers. No significant differences in fatty acids were found between organic and non-organic milk in 2013. However, overall TFA concentrations were higher compared to milk in 2011 mainly due to higher concentrations of vaccenic and palmitelaidic acid. Moreover, milk in 2013 has higher concentration of unsaturated fats (mainly oleic acid) and lower concentrations of saturated fats.

The second study was a cross- sectional observational study in avowed vegans who also avoid processed food. Vegans had lower SFA concentrations than participants in the randomised study, but similar TFA concentrations. Palmitelaidic acid was the predominant isomer found with negligible levels of other plasma TFA. The possibility that humans can bio-convert cis fatty acids to TFA cannot be ruled out and further research is needed to assess this.

Dairy food

Dairy is one of the most complex foods comprising more than 400 fatty acids, amino acids, organic acids and minerals. It is difficult to separate effects of specific fatty acids on health as this ignores possible interactions with all the components in dairy food. In observational studies, consumption of dairy food is associated with improved insulin resistance, less weight gain and lower blood pressure. However, dairy food intake is associated with confounders that are strongly associated with better health outcomes such as higher socio economic status and educational levels. Unfortunately, two diets shown to improve cardio metabolic risk and cardiovascular outcomes, the DASH and Mediterranean diets did not address the role of dairy food as part of a healthy eating plan.

A randomised study completed in 180 healthy adults showed that significantly changing dairy food intake for one month had little effect on cardiometabolic risk. However, a cross sectional study in vegans suggested that eschewing all animal fat is associated with more desirable blood pressure and lipid profiles. Vegans were found to have lower blood pressure, total cholesterol and LDL-c and their HDL-c was higher too. However, a strong confounder is that these vegans had other healthy behaviours, for example almost all avoided any form of processed food and exercised regularly.

A meta-analysis that included 20 studies with 1677 participants with an average duration of dietary change of 6 months and a mean increase in dairy food intake of 3.6 serves/day was completed. The meta-analysis revealed that most randomised studies are sponsored by industry and that these studies were more likely to show positive or little effects from dairy food on cardiometabolic risk factors.

Overall, the meta-analysis established that dairy food is associated with weight gain but no other significant effects on cardiometabolic risk factors. Interestingly, increased consumption of low fat dairy food compared to whole fat dairy food was associated with more weight gain. This apparent paradox has been also reported in weight loss studies when low fat diets are compared to high fat (Mediterranean-

style) diets. No other significant effect on a range of cardiometabolic risk factors including LDL-c, HDL-c, blood pressure, C- reactive protein and insulin resistance as observed suggesting that dairy food has little overall effect. This suggests that here are no cardiovascular health benefits of dairy food and it should not be promoted as helpful for weight loss or reducing insulin resistance.

Conclusion

Observational studies suggest that man-made trans fatty acids have negative effects on cardiovascular health, but that ruminant trans fatty acids are consumed in doses too low to have biological effects. However, this dissertation suggests that New Zealanders are exposed to relatively high levels of ruminant trans fats and that these may be associated with increased cardiovascular disease. Total trans fatty acid intake, regardless of source, may be more relevant when evaluating effects on cardiovascular health. In New Zealand there is a sizeable consumption of dairy food containing relatively high levels of trans fatty acids. Farming practices in New Zealand such as pasture feeding and the use of certain supplementary feeds may affect trans fatty acid content in dairy food. Modifying farming practice may be a pragmatic approach to reduce exposure to trans fatty acids in the New Zealand population. Increasing public awareness on unexpected sources of trans fatty acids, especially in high risk patients, could also be considered. Trans fatty acids are found in vegans who consume little or no processed food. The source of TFA in this population is hard to explain and requires further research.

It may, however, be more clinically relevant to evaluate the effects of whole dairy food rather than specific components like ruminant trans fatty acids on health. Focusing on one type of fatty acid ignores potential synergistic effects and interactions between all the components of the food. (202) Moreover, people eat a whole food and not one fatty acid. A randomised study showed that changing of dairy food consumption had no significant effects on cardio-metabolic risk factors and a meta-analysis performed suggests that increased intake of low fat, and to a lesser extent whole fat dairy food, was associated with weight gain but no significant effects on other cardio-metabolic risk factors. Traditionally the results of the randomized study would trump results from the observational study, however results of the observational study cannot

be completely discounted for a number of reasons; the randomized study cannot account for health effects that accumulate over long periods and adherence to dietary randomised studies is notoriously poor. Moreover, there is a small possibility that exposure to dairy food at crucial times in a person's life may have long lasting effects on risk factors, for example, cholesterol levels or endothelial function. These possibilities cannot be completely accounted for in a randomised study. Thus results from the observational study which contradict the randomized study have to be taken into account. Whilst small effects on cardiometabolic risks accumulating over a long period cannot be excluded by randomised studies, the evidence suggests that dairy food is neither harmful nor beneficial for cardiovascular health.

The lack of firm conclusions regarding clinical and public health implications in the thesis generally reflects the contrasting results from the thesis as well as emerging evidence. Further randomized studies assessing the long term effects of dairy food would better inform public health recommendations.

Appendix 1: Common fatty acids.

Common name	Systemic name	Structural Formula	Lipid Number
TRANS FATTY ACIDS			
Fumaric acid	trans-butenedioic acid	$C_4H_4O_4$	4:1 (n-2t)
Myristelaidic acid	9-trans-tetradecenoic acid	$C_{14}H_{26}O_2$	14-1 (n-5t)
10-trans-pentadecenoic acid	Methyl trans-10-pentadecenoic	$C_{15}H_{30}O_2$	15:1 (n-5t)
Palmitelaidic acid	9-trans-hexadecenoic acid	$C_{16}H_{30}O_2$	16:1 (n-7t)
Vaccenic acid	11- trans-octadecenoic acid	$C_{18}H_{34}O_2$	18:1 (n-7t)
Elaidic Acid	9-trans-octadecenoic acid	$C_{18}H_{34}O_2$	18:1 (n-9t)
Linoelaidic acid	9-trans,12- trans-octadecadienoic acid	$C_{18}H_{32}O_2$	18:2 (n-6t, 9t)
10-trans-nonadecenoic acid	Methyl 10 trans nonadecenoate	$C_{19}H_{38}O_2$	19:1 (n-10t)
11-trans-eicosenoic acid	Delta 11 trans-Eicosenoic acid	$C_{20}H_{38}O_2$	20:1 (n-9t)
SATURATED FATTY ACIDS			
Propionic acid	Propanoic acid	CH_3CH_2COOH	3:0
Butyric acid	Butanoic acid	$CH_3(CH_2)_2COOH$	4:0
Valeric acid	Pentanoic acid	$CH_3(CH_2)_3COOH$	5:0
Caproic acid	Hexanoic acid	$CH_3(CH_2)_4COOH$	6:0
Enanthic acid	Heptanoic acid	$CH_3(CH_2)_5COOH$	7:0
Caprylic acid	Octanoic acid	$CH_3(CH_2)_6COOH$	8:0
Pelargonic acid	Nonanoic acid	$CH_3(CH_2)_7COOH$	9:0
Capric acid	Decanoic acid	$CH_3(CH_2)_8COOH$	10:0
Undecylic acid	Undecanoic acid	$CH_3(CH_2)_9COOH$	11:0
Lauric acid	Dodecanoic acid	$CH_3(CH_2)_{10}COOH$	12:0

Tridecylic acid	Tridecanoic acid	$CH_3(CH_2)_{11}COOH$	13:0
Myristic acid	Tetradecanoic acid	$CH_3(CH_2)_{12}COOH$	14:0
Pentadecylic acid	Pentadecanoic acid	$CH_3(CH_2)_{13}COOH$	15:0
Palmitic acid	Hexadecanoic acid	$CH_3(CH_2)_{14}COOH$	16:0
Margaric acid	Heptadecanoic acid	$CH_3(CH_2)_{15}COOH$	17:0
Stearic acid	Octadecanoic acid	$CH_3(CH_2)_{16}COOH$	18:0
Nonadecylic acid	Nonadecanoic acid	$CH_3(CH_2)_{17}COOH$	19:0
Arachidic acid	Eicosanoic acid	$CH_3(CH_2)_{18}COOH$	20:0
Heneicosylic acid	Heneicosanoic acid	$CH_3(CH_2)_{19}COOH$	21:0
Behenic acid	Docosanoic acid	$CH_3(CH_2)_{20}COOH$	22:0
Tricosylic acid	Tricosanoic acid	$CH_3(CH_2)_{21}COOH$	23:0
Lignoceric acid	Tetracosanoic acid	$CH_3(CH_2)_{22}COOH$	24:0
Pentacosylic acid	Pentacosanoic acid	$CH_3(CH_2)_{23}COOH$	25:0
Cerotic acid	Hexacosanoic acid	$CH_3(CH_2)_{24}COOH$	26:0
Heptacosylic acid	Heptacosanoic acid	$CH_3(CH_2)_{25}COOH$	27:0
Montanic acid	Octacosanoic acid	$CH_3(CH_2)_{26}COOH$	28:0
Nonacosylic acid	Nonacosanoic acid	$CH_3(CH_2)_{27}COOH$	29:0
Melissic acid	Triacontanoic acid	$CH_3(CH_2)_{28}COOH$	30:0
Henatriacontylic acid	Henatriacontanoic acid	$CH_3(CH_2)_{29}COOH$	31:0
Lacceroic acid	Dotriacontanoic acid	$CH_3(CH_2)_{30}COOH$	32:0
Psyllic acid	Tritriacontanoic acid	$CH_3(CH_2)_{31}COOH$	33:0
Geddic acid	Tetratriacontanoic acid	$CH_3(CH_2)_{32}COOH$	34:0
Ceroplastic acid	Pentatriacontanoic acid	$CH_3(CH_2)_{33}COOH$	35:0
Hexatriacontylic acid	Hexatriacontanoic acid	$CH_3(CH_2)_{34}COOH$	36:0

MONOUNSATURATED FATTY ACIDS			
Undecenoic acid	cis10-undecenoic Acid	$C_{11}H_{20}O_2$	11:1(n-1c)
cis-10-dodecenoic acid	Cis-10-dodecenoic acid	$C_{12}H_{22}O_2$	12:1 (n-2c)
8-tridecenoic acid	8-tridecenoic acid	$C_{13}H_{24}O_2$	13:1 (n-5c)
Myristoleic acid	cis-9-tetradecenoic acid	$C_{14}H_{26}O_2$	14:1 (n-5c)
cis-10-pentadecenoic acid	cis-10-pentadecenoic acid	$C_{15}H_{28}O_2$	15:1 (n-5)c
Palmitoleic acid	cis-9-hexadecenoic acid	$C_{16}H_{30}O_2$	16:1 (n-7c)
Sapienic acid	cis-6-hexadecenoic acid	$C_{16}H_{30}O_2$	16:1 (n-10c)
Heptadecenoic acid	cis-10-heptadecenoic acid	$C_{17}H_{32}O_2$	17:1 (n-7c)
Oleic acid	cis-9 -octadecanoic acid	$C_{18}H_{34}O_2$	18:1 (n-9c)
OMEGA-3 POLYUNSATURATED FATTY ACIDS			
Hexadecatrienoic acid	2-Hydroxyethanesulfonic acid-3, 3-(carbonyldiimino) dibenzenecarboximidamide (1:1).	$C_{16}H_{22}N_6O_5S$	16:3 (n-3c, 6c, 12c)
Alpha-linolenic acid	all-cis-9,12,15-octadecatrienoic acid	$C_{18}H_{30}O_2$	18:3 (n-3c, 6c, 9c)
Stearidonic acid .	all-cis-6,9,12,15-octadecatetraenoic acid	$C_{18}H_{28}O_2$	18:4 (n-3c,6c ,9c, 12c)
Eicosatrienoic acid	all-cis-11,14,17-ecosatrienoic acid	$C_{20}H_{34}O_2$	20:3 (n-3c, 6c, 9c)
Eicosatetraenoic acid .	all-cis-8,11,14,17-eicosatetraenoic acid	$C_{20}H_{32}O_2$	20:4 (n-3c, 6c, 9c, 12c)
Timnodonic acid	all-cis-5,8,11,14,17-eicosapentaenoic acid	$C_{20}H_{30}O_2$	20:5 (n-3c, 6c, 9c, 12c, 15c)
Heneicosapentaenoic acid	all- cis-6,9,12,15,18- -heneicosapentaenoic acid	$C_{21}H_{32}O_2$	21:5 (n-3c, 6c, 9c, 12c, 15c)
Clupanodonic acid	all-cis-7,10,13,16,19 docospentaenoic acid	$C_{22}H_{34}O_2$	22:5 (n-3c, 6c, 9c, 12c, 15c)
Cervonic acid	all-cis- 4,7,10,13,16,19-docosahexaenoic acid	$C_{22}H_{32}O_2$	22:6 (n-3c, 6c, 9c, 12c, 15c, 18c)
Tetracosapentaenoic acid	all-cis-9,12,15,18,21-tetracosapentaenoic acid	$C_{24}H_{38}O_2$	24:5 (n-3c, 6c, 9c, 12c, 15c)

Nisinic acid	all-cis- 6,9,12,15,18, 21-tetracosahexaenoic acid	$C_{24}H_{36}O_2$	24:6 (n-3c, 6c, 9c, 12c, 15c, 18c)
OMEGA-6 POLYUNSATURATED FATTY ACIDS			
Linoleic acid	all-cis-9,12-octadecadienoic acid	$C_{18}H_{32}O_2$	18:2 (n-6c, 9c)
Gamma-linolenic acid	all-cis-6,9,12-octadecatrienoic acid	$C_{18}H_{30}O_2$	18:3 (n-6c, 9c, 12c)
Eicosadienoic acid	all-cis-11,14-eicosadienoic acid	$C_{20}H_{36}O_2$	20:2 (n-6c, 9c)
Dihomo-gamma-linolenic acid	all-cis-8,11,14-eicosatrienoic acid	$C_{20}H_{34}O_2$	20:3 (n-6c, 9c, 12c)
Arachidonic acid	all-cis-5,8,11,14-eicosatetraenoic acid	$C_{20}H_{32}O_2$	20:4 (n-6c, 9c, 12c, 15c)
Docosadienoic acid	all-cis-13,16-docosadienoic acid	$C_{22}H_{40}O_2$	22:2 (n-6c, 9c)
Adrenic acid	all-cis-7,10,13,16-docosatetraenoic acid	$C_{22}H_{36}O_2$	22:4 (n-6c, 9c, 12c, 15c)
Osbond acid	all-cis-4,7,10,13,16-docosapentaenoic acid	$C_{22}H_{34}O_2$	22:5 (n-6c, 9c, 12c, 15c, 18c)
Tetracosatetraenoic acid	all-cis-9,12,15,18-tetracosatetraenoic acid	$C_{24}H_{40}O_2$	24:4 (n-6c, 9c, 12c, 15c)
Tetracosapentaenoic acid	all-cis-6,9,12,15,18-tetracosapentaenoic acid	$C_{24}H_{38}O_2$	24:5 (n-6c, 9c, 12c, 15c, 18c)
OMEGA-9 POLYUNSATURATED FATTY ACIDS			
Oleic acid	cis-9-octadecenoic acid	$C_{18}H_{34}O_2$	18:1 (n-9c)
Gondoic acid	cis-11-eicosenoic acid	$C_{20}H_{38}O_2$	20:1 (n-9c)
Mead acid	all-cis-5,8,11-eicosatrienoic acid	$C_{20}H_{34}O_2$	20:3 (n-9c, 12c, 15c)
Erucic acid	cis-13-docosenoic acid	$C_{22}H_{42}O_2$	22:1 (n-9c)
Nervonic acid	cis-15-tetracosenoic acid	$C_{24}H_{46}O_2$	24:1 (n-9c)
CONJUGATED FATTY ACIDS			
Rumenic acid	9Z,11E-conjugated Linoleic Acid	$C_{18}H_{32}O_2$	18:2 (n9t, 11c)

α-Calendic acid	8E,10E,12Z-octadecatrienoic acid	$C_{18}H_{30}O_2$	18:3 (n-6c, 8t, 10c)
β-Calendic acid	8E,10E,12E-octadecatrienoic acid	$C_{18}H_{30}O_2$	18:3 (n-6t, 8t, 10t)
Jacaric acid	8Z,10E,12Z-octadecatrienoic acid	$C_{18}H_{30}O_2$	18:3 (n-6c, 8t, 10c)
α-Eleostearic acid	9Z,11E,13E-octadeca-9,11,13-trienoic acid	$C_{18}H_{30}O_2$	18:3 (n-9c, 11t, 3t))
β-Eleostearic acid	9E,11E,13E-octadeca-9,11,13-trienoic acid	$C_{18}H_{30}O_2$	18:3 (n-5t, 7t,9t)
Catalpic acid	9Z,11Z,13E-octadeca-9,11,13-trienoic acid	$C_{18}H_{30}O_2$	18:3 (n-5t,7c,9t)
Punicic acid	9Z,11E,13Z-octadeca-9,11,13-trienoic acid	$C_{18}H_{30}O_2$	18:3 (n-5c,7t,9c)

E = trans

Z =cis

Appendix 2: Food frequency questionnaire

GENERAL INSTRUCTIONS

- **Answer each question as best you can. Estimate if you are not sure. A guess is better than leaving a blank.**
- **Put an X in the box next to your answer.**
- **This questionnaire is based on you intake over 3 DAYS, please fill this in every day and bring it with you to clinic visit**

1. How often did you drink **milk as a beverage** (NOT in coffee, NOT in cereal)?

Day 1	Day 2	Day 3
☐ none	☐ none	☐ none
☐ once a day	☐ once a day	☐ once a day
☐ twice a day	☐ twice a day	☐ twice a day
☐ > twice a day	☐ > twice day	☐ > twice a day

2. Each time you drank **milk as a beverage**, how much did you usually drink?

Day 1	Day 2	Day 3
☐ Less than 1 cup (250ml)	☐ Less than 1 cup (250ml)	☐ Less than 1 cup (250ml)
☐ 1 to 1½ cups (250ml-375ml)	☐ 1 to 1½ cups (250ml-375ml)	☐ 1 to 1½ cups (250ml-375ml)
☐ More than 1½ cups (375ml)	☐ More than 1½ cups (375ml)	☐ More than 1½ cups (375ml)

3. What kind of **milk** did you usually drink?

Day 1	Day 2	Day 3
☐ Full cream milk (blue top)	☐ Full cream milk (blue top)	☐ Full cream milk (blue top)
☐ 2% fat milk (light blue top)	☐ 2% fat milk (light blue top)	☐ 2% fat milk (light blue top)
☐ Skim (green top)	☐ Skim (green top)	☐ Skim (green top)
☐ Soy milk	☐ Soy milk	☐ Soy milk
☐ Rice milk	☐ Rice milk	☐ Rice milk
☐ other	☐ other	☐ other

4. How often have you had milk over your cereal?

Day 1	Day 2	Day 3
☐ none	☐ none	☐ none
☐ once a day	☐ once a day	☐ once a day
☐ twice a day	☐ twice a day	☐ twice a day
☐ > twice a day	☐ > twice a day	☐ > twice a day

5. What kind of **milk** did you usually use?

Day 1	Day 2	Day 3
☐ Full cream milk (blue top)	☐ Full cream milk (blue top)	☐ Full cream milk (blue top)
☐ 2% fat milk (light blue top)	☐ 2% fat milk (light blue top)	☐ 2% fat milk (light blue top)
☐ Skim (green top)	☐ Skim (green top)	☐ Skim (green top)
☐ Soy milk	☐ Soy milk	☐ Soy milk
☐ Rice milk	☐ Rice milk	☐ Rice milk
☐ other	☐ other	☐ other

6. How often have you had milk in your coffee /tea?

Day 1	Day 2	Day 3
☐ none	☐ none	☐ none
☐ once a day	☐ once a day	☐ once a day
☐ twice a day	☐ twice a day	☐ twice a day
☐ 3-5 times a day	☐ 3-5 times a day	☐ 3-5 times a day
☐ >5 times a day	☐ >5 times a day	☐ >5 times a day

7. Each time milk was added to your coffee or tea, how much was usually added?

Day 1	Day 2	Day 3
☐ Less than 1 tablespoon	☐ Less than 1 tablespoon	☐ Less than 1 tablespoon
☐ 1-3 tablespoons	☐ 1-3 tablespoons	☐ 1-3 tablespoons
☐ >3 tablespoons	☐ >3 tablespoons	☐ >3 tablespoons

8. What kind of **milk** did you usually use?

Day 1	Day 2	Day 3
☐ Full cream milk (blue top)	☐ Full cream milk (blue top)	☐ Full cream milk (blue top)
☐ 2% fat milk (light blue top)	☐ 2% fat milk (light blue top)	☐ 2% fat milk (light blue top)
☐ Skim (green top)	☐ Skim (green top)	☐ Skim (green top)
☐ Soy milk	☐ Soy milk	☐ Soy milk
☐ Rice milk	☐ Rice milk	☐ Rice milk
☐ other	☐ other	☐ other

9. How often have you had dairy based meal replacement or high-protein beverages (such as Up 'n Go, Energize)?

Day 1	Day 2	Day 3
☐ none	☐ none	☐ none
☐ once a day	☐ once a day	☐ once a day
☐ twice a day	☐ twice a day	☐ twice a day
☐ 3-5 times a day	☐ 3-5 times a day	☐ 3-5 times a day

10. How often have you had cheese?

Day 1	Day 2	Day 3
☐ none	☐ none	☐ none
☐ once a day	☐ once a day	☐ once a day
☐ twice a day	☐ twice a day	☐ twice a day
☐ 3-5 times a day	☐ 3-5 times a day	☐ 3-5 times a day

11. Each time you had cheese, how much did you usually have?

Day 1	**Day 2**	**Day 3**
□ 1 tablespoon/ 1 thin slice (15g)	□ 1 tablespoon/ 1 thin slice (15g)	□ 1 tablespoon/ 1 thin slice (15g)
□ 2-3 tablespoons (30-45g)	□ 2-3 tablespoons (30-45g)	□ 2-3 tablespoons (30-45g)
□ >3 tablespoons	□ >3 tablespoons	□ >3 tablespoons

12. How often have you had ice cream?

Day 1	**Day 2**	**Day 3**
□ none	□ none	□ none
□ once a day	□ once a day	□ once a day
□ twice a day	□ twice a day	□ twice a day
□ 3-5 times a day	□ 3-5 times a day	□ 3-5 times a day

13. Each time you had ice cream, how much did you usually have?

Day 1	**Day 2**	**Day 3**
□ 1 scoop/ 1 small ice cream stick	□ 1 scoop/ 1 small ice cream stick	□ 1 scoop/ 1 small ice cream stick
□ 2-4 scoops / 1-2 large ice cream sticks	□ 2-4 scoops / 1-2 large ice cream sticks	□ 2-4 scoops / 1-2 large ice cream sticks
□ > 4 scoops	□ > 4 scoops	□ > 4 scoops

14. How often have you had butter?

Day 1	**Day 2**	**Day 3**
□ none	□ none	□ none
□ once a day	□ once a day	□ once a day
□ twice a day	□ twice a day	□ twice a day
□ 3-5 times a day	□ 3-5 times a day	□ 3-5 times a day

15. Each time you had butter how much did you usually have?

Day 1	**Day 2**	**Day 3**
□ Less than 1 teaspoon	□ Less than 1 teaspoon	□ Less than 1 teaspoon
□ 1-3 teaspoons	□ 1-3 teaspoons	□ 1-3 teaspoons

□ >3 tablespoons	□ >3 tablespoons	□ >3 tablespoons

16. How often have you had yogurt?

Day 1	**Day 2**	**Day 3**
□ none	□ none	□ none
□ once a day	□ once a day	□ once a day
□ twice a day	□ twice a day	□ twice a day
□ 3-5 times a day	□ 3-5 times a day	□ 3-5 times a day

17. Each time you had yogurt, how much did you usually have?

Day 1	**Day 2**	**Day 3**
□ less than 2 tablespoons	□ less than 2 tablespoons	□ less than 2 tablespoons
□ 2- 4 tablespoons	□ 2- 4 tablespoons	□ 2- 4 tablespoons
□ 1 tub	□ 1 tub	□ 1 tub
□ >2 tubs	□ >2 tubs	□ >2 tubs

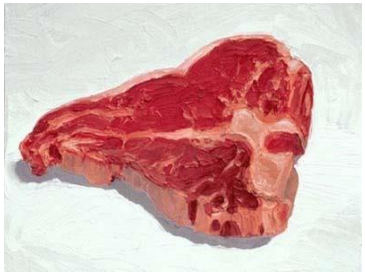
18. How often have you had cream?

Day 1	**Day 2**	**Day 3**
□ none	□ none	□ none
□ once a day	□ once a day	□ once a day
□ twice a day	□ twice a day	□ twice a day
□ 3-5 times a day	□ 3-5 times a day	□ 3-5 times a day

19. Each time you had cream, how much did you usually have?

Day 1	**Day 2**	**Day 3**
□ less than 2 tablespoons	□ less than 2 tablespoons	□ less than 2 tablespoons
□ 2- 4 tablespoons	□ 2- 4 tablespoons	□ 2- 4 tablespoons
□ 1 tub	□ 1 tub	□ 1 tub
□ >2 tubs	□ >2 tubs	□ >2 tubs

20. How often have you had red meat?



Day 1	**Day 2**	**Day 3**
□ none	□ none	□ none
□ once a day	□ once a day	□ once a day
□ twice a day	□ twice a day	□ twice a day
□ 3-5 times a day	□ 3-5 times a day	□ 3-5 times a day

21. Each time you had red meat, how much did you usually have?

Day 1	**Day 2**	**Day 3**
□ Less than 100g	□ Less than 100g	□ Less than 100g
□ 100-200g	□ 100-200g	□ 100-200g
□ >250g	□ >250g	□ >250g

Thank you *very much* for completing this questionnaire! Because we want to be able to use all the information you have provided, we would greatly appreciate it if you would please take a moment to review each page making sure that you:

- **Did not skip any pages and**
- **Crossed out the incorrect answer and circled the correct answer if you made any changes.**

Appendix 3: Study protocol for the randomised study

Trans fatty acid (TFA) is the common name for unsaturated fats that have a double bond in the trans configuration. Humans cannot create TFA so all found in human tissue comes from the diet. (332) Unlike other dietary fats, TFA are not essential for human health, conversely, evidence suggests that they are harmful to human health. Studies have shown a graded association between increased dietary intake of TFA, predominantly hydrogenated vegetable oil and the risk of cardiovascular disease. In a meta-analysis of three prospective cohort studies which included ~140 000 subjects, an increase in 2% of energy from TFAs (or a teaspoonful a day) estimated from detailed food-frequency questionnaires was associated with a ~25% increase in the risk of cardiovascular disease. (21)

Two sources of TFA are found in the human food chain. (333) Industrial TFA which is a by-product during the process of partial hydrogenation of vegetable oil, and 'natural' TFA found in by-products from ruminant animals.

Ruminant TFA

Ruminant TFA is created by bacteria in the ruminant gut. The amount and type of TFA in meat and milk is dependent on the feed of the animal. (16) Altering the feed changes the composition and level of fatty acids in milk e.g. adding sunflower oil to the feed increases the level of vaccenic acid and reduces saturated fat. (79) New Zealand cows are predominantly pasture fed and consequently have a higher level of TFA in their by-products than cows from Europe and the USA. Dietary surveys in New Zealand show that the majority of ruminant TFA are derived from dairy products rather than meat. (138) There is evidence that feed in New Zealand is supplemented with palm kernel, the waste product of the palm oil industry. This may result in increased levels of palmitoleic acid (16:1 (n-7c)), and possibly 16:1(n-7t) (palmitelaidic acid) in the by-products. A cross sectional study in 2004-2005 at Auckland City Hospital suggests that significant palmitelaidic acid is derived from ruminant sources (0.14 % of total fatty acids). (10)

Unlike the harmful industrial TFA, (10, 19, 23, 33, 334) ruminant TFA appear to have either little or no adverse effect on human health. (134, 334) This conclusion is based on studies in countries with relatively high industrial TFA intake and a very low ruminant TFA intake. (20, 23, 39, 219) A recent study at Auckland City Hospital suggests that dairy TFA, particularly palmitelaidic acid, is associated with increased risk of poly-vascular disease, and acute coronary syndrome.

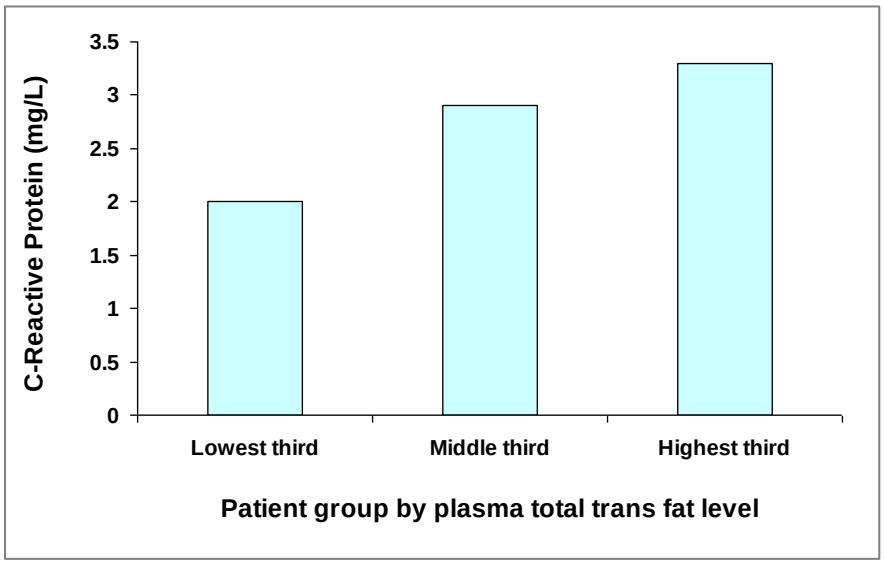
Biological effects of TFA

Studies have shown that TFA, *particularly industrial TFA*, have adverse effects on lipids, inflammation, (26, 33, 122) cell wall fluidity and endothelial function. (28, 30, 32, 33)

Metabolic and meta-analysis show a clear and consistent association between increased TFA intake and reduced HDL-c, increased LDL-s and triglyceride levels. (33) These studies have also shown that TFA increase lipoprotein (a) and reduce the particle size of LDL-c, both of which contribute to an increased risk of CVD. Changes in lipids caused by TFA do not completely account for the increased risk of CVD seen in epidemiological studies. Residual risk is due to effects on inflammation and endothelial function.

Effects on inflammation derive from epidemiological and experimental studies. A study of over 700 nurses showed that those in the highest quartile of trans fat consumption had blood levels of CRP that were 73% higher than those in the lowest quartile.(29) Other studies have also linked significant intake of TFA with increased circulating concentrations of inflammatory molecules such as interleukin-6, tumor necrosis factor-alpha, C-reactive protein, and monocyte chemo-attractant protein-1. A recent observational study in Auckland (currently under review for a peer review journal), showed increased plasma TFA levels, was associated with increased CRP levels (Figure1)

Figure 1: Total plasma TFA and CRP levels



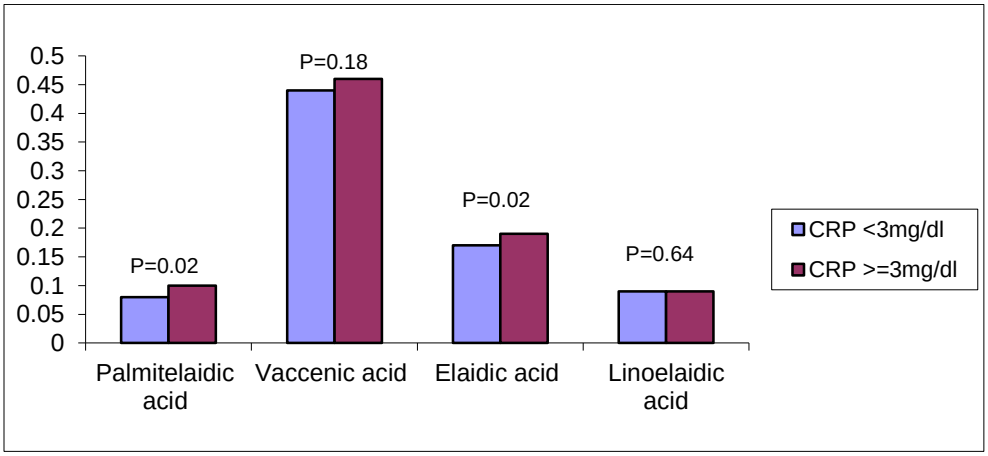
Several studies suggest that TFA cause endothelial dysfunction. After adjustment for other risk factors, greater intake of TFA was associated with increased levels of several markers of endothelial dysfunction, including soluble intercellular adhesion molecule 1, soluble vascular-cell adhesion molecule 1, and E-selectin. In another trial, consumption TFA impaired endothelial function, as reflected by a reduction in brachial artery flow-mediated vasodilatation by 29 percent, as compared with intake of saturated fat. (28)

Biological effect of ruminant TFA

There is paucity of data on the effect of ruminant fats on surrogate markers of cardiovascular disease. One Danish study (79) has investigated the effects of increasing vaccenic acid in milk on the levels of lipids, inflammatory markers and oxidative stress. Increasing vaccenic acid was achieved by adding sunflower oil to the animal feed. Participants were then given 115g/d from the test butter that was high in vaccenic acid or the control that was low in vaccenic acid. This study showed that the lipid profile was improved by the test butter, but this was felt to be due to the higher lever of monounsaturated fat and reduced saturated fat in that butter rather than the vaccenic acid. There was no effect on markers of endothelial dysfunction and oxidative stress.

A recent observational study in Auckland showed that there was a low intake of total TFA with most (75%) derived from ruminant sources. Correlation coefficients suggest that palmitelaidic acid was associated with vaccenic acid($r=0.83$, $p<0.0001$). The implication is that the ruminant TFA isomers found in this population were vaccenic acid and palmitelaidic acid. There was a dose response association with CRP in patients with heart disease (Figure 2).

Figure 2: Plasma trans fatty acid isomers with plasma C-reactive protein levels



Cardiovascular disease and trans fat intake

Studies have shown a graded association between increased dietary intake of TFA, predominantly industrial TFA, and the risk of cardiovascular disease. In a meta-analysis of three prospective cohort studies which included ~140 000 subjects, an increase in 2% of energy from TFAs (or a teaspoonful a day) estimated from detailed food-frequency questionnaires was associated with a ~25% increase in the risk of cardiovascular disease. (21)

The major evidence for the effect of TFA on CHD comes from the Nurses' Health Study (NHS); a cohort study that has been following 120,000 female nurses since its inception in 1976. In this study, data from 900 coronary events from the NHS population during 14 years of follow up was analyzed. CHD risk almost doubled for each 2% increase in TFA calories consumed.

Studies testing TFA tissue levels in serum, adipose tissue and erythrocytes have shown a strong positive correlation between risk for cardiovascular disease and tissue concentration of TFA. (17, 20,

39, 134) Two studies have linked the risk of sudden death from cardiac causes and levels of TFA, specifically the industrial TFA, Linoelaidic acid.

Studies are not consistent about whether specific TFA isomers have different effects on CVD risk. Elaidic acid has been associated with increased risk of sudden death, fatal ischemic heart disease and acute coronary syndrome.

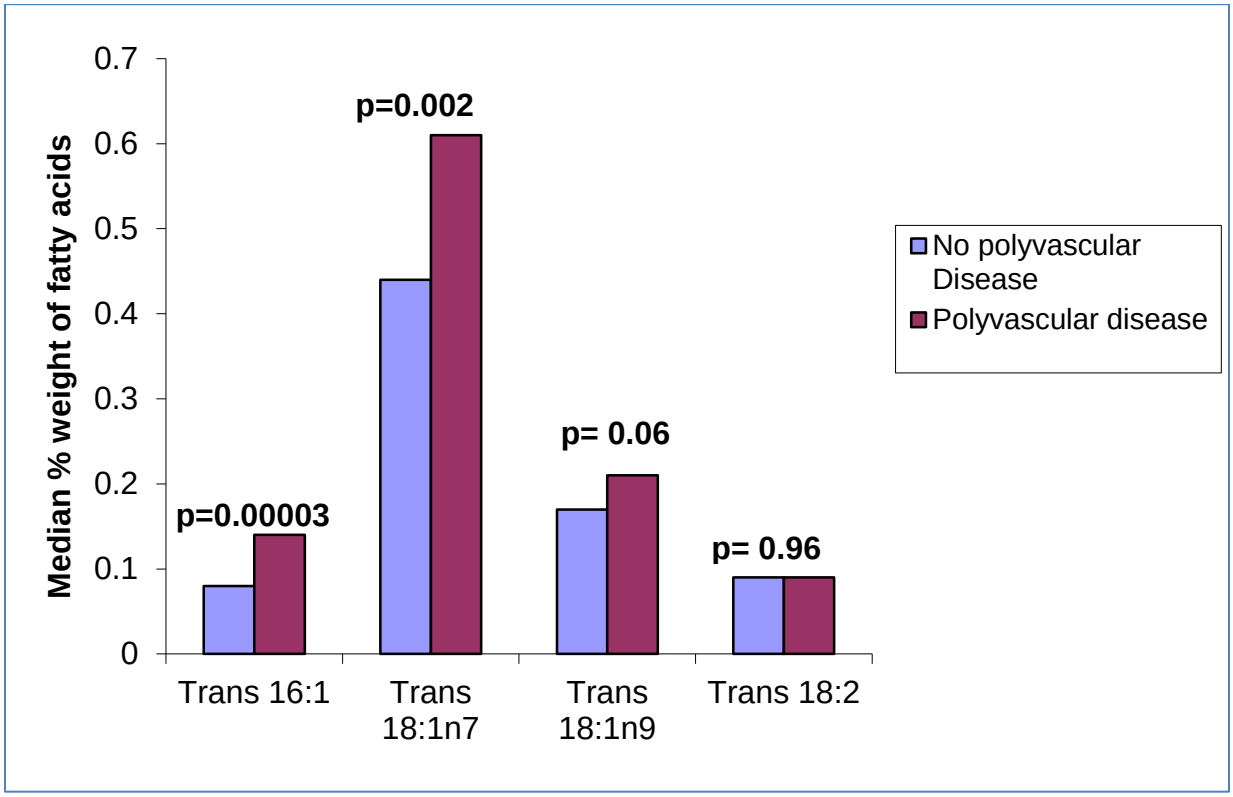
The data on effects of ruminant TFA is less convincing (18) with studies showing either neutral or adverse effects. he lack of biological effect of ruminant fats in these studies may in part be due to low levels of intake (typically less than 0.5 percent of total energy intake) in most Western countries.

Ruminant TFA and Cardiovascular disease

Current literature suggests that there is little or no adverse effect of ruminant TFA on cardiovascular disease. Two possible reasons are thought to account for this, firstly these 'natural' TFAs may not be harmful, and secondly, humans do not ingest these at a dose significant enough to cause a noticeable clinical effect.

A recent observational study done at Auckland City Hospital in patients with significant CVD showed a highly significant increased prevalence of polyvascular disease and acute coronary syndrome related to total TFA intake. There was also a significant association seen with ruminant TFA and polyvascular disease and increased CRP (Figure 3).

Figure 3: TFA isomers and polyvascular disease



A possible confounder of this study was that the TFA was derived from sources high in saturated fats. However, in this study, no relationship was found between CRP, polyvascular disease and acute coronary syndromes and fatty acids such as saturated and unsaturated fatty acids.

Dairy intake and insulin resistance

A growing body of evidence suggests an inverse relationship between dairy food intake and the development of the insulin resistance and type 2 diabetes mellitus (Type 2DM). (190, 288) In observational studies there is a consistent inverse association between dairy intake and the prevalence of insulin resistance, the metabolic syndrome and Type 2DM. In a systematic review of the observational evidence, the odds for developing insulin resistance was 0.71 (95% CI, 0.57-0.89) for the highest dairy intake (3-4 servings/d) vs. the lowest intake (0.9-1.7 servings/d). The Coronary Artery Risk Developments in Young Adults (CARDIA) study, (196) dietary intake of dairy and its relationship to various cardiac risk factors was assessed in 3157 people. The study found an inverse relationship

between dietary intake of dairy and insulin resistance (measured by HOMA model) in the overweight cohort.

Few interventional studies have evaluated the effects of dairy food intake on the management or prevention of insulin resistance, the metabolic syndrome or type 2DM. Some studies such as the Dietary Approaches to Stop Hypertension (DASH diet), (240) focused on general eating plans. The DASH diet examined the independent effects of dairy intake on specific metabolic components of insulin resistance including blood pressure and obesigenic parameters. Some favourable effects on BP and insulin resistance were shown. The mechanism for the inverse relationship has been postulated to be related to calcium, vitamin D, and the low glycaemic index of dairy and its ability to suppress appetite.

Ruminant TFA and insulin resistance

The Coronary Heart study (99) in 3736 adults in the US published this year has shown that circulating palmitelaidic acid in the US is primarily derived from dairy. This observational cross sectional study assessed TFA, meal diaries and insulin resistance. The cohort was assessed in 1992 and followed for 10 years with yearly clinic visits. One blood sample stored in 1992 was used to asses TFA levels, glucose and insulin. Circulating palmitelaidic acid was associated with lower insulin resistance ($p<0.001$), presence of atherogenic dyslipidaemia, and incident diabetes. The study has limitations. Conclusions were based on only one sample and the levels of palmitelaidic acid were very low (<0.8%). This raises the question as to whether this was the active compound or a marker for some other, unknown protective constituent of dairy or other ruminant foods.

Measure of insulin Resistance

The Homeostasis Model Assessment (HOMA) estimates steady state beta cell function (%B) and insulin sensitivity (%S), as percentages of a normal reference population. It has been widely validated and applied for quantifying insulin resistance and β-cell function. (256) The measures correspond well, but are not necessarily equivalent, to non-steady state estimates of beta cell function and insulin

sensitivity derived from stimulatory models such as the hyperinsulinaemic clamp, the hyperglycaemic clamp, the intravenous glucose tolerance test (acute insulin response, minimal model), and the oral glucose tolerance test (0-30 delta I/G).The equations have been used widely, particularly for estimates of beta cell function and insulin resistance in large-scale studies, but are not appropriate for use with currently available insulin assays.

The updated HOMA model (HOMA2) which takes account of variations in hepatic and peripheral glucose resistance, increases in the insulin secretion curve for plasma glucose concentrations above 10 mmol/L (180 mg/dL) and the contribution of circulating proinsulin. (256, 335, 336) The model has also been recalibrated to give %B and %S values of 100% in normal young adults when using currently available assays for insulin, specific insulin or C-peptide.

Summary

It is not clear whether ruminant TFA have significant effects on cardiovascular disease, diabetes and insulin resistance. This may be partly due to the fact that most of the data comes from countries (predominantly USA) with low intake of ruminant TFA and corn is the predominant supplement. The amount and type of ruminant TFA in dairy is dependent of feed which varies from country to country. New Zealand cows produce milk with higher TFA levels, and have increasingly been fed palm kernel extract which may affect TFA isomers. This study is designed to examine the types of ruminant TFA which is in the human food chain and the potential effects of these TFA on insulin resistance and markers of cardiovascular disease. The New Zealand population is ideal to assess this due to the relatively high dairy intake.

Aims of Research

This proposal is to evaluate associations between dairy food, TFA levels and cardiometabolic risk factors in a dietary intervention study.

Primary Objective

To determine whether dietary advice to increase or decrease dairy intake significantly changes plasma levels of vaccenic acid and palmitelaidic acid in participants. (Correlation with pentadecanoic and heptadecanoic levels will be done to ensure these TFA's are derived from dairy)

Secondary Objective

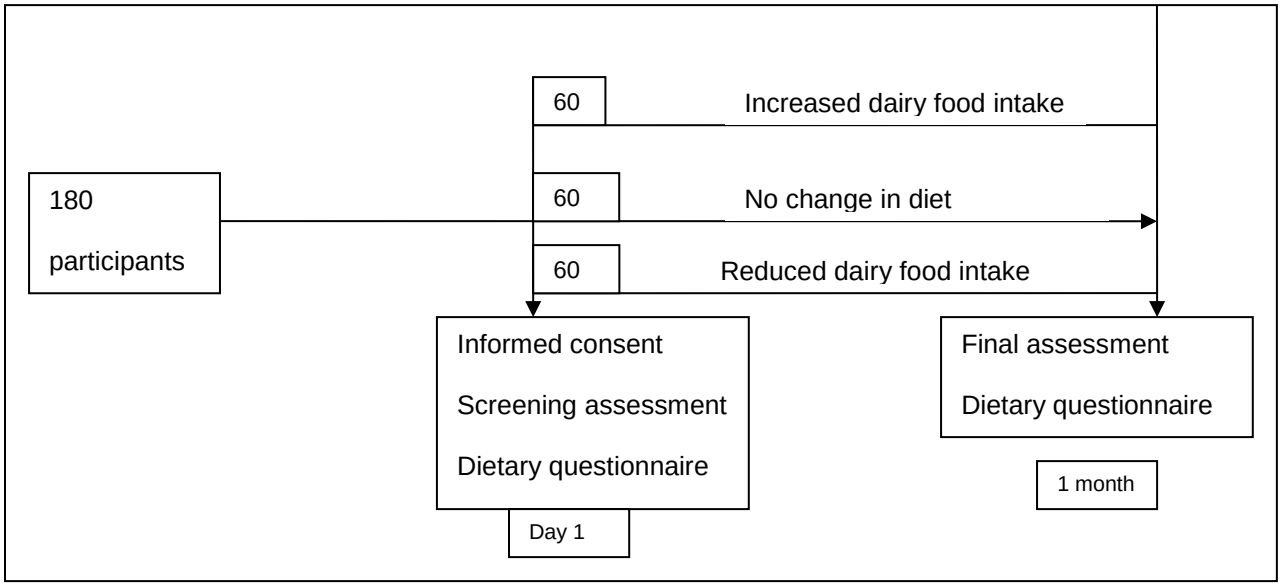
To determine whether dietary changes are associated with changes in plasma levels of C-reactive protein (CRP), lipid profile and blood pressure and insulin resistance.

Hypothesis

Changing dairy food intake will significantly change plasma levels of palmitelaidic and vaccenic acids. Changing dairy food intake will be associated with reduced blood pressure, weight and insulin resistance.

Design

This is a one month randomised clinical trial comparing a control group vs. reduced vs. an increase in dairy food intake for 4 weeks. 180 participants will be randomised to either increasing dairy intake, reducing dairy for one month or to maintain their normal diet.



The study is designed to evaluate effects of changing dairy intake on specific isomers of TFA. This 3 arm design has been chosen for a number of reasons:

1. A more robust assessment will be made if we show that both increasing and reducing TFA levels occur with dietary change.

2. A control group will ensure changes in TFA levels are not due to variability such as seasonal feeding habits affecting TFA in the dairy products.

Study population

180 normal volunteers who have at least a moderate intake of dairy products and willing to change their diet for the duration of the study. Those on a statin are excluded as this may affect lipid levels, those with known factors to affect CRP (inflammatory disease and CVD) are also excluded.

Inclusion criteria

1. Aged over 18 years of age
2. Able to give informed consent

Exclusion criteria

1. Unwilling to trial dietary intervention
2. lactose intolerant/milk allergy
3. pregnant/lactating
4. Known vitamin D deficiency or hypocalcaemia
5. Osteoporosis.
6. Inflammatory conditions e.g. rheumatoid arthritis
7. On a statin
8. Known cardiovascular disease (stroke, ischemic heart disease, peripheral vascular disease)

Study procedures

An advert is to be placed in the local paper/NOVA asking for adult individuals willing to be involved in a one month study looking at dietary interventions.

Day 1

1. Informed consent taken
2. Dietary questionnaire
3. BP, height, weight, hip and waist circumference
4. fasting bloods (lipids, CRP, plasma phospholipids, glucose and insulin)
    1. Randomised to:
        a. Elimination of dairy for 1 month

b. Increased dairy for 1 month

c. Continue normal diet for 1 month

Month 1

1. Dietary questionnaire
2. BP, height, weight, hip and waist circumference
3. fasting bloods (lipids, CRP, plasma phospholipids, glucose and insulin)

Intervention

Participants will be given dietary advice to either no change, increase or reduce dairy for 1 month.

1. Participants randomised to reduced dairy will be given dietary counselling to eliminate all sources of dairy from their diet including cheese, milk, yoghurt, ice cream and butter. The baseline food diary will be assessed to ensure that all sources of dairy are identified and eliminated. Information regarding alternate sources of calcium and protein will be given to help them achieve this.
2. Participants randomised to increased dairy will be asked to add ≥ 3 extra serving of dairy to their diet every day. The food diary will be used as a tool to ensure compliance to diet is maximised.
3. Participants randomised to the control group will be asked to maintain normal dietary habits.

Serving size
1 cup milk, 1/3 cup cottage cheese, 1 small pottle yoghurt, 2 Tbsp grated parmesan , 2 thin slices cheese, 1 scoop ice cream

Dietary questionnaire

A dietary questionnaire is to be administered at baseline and month 1. The questionnaire will consist of a tick box list of foods that contain dairy products. Participants will be asked how many servings of each product they have had per day over the last 48hours.

Blood tests

A+ laboratory is testing lipids; glucose, CRP, insulin .Serum and plasma are stored at -70 degrees.

The University of Auckland will be doing FC-MS on some of the stored plasma. This will allow us to assess all plasma fatty acids. This will enable us to assess the correlation of TFA with pentadecanoic and heptadecanoic acids, considered markers of dairy intake. (213) The rest of the plasma will be stored for further tests e.g. oxidised LDL-c.

Statistical Procedures

Sample Size

Based on the observational study at Auckland city hospital, a total of **180** patients will enter this intervention study. It is expected that a 30% difference in ruminant TFA levels will be seen with the intervention .The probability is 80% that the study will detect a treatment difference at a two sided 5% significance level, if the true difference between the treatments is 0.15 units.

Statistical analysis

The distribution of TFA is expected to not be normal.

1. The Kruskal Wallis test will be used to compare TFA levels between the 3 arms of the study.
2. The Wilcoxon signed rank test will be used to compare changes in TFA levels within the patient
3. The relationship with changes in TFA levels will be evaluated with CRP (log transformed) and lipid levels and association .Paired t-test will be used to compare the change of CRP levels, LDL-C, triglycerides, HDL-C within patients if the data is normally distributed, otherwise Wilcoxon Rank Sum test will be used where appropriate.
4. The relationship with changes in TFA levels will be evaluated with (insulin resistance) IR .Paired t-test will be used to compare the change IR within patients if the data is normally distributed, otherwise Wilcoxon Rank Sum test will be used where appropriate.

Randomisation

Participants will be allocated a number on enrolment. They will then been randomly assigned to either arm of the study. Treatment allocation will be non-blinded for the study coordinator and participant. However, the analysis of bloods and analysis of results will be blinded. Unblinding of treatment arm will occur after analysis is complete.

Appendix 4: Study protocol for meta-analysis

Background

Dairy food especially low fat dairy food is considered to improve cardio metabolic risk factors such as weight, blood pressure ad insulin resistance. This is based on observational studies that show increased dairy food consumption is associated with less weight gain, (198) reduced risk of diabetes (187, 288) and lower blood pressure. (188) Dairy food is the main source of saturated fats that are thought to increase cardiovascular risk and adversely affect lipids. (150) Subsequently most food guidelines recommend daily consumption of low fat dairy food so that consumers receive the 'health benefits' of dairy calcium and protein but avoid adverse health effects of saturated fats. These guidelines are however not based on evidence.

Dairy is a complex food which contains short, medium and long chain saturated fats which may have differing health effects. (4) Observational studies assessing dairy food intake are confounded by multiple factors for example dairy food intake is positively associated with healthier lifestyle behaviours and higher socio economic status. (234) Large randomised studies (240, 337) assessing the effects of dietary patterns on cardiovascular health have not focused on the effects of dairy food. The effects of dairy food on cardio metabolic risk factors have been assessed in multiple small studies. A recent meta-analysis (326) has assessed the effects of all dairy food on weight but did not compare effects of whole and low fat dairy food. No meta-analysis has assessed effects on other cardio metabolic risk factors.

Aim: To evaluate the effects of increased dairy food in a healthy population on cardio metabolic risk factors.

Objective: To see effects of increasing dairy on blood pressure, HOMA, weight, waist circumference, lipids, C- reactive protein

Methods: A meta- analysis using Rev Man 5 software assessing the effects of dietary interventions to increase dairy intake for at least on month will be performed. No date restriction will be applied. All randomised control studies with dairy as intervention that reported any of the outcome measures in healthy populations is eligible. All databases (science direct, Google, Cochrane database, Medline) will be searched.

Inclusion criteria

1. Randomised controlled study
2. Participants > 18 years of age
3. Intervention longer than 1 month
4. Control arm is usual diet or reduced dairy food
5. Increased whole or low fat dairy food

Exclusion Criteria

1. Observational or not randomised
2. Participants have diabetes, hypertension, heart disease
3. Participants on medications for lipids or blood pressure
4. Caloric restriction or other dietary intervention
5. Control arm is another intervention

Data extraction:

2 people will assess all articles for inclusion and exclusion criteria. Baseline characteristics of the participants, the intervention, effects on any cardio metabolic risk factor and funding source will be obtained. Each study will be scored using Jadad score. If any data is missing, corresponding authors will be contacted. All data will be converted to SI units and mean change and standard deviations will be used for analysis. Three people will then review every included study to ensure they meet inclusion and exclusion criteria. Every study will be assessed for study design and risk of bias.

Statistics

Cases will be compared only with controls within the same study. For those studies with 3 treatment groups, comparison will be made with control and the high dairy group. Differences by dietary intervention are calculated as weighted mean difference divided by variance, expressed as a sample weighted mean effect size (95% confidence interval). A test for homogeneity will be performed for each cardio metabolic risk evaluated. Stratified analysis will be performed if there is enough data.

Ethics approval:

Ethics approval for the study will be sought prior to any study procedure.

Appendix 5: Methodology for analysis of levels of fatty acids in milk and plasma

All samples of milk and plasma in the randomised study, and milk study in 2011 were analysed at the School of Biological Sciences, University of Auckland. A different column was used in 2013 that was better able to discriminate between cis and trans fatty acids. Unfortunately samples were not retrievable from 2011 to repeat the tests using the new column so direct comparison of unsaturated fatty acids levels between samples in 2011 and 2013 cannot be made The following methodology was used in 2011.

Internal standard addition and drying

Samples received by the laboratory were thawed and spun and aliquots of 300 µl (plasma) or 200 µl (milk) were collected. Internal standard (20 µl of 10 mM alanine-d4) was added to each aliquot and the samples were spun. Negative controls containing only alanine-d4 were also prepared. Samples were frozen at -80 °C and dried in a centrifugal concentrator with a -104 °C condenser at 0.8 kPa for 4 hours. Dried samples were stored at -80 °C.

Methods for analysis of milk and plasma samples for the randomised study in 2011

Extraction of metabolites and drying

Samples were kept on ice or dry ice throughout the extraction process.

Plasma samples: Extraction was carried out through the addition of 500 µL of cold methanol (1:1) to the sample, followed by spinning until the dried plasma was re-suspended. The samples were placed in a refrigerated centrifuge at -9° C and were centrifuged at 3500 rpm for 5 min, and then the supernatant was collected. The remaining pellets had 500 µL of cold methanol: water (4:1) added and were spun to re-suspend the pellet. The samples were centrifuged under the same conditions as previously and the supernatant was combined with the first extract. The final extraction used the addition of 500 µl of 100 % methanol to re-suspend the pellet obtained from the previous step, followed by centrifugation at -9C

and at 3500 rpm for 5 min, after which the supernatant was added to the extracts from the previous steps. After extraction, the pellet was discarded, and the extract was dried in a centrifugal concentrator with a -104 °C condenser at 0.8 kPa for 4 hours then stored at -80°C.

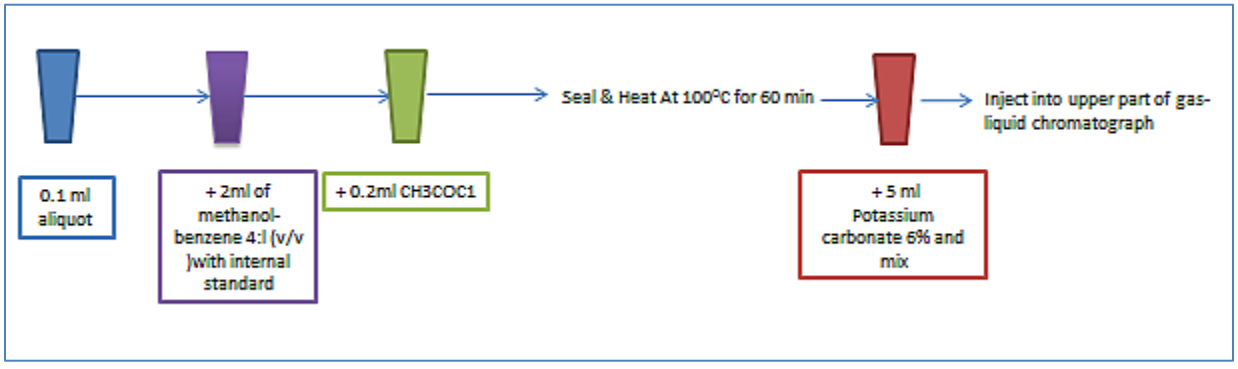
Milk samples: Samples were extracted using the same protocols as the plasma samples (above), except that metabolites were first extracted with 80 % methanol followed by 50 % methanol.

Derivation with methylchloroformate (MCF)

Controls were also prepared for the derivation process. They comprised a derivation negative control (empty tube) and a positive control containing 14 reference standard amino acids with an injected concentration of 0.5 mM. (338)

The samples, mixes and controls were re-suspended in a methanol-benzene mixture at a ratio of 4: l. Tubes were tightly closed with Teflon-lined caps and subjected to methanolysis at 100°C for 1 hour. After the tubes were cooled in water, 5 ml of 6% K_2C0_3 (potassium carbonate) solution was slowly added to stop the reaction and neutralize the mixture. The tubes were then shaken and centrifuged at 2500 rpm for 5 min at 6° C. An aliquot of the sample was injected into the chromatograph. (339)

Figure 1: Schematic diagram of the procedure for sample preparation for Gas Chromatography-Mass Spectrometry.



Gas Chromatography-Mass Spectrometry parameters

GC-MS was used for identification and semi-quantitation of polar and non-polar fatty acids. GC-MS instrument parameters were based on Smart et al. (338) The instrument used was an Agilent 7890A gas chromatograph coupled to an 5975C inert mass spectrometer with a split/splitless inlet. One microliter of sample was injected using a CTC PAL auto sampler into a glass split/splitless 4mm ID straight inlet liner packed with deactivated glass wool. The inlet was set to 290°C, splitless mode with a column flow of 1.0 mL/min. Purge flow was set to 25 mL/min 1 min after injection.

The column was a fused silica ZB-1701 (stationery phase: 86% dimethylpolysiloxane, 14% cyanopropylphenyl, Phenomenex) 30m long, 0.25mm i.d., 0.15 µm film. Carrier gas was instrument grade helium (99.99%, BOC). The GC oven temperature programming started isothermally at 45°C for 2 min, increased 9°C/min to 180°C, held 5 min; increased 40°C/min to 220°C, held 5 min; increased 40°C/min to 240°C, held 11.5 min; increased 40°C/min to 280°C, and held 10 min. The transfer line to the MS was maintained at 250°C, the source at 230°C and the quadropole at 150°C. The detector was turned on 5.5 min into the run. The detector was run in positive-ion, electron-impact ionisation mode, at 70 eV electron energy, with the electron multiplier set with no additional voltage relative to the autotune value. Chloroform blanks were run for every 10-12 samples to monitor instrument carryover. Mass spectra were acquired in scan mode from 38 to 550 atomic mass units, with detection threshold of 100 ion counts. "

Data analysis

Data analysis was automated, (338) and then manually verified. The raw data output from the GCMS was converted to AIA format (.cdf) and analysed using Automated Mass Spectral Deconvolution and Identification System (AMDIS-NIST) against an in-house library of 165 methyl chloroformate derivedd compounds (excluding arginine). The reference ion used as a measure for the quantity of for each compound is the most intense fragment in the mass spectrum for that compound. As the output from AMDIS returns zero values that are not suitable for statistical analysis, an in-house R-script was used in conjunction with the AMDIS output to produce data that includes trace levels of metabolites normally

excluded by AMDIS. The values are generated from the maximum height of the reference ion for the compound peak. Unlike peak area, peak height can be affected by chromatographic disturbances such as column contamination. Data was checked against negative controls and any data that was obviously caused contamination or artefacts has been highlighted in the uncorrected results and removed in the corrected results. Values for negative controls have not been subtracted from data. Co-eluting peaks have been highlighted, and corrected whenever they were able to be separated using single-ion extraction. Where two identifications were equally likely for one peak, both identifications have been reported. For some unknown peaks, mass spectra were checked against the National Institute of Standards and Technology (NIST) 2005 mass spectral database, and the major ion fragments and intensities are reported. Data was normalised to the internal standard alanine-d4. The normalised values are not concentrations but are ratios of the GC-MS response of compounds relative to the internal standard alanine-d4.

Methods for analysis of milk and plasma samples in vegans in 2013

Sample preparation

The sample extraction and derivatisation method was based on LePage & Roy.(340) The samples were kept at -80°C until preparation for analysis. Milk and plasma samples were thawed on ice, vortexed, then aliquoted into screw-cap glass culture tubes (Kimax). Due to the rich fatty acid profile in milk, less milk (50 µL) was analysed than plasma (200 µL).

Extraction solution (2 mL of methanol:toluene 4:1 v/v, Analytical Grade, Merck) containing internal standards (Nonadecanoic acid 55 µg/mL and Tridecanoic acid 53 µg/mL, Nuchek Inc.) was added to each sample. Magnetic stirring bars were added to each tube. Acetyl chloride (200 mL, ECP) was added slowly, dropwise to each sample over a period of 1 min. The tube was closed tightly and teflon tape was wrapped around the outside in order to provide visual confirmation of leakage, if it occurred. The tubes were placed in a heating and stirring dry block at 100°C for 1h. After 1h the tubes were cooled in water and 5 mL of an aqueous solution of 6% potassium carbonate was added to each tube.

The tubes were vortexed, then centrifuged at 2500 rpm for 5 min at room temperature. The upper toluene phase was recovered for analysis by GC-MS

Gas Chromatography-Mass Spectrometry parameters

Gas Chromatography-Mass Spectrometry was used for identification and quantitation of fatty acids. Instrument analytical parameters were based on those developed by Kramer & Hernandez.(341)

The instrument used was an Agilent 7890A gas chromatograph coupled to an 5975C mass spectrometer with a split/splitless inlet. One microliter of sample was injected using a CTC PAL autosampler into a glass 4mm ID straight inlet liner packed with deactivated glass wool (Restek Sky®). The inlet temperature was 250°C, in splitless mode, the column flow was set at 1 mL/min, with a column head pressure of 9 psi, giving an average linear velocity of 19 cm/sec. Purge flow was set to 50 mL/min 1 min after injection.

Column selection was based on the recommendations from the Official Methods for the determination of trans fat (American Oil Chemists Society).(342) The column was a fused silica Rtx-2330 100 m long, 0.25 mm internal diameter, 0.2 µm highly polar stationary phase (90% biscyanopropyl 10% cyanopropylphenyl polysiloxane, Shimadzu). Carrier gas was instrument grade helium (99.99%, BOC). The GC oven temperature programming started isothermally at 45°C for 2 min, increased 10°C/min to 215°C, held 35 min; increased 40°C/min to 250°C and held 10 min. The transfer line to the mass spectrometric detector (MSD) was maintained at 250°C, the MSD source at 230°C and the MSD quadropole at 150°C. The detector was turned on 14.5 min into the run. The detector was run in positive-ion, electron-impact ionisation mode, at 70 eV electron energy, with electron multiplier set with no additional voltage relative to the autotune value. Identification of compounds was carried out using mass spectra acquired in scan mode from 41 to 420 atomic mass unit, with detection threshold of 100 ion counts

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