To Investigate Associations Between Postprandial Meal Response and Genetic Variation in Young Healthy Male Adults

A Pilot Study

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Abstract

The science of nutrigenomics assesses gene–nutrient interactions using nutrient-related genetic markers or site-specific genetic variations in genes known as single nucleotide polymorphisms (SNPs). SNPs are one aspect of genetic variability that can impact an individual's response to food, including lipid and glucose metabolism, insulin response and appetite. In general, genetic differences can influence absorption, metabolism, uptake, utilisation and excretion of nutrients, ultimately affecting several physiological and nutritional outcomes. The effect of genetic differences can be assessed by changes in physiological outcomes during the postprandial state. The literature reports known associations between a particular SNP and a change in a physiological outcome; the robustness of many of these associations is uncertain.

This study aimed to investigate the association between a range of physiological measures and the related 26 SNPs located in specific genes to consider the strength of their relationship during the postprandial digestive response to a standardised breakfast meal. This included: *plasma concentrations of* vitamin D and the cytochrome P450 family 2 subfamily R member 1 gene (CYP2R1) and the group-specific component vitamin D binding protein gene (GC); iron and the homeostatic iron regulator protein gene (HFE) and the solute carrier family 17 member 1 (SLC17A1) and the transmembrane protease serine 6 gene (TMPRSS6) and the type-2 transferrin receptor gene (TRF2) and the transferrin coding gene (TF); zinc and the solute carrier family 30 member 3 gene (SLC30A3); saturated fat and the apolipoprotein A-II gene (APOA2); total cholesterol and the apolipoprotein A5 gene (APOA5); low-density lipoprotein and the ATP-binding cassette subfamily G member 8 gene (ABCG8); high-density lipoprotein and the ATP-binding cassette subfamily A member 1 gene (ABCA1); triglycerides and the angiopoietin-like 3 gene (ANGPTL3); glucose and the adenylate cyclase 5 gene (ADCY5); insulin and the insulin-receptor substrate 1 gene (IRS1); *dietary intake of* omega-6 & -3 and the fatty acid desaturase 1 gene (FADS1); *nutrients to assess* energy balance and the mitochondrial uncoupling protein 1 gene (UCP1); total fat and the transcription factor 7-like 2 gene (TCF7L2); saturated and unsaturated fat and the fat-mass and obesity-related alpha-ketoglutarate dependent dioxygenase gene (FTO); monounsaturated fatty acids and the peroxisome proliferator-activated receptor γ2 gene (PPARγ2); protein and FTO gene; *appetite scores for* fat-taste perception and the cluster

determinant 36 gene (CD36); sugar preference and the glucose transporter type 2 gene (GLUT2); hunger and the neuromedin beta gene (NMB).

Thirty young, healthy males (20–34 years) participated in an experimental study and consumed a standardised breakfast meal. Blood samples were collected before and hourly for 4 hours after a meal. Plasma samples were used to assess nutrient concentrations or physiological biomarker status. Buccal swabs were collected and analysed using the Illumina assay technique to assess SNPs. An online visual analogue 100-point scale was used to assess appetite scores upon arrival, immediately following ingestion, 30 minutes after ingestion and hourly for 4 hours after ingestion.

There was a positive association between the insulin-signalling IRS1 gene variant rs2943641, the typical risk (TT) compared to the increased risk (CT or CC), in relation to postprandial insulin levels, $\chi^2 = 1$, N = 30, $P = 0.0025$, 95% confidence interval (CI) [1.61, 4.93]. The UCP1 gene variant -3826 rs1800592, the typical risk (AA) compared to the increased risk (GG or GA), was positively associated with participants' body-mass index (BMI), $\chi^2 = 1$, N = 30, $P = 0.011$, 95% CI [0.081, 0.757]. The "sugar preference" GLUT2 gene variant rs5400 was insignificant between the typical risk (CC) compared to the increased risk (CT or TT) in relation to an elevated preference for sugar intake, $\chi^2 = 1$, N = 30, $P = 0.07$, 95% CI [0.94, 19.81]. However, a larger sample size may have revealed differences as significant. The remaining measures (vitamin D, iron, zinc, omega-6 and -3, protein, total fat, unsaturated and monounsaturated fat, cholesterol, low density lipoprotein, high density lipoprotein, triglycerides, fat taste, hunger and glucose) did not appear to associate with the genetic variants.

The findings of this study suggest a significant relationship between the associated SNP and digestive responses for the IRS1 gene variant rs2943641 and the UCP1 gene variant -3826 rs1800592. Despite scientific literature indicating statistically significant associations between other genetic variants and physiological outcomes, this study did not confirm the associations. However, as a pilot experimental study, we acknowledge that the power to determine other associations may be too small due to the limited sample size and the complexity of the genetic assessment. This study has emphasised many known associations between a particular SNP and a change in a physiological outcome, whilst providing information on how a genetic variant could increase health risks. Future research to establish the robustness and statistically significant associations between genetic variation and related psychological outcomes is needed. Whether the science of nutrigenomics is the key to

producing the “perfect diet,” the efficacy and utility of nutrient-related genetic markers are still under investigation.

Key Words: digestive responses, gene–nutrient interaction, nutrigenomics, postprandial, SNPs

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List of Abbreviations

AA	Amino acid
AI	Adequate intake
ALA	Alpha-linolenic acid
AUC	Area under the curve
BAT	Brown adipose tissue
BMI	Body mass index
BP	Blood pressure
CAD	Coronary artery disease
CI	Confidence intervals
CMRF	Chylomicron-rich fraction
DBP	D binding protein
DEXA	Dual-energy X-ray absorptiometry
DGI	Diabetes Genetics Initiative
DTC	Direct-to-consumer
EER	Estimated dietary energy requirement
FADS	Fatty acid desaturase
FAME	Fatty acid methyl esters
FFQ	Food frequency questionnaire
FUSION	Finland-United States Investigation of NIDDM
GINA	Genetic Information Non-discrimination Act
GNA	Genetic Non-discrimination Act
GWA	Genome-wide association
HDL	High-density lipoprotein
HFD	High-fat diet
HFE	Human factors engineering
HH	Hereditary hemochromatosis
HPD	High-protein diet
HR	Hazard ratio
KED	Kinetic energy discrimination
LA	Linoleic acid
LCPUFA	Long-chain polyunsaturated fatty acid

LDL	Low-density lipoprotein
LFD	Low-fat diet
LOLIPOP	London Life Sciences Prospective Population
LPD	Low-protein diet
MFC	Macrophage foam cells
MI	Myocardial ischemia
MS	Metabolic syndrome
NCD	Noncommunicable diseases
OR	Odds ratio
PAL	Physical activity levels
PREDICT	Personalised Response to Dietary Composition Trial
RDI	Recommended dietary intakes
REE	Resting energy expenditure
RI	Reference intervals
RMR	Resting metabolic rate
ROS	Reactive oxygen species
RT	Room temperature
RV	Risk variant
SNP	Single nucleotide polymorphism
SNS	Sympathetic nervous system
TC	Total cholesterol
TS	Transferrin
UL	Upper limit
VAS	Visual analogue scale
VDR	Vitamin D receptor
WHO	World Health Organisation
ZnT	Zinc transporters

Chapter 1: Introduction

1.1 Research Problem

Differences in human genomes affect how genes instruct cells during protein synthesis, termed gene expression (Kohlmeier, 2015). Gene variants can cause dysfunction of this process by a mutation in genes as deletion or insertion of nucleotides in DNA or site-specific variations in genes named single nucleotide polymorphisms (SNPs) (Barnes, 2008; Camp & Trujillo, 2014; Liu, 2007). Gene variants can cause individuals to be more susceptible to diseases, especially if combined with a nutrient that could increase their susceptibility (M. N. Mead, 2007). The poor understanding of gene–nutrient interaction is partly due to the limited number of investigations and the complexity, in that gene–nutrient interactions that cause disease in one individual will not necessarily cause disease in another individual. Genetic and nutrient factors for maintaining normal health conditions have been proposed to help tailor individual dietary requirements (Kohlmeier, 2015; M. N. Mead, 2007; Paoloni-Giacobino et al., 2003). Increasingly, postprandial responses are considered independent risk factors for health and disease and may represent health more than a fasting blood assay (Berry et al., 2020). There are also limited data on digestion and physiological response to dietary foods and inherent genetic variability that may influence eating habits (Klementova et al., 2019).

Therefore, the power of nutrigenomics to understand gene–nutrient interaction for the prevention of disease is gaining significant interest, and will ultimately be used in a longer term lifestyle plan by producing personalised nutritional interventions to help individuals to reduce their risk of disease and modify their eating habits (Grimm & Steinle, 2011; Lopez-Miranda & Marin, 2010). Nevertheless, evidence from well-designed nutritional studies that can translate easily into health benefits through a clinical application is lacking (Ordovas, 2008).

1.2 Background of the Research Problem

SNPs are one aspect of genetic variability that can impact an individual's digestive response to a meal, including lipid and glucose metabolism, insulin response and appetite. The process of the postprandial digestive response depends on an individual's metabolic efficiency and subtle differences in genetic variability. This variability is due to different allele forms caused by genes' polymorphisms. The most common form is site-specific variations or SNPs, used as nutrient-related genetic markers. SNPs are helpful to study the

relationship between genetic variation and digestive response influenced by diet in an individual (Vincent et al., 2002).

Subsequently, the effect of genetic differences can be assessed by changes in physiological outcomes during the postprandial state. Evaluating the postprandial digestive response to a meal is considered a relevant assessment for identifying the changes in digestion and metabolism as it enables the evaluation during the whole digestive response to a meal (den Hoed et al., 2008; den Hoed et al., 2009; Ellis et al., 2021; Grimm & Steinle, 2011; Monrroy et al., 2019; Zeevi et al., 2015).

Much of the nutrigenomic research has focused on the interaction of SNPs and singular physiological outcomes. Many postprandial investigations focus on a specific population with the same polymorphism related to a dietary nutrient-related genetic marker and resulting in individual disease-risk factors (Lairon et al., 2007; Lopez-Miranda & Marin, 2010).

Whilst most nutrigenomic research reports a known association exists between the SNPs and related physiological outcomes, this research will question the robustness of these interactions.

1.3 Research Question

Previous nutrigenomic research reports positive associations between a particular SNP and a related physiological or nutritional outcome change, but how robust are these interactions?

1.4 Study Aim and Objectives

This thesis aimed to investigate the unique genetic background of an individual and consider whether there are any associations between a range of physiological measures and the related 26 SNPs located in genes (Table 1).

Table 1

Investigated Physiological Measures With the Related Dietary Components Justified Alongside the Published Related Gene–Nutrient Interaction

Associated gene	Published gene–nutrient interaction[a]	Gene abbreviation	SNP reference marker	Physiological measure tested in the thesis
Cytochrome P450 family 2 subfamily R member 1	Involved in activation of vitamin D	CYP2R1	rs10741657	Vitamin D
Group-specific component vitamin D binding protein	Involved in vitamin D transport	GC	rs2282679	Vitamin D
Homeostatic iron regulator protein	Involved in iron transport (used to assess iron overload)	HFE	variant H63D-rs1799945 & variant C282Y-rs1800562	Iron
Solute carrier family 17 member	Involved in iron transport (used to assess iron overload)	SLC17A1	rs17342717	Iron
Transmembrane protease serine 6	Involved in absorption of iron (used to assess low iron)	TMPRSS6	rs4820268	Iron
Type-2 transferrin receptor	Involved in iron transport (used to assess low iron)	TRF2 gene	rs7385804	Iron
Transferrin coding gene	Involved in iron transport (used to assess low iron)	TF gene	rs3811647	Iron
Solute carrier family 30 member 3	Involved in zinc transport	SLC30A3	rs1126936	Zinc
Fatty acid desaturase 1	Involved in activation of omega-6 & -3 long-chain polyunsaturated fatty acid	FADS1	rs174547 & rs174546 in direct linkage with rs174547	Long-chain polyunsaturated fatty acids
Mitochondrial uncoupling protein 1	Involved in stimulating oxidation of fatty acid and increasing energy expenditure (used to assess energy balance; intake, expenditure, storage)	UCP1	rs1800592	Body-mass index (BMI) measures
Transcription factor 7-like2	Involved in response to dietary intake of fat	TCF7L2	rs7903146	BMI measures
Apolipoprotein A-II	Involved in response to dietary intake of saturated fat	APOA2	rs5082	Low-density lipoprotein (LDL) cholesterol
Fat-mass and obesity-related alpha-ketoglutarate-dependent dioxygenase	Involved in response to dietary intake of food (used to assess saturated and unsaturated fat)	FTO	rs9939609	BMI measures
Fat-mass and obesity-related alpha-ketoglutarate-dependent dioxygenase	Involved in response to dietary intake of food (used to assess protein)	FTO	rs9939609	BMI measures
Peroxisome proliferator-activated receptor γ2	Involved in fat cell formation in adipose tissue (used to assess monounsaturated fatty acids)	PPARγ2	rs1801282	BMI measures
Apolipoprotein A5	Involved in lipid metabolism (used to assess total cholesterol)	APOA5	rs662799	Total cholesterol
ATP-binding cassette subfamily G member 8	Involved in cholesterol uptake (used to assess LDL cholesterol)	ABCG8	rs6544713	LDL cholesterol
ATP-binding cassette subfamily A member 1	Involved in cholesterol metabolism (used to assess HDL cholesterol)	ABCA1	rs1883025	HDL cholesterol
Angiopoietin-like 3	Involved in release of fatty acids and glycerol from adipose tissue (used to assess triglycerides)	ANGPTL3	rs10889353	Triglycerides
Cluster determinant 36	Involved in lipid absorption and response to fat detection (used online visual analogue 100-point scale (OVAS) to assess fat-taste perception)	CD36	rs1761667	Appetite scores
Glucose transporter type 2 gene	Involved in glucose homeostasis and insulin release (used OVAS to assess sugar preference)	GLUT2	rs5400	Appetite scores
Neuromedin beta	Involved in eating behaviours (used OVAS to assess susceptibility to hunger)	NMB	rs1051168	Appetite scores
Adenylate cyclase 5	Involved in fasting glucose levels	ADCY5	rs11708067	Glucose
Insulin-receptor substrate 1	Involved in insulin levels	IRS1	rs2943641	Insulin

[a] The SNPs listed have been suggested by previous genome-wide association (GWA) studies to be associated with disease risk in a population (Appendix A) (Patron et al., 2019).

To achieve the overarching aims, the objectives of this study will include investigating dietary-nutrient-related genetic markers with the measured parameters reflecting a participant's postprandial digestive response to a standardised breakfast meal.

1.5 Overview of the Methodology

Postprandial digestive responses were analysed using each participant's physiological outcome measures or markers that correspond to the dietary component and correlated with the qualitative genetic SNP associated with published literature (Appendix A, Table 1).

Participants' plasma samples at baseline / premeal (t-pre) were used to assess the physiological measure for concentrations of vitamin D, iron, zinc, total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), triglycerides (TG), glucose and insulin.

Participants' postprandial changes in long-chain polyunsaturated fatty acid (LCPUFA) (18:2 n-6, 18:3 n-6, 18:3 n-3) were used to assess the physiological measure for concentrations in the chylomicron-rich fraction (CMRF) for dietary intake of omega-6 and -3.

An online visual analogue 100-point scale was used to assess the physiological measure for each participant's postprandial appetite changes associated with fat-taste perception, sugar preference and susceptibility to hunger.

Participants' body-mass index (BMI) was used as the physiological marker related to body composition and energy balance. The categories of BMI were: Underweight < 18.5 kg/m^2, Healthy/Normal 18.5–24.9 kg/m^2, Overweight 25–29.9 kg/m^2 and Obese > 30 kg/m^2 (National Heart Foundation of New Zealand, 2022).

The Oragene DNA ON-500 self-collection kits were used to take buccal samples from each participant for their genetic analysis. The samples were sent to and analysed by Nutrigenomix (2020) using the Illumina assay technique to assess 26 SNPs. Nutrigenomix is a multinational biotechnology company with over 10,000 accredited healthcare practitioners who practise in 40 countries. Nutrigenomix provides genetic-test kits and a direct-to-practitioner customised nutritional-information report based on genetic variation and genotype. The report describes how each individual is susceptible to food-related traits based on their risk variant.

1.6 Research Roles and Responsibilities

This thesis is a small subsection of a larger randomised crossover trial to capture the biological difference in postprandial nutrient dynamics of the effect of red meat on

postprandial meal response. The trial compared the acute nutritional effects of iso-caloric, blinded meals. The trial was conducted at the Auckland Clinical Research Centre, and the writer's duties and responsibilities were: the purchasing of most of the meal ingredients and supporting meal preparation; the cooking and serving of hot meals to participants who made four consecutive visits to the clinic over 9 weeks; the taking of vital signs for baseline data (height, weight, blood pressure, heart rate) (Appendix B); encouraging participants' completion of the ASA 24-hour dietary recall and questionnaires; gaining consent for the collection of genetic samples; the coordination and collection of buccal samples for genetic testing; the management of data cleaning and manipulation for the extensive trial; and the interpretation and write up of outcomes for this thesis.

1.7 Impact

Genetic variation is one factor that can impact an individual's digestion and physiological response to foods. Dietary components can impact health by affecting gene expression, necessitating an understanding of gene–nutrient interactions (Klementova et al., 2019). The postprandial state presents independent health-risk factors due to elevated blood glucose and lipids which cause oxidative stress, inflammation, and endothelial damage (Berry et al., 2020). Therefore, investigating an individual's inherent genetic variability and their postprandial digestive responses can be used to inform the individual about their disease risk and ultimately translate into effective preventive longer term lifestyle strategies using personalised dietary interventions (Ellis et al., 2021; Nielsen & El-Sohemy, 2014; Ordovas, 2008). These investigations will help explain why certain nutrients may benefit one individual more than another and support individuals with unhealthy diets and noncommunicable diseases such as obesity, Type 2 diabetes mellitus (T2DM) and cardiovascular disease (CVD) (Barnes, 2008; Berry et al., 2020; Binia et al., 2014; Lopez-Miranda & Marin, 2010).

A good example of gene–nutrient interaction is between the "fat mass and obesity-associated" (FTO) genes and how they can impact the body's response to saturated fat (Rodrigues et al., 2015). Overconsumption of saturated fat causes weight gain leading to higher BMI and obesity. Obesity is a causal factor in developing insulin resistance, T2DM, and metabolic disease. Obesity has been linked with a common variant in the FTO gene variant rs9939609 genotype AA or TA (C. M. Phillips et al., 2012). The frequency of the A allele of the rs9939609 SNP is 0.4 in European-descent population and closer to 0.15 in Asian descent (National Center for Biotechnology Information [NCBI], 2005). Carriers of the

FTO A allele who consume larger quantities of saturated fat (commonly found in animal products) compared to polyunsaturated fatty acid (PUFA) increase their risk of obesity and CVD. Individuals with the high-risk allele may respond positively to a weight-loss plan that is high in PUFA and low in saturated fats (den Hoed et al., 2009; C. M. Phillips et al., 2012; Rodrigues et al., 2015). Therefore, future research on the FTO gene variant rs9939609 and other gene–nutrient interactions alongside an individual's dietary intake could help translate into their personalised dietary plan.

Another example of gene–nutrient interaction studies could impact carriers with specific genotypes as they may be more at risk of disease and more susceptible to dietary factors. For example, 57% of Indigenous Indians of America are carriers of the MTHFR gene TT genotype and are more at risk of colon cancer, but folate supplementation can help to reduce this risk (Binia et al., 2014).

This thesis aimed to investigate the unique genetic background of individuals and consider whether there are any associations between a range of physiological measures and the related 26 SNPs (Table 1). The investigated physiological measures were selected alongside the corresponding dietary components and justified with the published related gene–nutrient interaction. The investigated gene–nutrient interactions with the measured parameters reflected a participant's postprandial digestive response to a standardised breakfast meal. The "Personalised Response to Dietary Composition Trial" (PREDICT) helped justify using a postmeal analysis as a good way of investigating nutrition response, rather than what most studies do, which is a fasting blood sample (Berry et al., 2020).

Chapter 2: Literature Review

2.1 The Emerging Science Behind Nutrigenomics

The science of nutrigenomics assesses the gene–nutrient interactions using nutrient-related genetic markers or site-specific genetic variations in genes known as SNPs. The term *nutrigenomics* first made an appearance around 2001 in literature by Dr Nancy Fogg-Johnson, who, in her opinion, would "revolutionise how people manage nutrition and dietary components" (Peregrin, 2001, p.1). Fogg-Johnson believes nutrigenomics will allow gene variants in metabolic diseases to be successfully managed through dietary intervention. Today, there is a general acceptance of the notions that genetic variation plays a role in the health and longevity of an individual and that a perfect diet may differ depending on genetic background (Passarino et al., 2016). Whether or not the science of nutrigenomics is the key to producing the "perfect diet," the efficacy and utility of nutrient-related genetic markers are still under investigation.

2.1.1 Nutrigenomics and Genetic Variation

Nutrigenomics is a subbranch of nutritional genomics or gene–nutrient interaction (the other branches being nutrigenetics and nutritional epigenetics) and incorporates the human genome, nutrition and health. Genomic technologies and genetic information are used in nutrigenomics to study how the body responds to nutrients (Farhud et al., 2010; M. N. Mead, 2007). Nutrigenomics utilises techniques such as proteomics, including gene expression and metabolism profiling (metabolomics), to investigate the role of an individual's gene–nutrient interaction (M. N. Mead, 2007; Paoloni-Giacobino et al., 2003). Combined with individual dietary interventions, nutrigenomics is helping shift from a "one-size-fits-all" approach to diet towards an individualised, personalised diet for effective wellness (Nielsen & El-Sohemy, 2014; Zeisel, 2007).

The Human Genome Sequencing Project, started in 1990 and completed in 2003, made it possible to identify SNPs (Chial, 2008; Karczewski et al., 2020). Data analysis from the project has allowed the referencing of SNPs using the prefix "rs" followed by a unique number (gnomAD, n.d.; Karczewski et al., 2020; NCBI, 2005; Sherry et al., 2001). This project provides a detailed description of genetic variation across populations. Everyone's human genome is identical apart from approximately 0.001% of individual genetic variation in one's DNA, chromosomes, or genes. Variations known as polymorphism could cause a genetic risk and increase the susceptibility to a disease or disorder (Collins et al., 2003). SNPs

are the most common polymorphisms found within genes and promising biomarkers for disease susceptibility (Farhud et al., 2010; M. N. Mead, 2007).

2.1.2 Single Nucleotide Polymorphisms

With the arrival of nutrigenomics and GWA studies, much research has been centred around SNPs, as they are associated with responses to diet and influence health and disease (Fenech, 2005).

SNPs are a common type of genetic variation among individuals and constitute a single nucleotide variation at a specific position in genes located in an individual's DNA sequence (den Hoed et al., 2008; den Hoed et al., 2009; Eny et al., 2008; Melis et al., 2015). DNA comprises a string of four different types of nucleotide bases: adenine, A, cytosine, C, guanine, G, and thymine, T. Alleles are the possible bases of a genetic variant and in SNPs are comprised of the single bases A, T, C or G. SNPs contain only two possible alleles, for example, T or C, and three possible genotypes: homozygous dominant, homozygous recessive, or heterozygous, making them easily assayed (Hinds et al., 2005; Sachidanandam et al., 2001). There are many types of SNP assay methods including polymerase chain reaction: resistive fault locates (PCR:RFL) -based genotype; single-strand conformation polymorphism; restriction fragment length polymorphism; DNA sequencing; DNA microarray; SNP chip analysis; capillary electrophoresis, and single base extension (Liu, 2007).

SNPs are genetic mutations caused by environmental factors, poor lifestyle, diet or stressful conditions. SNPs alter gene transcription and protein formation and act as genetic markers to locate genes associated with a disease. When SNPs interact with nutrients, initiating the turning off or on of gene expression, they can modify the behaviour of the molecular function and signalling pathways in cells (Camp & Trujillo, 2014). Failure of gene expression could cause damage to the cells and trigger the initiation of disease (Paoloni-Giacobino et al., 2003; T. Phillips, 2008). Therefore, gene–nutrient interactions can profoundly affect dietary implications and health outcomes by influencing gene expression (Ramos-Lopez et al., 2017). The nutritional impact of the evidence of gene–nutrient variants supports healthy dietary intake to reduce the risk of disease (Paoloni-Giacobino et al., 2003).

Throughout the population, 99% of the human genome is identical; the remaining 1% carries the same nucleotide variation or SNPs and there are about 10 million SNPs spaced approximately 300 nucleotides apart (Hinds et al., 2005). Of the 10 million SNPs, the most common ones are found in about 5% to 50% of the population (Hinds et al., 2005).

Advancements in biotechnology have allowed SNPs to be readily assayed using noninvasive techniques, thus, making discovering their risk variants accessible for individuals. Many companies now provide SNP analysis services and focus on the most common risk variants to report how susceptible an individual is to food-related traits that can increase health risks and cause disease (Zeisel, 2007).

The location and identification of gene variants are currently being investigated and will help explain how different nutrients affect health and predict a person's risk of disease (M. N. Mead, 2007). Dietary interventions can then be introduced as a treatment to reduce risk due to understanding gene–nutrient interaction (Afman & Müller, 2006; Paoloni-Giacobino et al., 2003).

Much research has reported disease-risk variants associated with gene–nutrient interaction. An example of a gene–nutrient interaction is a low intake of folate (B9), B6 and B12, which is associated with the methylenetetrahydrofolate reductase (MTHFT) gene variant rs1801133. This gene variant regulates homocysteine metabolism and increases the risk of breast cancer (Jiang-Hua et al., 2014). Another example is low calcium intake, affecting vitamin D availability and bone mineral density linked to the vitamin D receptor (VDR) gene variant rs1544410. This gene variant is associated with osteoporosis in postmenopausal women (Stathopoulou et al., 2011). The lipoproteins encoding genes, APOC3 gene variant rs5128, and the APOA1 gene variant rs670 and rs5069 are associated with a higher risk of metabolic syndrome (MS) in Tehranian adults (Hosseini-Esfahani et al., 2015; Ramos-Lopez et al., 2017).

The association of nutrient-related genetic markers and risk variants could be linked to multifactorial diseases like heart disease, cancer, and diabetes (M. N. Mead, 2007; Paoloni-Giacobino et al., 2003). M. N. Mead (2007) has reported that "the selective use of genome-protective nutrients in individuals with specific gene variants could potentially result in improved resistance towards these major diseases" (p. 584). Fenech (2005), who initiated the concept of nutrigenomics, has described the link between genome instability and nutritional deficiency as dependent on "genetic polymorphisms that can alter the function of genes" (p. 255) with unfavourable health outcomes. Therefore, certain nutrients could provide protection in individuals with a particular gene variant (Fenech, 2005).

The current thesis has a focus on SNPs as the basis for the genetic information; however, we acknowledge that epigenetic changes mean that our food is not just an input to the body system, as common with SNPs variants, but can also change how that system functions. Further, genetic variation can also influence epigenetic modifications, adding

another layer of complexity. While nutritional epigenomics is a relatively new field, it is clear that epigenetic processes play essential roles in how our bodies interact with food and other bioactive compounds. It may also partially explain the "missing heritability" problem of GWA studies, i.e., those genetic variants typically only explain a small fraction (5%–10%) of a phenotype's heritability (Mullins et al., 2020).

2.1.3 The Postprandial State and the Digestive Response

Dietary advice to help control blood lipids and glucose levels are similar for all people. Recent research suggests that particular diets recommended to support optimal health can have the opposite effect on some people (Zeevi et al., 2015). Contrary to population-level guidance, we know there are considerable interpersonal variations in the glycaemic and lipid response to a meal and differences in how individuals respond to particular diets. Interestingly, while significant variation exists between different individuals, meal response is reasonably predictable from meal to meal (Monrroy et al., 2019; Zeevi et al., 2015).

The postprandial state is the period between the ingestion of food and the postabsorptive state when the increase in plasma fatty acids, amino acids and appetite response occurs (den Hoed et al., 2008; den Hoed et al., 2009). Many dietary interventions have focused on taking measurements during the fasting state, but most adults spend more time in a dynamic postprandial state. For example, it can take up to 6 hours for triglycerides, lipoproteins, fatty acids, glucose and insulin response levels to return to preprandial or baseline levels (den Hoed et al., 2008; den Hoed et al., 2009; Desmarchelier et al., 2013; Lopez-Miranda & Marin, 2010). Therefore, studies should focus on assessing the relationship between digestion, metabolism, and genetic variants' influence on the whole digestive response to a meal. This relationship is an important and under investigated topic in many dietary interventions.

During the postprandial state, there are fluctuations in physiological parameters, including glucose, insulin, lipids and appetite response which can be analysed using physiological variables (den Hoed et al., 2008; den Hoed et al., 2009; Desmarchelier et al., 2014; Mortensen et al., 2012). Therefore, the postprandial digestive response may be more relevant to identifying the differences in digestion and metabolism than measures taken in the fasting state (Azpiroz et al., 2014; Lopez-Miranda & Marin, 2010). Dietary intervention studies that measure the fasting state cannot accurately predict the peak changes accompanied by postprandial digestive responses during the test period. The changes can be calculated

using the trapezoidal method for the area under the curve (AUC) time-point differences during the postprandial digestive response (Lairon et al., 2007).

2.2 Biotechnology Companies Offering Nutrigenomic Testing

Recently with the explosion of nutrigenomics research and the demand for genetic data, biotechnology companies have developed individual genetic mapping relating to ancestry and to "optimise health." These companies can assess an individual's SNPs, identify their risk variant, and produce customised reports commenting on health and well-being insights (Dib et al., 2019; Mullins et al., 2020). Customised reports have become possible due to the genetic mapping used in the Human Genome Project and HapMap Project to identify genetic variations which may carry common traits and disorders (Vimaleswaran & Loos, 2010). Genetic mapping has enabled the identification of 38 million SNPs, opening the floodgates for credible GWA population-based studies that have linked genetic variants to certain diseases and health risks (Moore, 2020). Personalised dietary nutrition plans have followed and been tailored to an individual's genetic data to optimise health and well-being and reduce disease risk.

Since 2001, due to growing public interest, there has been an increase in the publication of articles in the PubMed database on "stratified nutrition," "nutrigenetics," "personalised nutrition," and "precision nutrition" (Moore, 2020). This interest has fuelled increased spending in the personal genomic biotechnology industry and decreased genotyping costs. The reduction in costs has allowed for the exponential growth since 2016 of nutrigenomic tests available to the public, and consumer numbers have surpassed 10 million (Khan & Mittelman, 2018; Moore, 2020). Growth to date, 2022, has seen investment in biotechnology being very profitable. Consequently, Illumina, a gene-sequencing company giant with a market cap of US$54.98 billion as of March 2022, has amalgamated with investors to form Helix, a new company that includes health and well-being consumer reports (Khan & Mittelman, 2018; Macrotrends, 2022), attracting many individual consumers who want to seek out health information and the risk of getting a disease (Khan & Mittelman, 2018).

Several issues exist with these biotechnology companies' reported data on gene–nutrient interaction. A lot of the information regarding the gene–nutrient interaction is derived from GWA studies performed on healthy individuals of similar ethnicity and does not account for changes in different environmental and stress conditions (Dib et al., 2019). Trying to untangle the gene–nutrient interaction, which involves a complex biosynthesis

process, will take time. Therefore, precision nutrition can only be successful when more rigorous research on individual differences associated with physiological outcome responses to nutrients is pursued (Dib et al., 2019; Khan & Mittelman, 2018).

The Academy of Nutrition and Dietetics's takes an evidence-based approach to nutrigenetics, and believes that dietary intake and genetics affect phenotype and can, together, effect health (Braakhuis et al., 2021). Nutrigenetics can be used in clinical practice, registered dietitians can interpret and convey the reported outcomes to individuals. Even though much research investigates gene–nutrient interactions, this has not led to successful facilitation in many clinical practices (Ellis et al., 2021). This is mainly due to practitioners' lack of guidance and knowledge on applying personalised nutrition based on a patient's genetic variation. Evidence-based guidelines called "nutrigenomic care maps" are being developed to help practitioners implement personalised nutrition. These care maps will help increase practitioners' knowledge of nutrigenomics, genetic testing, and personalised nutrition (Horne et al., 2021). However, continued research is needed as polygenic traits are associated with more than one gene that can influence nutritional status. The efficacy of using nutrigenomics for multifactorial diseases such as obesity, T2DM and CVD to improve health outcomes is still under investigation (Camp & Trujillo, 2014; Ellis et al., 2021).

2.2.1. Ethical Considerations

Factors holding back individuals from sending off their DNA sample are privacy concerns about what happens to their sample and who owns the data once analysed. To stop the distribution of genetic information for other than private use, the USA passed the Genetic Information Nondiscrimination Act of 2008 (GINA), and Canada, the Genetic Non-Discrimination Act 2017 (GNA), formally known as Bill S-201 (Sterling, 2008).

Therefore, before submitting DNA samples, it is vital to know the biotechnology company's privacy and data-handling policy and if they adhere to the GINA or GNA. Dr Ahmed El-Sohemy, professor and Canadian research chair in nutrigenomics at the University of Toronto and founder and chief science officer of Nutrigenomix, started in 2011, ensures the anonymity of all samples handled (Nutrigenomix, 2020). El-Sohemy assures consumers that his company uses the "most stringent standards for secure data transfer, privacy and security" as only registered dietitians or physicians can obtain genetic testing kits for their patients (Nutrigenomix, 2020, p. 1). Therefore, healthcare professionals, acting in their patient's best interest, can provide an analysis of the Nutrigenomix results. Other nutrigenomic companies do not give the same "direct-to-practitioner" assurance. Sterling

(2008) reported this in his research on websites promoting biotechnology companies offering nutrigenomic testing. He found only 44 companies that had a privacy statement; 19 addressed the issue of privacy concerns regarding specimens and data results and only 14 of these specifically detailed their confidentiality procedure with the destruction of samples. Eleven other companies stated they would use data results for "future research."

2.2.2 Direct-to-Consumer Genetic Tests

The evidence for and against the use of direct-to-consumer (DTC) biotechnology companies is that the genetic testing kits are easy to use for individuals to gain information concerning their nutrient metabolism, eating habits, food tolerance or health (Oh, 2019). DTC reports do not need approval from a healthcare practitioner and are less expensive than direct-to-practitioner reports. But caution is required when analysing results that suggest the consumer is more susceptible to a disease or health condition (Jansen et al., 2019; Oh, 2019). The results may cause undue stress and anxiety to the consumer, but it could lead to lifestyle changes for others. A negative test is not conclusive, and a disease or health condition may develop irrespectively. The information from the tests becomes the biotechnology company's property. For example, 23andMe, a DTC biotechnology company in the USA, collects and uses the data for further research (Jansen et al., 2019). Therefore, DTC tests are not always in the consumer's best interest as they do not provide guidance or support, especially when analysing the results, which can be complex. Therefore, consultation with a healthcare practitioner is essential when consumers make health changes based on their genetic variants (Oh, 2019).

2.3 Vitamins and Essential Minerals and Associated Genetic Risk Variant

This section will investigate the association between vitamin D, iron overload, low iron, zinc and the related SNP variant located in genes: cytochrome P450 family 2 subfamily R member 1 gene (CYP2R1) and group-specific component vitamin D binding protein gene (GC), homeostatic iron regulator protein gene (HFE) (C282Y, H63D) and solute carrier family 17 member 1gene (SLC17A1), transmembrane protease serine 6 gene (TMPRSS6), type-2 transferrin receptor gene (TFR2) and transferrin coding gene (TF), and solute carrier family 30 member 3 gene (SLC30A3), respectively.

2.3.1 Vitamin D and the CYP2R1 and GC Gene (rs10741657 and rs2282679)

Vitamin D is a fat-soluble steroid hormone and has two forms: endogenous, manufactured by the skin's exposure to UV light producing D3 (cholecalciferol), and

exogenous, consuming dietary components found as D2 (ergocalciferol) (Speeckaert & Delanghe, 2021). Vitamin D circulates in the blood as 25-hydroxyvitamin D (25[OH]D), the inactive precursor hormone used as a biomarker, and 1,25-dihydroxyvitamin D or calcitriol (1,25[OH]$_2$D), the active hormone or metabolite involved in many physiological processes, such as maintaining immune function, cell growth and apoptosis (Christakos et al., 2011; Tomei et al., 2020). The primary function of vitamin D is to regulate levels of the minerals calcium and phosphorus by enhancing their absorption from the small intestines into the blood. Chylomicrons transport vitamin D to the liver via the bloodstream, where 25(OH)D can be circulated, attached to a vitamin D binding protein (DBP), and delivered to target tissues (Kohlmeier, 2015; Speeckaert & Delanghe, 2021). DBP allows calcium and phosphorus deposit into bone tissue as the mineral hydroxyapatite. This mineral is essential for building the bone matrix to increase bone density, bond strength and reduce the risk of bone diseases, including osteoporosis, rickets, osteomalacia and bone stress fractures (Alathari et al., 2020; Slater et al., 2017). The deficiency of vitamin D is associated with the risk of other multifactorial conditions such as diabetes, CVD, and cancer (T. J. Wang et al., 2010).

Not only do diet and seasonal conditions affect vitamin D deficiencies, but plasma concentrations of 25(OH)D might be heritable (Shea et al., 2009). Using 1,762 participants from the Framingham Heart Study, a cross-sectional study by Shea et al. (2009) found the heritability of 25(OH)D to be statistically significant (estimated at 28.8 ± 11.3% in multivariable-adjusted analysis, $P = 0.003$).

Several studies are reporting a growing concern at the number of New Zealanders with insufficient levels of vitamin D (Baghurst & Record, 2002; Green et al., 2004). New Zealand has reported that 27.6% of adults have low vitamin D levels (< 37.5 nmol/L) and 2.8% have very low (< 17.5 nmol/L) (Green et al., 2004). Therefore, to help boost vitamin D levels, it is commonly added to milk products (Baghurst & Record, 2002; Holick, 2001; Vanlint, 2005).

A cross-sectional study on elderly women reported low vitamin D levels, resulting in increased falls leading to fractures that happened more often during winter (Pasco et al., 2004). Vitamin D levels in winter were less than 58 nmol/L compared to less than 70 nmol/L in summer. The study reported seasonal variation in the number of fractures sustained in the winter months (67.5%; 95% CI [64.2, 70.8]) compared to the summer months (58.2%; 95% CI [54.6, 61.8], $P < 0.001$) and that low vitamin D reduces bone strength (Pasco et al., 2004).

Some studies have linked three gene variants to vitamin D status and low plasma levels of 25(OH)D (Tomei et al., 2020; T. J. Wang et al., 2010). The first is the protein-coding gene, 7-dehydrocholesterol reductase (DHCR7). The DHCR7 gene synthesises cholesterol from 7DHC, a cholesterol precursor and a vital precursor essential for cholecalciferol (D3); 7DHC is then converted to vitamin D and D3 by a photochemical process in the skin (Tomei et al., 2020; T. J. Wang et al., 2010).

The CYP2R1 gene is thought to encode the microsomal enzyme 25-hydroxylase in the hepatic system, where it gets hydroxylated to 25(OH)D and sequentially converted to the active form 1,25$(OH)_2$D in the kidneys (Slater et al., 2017; Speeckaert & Delanghe, 2021). The CYP2R1 gene variant rs10741657 (genotypes GG and GA) is associated with low plasma levels of 25(OH)D (Slater et al., 2017; Speeckaert & Delanghe, 2021; Tomei et al., 2020; T. J. Wang et al., 2010).

The GC gene is involved in the transport of vitamin D by encoding the vitamin D binding protein (DBP) and binding of the vitamin D metabolites, 25(OH)D and 1,25$(OH)_2$D, in the plasma (Kohlmeier, 2015). Therefore, low plasma levels of 25(OH)D and vitamin D are associated with low concentrations of DBP produced by the GC gene variant, rs2282679 (genotype GG) (Slater et al., 2017; Speeckaert & Delanghe, 2021; Tomei et al., 2020; T. J. Wang et al., 2010).

A cross-sectional study conducted by Slater et al. (2017) reported that of the 180 participants taking similar supplements, 140 (78%) had low levels of 25(OH)D (< 75 nmol/L) and were linked to an increasing frequency of the CYP2R1 gene variant, rs10741657 (genotypes GG and GA) and the GC gene variant, rs2282679 (genotype GG). Participants with the CYP2R1 gene variant (rs10741657 GG or GA) were 3.67 times more likely (OR = 3.67, 95% CI [1.35, 9.99]) to have low vitamin D, whilst participants with the GC gene variant (rs2282679 GG) were 0.5 times more likely (OR = 0.42, 95% CI [0.18, 0.93]) to have lower levels of vitamin D. The results suggest genetics has a more significant impact on vitamin D status compared to supplementation (Slater et al., 2017).

Another study conducted by T. J. Wang et al. (2010) using 33,996 European-descent subjects from the SUNLIGHT Consortium (Study of Underlying Genetic Determinants of Vitamin D and Highly Related Traits from the United Kingdom, United States, Canada, Netherlands, Sweden, and Finland) used 15 cohorts. T. J. Wang et al. (2010) found genetic variants identified at three different loci were significant in one cohort and confirmed in another. These genetic variants had a combined total of $P = 2.9 \times 10^{-109}$ for SNP rs2282679 near GC, $P = 3.3 \times 10^{-20}$ for SNP rs10741657 near CYP2R1 and $P = 2.1 \times 10^{-27}$ for SNP

rs12785878 near DHCR7 and explain 1%–4% variation in 25(OH)D levels. The study assessed the influence of the SNPs and how they affected low vitamin D levels using 25(OH)D of less than 75 nmol/L or less than 50 nmol/L. Findings reported that participants who had all three variants from the top quartile compared to the lower quartile had increased odds by 2–2.5-fold associated with insufficient vitamin D (25(OH)D < 20 nmol/L, adjusted odds = 1.43, 95% CI [1.13, 1.79], $P = 0.02$) (T. J. Wang et al., 2010).

The studies by T. J. Wang et al. (2010) and Slater et al. (2017) have associated genetic variants with insufficient vitamin D levels. Still, there are confounding issues as vitamin D levels are dependent on biological nongenetic factors and environmental conditions. These factors include dark skin complexions, low sunlight exposure, low vitamin D dietary intake, obesity, and low magnesium levels, and may account for the low vitamin D in the studies. Suggesting CYP2R1 and GC genetic variants may not be the only causal effect of low vitamin D, and further investigation to enhance the association is needed (Al-Khalidi et al., 2017; Tomei et al., 2020).

2.3.2 Iron Overload and the HFE Gene (rs1799945, rs1800562) and SLC17A1 Gene (rs17342717)

The HFE gene synthesises the human factors engineering protein that binds to transferrin receptor-1 (TfR1) (Holmström et al., 2002). Mutation of the HFE protein reduces its affinity to bind to transferrin receptors to form diferric transferrin and transport iron, absorbed from the diet into cells. This lack of binding results in iron overload as iron cannot be released into cells causing ferritin levels to elevate. Surplus iron is then deposited into tissues due to transferrin saturation (TS), causing a detrimental effect on organs, especially the liver, resulting in liver fibrosis or cirrhosis (Jacobs et al., 2009). Iron overload is associated with fatigue, aching joints, cancer, heart failure, diabetes, and hemochromatosis. The imbalance of iron homeostasis is worse for homozygous C282Y carriers at greater risk of developing autosomal recessive hereditary hemochromatosis (HH), commonly caused by a mutation in the HFE gene. (Allen et al., 2008; Emanuele et al., 2014; Holmström et al., 2002).

Studies have identified three-nucleotide sites where HFE mutation might occur (Emanuele et al., 2014). The most common is the G to A allele mutation forming the AA genotype at amino acid (AA) 282, resulting in cysteine substitution. Homozygous carriers of the C282Y gene variant, rs1800562, constitute 82%–90% of HH sufferers (Emanuele et al., 2014; Garcia-Tsao et al., 2007). The second is the G to C allele substitution at AA 63,

resulting in histidine substitution, H63D gene variant, rs1799945. Last is the A to T allele mutation at AA 65 and serine substitution, S65C (Allen et al., 2008; Ganesh et al., 2009; Holmström et al., 2002; Katsarou et al., 2019). The American Association for the Study of Liver Disease has recommended that homozygous C282Y carriers with elevated TS reading of $\geq 45\%$ should get a test for HH (Garcia-Tsao et al., 2007).

Jacobs et al. (2009) conducted a cohort study using participants' information from the Hemochromatosis Family Study. They confirmed a genetic risk for relatives of developing HH from proband carriers of homozygous C282Y who have elevated serum iron. Still, a positive genetic test does not suggest the existence of HH. H63D and S65C carriers have a low level of risk of HH, and heterozygous S65C carriers have a low risk of developing HH (Emanuele et al., 2014; Pedersen & Milman, 2009).

Studies investigating HFE mutations include Allen et al. (2008), a 12-year project titled The Melbourne Collaborated Cohort Study that followed 31,192 European participants, with 29,676 genotyped successfully. A random sampling of 1,438 participants with the HFE genotype AA included 203 homozygous C282Y carriers (rs1800562). The sample was then stratified, and investigators were blinded to the participant's genotype; 1,054 out of 1,325 participants completed the study (79.5%). Out of the 203, 74 males completed the study, and 21 had iron-overload-related diseases (28.4%; 95% CI [18.8, 40.2]) compared to 84 females, of whom one had an iron-overload-related disease (1.2%; 95% CI [0.03, 6.5]). The study concluded that homozygous C282Y carriers, especially males with a serum ferritin level of ≥ 1,000 μg/L, are at a greater risk of disease caused by the HFE gene. The study's limitations are that the 95% CI range in both males (18.8, 40.2) and females was too wide (0.03, 6.5), making the results less reliable. Validity is in question due to the high attrition rate and the small number of participants with homozygous C282Y that completed the study. Therefore, the study has low power making the result not statistically significant. Nevertheless, Allen et al. (2008) confirmed Guyader et al.'s (1998) earlier finding that elevated serum ferritin levels may be associated with cirrhosis and HH in homozygous C282Y carriers.

A meta-analysis study performed on an Italian cohort found a common variant in the TFR2 gene associated with serum iron and the HFE gene variant H63D, rs1799945 (P = 0.001) (Pichler et al., 2011). For ferritin, they found an association with TFR2 and the SLC17A1 gene variant, rs17342717, C/T allele ($P = 8.0\text{x}10^{-6}$), and this reflected an association with HFE gene variant C282Y, rs1800562 and the hereditary disease, HH (HapMap *CEU* r2 = 0.42, P = 0.417). The analysis presented as forest plots confirmed an association between serum iron levels and TRF2 and SLC17A1 in linkage disequilibrium

with the HFE gene. Therefore, the HFE gene influences serum iron, transferrin, TS, and ferritin levels and may lead to iron overload and HH. Homozygous C282Y carriers may have an increased iron overload and HH risk. (Pichler et al., 2011).

2.3.3 Low Iron and the TMPRSS6 Gene (rs4820268,), TRF2 Gene (rs7385804) and TF Gene (rs3811647)

Iron is an essential mineral for blood production and is a primary component of haemoglobin found in red blood cells and myoglobin found in muscle cells. (Pichler et al., 2011). The remainder iron is either stored in the liver as ferritin, and is necessary for cellular processes and DNA synthesis, or used in cytochromes, and various enzyme activities involved in metabolic processes. Low iron causes a reduction in oxygen flow and results in anaemia with symptoms of fatigue, dizziness, shortness of breath and rapid heartbeat. Iron deficiency is a common disorder and, if left untreated, causes an imbalance of iron homeostasis that can result in serious diseases, including T2DM and CVD. (Ganesh et al., 2009; Pichler et al., 2011). To increase the amount of iron in the diet, nutritionists and dietitians will advocate that the patient consumes a range of foods rich in haem iron found in fish, red meat, poultry and wholemeal cereals (Timoshnikov et al., 2020). Plant sources have less bioavailable iron because they contain nonhaem iron that cannot be absorbed efficiently through the intestinal barrier. Therefore, if following a restricted diet to help increase nonhaem-iron absorption, it is advisable to include vitamin C (ascorbic acid) into the diet (Australian Government, National Health and Medical Research Council, New Zealand Ministry of Health, 2006). Nonhaem iron contains mainly ferric ions (Fe^{3+} ions), and, in acidic conditions, ascorbic acid forms a chelate with Fe^{3+} ions to maintain its solubility when entering alkaline conditions in the duodenum. Conversely, zinc, calcium, the stored form of phosphorous (phytate) and polyphenols can hinder iron absorption (Benyamin et al., 2009; Timoshnikov et al., 2020).

Genetic testing cannot determine iron deficiency, but research has reported the association between gene variants and low-iron status (Benyamin et al., 2009). There are three major genes associated with iron deficiency: TMPRSS6 gene that has links to hepcidin levels for iron absorption, the TRF2 gene for iron transport into liver cells, and the TF gene for iron transport around the body (Benyamin et al., 2009; Ganesh et al., 2009; Pichler et al., 2011).

Pichler et al. (2011) investigated the regulation of iron levels in a European and USA five-population-based GWA study using meta-analysis. The study aimed to analyse serum

iron levels and iron status markers, including transferrin, soluble transferrin receptors (sTfR), ferritin and sTfR-ferritin index, to find an association with gene loci. Pichler et al. reported a significant association of iron levels with the TRF2 gene variant rs7385804 (CC genotype) and expression of the TF gene variant rs3811647 when analysed by the Bonferroni correction for multiple testing, along with iron homeostasis imbalance linked with the TF gene variant rs3811647 (AA genotype), the TMPRSS6 gene variant rs4820268 and the HFE gene variant rs1799945. The results showed an association of effect size in the same direction and magnitude of the TMPRSS6 gene variant rs4820268 (GG genotype) on hepcidin RNA found in blood levels and hepcidin in urine levels. Hepcidin, a peptide hormone, is one of the primary regulators of iron as it controls iron recycling and absorption and is involved in the manufacture of erythrocytes (Ganesh et al., 2009; Pichler et al., 2011).

Consequently, chronically high levels of hepcidin cause iron deficiency, with low levels of hepcidin causing iron overload, potentially leading to HH (Pichler et al., 2011). Therefore, the TMPRSS6 gene variant rs4820268 may influence the hepcidin iron feedback loop and suppress hepcidin and iron absorption. Thus, homozygous G carriers are more at risk of iron deficiency. The study needs future investigation using larger samples to produce higher power results to prove statistically significant findings.

2.3.4 Zinc and the SLC30A3 Gene (rs1126936)

Zinc is the second-most abundant essential mineral found in > 85% of human bones and muscle (Huang & Tepaamorndech, 2013). Over 300 enzymes use zinc (in the form of zinc ions Zn^{2+}) as a cofactor, called zinc metalloenzymes and used as catalysts to assist in protein synthesis and help maintain structure and stability. Zinc has antioxidant properties, is essential for the smooth running of the immune system and is involved in DNA production and metabolic processes. Zinc is found mainly in oysters, red meat, chicken, beans, fortified cereals, and dairy. It is not readily available as Zn^{2+}, limiting its bioavailability, affecting its absorption in the digestive process, and binding affinity to protein. Iron supplements may counteract dietary zinc absorption, with severe zinc deficiency causing poor appetite, metabolic disorders, cognitive and immune dysfunction and alopecia (Australian Government, National Health and Medical Research Council, New Zealand Ministry of Health, 2006). To improve zinc absorption, consuming a diet high in animal proteins instead of a diet high in plant proteins is beneficial. Therefore, it is vital for individuals on a restricted diet to check their consumption of dietary zinc to maintain zinc homeostasis at the cellular level (Huang & Tepaamorndech, 2013).

Zinc homeostasis is controlled by two zinc transporters (ZnT) or functional membrane proteins that help reduce intracellular zinc concentration (da Rocha et al., 2014). ZnT are encoded by the solute carrier family 30 member 3 (SLC30A) gene and the solute carrier family 39-member 14 gene (SLC39A). The SLC30A gene lowers cytosolic zinc levels by transporting zinc out of cells. Therefore, the solute carrier SLC30A gene can be used as a biomarker for zinc serum concentration levels.

A cross-sectional study by da Rocha et al. (2014) examined 56 Brazilian females and 14 Brazilian males aged 50 years and over to investigate the influence of SNP rs11126936 and the solute carrier gene SLC30A3 on zinc serum concentrations. The study found that zinc levels were statistically significantly lower ($P = 0.014$) in participants with the SLC30A3 gene variant rs11126936, genotype CC recorded at 0.74 ± 0.30 mg/L compared to carriers of the genotype AA or AC recorded at 0.89 ± 0.28 mg/L. They also found that zinc serum levels negatively correlated with age ($P = 0.008$), and the genotype CC carriers most often had lower zinc levels (34.5%) compared to the genotype AA and AC carriers (18.2%, $P = 0.024$). The study's limitations were small sample size, low power, no food-frequency questionnaires, diet recall, or anthropometric data. Therefore, this study needs to be interpreted with caution and will depend on future studies to further establish the influence of SNP rs11126936 and zinc serum levels alongside the mechanism of action.

Another study by Fujihara et al. (2018) examined zinc levels on 102 autopsied Japanese subjects and agreed with da Rocha et al. (2014) that the SNP rs 11126936 had an association with zinc serum levels. Still, further study is needed to verify this study's finding.

2.4 Dietary Fats and Associated Genetic-Risk Variant

This section will investigate the association between omega-6 and -3, energy balance, total fat, saturated fat, saturated and unsaturated fat and monounsaturated fat and the related SNP located in genes; fatty acid desaturase 1 gene (FADS1), mitochondrial uncoupling protein 1 gene (UCP1), transcription factor 7-like2 gene (TCF7L2), apolipoprotein A-II gene (APOA2), fat mass and obesity-related alpha-ketoglutarate dependent dioxygenase gene (FTO) and peroxisome proliferator-activated receptor γ2 gene (PPARγ2), respectively.

2.4.1 Omega-6 and -3 Polyunsaturated Fatty Acids and the FADS1 Gene (rs174547)

Omega-6 long-chain (≥ C20) polyunsaturated fatty acids (n-6 PUFA) in the form of linoleic acid (LA) and omega-3 (n-3 PUFA) in the form of alpha-linolenic acid (ALA) are essential fatty acids and obtained from the diet (Hoppenbrouwers et al., 2019). Oily fish and

fish supplements contain n-3 PUFA, and the two main ones are eicosapentaenoic acid (EPA, C20:5n-3) and docosahexaenoic acid (DHA, C22:6n-3). EPA and DHA are known for their anti-inflammatory effects and increased amounts in the diet may help reduce triglyceride (TG) levels (Hoppenbrouwers et al., 2019; Kaur et al., 2011).

N-3 PUFAs are TG and are broken down into fatty acids and incorporated into phospholipids, leading to numerous outcomes, including lower blood pressure and an increased lipoprotein lipase that helps break down TG, reducing serum triglyceride (Bäck, 2017). Pure EPA can help decrease LDL cholesterol, improve endothelial and arterial function, reduce platelet aggregation, increase plaque stability, and protect against myocardial ischemia, indirectly reducing CVD risk (Bäck, 2017; Chang & Deckelbaum, 2013).

PUFAs have pleiotropic functions in cells, including influencing gene regulation, energy production, and forming eicosanoids, the signalling molecules for signalling pathways (Jump et al., 2013). The retina photoreceptor cells and phospholipids in the brain's grey matter have high concentrations of PUFA, making them essential for vision, brain development, and the central nervous system (Innis, 2008; SanGiovanni & Chew, 2005).

Therefore, the human diet must contain sources of PUFA for beneficial health benefits, including brain function and development; deficiency when young can impair growth and produce a dry skin rash (Burdge & Calder, 2005; Innis, 2008). Substituting saturated fatty acids with n-6 PUFA can help lower total blood cholesterol and reduce CVD risk (Hoppenbrouwers et al., 2019). However, a high dietary intakes of n-6 PUFA, such as sunflower seeds, Brazil nuts, corn oil and sesame oil, and low intake of n-3 PUFA can raise TG levels and lower HDL levels and cause adverse health effects (Hoppenbrouwers et al., 2019; Kaur et al., 2011).

The FADS1 and fatty acid desaturase 2 (FADS2) genes in the fatty acid desaturase (FADS) gene cluster region encode the rate-limiting enzymes delta-5 and delta-6 desaturases (Tosi et al., 2014). Delta-5 and delta-6 are important enzymes for the biosynthesis of PUFA (18 carbon chain) to active long-chain PUFA (≥ 20 carbon chain) (Rahbar et al., 2018). Alteration of the enzyme's activity can affect fatty acids associated with T2DM and CVD (Lu, Feskens, Dollé et al., 2010; Tosi et al., 2014).

In a cohort study of 3,575 participants, measurements were performed on three variants in the FADS1 gene cluster region and analysis of n-6 and n-3 PUFA intake on plasma cholesterol levels (Lu, Feskens, Dollé et al., 2010). The results confirmed an association between the FADS1 gene variant rs174546, C allele (in linkage disequilibrium

with rs174547) and HDL and total cholesterol levels (*P* = 0.02) in participants with a high intake of n-6 PUFA (> 5.26% energy). Furthermore, participants with the CC genotype compared to the TT genotype (*P* = 0.004) had a lower HDL concentration than participants with a high intake of n-6 PUFA. Even though the study had a large sample size, there was no statistical significance between n-3 PUFA and the FADs1 gene variant rs174546, genotype TT, on cholesterol levels. More research on the FADS1 gene and its links to n-3 PUFA and n-6 PUFA performed on larger groups is needed to confirm the association.

A cohort study by Dumont et al. (2018) tested cholesterol levels of 3,069 individuals and found an association with the FADS1 gene variant rs174547, CC genotype (β = -0.05 mmol/L, *P* = 0.0002). The study reported that individuals with the CC genotype had lower levels of HDL cholesterol when intakes of n-6 PUFA (LA) were greater than 9.5 g/day compared with individuals with the TT genotype. Thus, individuals with the CC genotype might benefit by lowering their n-6 PUFA (LA).

Hellstrand et al. (2012) supported Dumont et al.'s (2018) results with their study on 4,635 subjects. They confirmed the C allele link to low levels of HDL-cholesterol levels (*P* = 0.03) in individuals with high n-6 PUFA levels (high ratio of n-6 PUFA: n-3 PUFA). Therefore, studies have confirmed the FADS1 gene encodes for the rate-limiting enzymes delta-5 and delta-6 desaturases, linked to n-3 PUFA and n-6 PUFA and HDL-cholesterol levels (Dumont et al., 2018; Hellstrand et al., 2012; Lu, Feskens, Dollé et al., 2010).

2.4.2 Energy Balance and the UCP1 Gene (rs1800592)

Brown adipose tissue (BAT) is a type of body fat that oxidises high amounts of lipids and glucose (Dalgaard & Pedersen, 2001). BAT produces heat energy and is activated to maintain thermoregulation and decrease reactive oxygen species (ROS). The UCP1 gene, classed as a 33 kDa integral mitochondrial protein, is found in BAT and helps control nonshivering thermogenesis (Cannon & Nedergaard, 2004).

The UCP1 facilitates proton transport across the mitochondrial inner membrane. During this process, the UCP1 uncouples respiration, increases the conductance across the membrane whilst dissipating excess heat energy and reducing ATP synthesis. (Cannon & Nedergaard, 2004; Dalgaard & Pedersen, 2001). Reducing ATP allows the maintenance of energy expenditure by stimulating the oxidation of fatty acids whilst protecting against oxidative stress caused by ROS (Brondani et al., 2012; Cannon & Nedergaard, 2004). There are different types of energy expenditure: resting metabolic rate (RMR) or basal/ resting energy expenditure (REE), physical exercise energy expenditure and thermogenesis energy

expenditure. RMR or REE is dependent on body fat, the concentration of hormones, genetic variation and the sympathetic nervous system (Brondani et al., 2012; Dalgaard & Pedersen, 2001).

Research suggests that the BAT function and UCP1 gene increases energy expenditure and decreases the mitochondrial membrane potential due to the UCP1 gene polymorphism of rs1800592 (-3826 GA, -1766GA, and -112AC) in the intraperitoneal adipose tissue (Brondani et al., 2012; Nagai et al., 2011; Vimaleswaran & Loos, 2010). Brondani et al. (2012) and Vimaleswaran and Loos (2010) suggested that carriers of the UCP1 gene polymorphisms rs1800592 (-3826 GA, -1766 GA, and -112AC) are at greater risk of multifactorial diseases, including obesity and T2DM; however, findings are still ambiguous.

Other studies have reported that UCP1 gene expression in the intraperitoneal fat lining of the abdominal cavity walls in obese weight subjects is 50% lower than normal-weight subjects, even though BAT in adults is commonly low (Virtanen & Nuutila, 2011). BAT activity and mass decline with age, especially in males, as they burn fewer calories than females (Pfannenberg et al., 2010). Therefore, the lack of clarity in outcomes of UCP1 genetic studies may be explained by differences in participants' age, sex, ethnicity, muscle mass, weight and lifestyle. Also, multifactorial influences of other genes might account for the abundance of controversial conclusions (Brondani et al., 2012).

A cohort study conducted by Nagai et al. (2011) used 82 Japanese females aged 20–22 years from the same university campus in Japan and genotyped for the UCP1 gene polymorphism -3826 GA (rs1800592). Nagai et al. explored the links of the UCP1 gene polymorphism -3826 GA (rs1800592) to REE and thermoregulation. The study monitored each participant's heart rate for their thermoregulatory activity of the sympathetic nervous system. Recorded results of the frequency rate of genotype AA was 0.27, genotype GA was 0.45, and genotype GG was 0.28. The fat-energy consumption in carriers of genotype GG was lower (26.0 ± 1.2%) compared to genotype AA (30.7 ± 1.1%) and genotype GA (31.3 ± 1.0%) with $P < 0.01$. The thermoregulatory activity of the sympathetic nervous system of carriers of genotype GG was the lowest (185) compared to genotype GA (333) and AA (313) with $P < 0.05$. The conclusion was that carriers of the G allele had lower REE/RMR (energy expenditure) and their energy needs were lower than the A allele carriers. A reduction in the thermoregulatory activity of the sympathetic nervous system in carriers with the genotype GG is linked to lower expression of the UCP1 gene. The association of the G allele to high LDL levels and low HDL levels suggests that the G allele influences lipid metabolism and

could affect appetite and food preference due to lipids usage in the BAT. Further investigation is needed using larger cohorts and a wider range of participants, especially male subjects who record stronger effects of BAT activity (Nagai et al., 2011; Pfannenberg et al., 2010).

2.4.3 Total Fat and the TCF7L2 Gene (rs7903146)

Dietary fat is an essential source of fatty acids and an energy-dense macronutrient vital for absorbing fat-soluble vitamins such as A, D, E and K (Ravisankar et al., 2015). Lipid triglycerides are the main component of fatty adipose tissue, and hydrolysis happens in the body during exercise or fasting in a process called lipolysis (Piers et al., 2003). During lipolysis, TGs are converted into fatty acids and glycerol and contain twice the caloric energy (37.6 kJ/g) of protein or carbohydrates (16.7 kJ/g) (Rolls, 2000). There are different types of fatty acids: saturated, monounsaturated, polyunsaturated (PUFA) and trans-unsaturated (Marchand, 2010). Studies have reported that consuming too much saturated or trans-fat can increase fatty adipose tissue leading to obesity and raised LDL levels. Elevated LDL levels clog up arteries, eventually causing atherosclerosis and increasing the risk of myocardial infarction or CVD. Trans-fat causes the decrease of HDL used to carry cholesterol away from arteries to the liver and excreted as bile into the intestinal tract (Li et al., 2015; Piers et al., 2003).

Therefore, the World Health Organisation has advised consuming more unsaturated fats, especially n-3 PUFA; less saturated fat; and eliminating or limiting trans-fat to less than 2.2 g / day (Ghebreyesus & Frieden, 2018; Marchand, 2010). Human deaths since 2010 from CVD linked to consuming manufactured trans-fat have risen to 537,200 per year and worldwide 7.7% (T. J. Wang et al., 2016).

Dietary fat is easy to overconsume, which is thought to be due to the influence of genes on satiety (sensation of fullness between meals) and satiation (sensation of fullness that develops while eating a meal) compared to protein or carbohydrates (S. B. Roberts, 2000; Rolls, 2000). The TCF7L2 gene has been linked to T2DM and obesity and may influence how a body responds to weight-loss diets when consuming different amounts of fats in the diet (Grau et al., 2010; Mattei et al., 2012). The TCF7L2 transcription gene for the proglucagon gene produces proglucagon to form glucagon-like peptide 1 (GLP-1) (Yi et al., 2005). GLP-1 is stimulated during consumption of food, and its main action is to secrete insulin and inhibit glucagon activity. Limiting glucagon reduces its breakdown in the liver to form glucose and thus lowers postprandial glucose release. GLP-1 regulates food intake and

controls hunger and features in T2DM research (Yi et al., 2005). Fat consumption appears to stimulate more GLP-1 as opposed to carbohydrates, whilst overstimulus of GLP-1 is thought to lead to hypoglycaemia and understimulus to obesity (Holst, 2007).

A 10-week RCT focused on the influence of the TCF7L2 gene variant rs7903146, and total fat consumption, examined the effect on body weight and insulin resistance (Grau et al., 2010). The study randomly selected 739 European obese participants aged 20–50 years from the more extensive Nutrient-Gene Interaction in Human Obesity Study. The female participants (75%) consumed either a high-fat diet (HFD) containing 40%–45% fat energy or a low-fat diet (LFD) containing 20%–25% fat energy. Both diets contained the same quantity of protein (15%) and carbohydrates (60%–65%). Results found that carriers of the genotype TT who consumed the LFD lost 2.57 kg less than carriers of the genotype TT who consumed the HFD. Consumers of the HFD with genotype TT lost 2.08 kg less than consumers of the HFD with the genotype CC or CT. Participants with genotype CC or CT recorded no weight loss when consuming either the LFD or HFD ($P = 0.35$). Therefore, individuals with the SNP rs7903146 and genotype TT respond more to LFD than HFD (Grau et al., 2010). This study presented factors that were difficult to control. For example, participants may have changed their behaviour during the study, termed the *Hawthorne Effect* (Mattei et al., 2012; Sedgwick & Greenwood, 2015). Keeping to the strict diet regime throughout the study period would have been challenging for participants (Grau et al., 2010). Thus, this research used post hoc analysis, and the interpretation of results could have been skewed, and further studies to support or refute these claims are needed.

A 2-year RCT examined 588 White adults aged 30–70 years using GWA data from the Preventing Obesity Using Novel Dietary Strategies intervention and studied the influence of the TCF7L2 gene variant rs7903146 on body composition (Mattei et al., 2012). Participants were randomising to different macronutrient diets which ranged in the percentage of fat, protein and carbohydrates as follows: LFD 20%, 15%, 65%; LFD 20%, 25%, 55%; HFD 40%, 15%, 45%, and HFD 40%, 25%, 30% respectively. During the trial, a dual-energy X-ray absorptiometry or DEXA scanner provided information on the changes in lean body mass (muscle mass, bones, and bodily fluids) versus fat mass. Findings reported a significant difference between the LFD participants with genotype CC who lost more lean mass at 6 months ($P = 0.035$) compared with the HFD participants with genotype CC. Therefore, lean fat maintenance and carriers of the genotype CC would not benefit from a LFD. There was no significant effect for carriers of the genotype TT on either the LFD or HFD. The Preventing Obesity Using Novel Dietary Strategies is one of the largest weight-

loss programmes and means the gene–nutrient interaction RCT could control for confounding factors such as medication, smoking and alcohol consumption. The study's limitations were that daily dietary values were not always complied with, and participants were mainly Caucasian adults.

2.4.4 Saturated Fat and the APOA2 Gene (rs5082)

The common apolipoprotein A-II protein, encoded by the APOA2 gene, is thought to impair the removal of excess cholesterol called reverse-cholesterol transport, out of cells, mediated by HDL (Corella et al., 2009). RCTs and epidemiologic studies have linked increased risk of atherosclerosis, heart disease, and obesity with saturated-fat consumption but only recently associated these risks with the APOA2 gene. Many gene–nutrient interaction studies have linked the APOA2 gene with saturated fat and its effect on hormonal control of food intake and weight management. (Zaki et al., 2013).

A meta-analysis study by Corella et al. (2009) examined the interaction of the APOA2 −265T>C gene variant on saturated-fat intake and obesity. The subjects, aged between 26–80 years, were from three independent study populations, the Framingham Offspring Study (FOS) (1,454 non-Hispanic White participants), the Genetics of Lipid-Lowering Drugs and Diet Network (GOLDN) (1,078 European participants) and the Boston-Puerto Rican Centers on Population Health and Health Disparities (BPR) (930 Puerto Rican participants). The results recorded were a positive association with the genotype CC and the occurrence of obesity among participants who consumed > 22 g of saturated fat daily in all three strata compared to genotype TC and TT. The odds ratio (OR) between the genotype CC was significant at 1.84 (95% CI [1.38, 2.47], $P < 0.001$) compared to genotype TC and TT when consuming a high-saturated-fat diet of > 22 g/day. The results only infer a gene–nutrient interaction between BMI and obesity in individuals who carry the APOA2 gene variant rs5085 (CC genotype) when combined with consuming a high-saturated-fat diet of > 22 g/day.

Zaki et al. (2013) conducted a cross-sectional study on the metabolism of lipids, body-fat distribution and the risk of obesity in a sample of 303 Egyptians aged 16 to 19. The study found that the CC carriers of the APOA2 gene variant −265T>C (renamed -492T>C) had a higher level of visceral adipose tissue, food consumption, waist, and body circumference than the TT or TC carriers.

Therefore, the influence of the APOA2 gene variant −265T>C and high-saturated-fat diets are possibly linked and fuel more nutrigenomic studies (Corella et al., 2009; Zaki et al.,

2013). These studies enhance the understanding of gene–nutrient interactions and are especially important for individuals with obesity who may benefit from this knowledge.

2.4.5 Saturated and Unsaturated Fat and the FTO Gene (rs9939609)

The FTO gene is associated with the body's response to saturated and unsaturated fat (C. M. Phillips et al., 2012; Rodrigues et al., 2015). Unsaturated fats contain double bonds and are usually of plant origin and liquids at room temperature (RT). In contrast, saturated fats have no double bonds and are typically of animal origin and solid at RT. Therefore, saturated-fat concentration is high in animal red meats and dairy products and linked to multifactorial diseases such as obesity, T2DM and CVD (C. M. Phillips et al., 2012). These diseases are associated with diet, environmental and polygenic traits, with studies showing that the FTO gene variant, rs9939609, has the strongest association with food intake (C. M. Phillips et al., 2012). Increased food intake of mono- and polyunsaturated fats found in seed oils has been linked to a plethora of health benefits as they reduce the risk of many multifactorial diseases (Australian Government, National Health and Medical Research Council, New Zealand Ministry of Health, 2006).

C. M. Phillips et al. (2012) conducted a prospective case-control study using 1,754 participants to investigate the FTO gene variant rs9939609 and its links to obesity and metabolic syndrome (MS). Participants were part of the French Diet, Genome and Metabolic Syndrome Project (LIPIENE). Diagnosis of MS uses measurement from three or more indicators: BMI, saturated-fat intake, waist circumference, TG, fasting blood sugar, blood pressure (BP) and HDL levels (C. K. Roberts et al., 2013). Measurements were taken for 2 months and followed up at 7.5 years. The study concluded that the carriers of the A allele had higher MS measurements (OR = 1.66, 95% CI [1.07, 2.57], $P = 0.02$) and carried a greater risk of obesity than the TT genotype. Waist circumference ($P = 0.04$) and BMI of ≥ 25 kg/m^2 ($P = 0.02$) were higher in the AA and AT genotype participants whose saturated-fat intake was ≥ 15.5%, and the saturated-fat ratio was > 0.38 compared to the TT genotype. C. M. Phillips et al. (2012) concluded MS measurements were higher when on a high-saturated-fat diet, making participants more at risk of obesity. Thus, AA and AT genotype participants were more sensitive to low-saturated-fat diets than genotype TT, suggesting a gene–nutrient interaction between saturated fat and the FTO gene.

C. M. Phillips et al. (2012) found gender-specific links and only observed statistically significant results for males genotyped AA and AT compared with male genotyped TT. The

female results were not significant. Due to the study using self-reporting questionnaires, it was prone to memory loss and inaccurate dietary accounts.

2.4.6 Monounsaturated Fatty Acids (MUFA) and the PPARγ2 Gene (rs1801282)

Oleic acid is the main MUFA and contains one (mono) double bond and is found in olive, peanut and canola oils, avocados, and nuts (Al-Goblan et al., 2014). Consuming foods high in MUFA may decrease the risk of obesity, insulin resistance and CVD by lowering LDL cholesterol. Insulin resistance results in a high concentration of nonesterified fatty acids (NEFAs) in plasma and low movement of glucose into the muscle tissues, eventually leading to T2DM (Al-Goblan et al., 2014; Australian Government, National Health and Medical Research Council, New Zealand Ministry of Health, 2006; Brassard et al., 2008).

The PPARγ2 gene polymorphisms of the ALa allele in Pro12Ala are associated with fat cells' formation in adipose tissue (Garaulet et al., 2011). Consequently, insulin resistance, obesity, body composition and weight loss have links to the gene PPARγ2.

Garaulet et al. (2011) analysed the possibility of PPARγ2 polymorphism Pro12Ala (rs1801282) interaction with fat intake and BMI by studying 1,465 overweight and obese Spanish subjects (89% completed the study). The subjects enrolled in a dietary, behavioural, nutritional education and activity treatment programme. The programme included support groups, a controlled daily Mediterranean-style diet containing 35% fat (10% saturated and 20% MUFA), 50% carbohydrates and 15%–20% protein and exercise scheduled 2–3 times per week. After the intervention, results indicated a direct gene–nutrient interaction between subjects with the PPARγ2 polymorphism Pro12Ala and MUFA intake associated with fat loss. Subjects with genotype GG or GC had a slower fat loss rate than genotype CC ($P = 0.003$). Along with this, it was detected that genotype GG or GC were less obese and had less body fat if the proportion of fat consumed consisted of ≥ 56% of MUFA compared to genotype CC (BMI $P = 0.039$ and body fat $P = 0.02$). Therefore, Garaulet et al. concluded that when genotype GG or GC consumed a MUFA diet comprising ≥ 56%, there was a more positive response to weight loss than genotype CC. However, the results were not statistically significant for all genotypes on a low-MUFA diet who consume less than 56% MUFA ($P = 0.75$). Based on these results, it is clear that there is no evidence that *PPAR*γ2 influences weight reduction in response to dietary treatment.

2.5 Protein and Associated Genetic-Risk Variant

Proteins are macronutrients made from long-chain polypeptides from a combination of 20 amino acids; some are essential, for example, histidine, isoleucine, leucine, and phenylalanine (Moon & Koh, 2020). Protein is found in animal and plant food and is vital for the human body for energy, growth and the repair of cells. Protein is essential for muscle mass (especially for the heart and brain), tissue function, a healthy immune system and gut function (Moon & Koh, 2020; Wolfe et al., 2018). Protein has been associated with the FTO gene variant rs9939609 (Wolfe et al., 2018).

2.5.1 Protein and the FTO Gene (rs9939609)

Consuming a high-protein diet (HPD) has been linked to lower body weight, decreased fat mass and increased satiety (Wolfe et al., 2018). HPD and increased satiety are associated with increased anorexigenic hormones and reduced orexigenic hormones. HPD is, therefore, considered a successful and safe method for weight loss and could benefit obese and overweight individuals who are at risk of T2DM and CVD (Australian Government, National Health and Medical Research Council, New Zealand Ministry of Health, 2006; Moon & Koh, 2020; Wolfe et al., 2018).

GWA studies have recognised and associated gene loci with an increased risk of obesity (Zhang et al., 2012). One of the most robust findings is the fat-mass and obesity-associated FTO gene linked to food intake. The FTO gene, expressed in the nuclei of the paraventricular (PVN) and dorsomedial (DMH) hypothalamus region of the brain, may impact metabolism and energy homeostasis in an individual (Pausova et al., 2009; Zhang et al., 2012). One of the hypothalamus's key roles in regulating appetite and balancing food intake is controlled by a complex signalling pathway of neuropeptidergic neurons (Zhang et al., 2012).

A 2-year RCT using participants from the Preventing Obesity Using Novel Dietary Strategies intervention used four different diets to investigate 742 obese adults (642 completing the trial), 61% women, 80% white, 15% black, 3% Hispanic, 2% Asian, aged 30–70 years (Zhang et al., 2012). Each participant was a carrier of the FTO gene variant rs1558902, which has linkage disequilibrium with the FTO gene variant rs9939609. The participants' tomography of body composition and fat were measured using DEXA. The study found that the intervention with genotype AA, who received 25% energy as protein, displayed the most modifying effects and recorded the most changes in their fat mass and adipose tissue (P = 0.05). Over the 2 years, participants with the genotype AA receiving the

25% HPD lost 1.5 kg ($P = 0.01$) compared to the individuals with the genotype TT or AT. The participants with genotype AA who consumed a lower 15% protein diet than the genotype AA on the 25% HPD did not lose as much body fat. Therefore, the study found that the FTO gene variant rs9939609 and a HPD were linked but not to the HFD, but how protein interchanges with the FTO gene are still unclear. Zhang et al. (2012) concluded that individuals with the genotype AA who consumed a HPD (25% energy) compared to those with AA on a low-protein diet lost more visceral adipose tissue over 2 years. The study had 80% White participants, whilst overadjusted statistical comparisons increased the type II error. Therefore, future studies comparing the FTO gene on obese individuals consuming different diets, using larger diverse ethnic groups, are needed.

An intervention followed 146 obese patients 5 years after bariatric surgery and found all patients to have a high frequency of the FTO gene variant rs9939609 (Rodrigues et al., 2015). Twenty-two percent of patients had a BMI of over 30 kg/m^2 and the AA genotype. Those with the FTO gene variant AA and AT had higher BMI than the genotype TT 3 to 5 years after surgery. Two years after surgery, the patients who achieved nonobesity, who had the genotype TT, were negatively related to genotypes AA and AT and associated with the FTO gene variant rs9939609. Therefore, participants with genotypes AA, AT, and TT influence bariatric surgery success and long-term weight loss (Rodrigues et al., 2015).

Other studies that have focused on the FTO gene of obese individuals include the Finnish Diabetes Prevention Study which reported no statistically significant results with changes in BMI or body fat when reducing fat or sugar and increasing fibre in a diet (Haupt et al., 2008; Lappalainen et al., 2009; Müller et al., 2008).

2.6 Lipoproteins and Associated Genetic-Risk Variant

This section will investigate the association between total cholesterol (TC), LDL cholesterol, HDL cholesterol, TG and the related SNP located in genes: apolipoprotein A5 gene (APOA5), ATP-binding cassette subfamily G member 8 gene (ABCG8), ATP-binding cassette subfamily A member 1 gene (ABCA1) and angiopoietin-like 3 gene (ANGPTL3), respectively.

Chylomicrons (CM), the most significant primary class of lipoproteins, are formed in the intestines and made from apolipoprotein components (Feingold & Grunfeld, 2015). CMs are involved with the absorption and transportation of dietary insoluble hydrophobic lipids from the small intestines into the bloodstream and then onto the muscles and adipose tissue. The lipids are then metabolised to fatty acids and glycerol and used for energy and fat

storage, with surplus cholesterol returning to the liver (Bayly, 2014; Feingold & Grunfeld, 2015).

Each lipoprotein transports certain types of lipids, CM transports triglycerides (TG) and cholesterol, VLDL transports TG; LDL transports most of the cholesterol (LDL-C) and are cholesterol-rich. HDL transports cholesterol (HDL-C) and removes cholesterol from the body by reverse-cholesterol transport (Bayly, 2014; Feingold & Grunfeld, 2015).

Lipoprotein profile is a measure of total cholesterol (total LDL-C and HDL-C), HDL-C, LDL-C, TG, total/HDL ratio and non-HDL-C that measures artery-clogging apoB particles such as VLDL, IDL and LDL (Auckland District Health Board, 2022).

For a healthy heart, it is advisable to keep total cholesterol < 5.0 mmol/L; total/HDL ratio < 4.5 mmol/L; LDL cholesterol < 3.4 mmol/L; HDL cholesterol > 1.0 mmol/L in men and > 1.3 mmol/L in women; TG < 2.0 mmol/L and non-HDL-C < 4.2 mmol/L. If a measurement of non-HDL-C is > 5.7mmol/L, this could indicate signs of genetic dyslipidaemia (Auckland District Health Board, 2022). Obesity and T2DM can also lead to secondary dyslipidaemia, and high levels of LDL or TG can result in hyperlipidaemia; therefore, routine lipoprotein profile checks are essential in adults to minimise the risk of coronary artery disease (CAD) or CVD (Lee et al., 2017).

Research has suggested SNPs can modify postprandial lipid metabolism that can be assayed by measuring apolipoproteins during the digestive process (Desmarchelier et al., 2013; Nakajima et al., 2014). Measuring the progress of the apolipoproteins during postprandial lipid metabolism whilst analysing an individual's SNPs could reveal interesting information about the interaction (Desmarchelier et al., 2014; Nakajima et al., 2014). For example, this information could help identify dyslipidaemia (high levels of LDL cholesterol or low levels of HDL cholesterol) in individuals who are more susceptible to the risk of heart disease or atherosclerosis, the leading factor of deaths globally (Rafieian-Kopaei et al., 2014).

2.6.1 Total Cholesterol (Total LDL-C and HDL-C) and the APOA5 Gene (rs662799)

Cholesterol is a vital component for cells' membranes, formation of vitamin D and a precursor of hormones and bile-acid synthesis (Röhrl & Stangl, 2013). Cholesterol is manufactured endogenously and is, therefore, not needed as a food source but is consumed by many in the form of dairy, animal, cakes, biscuits and pastry products (Cerqueira et al., 2016).

Cholesterol is a lipid attached to a protein to form lipoprotein and is circulated in the bloodstream by HDL and LDL particles (Cerqueira et al., 2016). HDL is helpful as it

transports surplus cholesterol found in peripheral cells, including macrophage foam cells (MFC so-called due to them being saturated with lipids) back to the liver in a process called reverse-cholesterol transport. MFC are one of the leading causes of atherosclerosis as surplus cholesterol causes high quantities of LDL-C and results in the build-up of atherosclerotic plaque in arteries leading to atherosclerosis and eventually CAD. Therefore, when total cholesterol is low, this is a favourable biomarker for individuals; and when the ratio of HDL-C levels are high, and LDL-C levels are low, this indicates a reduced risk of atherosclerosis, CAD and CVD (Mora et al., 2009; Rafieian-Kopaei et al., 2014; Röhrl & Stangl, 2013).

High total-cholesterol levels are associated with an unhealthy diet, lack of exercise, and genetic variation resulting in low lipid metabolism (X. Su et al., 2018). A critical gene for lipid metabolism is the APOA5 gene, a subunit of lipoproteins. The APOA5 gene is involved with the lipolysis of TG and is found in HDL, VLDL and chylomicrons circulating in the blood. There is increasing evidence that polymorphisms of the APOA5 gene may increase total cholesterol-plasma levels, leading to obesity, insulin resistance, MS, and eventually T2DM and CVD. Reports indicate that low quantities of plasma APOA5 have a negative correlation in obese individuals (Lu, Feskens, Boer, et al., 2010; X. Su et al., 2018).

Lu, Feskens, Boer, et al. (2010) used a longitudinal cohort study to examine SNPs across 243 genes in cholesterol metabolism by following 1,668 European participants over 11 years, taking three nonfasting cholesterol blood readings. The study concluded that participants with the APOA5 gene variant rs662799 (genotype CC and TC) had a total average cholesterol reading of 0.18 mmol/L higher than genotype TT. When analysing only genotype CC, the magnitude of readings was double ($P = 0.0066$) compared to genotype TC and TT (Lu, Feskens, Boer, et al., 2010). The study did not provide clear evidence for the relationship between the APOA5 gene variant rs662799 and total cholesterol levels, as environmental exposures of the participants could have influenced the results. Therefore, future studies performed on different ethnic groups and controlled for environmental exposures are needed to ascertain more proof.

2.6.2 LDL Cholesterol (LDL-C) and the ABCG8 Gene (rs6544713)

High LDL-C levels or “bad cholesterol” can lead to atherosclerosis and eventually CAD or CVD as the lipoproteins that carry LDL-C are cholesterol-rich (Röhrl & Stangl, 2013). Studies have shown that LDL-C levels and lipoprotein metabolism have links to diet, lifestyle and genetic factors, and research on the ABCG8 gene has inferred this. (Kathiresan et al., 2009). ATP-binding cassette SGM8, encoded by the ABCG8 gene, is a half-transporter

protein found in the liver and intestines. ABCG8 protein predominately moves cholesterol and plant sterols into the liver, which get absorbed into the bile acids. Excretion of the ABCG8 gene is via the intestines, and during this process, cholesterol absorption from the intestines reduces (Feingold & Grunfeld, 2015). The ABCG8 gene variant rs6544713, T allele, is associated with high levels of LDL-C. Therefore, the ABCG8 gene variant rs6544713 can elevate cholesterol uptake and lower secretion from the intestines (Acalovschi et al., 2006; Schroor et al., 2021).

Kathiresan et al. (2009) conducted a large-scale study, using data from seven GWA studies, the Framingham Heart, the London Life Sciences Prospective Population (LOLIPOP), Supplémentation en Vitamines et Minéraux Antioxydants (SUV1MAZ), Invecchiare in Chianti (inCHIANTI), Diabetes Genetics Initiative (DGI), Finland-United States Investigation of NIDDM Genetics (FUSION) and Sardinia Study of Aging (SardiNIA). The study screened 19,840 participants alongside a replication study of 20,623 participants. Meta-analysis results confirmed the link of 11 distinct loci, with LDL-C being close to several common variants; one was the ABCG8 gene variant rs6544713. The research concluded that the T allele carriers (TT and TC) were more at risk of high LDL-C than CC carriers. Also, that polygenic hypercholesterolaemia was associated with several common variants. The study implied that genetic variants are associated with cholesterol levels. Therefore, further investigations, involving different ethnicities, with rigorous experiment procedures are needed. Once this happens, the SNP rs6544713 can be used as an indicator for clinical application to help at-risk individuals with CAD and CVD.

A cross-sectional study by Schroor et al. (2021) investigated SNPs from 456 individuals, aged 18 years and over, associated with cholesterol absorption in the intestines. The study found that the ABCG8 gene variant rs6544713 significantly deviated from Hardy-Weinberg equilibrium ($P = 0.05$). The study's limitations were that it had low power due to the small sample size and the participants had differences in baseline characteristics. As the study only examined European participants, the small heterogeneity of the study meant the results were biased. Therefore, further research is needed to follow up on these findings.

2.6.3 HDL Cholesterol (HDL-C) and the ABCA1 Gene (rs1883025)

The medical literature has well reported that cholesterol plaque build-up on artery walls inhibits blood flow and causes atherosclerosis that usually has minimal symptoms until the plaque ruptures and forms a blood clot (Stewart et al., 2017). The blood clot can cause CAD and includes heart attacks, strokes or sudden death. Reducing CAD risk by following a

healthy diet and weight management plan is the best prevention (Feig et al., 2014; Stewart et al., 2017).

ATP-binding cassette transporter A1 is a protein encoded by the ABCA1 gene (Feingold & Grunfeld, 2015). The ABCA1 gene is involved in cholesterol metabolism, the cholesterol pump (involved in reverse-cholesterol transport), removal of excess cholesterol from cells and transfer of atherosclerotic plaques to HDL particles. Surplus cholesterol can then get transported through the plasma to the liver, and eventually excreted. Therefore, high HDL-C levels are negatively associated with atherosclerosis. HDL is involved with many other biological processes, including its interlinkage with TG and its antioxidant effects (Bandeali & Farmer, 2012; Feingold & Grunfeld, 2015).

Research has linked the ABCA1 gene variant rs1883025 with HDL-C plasma levels (Kathiresan et al., 2009). Using population data from GWA studies, Kathiresan et al. (2009) analysed 40,463 European individuals and associated individuals who were carriers of the T allele (TT and TC). Results showed that TT and TC carriers had a 0.08 standard deviation lower HDL-C levels ($P = 0.001$) than individuals with the CC genotype.

Nishida et al. (2020) conducted a cross-sectional observational study, part of the Japan Collaborative Cohort Study, and examined 2,231 Japanese men aged 35–69 years. They found that the ABCA1 gene variant rs1883025 and carriers of the C allele (TC and CC) who had high HDL-C had reduced function in men who performed low physical activity ($\beta = 0.008$) compared to men with medium ($\beta = 0.032$) or high levels ($\beta = 0.034$) of physical activity. Therefore, the C allele may not be beneficial in men with low activity schedules. Still, conversely, men who carry the C allele and have active physical programmes have high HDL-C levels. Further research on different ethnic populations is needed to provide more evidence for the association between the gene variant rs1883025 and the genotypes CC, TC and TT and to confirm causal inference.

2.6.4 Triglycerides (TG) and the ANGPTL3 Gene (rs10889353)

TG are tri-esters produced from glycerol and three (tri) fatty acids found in approximately 95% of dietary fats: saturated, unsaturated, PUFA (linoleic and linolenic acid), and trans-fats (Feingold & Grunfeld, 2015,). They are the main components of body fat and, once consumed, carried in CM particles secreted from the intestines into the blood and stored in the adipose tissues as fat. Fatty-acid excess is converted into TG and transported by VLDL particles from the liver to peripheral tissues and used in muscles or stored in adipose tissue.

Elevated levels of TG can lead to dyslipidaemia, obesity and an increased prevalence of CAD and CVD (Chen et al., 2021; Feingold & Grunfeld, 2015; Lee et al., 2017).

A prospective cohort study of 3,216 adult American Indians from the Strong Heart Study found that elevated TG levels and low HDL-C increased their risk of CAD and CVD (Lee et al., 2017). The findings found a 1.32-fold increase in the hazard ratio (HR) (95% CI [1.06, 1.64]) for CAD compared to adults with normal TG and high HDL levels. There was an even greater health risk of CAD for adults with diabetes of a 1.54-fold HR (95% CI [1.15, 2.06], $P = 0.003$) than for adults without diabetes.

The angiopoietin-like protein 3 (ANGPTL) encoded by the ANGPTL3 gene has been linked to TG levels as it helps to promote the release of fatty acids and glycerol from adipocytes found in adipose tissues (Chen et al., 2021). The ANGPTL regulates lipoprotein metabolism and inhibits lipoprotein and endothelial lipase activity, a catalyst involved in the hydrolysis of TG to fatty acids and glycerol. Therefore, endothelial lipase is an enzyme crucial for lipoprotein metabolism. Prevention of the lipase catalysts will slow the hydrolysis of TG-rich lipoproteins resulting in higher levels of TG and LDL-C in the blood plasma, linked to atherosclerosis and CVD (Chen et al., 2021; J. R. Mead et al., 2002; X. Su et al., 2018).

The ANGPTL3 gene variant rs10889353 was examined by Kathiresan et al. (2009) using population data from 40,463 European individuals. The study found that individuals who were carriers of the A allele (AA and CA) had a 0.05 standard deviation higher TG levels ($P < 0.0001$) than individuals with the CC genotype.

2.7 Eating Habits and Associated Genetic Risk Variant

This section will investigate the association between fat-taste perception, sugar preference and susceptibility to hunger and the related SNP located in genes: cluster determinant 36 gene (CD36), glucose transporter type 2 gene (GLUT2) and neuromedin beta gene (NMB), respectively.

Individuals consume a meal when they feel hungry until reaching fullness or satiation (Robinson et al., 2014). The hunger-satiation feedback mechanisms trigger many physiological and metabolic processes during digestion. This feedback begins with oro-sensory signals in the mouth, including food taste, texture, flavour, duration and quality, and is experienced during mastication of food, affecting food intake (Lasschuijt et al., 2021). During mastication, sensory signals are delivered to the higher cortex regions in the brain for taste and reward, influencing how much food an individual can consume until satiation is

reached (Bolhuis et al., 2012; Lasschuijt et al., 2021). Consequently, oro-sensory exposure is associated with regulation of food, weight management and eating habits. Observational studies have linked earlier satiation to more prolonged oro-sensory exposure when consuming certain textures or different tastes of foods (Robinson et al., 2014).

Research has shown that food intake may reduce when foods are highly sweet or salty due to earlier satiation (Bolhuis et al., 2012). With the rising rate of obesity and T2DM, there is a need to understand the mechanisms of action involved in food-consumption regulation and the influence of genetic variants (Eny et al., 2008).

2.7.1 Fat-Taste Perception and the CD36 Gene (rs1761667)

Oro-sensory signals influence how much fatty foods an individual can consume before feeling satiated, affecting a person's nutritional status (Melis et al., 2015). Fatty-food intake, fat-taste perception, and fat absorption influence the oral marker 6-*n*-propylthiouracil (PROP, genetic ability to taste bitterness) and the CD36 gene variant rs1761667 (Melis et al., 2015; Sollai et al., 2019). The CD36 gene is found in many cells and is involved in the lipid absorption and processing of fatty acids. The CD36 rs1761667 polymorphisms affect GA substitution and excess fatty-acid intake, and both are associated with the accumulation of fatty acids and dysfunction of metabolic processes resulting in MS (Bajit et al., 2020; Pepino et al., 2014).

During mastication of dietary fat to fatty acids, taste receptor "bud" cells express the CD36 gene and release lingual lipase, a salivary digestion enzyme (Keller, 2012). Therefore, the CD36 gene could influence physiological response to fat-taste perception (Keller, 2012; Pepino et al., 2014). The CD36 gene is also involved with dispensing oleic acid in the small intestines that turn into oleoylethanolamide and subsequently lowers food intake and production of chylomicrons (Pepino et al., 2014).

Bajit et al. (2020) performed a case-control study on obese individual carriers of the CD36 gene variant rs1761667, genotype AA. Results suggested that participants with the genotype AA had a lower fat-taste perception than genotype GA and GG. Keller's (2012) study reported that individuals with the genotype AA tasted more creaminess and wanted more fat added to salad dressings than genotype GA and GG. Therefore, genotype GA and GG individuals have been classed as "supertasters" as their oro-sensors can detect lower levels of fat and oil in foods consumed than AA genotype who are "typical-tasters" (Bajit et al., 2020; Keller, 2012; Pepino et al., 2014). These findings indicate that genotype AA carriers might be more at risk of obesity due to having a lower perception of fat detection.

Melis et al. (2015) conducted a RCT study on 64 Italian individuals aged 27 ± 0.85 years using a randomised taste test to examine an association between oro-sensory exposure to oleic acid and the association with the CD36 gene variant rs1761667. They used paper filters soaked in three different concentrations of oil with two containing oil and one containing oleic acid ranging from 0.0015 to 10 μL. Individuals were sorted into taster status according to the oral marker PROP and genotyped. Results indicated that genotype GG supertasters had a 5-fold lower detection of oleic acid ($P = 0.041$), and GA "intermediate-tasters" could detect low levels of oleic acid compared to genotype AA typical tasters. Therefore, there is a reduction of CD36 gene expression in genotype AA carriers. The study did have low power, but these observations could indicate that genetic variation in the CD36 gene might impact fat-taste perception. Still, more research is needed to investigate if this is linked to supertasters reducing dietary-fat intake due to earlier satiation (Melis et al., 2015).

A cross-sectional study by Lopez-Ramos et al. (2005) examined 441 West Mexican adults. Findings inferred an association between the CD36 gene variant rs1761667 (genotype AA) and consuming a HFD. Results indicated that cholesterol serum levels were higher overall in participants with genotype AA than in genotype GG or GA who did not have high cholesterol levels (OR = 2.75, 95% CI [1.33, 5.69], $P = 0.005$). This study has shown a direct link between fat intake and fat-taste preference with carriers of the genotype AA (Lopez-Ramos et al., 2005). Still, environmental factors, including consumption of alcohol, may enhance CD36 expression. Therefore, further research is needed to associate the CD36 gene and dietary-fat intake.

2.7.2 Sugar Preference and the GLUT2 Gene (rs5400)

Sugar or glucose is a vital fuel for the brain, which needs a constant supply. The hypothalamus controls blood glucose levels via glucose-sensing neurons, first pioneered by Mayer in 1955 with his glucostatic theory (Mayer, 1955; Routh et al., 2014). The hypothalamus and hormones such as insulin and leptin control the rate of glucose metabolism, releasing glucose from the bloodstream when needed and using fatty acids and proteins if glucose levels are too low. If glucose homeostasis becomes unbalanced due to low glucose levels, a neuroendocrine response is activated to release more glucose into the bloodstream to prevent hypoglycaemia. If blood glucose levels are high, insulin is released to move more glucose into cells or store it as glycogen in the liver or muscles to prevent hyperglycaemia (Mayer, 1955; Pénicaud et al., 2002; Routh et al., 2014). As glucose gets depleted in the body, the body responds similarly to feelings of hunger. Therefore, high

carbohydrate intake has been linked to reaching satiation quicker and reducing food intake sooner (Eny et al., 2008).

The solute carrier family 2 member gene (2SLC2*a*2) codes for the glucose transporter type 2 gene (GLU2) expressed in the hypothalamic glucose-sensing neurons and various other organs in the body (Eny et al., 2008). The GLU2 gene is associated with glucose homeostasis and insulin release during the postprandial state (Pénicaud et al., 2002). Research has focused on the association of the GLU2 gene variant rs5400 with the increased risk of T2DM, especially among carriers of the genotype TT or TC who have a greater desire for sugary foods (Eny et al., 2008; Pénicaud et al., 2002).

Eny et al. (2008) compared two Canadian populations, one (P1) comprised 50 men and 50 women, aged 42–75 years, with BMI 30.7 ± 4.2 kg/m^2 from the Diabetes Multicentre Intervention Study, who had early T2DM. Two (P2) comprised 182 men and 405 women, aged 20–29 years, part of the Toronto Nutrigenomics and Health Study. Eny et al. (2008) reported that P1 individuals with the GLUT2 gene variant rs5400, genotype CT or TT had a greater daily sugar consumption than genotype CC. Participants were assessed over a 3-day food diary, a food-frequency questionnaire (FFQ) and two on-site visits 2 weeks apart (*visit 1*: 112 ± 9 vs 86 ± 4 g/day, $P = 0.01$; *visit 2*: 111 ± 8 vs 82 ± 4 g/day, $P = 0.003$). P2 participants with the genotype CT or TT had a higher consumption of sugar over 1 month (131 ± 5 vs 115 ± 3 g/day, $P = 0.007$) compared to genotype CC. The study concluded that the GLUT2 gene variant rs5400 is associated with increased sugar consumption and could account for sugar craving in certain individuals (Eny et al., 2008). The oro-sensory signals that control the regulation of glucose intake need further investigation as elevated sugar craving has links to other factors, including mood, environment, culture, lifestyle and pleasure-generated response (Routh et al., 2014). Further RCT-type research is needed to control individual food intake as FFQ are sometimes unreliable.

2.7.3 Susceptibility to Hunger and the NMB Gene (rs1051168)

Much research focused on eating behaviours and obesity compares different diets, such as low-GI diet versus high-GI carbohydrate diet (Rashid et al., 2015). More recent studies have turned to genes linked to eating disorders (S. B. Roberts, 2000). One stressor resulting in an eating disorder such as obesity is hunger which is negatively associated with weight-control success (Pekkarinen et al., 1996). Hunger is a complicated process that is not fully understood; the hypothalamus partly controls hunger, dependent on glucose levels, the

emptiness of the stomach and intestines, and ghrelin, the “hunger hormone” (Pénicaud et al., 2002; Rashid et al., 2015).

Physiological responses that downregulate eating behaviours have links to the NMB gene (Bouchard et al., 2004). The NMB protein, encoded by the NMB gene, is linked to eating behaviours and could affect the ability to maintain weight management, leading to obesity. The NMB receptor proteins get expressed in the visceral adipocyte cells in adipose tissue, suggesting that fat deposits could regulate food intake. (Bouchard et al., 2004; Yang et al., 2003).

A study conducted by Bouchard et al. (2004) focused on genes associated with eating habits and obesity and used the three-factor eating questionnaire (3FEQ). Stunkard and Messick (1985) created the 3FEQ in 1985 to study eating behaviours by measuring three components: dietary restraint, disinhibition and hunger. Overweight and obese individuals usually score higher on the disinhibition and hunger questions than normal-weight individuals. Bouchard et al. (2004) used participants from the Quebec Family Prospective Study that involved 274 men and 386 women aged 27–58 years with BMIs of 20.3 to 37.9 kg/m^2. The study aimed to identify numerous trait loci involved in eating behaviours. The study reported that the most significant linkage between locus and susceptibility to hunger ($P < 0.0001$) was the NMB gene variant rs1051168, genotype TT, compared to genotype GG or GT. The study revealed a missense mutation resulting in a genetic variation within the NMB gene located on SNP rs1051168. This SNP was positively associated (OR = 1.9, 95% CI [1.15, 3.06], $P = 0.01$) with eating behaviours in obesity phenotypes. The study’s 6-year follow-up reported that carriers of the genotype TT gained more than twice as much body fat than those carrying GG or GT genotype (3.6 compared with 1.5 kg; $P < 0.05$). The results show a significant association between the NMB gene variant rs1051168, genotype TT and eating behaviour with a predisposition to obesity. The limitation of the study was the use of FFQ which are prone to recording errors by participants. Also, for complex behaviours such as eating, it is unlikely that a single SNP could account for these results fully. Therefore, more research is needed to identify other mutations in the NMB gene, or in that location, linked to eating habits and hunger.

2.8 Regulation of Blood Glucose and Associated Genetic-Risk Variant

This section will investigate the association between glucose and insulin and the related SNP located in genes: adenylate cyclase 5 gene (ADCY5) and insulin-receptor substrate 1 gene (IRS1), respectively.

The pancreas's endocrine hormones regulate blood glucose levels in the body (Almgren et al., 2017). Within minutes of eating, the body secretes hormones: glucagon, insulin, somatostatin, amylin, gut hormones and incretins from enteroendocrine cells. These hormones enter the bloodstream to help regulate blood glucose balance by negative feedback. Insulin increases glucose uptake by signalling the muscles to use the glucose for energy or transport it to the liver, where excess glucose gets stored as glycogen in fat cells. If the body does not produce enough insulin, Type 1 diabetes (T1DM) develops. When the body does not respond to insulin, the body becomes insulin resistant, and insulin-producing cells called pancreatic beta cells are less responsive. If the body has a high fasting insulin level, it will, if left untreated, result in T2DM (Almgren et al., 2017; Dupuis et al., 2010). Much research has been focused on Westernised populations as there is a higher frequency of elevated glucose levels, which increases the risk of T2DM (Dupuis et al., 2010). Obesity is a causal factor in developing insulin resistance, T2DM and MS (C. K. Roberts et al., 2013).

2.8.1 Fasting Glucose and the ADCY5 Gene (rs11708067)

The ADCY5 gene encodes the enzyme adenylate cyclase, which catalyses cyclic AMP 5 (Dupuis et al., 2010). Signalling in the pancreas triggers insulin secretion, dependent on the generation of cyclic AMP. The ADCY5 gene variant rs11708067 has been linked to elevated fasting glucose levels (Almgren et al., 2017; Dupuis et al., 2010).

Dupuis et al. (2010) combined 21 GWA studies, using 46,186 European nondiabetic subjects, that included loci associated with fasting glucose near the ADCY5 gene. Meta-analysis after adjustments for BMI demonstrated that the ADCY5 gene was associated with elevated fasting glucose levels of 0.027 mmol/L in A allele carriers ($P = 0.0001$). Therefore, A allele carriers had an increased risk of T2DM compared to G allele carriers. The study's limitations are that the findings could have been due to other factors influencing the results, such as the diet and lifestyle of the patients, and a cause-and-effect relationship may not be solely due to the rs11708067 SNP.

2.8.2 Fasting Insulin and the IRS1 Gene (rs2943641)

A study examining adipocytes reported that in obese individuals, their IRS1 gene mRNA concentration was less than in leaner individuals (Kovacs et al., 2003). Further investigation on the IRS1 gene has reported its possible association with insulin levels and T2DM.

Almgren et al. (2017) performed a population-based cohort study using 3,344 Swedish participants born between 1923 and 1950 and 4,905 Finnish participants. The study compared circulating insulin with identical phenotypes using an oral glucose tolerance test and blood samples after 30 and 120 minutes. The association of SNPs with insulin levels was analysed using linear regression models, and Cox regression models for prediction of risk of T2DM, and adjusted for sex and age. The study searched for a link between nondiabetic participants and fasting insulin levels and found that a location near the IRS1 gene variant rs2943641 showed a significant association ($P = 2.4 \times 10^{-7}$). Almgren et al. concluded that participants who carried the CT or CC genotypes had greater fasting insulin concentrations than the TT genotype. The study's finding could have been due to other factors such as the diet and lifestyle of the patients, and a cause-and-effect relationship may not be solely due to the rs2943641 SNP.

2.9 Conclusion

The Human Genome Project has provided information about the genome's structure and function and identified around 25,000 protein-coding genes (Chial, 2008; Karczewski et al., 2020; Moore, 2020). Many factors, including specific nutrients, cause genetic polymorphisms, producing gene variants. The nutrigenomics approach to examining individual variation and identifying nutrition response and risk has prompted much research (Moore, 2020). Developing SNP arrays to detect polymorphisms due to gene–nutrient interactions has resulted in extensive GWA studies on different genotypes. These studies have linked many gene–nutrient interactions, such as the FTO gene related to lipid profile which could help prevent overweight individuals from becoming obese and developing MS (Reddy et al., 2018).

Many of the observational studies using genome-wide genetic variants data discussed in the literature review had limitations. The loss of participants and the low occurrence rate of the genetic variant in the population resulted in bias, reducing the validity of results and causing low statistical power. For example, Zhang et al. (2012) performed a 2-year RCT, part of the Preventing Obesity Using Novel Dietary Strategies intervention, and concluded that the FTO gene and a HPD were linked. This RCT started with 742 participants but lost 100, an attrition rate of 13.5%. An attrition rate of $< 5\%$ results in slight bias, whilst $> 20\%$ has validity issues, but every participant lost can cause significant bias (Nunan et al., 2018).

Therefore, increasing statistical power for observational GWA studies may require the enrolment of many hundreds of participants to detect a difference. However, if the genetic

variant has low occurrence, this could still result in low numbers of carriers with the required genotype. For example, Allen et al. (2008) studied iron-overload-related diseases and their association with the C289 gene variant rs1800562. From a genome-wide sample set of 31,192, they successfully genotyped 29,676 for this study. A random stratified sample of 1,438 generated only 203 individuals (74 men and 84 women) who were C289 homozygous carriers. By the end of the study, there was a positive correlation between 21 men and one woman who had iron-overload-related diseases associated with the C289 gene variant rs1800562. The results highlight the problem when examining high-risk SNP variants in a cohort that only exists in a low frequency in the total population, making it difficult to adequately power the studies to detect effects. As is evident in Allen et al.'s (2008) study, only 0.05% of the population are carriers of the high-risk C289 gene variant rs1800562, A allele (gnomAD, n.d.; NCBI, 2005).

The studies involving food-consumption measures, such as Grau et al.'s (2010) examining total fat, proved challenging to monitor, especially when subjects were off-site. Another challenge is controlling participants' behaviour when they participate in a study, termed the Hawthorne Effect; and maintaining strict adherence to the protocols throughout the study period is difficult (Eny et al., 2008; Sedgwick & Greenwood, 2015). Self-reporting questionnaires are prone to memory loss and inaccurate dietary accounts. Some studies only used young, healthy subjects, for example, Nagai et al.'s (2011) study on energy balance. Some studies had confounding variables, as demonstrated in the vitamin D studies, as levels are dependent on other factors such as biological nongenetic factors and environmental conditions (Slater et al., 2017; T. J. Wang et al., 2010). Therefore, the results are unreliable and need to be replicated in a larger population.

Most of the studies reviewed needed further investigation involving different ethnicities and population groups and tighter, more rigorous experiment procedures. For example, the studies conducted by Kathiresan et al. (2009) on LDL-C, HDL-C and TG only used participants of European ancestry.

Due to the ever-increasing amount of epidemiological research performed all over the globe, on similar topics, using meta-analysis or systematic reviews to integrate findings from nutrigenomic studies would be beneficial (Page et al., 2020).

Chapter 3: Methodology

3.1 Study Design

This study is a small subsection of a more extensive investigation: An acute, blinded, randomised cross-over design intervention to compare beef, lamb and a meat analogue on digestive, metabolic and nutritional outcomes, 31 May 2022, PREPRINT (Version 1) available at Research Square (https://doi.org/10.21203/rs.3.rs-1640468/v1). The extensive investigation was registered as a universal trial number: U1111-1244-9426. The clinical trial prerecruitment (Ref: NCT04545398) was carried out under the auspices of the University of Auckland. The study was approved by the New Zealand Ministry of Health's Health and Disability Ethics Committees (Ref: 19/STH/226) and conducted following the ethical standards in the 1964 Declaration of Helsinki (Appendix C).

Thirty young, healthy males (20–34 years), 20 normal weight, nine overweight and one obese, participated in an experimental study, and they consumed a standardised breakfast meal. Blood and plasma samples were collected and analysed before and hourly for 4 hours after the meal to assess nutrient concentrations. Buccal swabs were collected and analysed using the Illumina assay technique to assess SNPs. An online visual analogue 100-point scale was used to assess appetite scores upon arrival, immediately following ingestion, 30 minutes after ingestion and then hourly for 4 hours after ingestion.

This thesis describes a pilot experimental study to examine the present and emerging knowledge of genetic variation and determine whether this may influence postprandial digestive responses to a meat meal. In particular, it assessed the participants' genetic variants and considered whether they influenced an individual's postprandial digestive response to a standardised breakfast meal.

3.2 Study Setting

The trial was conducted between October and December 2020 at the University of Auckland Clinical Research Centre. The study setting was used to examine the digestion and metabolism of essential nutrients and subjective qualities of the meal experience, such as appetite and gastrointestinal scores.

3.3 Eligibility Criteria

All participants were omnivores willing to consume a standardised breakfast meal (Appendix D). The study excluded participants with chronic health conditions,

hyperlipidaemia, BMI ≥ 30 kg/m^2, use of medications (except occasional use of nonsteroidal anti-inflammatory drugs and antihistamines), history of anosmia and ageusia (issues with taste and smell), current dieting or disordered eating pattern, smokers and recreational drugs users. Participants completed an online screening which included the Three-Factor Eating Questionnaire-R18 (TFEQ) and a health survey. Participants with a TFEQ score greater than 75% were excluded because underlying psychological issues potentially influence their perception of food (Appendix D).

3.4 Recruitment and Informed Consent

Males were recruited from the millennial generation (20–34 years), as males typically have a greater postprandial lipid response than females (Chan et al., 2013). In general, millennials were chosen as this population demographic has been demonstrated to have the most significant variation in meat intake (Lairon et al., 2007). Recruitment occurred via posters around the University of Auckland and social media sites (Facebook). Informed consent was collected by research staff following participant inquiry and provision of information (Appendix E).

3.5 Sample Size

The current thesis is a pilot investigation with a sample size of 30 participants and was based on the principal biomarker for the more extensive investigation for postprandial change in LCPUFA concentrations in blood chylomicrons (namely 20:4 n-6, 20:5 n-3, 22:5 n-3, 22:6 n-3). An estimate of 29 enabled detecting the slightest worthwhile change of 0.5 mmol/L from baseline to 5 hours postmeal consumption (Linderborg et al., 2013). The current investigation is observational by design but includes a range of SNPs. The study may not be sufficiently powered to detect weak gene–nutrient association, and, as such, we consider this investigation a pilot study.

3.6 Randomisation and Blinding

The design was an experimental study, and participants consumed a standardised breakfast meal. Staff responsible for meal preparation, blood collection and analysis were blinded to the intervention, as were participants.

3.7 Meal Preparation

The breakfast meal was a burrito wrap of grain-fed beef with vegetables and sauce, served hot. The meat was a 220 g raw serving of minced beef (approximately 160 g cooked),

in line with the World Cancer Research Fund International (n.d.). The agency recommendation on red meat consumption suggests limiting the weekly red meat consumption to 350–500 g cooked. A minimum quantity of 100 g of cooked meat is required to ensure adequate fat intake to assess postmeal lipid dynamics (Linderborg et al., 2013).

The breakfast meal was prepared according to standardised recipes by the research dietitians and served at the test kitchen site in the University of Auckland Clinical Research Centre. The recipe was analysed using the New Zealand food database for macronutrient status using Foodworks 10 Professional software (Xyris). The meat was grain-fed New Zealand beef, specifically slaughtered, minced, packaged and stored at the research centre for this trial. The meat was minced to ensure homogeneity between participants, and all other food items were purchased at a local supermarket. The nutritional value of the standardised meal is provided in Table 6 and the nutrient composition per 100 g of the cooked meal is provided in Table 7.

All participants fasted for 10 hours prior to consuming the test meal, which was given in the morning, as postprandial lipid responses are greatest at this time (Lairon et al., 2007).

One researcher, who was not involved with the meal preparation, took the allocated mince (220 g per person) from the freezer the day prior and placed it in the test kitchen refrigerator. They added 2 teaspoons of brown sugar per 220 g to the meat as a requirement to produce isocaloric meals for the larger investigation (data not reported in this thesis). A researcher who was blind to the allocation prepared the meal. The food preparation strictly adhered to food safety regulations (Australian Government, National Health and Medical Research Council, New Zealand Ministry of Health, 2006). Each participant had the same breakfast meal prepared in the university kitchen. Salter scales were used to measure the exact quantities per person: 220 g of mince, 54 g of chopped brown onion, 72 g chopped red capsicum, 67 g of canned corn kernels, two jumbo tortilla wraps, 1/6 jar of salsa and salt and pepper for seasoning. The onions were fried in a teaspoon of oil, using an electric wok, until tender, before adding the mince. Cooking of the mince continued until it reached a temperature of at least 70°C (checked with a PUREQ solo probe food thermometer). Next was the addition of the capsicum, corn, salsa and seasoning. The meal was left to simmer for 10 minutes before equally dividing the mixture into two portions onto two flat tortillas. The tortillas were folded and placed under a sandwich grill until toasted and then wrapped in aluminium foil and served. After the trial, a sample test meal was sent for nutritional analysis (Tables 5 & 6).

3.8 Collection of Data

Participants were asked to maintain a normal lifestyle and physical activity schedule before the study. Reminder text messages were sent to participants the night before the visit to ask them to remain fasted from 9 pm (only water was allowed for the rest of the evening). Participants arrived at the clinical research facilities at 7.30 am. A researcher carried out the collection of baseline data, before food consumption, on weight (kg), height (cm), BP (systolic and diastolic mm/Hg taken using a HEM7130 digital meter) and heart rate (beat/min) (Table 4; see also Appendix B). An Automated Self-Administered Recall System (ASA24 24-hour Australia 2016 edition, https://asa24.nci.nih.gov) food-recall questionnaire and appetite scores using an online visual analogue 100-point scale were completed upon arrival, immediately following ingestion, 30 minutes after ingestion and hourly for 4 hours after ingestion.

A cannula was inserted into the antecubital vein of the forearm. Venous blood was collected into ethylenediaminetetraacetic acid (EDTA) tubes immediately prior to the meal (t-pre) and at four-time points postprandially (60, 120, 180, and 240 min). Participants were instructed to consume the provided breakfast meal within 15 minutes. Blood samples were centrifuged at 1,500 g for 15 min at 4°C. Plasma samples were aliquoted and stored at -80°C for later analysis.

Participants recorded their 24-hour dietary food recall using the ASA24, using a specific password. ASA24 provides analysis on energy and essential macro- and micronutrients and is a proven valid method (Kirkpatrick et al., 2014). Nutrient composition of participants' dietary intake the day prior to the clinic visit based on 24-hour dietary recall is provided in Table 5.

3.9 Genetic Analysis

A direct-to-practitioner biotechnology company, Nutrigenomix (2020), analysed each participant's buccal swab sample. A customised nutritional report details nutrient metabolism, eating habits, and food intolerances based on genetic variation and genotype. The report indicated how susceptible a person is to food-related traits based on their risk variant. According to company literature, the accuracy of the genetic-test results is between 99.7%–100%.

Oragene DNA ON-500 collection kits were used to collect DNA from each participant's cheek cells and used for their genetic analysis. The buccal swab stick was taken out of the blue top screw cap collection tube and held in the middle, while the tip ends were

rubbed on the inside of the participant's cheek about six times. The swab was then placed back into the collection tube and sealed. The researcher labelled each collection tube with the participant's research number and the unique identification collection tube number was noted. The researcher then sent all the specimens to the Nutrigenomix CLIA-certified and CAP-accredited (College of American Pathologists) laboratory centre at the University of Sydney, Australia. The iPLEX Gold assay with mass spectrometry-based detection on the Sequenom MassARRAY platform (Agena Bioscience) was used for all genotyping. The Illumina assay technique is used to find mutations in the DNA by identifying different insertions of the addition or deletion of a base to determine the risk variant (Sachidanandam et al., 2001).

The qualitative genetic risk results from Nutrigenomix were low, typical, elevated, enhanced, or diminished genetic risk. Low or typical risk indicates a low or normal response to a genetic variant, elevated, enhanced, or diminished genetic risk indicates an increased response to a genetic variant.

3.10 Subjective Analysis

An online visual analogue 100-point scale was used to assess the physiological measure for each participant's postprandial appetite changes associated with fat-taste perception, sugar preference, and hunger, and correlated with the qualitative genetic risk.

Appetite scores were analysed using an online visual analogue scale previously validated for use in single-meal investigations (Flint et al., 2000). The participants completed appetite scores upon arrival, immediately following ingestion, 30 minutes after ingestion and then timed with blood sampling for 4 hours.

3.11 Digestive and Biochemical Analysis

Postprandial digestive responses were analysed using each participant's physiological outcome measures, or markers that corresponded to the dietary component, and correlated with the qualitative genetic SNP associated with published literature and after review by the research team (Tables 2 & 3; see also Appendix A).

Participants' plasma samples at baseline / premeal (t-pre) were used to assess the physiological measure for concentrations of vitamin D, iron, zinc, total cholesterol, LDL, HDL, triglycerides, glucose and insulin (Table 2).

Participants' postprandial changes in LCPUFA (18:2 n-6, 18:3 n-6, 18:3 n-3) were used to assess the physiological measure for concentrations in the CMRF for dietary intake of omega-6 and -3 (Table 2).

Participants' BMI was used as the physiological marker related to body composition and energy balance (Table 2). The categories of BMI were: Underweight < 18.5 kg/m^2, Healthy/Normal 18.5–24.9 kg/m^2, Overweight 25–29.9 kg/m^2 and Obese > 30 kg/m^2 (National Heart Foundation of New Zealand, 2022).

Table 2

Gene and Associated, Reference Marker, Genetic Variant, Gene Frequency, RDI, Physiological Measures and Reference Intervals (RI 95% Prediction Interval)

Gene	Variant and risk ref. marker	Genetic variant[a]	Ref[b] allele Alt[c] allele & frequency in global population	Recommend daily intake (RDI)	Physiological outcome measures	Ref. intervals
CYP2R1	rs10741657	AA typical GG or GA elevated risk	Ref A = 0.38 Alt G = 0.62	Meet RDI 5.0 µg	Resting premeal vitamin D conc.	< 25mod-severe deficiency 25–50 mild dif. 50–150 normal > 250 intoxication nmol/L
GC	rs2282679	TT or TG typical risk GG elevated risk	Ref T = 0.72 Alt G = 0.28	Meet RDI by increasing bioavailable sources		
SLC17A1	rs17342717	CC low risk CT typical TT elevated	Ref C = 0.92 Alt T = 0.84	If elevated risk does not exceed RDI of 8 mg iron, keep vit C to minimum monitor serum iron levels	Resting premeal plasma iron conc. umol/L	Hb < 90 mg/L Hb Medscape Male: 14–18 g/dL or 8.7–11.2 mmol/L Fe 10–30 umol/L (adults) Fe Medscape: Male: 80–180 mcg/dL or 14–32 µmol/L
HFE (C282Y)	rs1800562	GG low risk AG typical AA elevated	Ref G = 0.95 Alt A = 0.05			
HFE (H63D)	rs1799945	CC Low risk GC typical GG elevated risk	Ref C = 0.86 Alt G = 0.14			
TMPRSS6	rs4820268	GG or GA typical risk AA elevated	Ref G = 0.46 Alt A = 0.54	If elevated meet RDI of 8 mg iron + vit C	Resting premeal plasma iron conc. umol/L	Hb < 90 mg/L Fe 10–30 umol/L (adults)
TFR2	rs7385804	CA typical CC or AA elevated risk	Ref C = 0.36 Alt A = 0.64			
TF	rs3811647	GA or GG typical risk AA elevated	Ref G = 0.67 Alt A = 0.33			
SLC30A3 Zinc transporter	rs11126936	AA or AC typical CC elevated risk	Allele freq. G-T 0.35	Meet RDI 14 mg. If elevated keep to RDI by increasing bioavailable sources	Resting premeal zinc conc. mg/L	10.7–18.3 umol/L or 2.5–4.3 mg/L fasting
FADS1	rs174547	CC or CT elevated risk	Ref T = 0.67 Alt C = 0.33	Reduce ratio of n-6 PUFA: n-3 PUFA aim for RDI n-3 (ALA) of 1.3 g & n-6 (LA) 13 g	AUC essential fatty-acid concentration of C18:2.n-6, C18:3.n-6 & C18:3.n-3	No RI used median AUC
UCP1	rs1800592	AA typical risk. GG or GA diminished low resting metabolic rate (RMR) calorie needs are lower	Allele freq. T-C 0.39	If BMI > 25 keep to estimated energy requirements (EER) = basal metabolic rate (BMR) x physical activity levels (PAL) or lower daily kcal by 200–400 daily	Body mass index	Underweight < 18.5 kg/m2 Normal weight 18.5–24.9 kg/m2 Overweight 25–29.9 kg/m2 Obese 30+ kg/m2
FTO	rs9939609	TT or AT typical risk AA enhanced risk	Ref T = 0.60 Alt A = 0.40	Protein diet 15%–25% or RDI 52g If BMI > 25 aim for HPD 25%–35%	Body mass index	See above
TCF7L2	rs7903146	CC or CT typical risk TT enhanced risk	Ref C = 0.71 Alt T = 0.29	20%–35% fat energy/day If BMI > 25 aim for LFD 15%–25% fat energy/day	Body mass index	2.6–24.9 (fasting) mU/mL (mU/L x 6.95 = pmol/L)
APOA2	rs5082	TT or TC typical CC elevated risk	Allele Freq G-A 0.69	Saturated & Trans-fat 8%–10%/day essential for genotype CC & keep to total-fat RDI	Resting premeal LDL cholesterol mmol/L	< 3.4 mmol/L
FTO	rs9939609	TT typical risk AA or AT enhanced risk	Ref T = 0.60 Alt A = 0.40	Saturated & Trans-fat 8%–10%/day essential for genotype TA or AA and 5% PUFA and keep to total-fat RDI	Body mass index	See above

Gene	Variant and risk ref. marker	Genetic variant[a]	Ref[b] allele Alt[c] allele & frequency in global population	Recommend daily intake (RDI)	Physiological outcome measures	Ref. intervals
PPARγ2	rs1801282	CC typical risk GG or GC enhanced risk	Ref C = 0.90 Alt G = 0.10	Aim for balance of saturated, MUFA & PUFA. If BMI > 25 aim for 50% of total fat intake from MUFA	Body mass index	See above
APOA5 component of HDL	rs662799	TT typical risk CC or TC elevated risk	Allele freq. G-A 0.90	Typical risk of high total cholesterol Increased risk of high total cholesterol and if > 5mmol/L & adjust lifestyle	Resting premeal total cholesterol mmol/L	< 5.0 mmol/L
ABCG8 cholesterol transporter	rs6544713	CC typical risk TT or TC elevated risk	Ref T = 0.30 Alt C = 0.70	Typical risk of high LDL-C If elevated increased risk of high LDL-C	Resting premeal LDL-C mmol/L	< 3.4 mmol/L
ABCA1 cholesterol transporter	rs1883025	CC typical risk TT or TC elevated risk	Ref C = 0.74 Alt T = 0.26	Typical risk of low HDL-C Elevated risk of low HDL-C	Resting premeal HDL-C mmol/L	Men > 1.0mmol/L Women > 1.3mmol/L
ANGPTL3 lipid metabolism	rs10889353	CC typical risk AA or CA elevated risk	Ref A = 0.68 Alt C = 0.32	Typical risk of high TG Elevated risk of high TG	Resting premeal TG mmol/L	< 2.0 mmol/L
CD36	rs1761667	AA typical taste GG or GA enhanced taste	Ref G = 0.48 Alt A = 0.52	Typical ability to taste fat Enhanced ability to taste fat	AUC fat-craving questionnaire (appetite)	Genotype AA carriers might be more at risk of obesity especially if BMI 25+ as they have lower fat-taste perception
GLUT2	rs5400	CC typical risk CT or TT elevated risk	Allele Freq G-A 0.15	Typical risk for high sugar intake Elevated risk for high sugar intake	AUC sugar-craving questionnaire (appetite)	Genotype CT or CC might be more at risk of obesity especially if BMI 25+as they have elevated sugar intake preference
NMB regulates eating behaviour	rs1051168	GG or GT typical risk TT elevated risk	Ref G = 0.73 Alt T = 0.27	Typical susceptibility to hunger Elevated susceptibility to hunger	AUC fullness, hunger and how much can you eat questionnaire (appetite)	Genotype TT might be more at risk of obesity especially if BMI 25+ as they have elevated susceptibility to hunger
ADCY5 insulin secretion	rs11708067	GG typical risk GA or AA elevated risk	Ref A = 0.79 Alt G = 0.21	Typical risk of high fasting glucose Elevated risk of high fasting glucose	Resting premeal glucose plasma conc. mmol/L	<2.8 hypoglycaemia (HG) mmol/L 2.8–3.4 mild HG 3.5–5.4 normal fasting 5.5–6.0 borderline 6.1–6.9 prediabetes < 7.0 diabetes
IRS1 insulin signalling	rs2943641	TT typical risk CT or CC elevated risk	Ref T = 0.34 Alt C = 0.66	Typical risk of high insulin Elevated risk of high insulin	Resting premeal insulin conc. μU/mL	2.6–24.9 (fasting) mU/mL (mU/L x 6.95 = pmol/L)

Note. Dietary component reference intervals retrieved from Auckland District Health Board (2022). BMI retrieved from National Heart Foundation of New Zealand (2022). Data for reference allele, alternative allele and frequency allele retrieved from gnomAD (n.d.); and NCBI (2005), from population groups, European, African, African others, African American, Asian, East Asian, Other Asian, Latin American 1, Latin American 2, South Asian European.

[a]The qualitative genetic risk results were low, typical, elevated, enhanced, or diminished genetic risk. Low or typical risk indicates a low or normal response to a genetic variant, elevated, enhanced, or diminished genetic risk indicates an increased response to a genetic variant.

[b]Reference (Ref) allele frequency is the base found in the reference genome and is not always the major allele.

[c]Alternative (Alt) allele frequency is the base found at the locus, other than the reference allele.

For blood and plasma analyses, blood samples were centrifugated at 1,500 g for 15 minutes at 4°C. An aliquot of plasma was maintained at 4°C for the CMRF separation to occur within 6 hours with the remaining stored at -80°C until analysis.

The fatty-acid composition of the CMRF was analysed by the fatty-acid methyl esters (FAME) assay (Milan et al., 2016; M. Su et al., 2019).

The analysis of plasma vitamin D used ultra-high-performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) at the Liggins Institute, Auckland, New Zealand (Sharma et al., 2019).

Plasma samples were prepared under the fat-soluble vitamin procedure and analysed using the fat-soluble vitamin liquid chromatography-mass spectrometry (LC-MS) method (Khaksari et al., 2017) at AgResearch, New Zealand.

The inductively coupled plasma mass spectrometry (ICP-MS) process assessed iron and zinc at Analytica Laboratories, Hamilton, New Zealand. The plasma samples were digested in aqua regia on a hot block for 2 hours. Following digestion, Type 1 water was added to dilute the sample 50-fold total. Samples were analysed on a Perkin Elmer ICP-MS fitted with a CETAC autosampler. Internal standard and carrier solution were introduced into the instrument using an ESI peristaltic pump, and were combined with the sample prior to injection into the instrument by the nebuliser. The plasma was formed using argon gas. Both standard and kinetic energy discrimination (KED) modes were used, with helium gas being introduced into the collision cell for operation of KED mode, to remove polyatomic interferences where required. The instrument was calibrated using a 1-point calibration, and this calibration was verified with a range of internal quality controls.

Plasma glucose, cholesterol (total, LDL, HDL) and triglyceride were measured using a Roche Cobas c311 by enzymatic colorimetric assay and insulin was measured by an electrochemiluminescence immunoassay.

3.12 Statistical Analysis

Results were analysed using contingency tables and a chi-square test to compare the participants' genetic risk with the calculated median value from the 30 participants' corresponding physiological measures (Appendix F). Count scores of the low and high physiological values were recorded, along with the high- and low-risk genetic variants. The median value was used to split and triage the data into two halves and is considered more robust to outliers (del Campo-Albendea & Muriel-García, 2021).

The choice of physiological variables was considered the best matched for the associated SNP as reported in the literature review and after review by the research team (Table 3 and Appendix A).

A chi-square test for independence was used to determine if the physiological measure and the genetic risk of the associated SNP gene variant were related. This method uses a 2 x 2 contingency table for comparing two variables to see if they are related. The data calculated in the table must be 5 or greater to test for statistical significance established as $P \leq 0.05$ (McDonald, 2014). If they are not related, the variables are independent and have no influence. If they are related the variables are dependent on each other.

Median values were calculated using each participant's physiological parameters (n = 30) from their baseline / premeal (t-pre) recorded measures or BMI measures or the AUC measures. The AUC values were calculated using all-times recorded data from each participant's physiological measure. The data was imported into GraphPad Prism (Version 9.2), which used the trapezoidal method for AUC time-point differences and corrected for baseline values.

Each participant's quantitative physiological measures were categorised as either greater than or less than/equal to the median value. Their qualitative genetic risk was categorised as either normal or increased risk and values input into a 2 x 2 contingency table. A chi-square test was then used to determine if there was a statistically significant relationship between the physiological measure and the genetic risk of the SNP genetic variant.

3.13 Digestive Response and Corresponding Genetic Risk Variant Based on Published Associations

Table 3

Physiological Output Measures and the Corresponding Genetic-Risk Variant Based on Published Literature and Nutrigenomix, 2020.

Physiological measure & related gene variant	Analysis of genetic response based on published associations for participants in this study	Analysis of digestive response for participants in this study
Vitamin D and the CYP2R1 and GC gene (rs10741657 and rs2282679)	We might expect participants with the CYP2R1 gene variant AA (rs10741657) and the GC gene variant TT or TG (rs2282679) to have a low risk of vitamin D deficiency; they should follow the RDI of 5.0 µg/day for males aged 19–50 years. Participants with CYP2R1 gene variant GG or GA (rs10741657) and the GC gene variant GG (rs2282679) are at an elevated risk of vitamin D deficiency. To reduce this risk, recommendation of the RDI and the consumption of food sources rich in vitamin D.	CYP2R1 gene variant AA and the GC gene variant TT or TG typical variants, we might expect baseline plasma vitamin D levels to be 50–150 nmol/L.
Iron overload and the HFE gene (rs1799945, rs1800562) and SLC17A1 gene (rs17342717)	We might expect participants with the HFE gene variant SLC17A1, rs17342717 or C282Y, rs1800562 to be at a high to medium risk of iron overload and HH. These participants should not exceed the RDI of 8 mg/day for males aged 19–70 years and should keep vitamin C to a minimum as this enhances absorption of iron from the diet and increases iron overload. Participants with HFE variant H63D, rs1799945, who have a low risk of iron overload, should keep to the RDI of iron. For participants who have the SNPs rs17342717, rs1800562, or rs1799945, consultation with their doctor and monitoring serum iron levels is advisable.	We might expect typical baseline plasma levels of whole blood haemoglobin to be less than 90 mg/L and plasma iron levels to be 10–30 µmol/L.
Low iron and the TMPRSS6 gene (rs4820268,), TRF2 gene (rs7385804) and TF gene (rs3811647)	We might expect participants with the gene variants; TMPRSS6 gene variant GG or GA (rs4820268) could influence hepcidin RNA found in blood levels and hepcidin in urine levels. Whilst the TRF2 gene variant CC (rs7385804) could affect iron serum levels, and TF gene variant AA (rs3811647) could affect iron homeostasis, consequently, all are at risk of low-iron status. Therefore, all participants should follow the RDI of 8 mg/day for males aged 19–70 years. Participants with low iron need to increase iron levels by the consumption of food sources rich in haem iron and vitamin C to enhance iron absorption. If following a restricted diet, supplementation may be advisable but do not go above the UL of 45 mg/day.	We might expect typical baseline plasma levels of whole blood haemoglobin to be less than 90 mg/L and plasma iron levels to be 10–30 µmol/L.
Zinc and the SLC30A3 gene (rs1126936)	We might expect participants in our investigation with the SLC30A3 SNP rs11126936 AA or AC genotype to have a typical risk of having low zinc-plasma levels. These participants should follow the RDI of 14 mg/day. Participants with genotype CC could be at an elevated risk of low zinc-plasma levels and, if following a vegetarian diet, are most at risk. To reduce this risk, they should consume foods high in zinc.	With the typical variant genotype AA or AC, we might expect fasting plasma zinc levels to be 10.7–18.3 µmol/L.
Omega-6 and omega-3 polyunsaturated fatty acid and the FADS1 gene (rs174547)	We might expect participants in our investigation with the FADS1 gene variant CC or CT (rs174547) to have lower circulating HDL levels. HDL levels will be lower still if the ratio of dietary intake of n-6 PUFA is high compared to dietary intake of n-3 PUFA. Therefore, participants should consume adequate intake (AI) of n-3 PUFA (ALA) of 1.3 g/day and for n-6 PUFA (LA) of 13 g/day. If they have the elevated risk variant CC or CT, reducing the n-6 PUFA: n-3 PUFA ratio will be beneficial.	We might expect plasma levels of n-6 PUFA to be higher than n-3 PUFA.
Energy balance and the UCP1 gene (rs1800592)	We might expect participants in our investigation with the UCP1 gene variant -3826 GG or GA (rs1800592) to have a lower energy expenditure (REE/RMR) and their energy needs to be lower than AA genotype. Participants with GG or GA genotype may need to reduce their intake of calories if overweight or obese according to their BMI, whilst increasing their output energy (Nagai et al., 2011; Pfannenberg et al., 2010). If the participants are overweight or obese, aiming for energy balance strategies and healthy lifestyle choices is recommended to maintain healthy body weight (Hill et al., 2012). To work out an individual's estimated dietary energy requirement (EER), calculate basal metabolic rate (BMR) x physical activity levels (PAL) (Australian Government, National Health and Medical Research Council, New Zealand Ministry of Health, 2006).	With the typical variant, AA genotype, we might expect a normal BMI if following a healthy diet and active lifestyle.

Physiological measure & related gene variant	Analysis of genetic response based on published associations for participants in this study	Analysis of digestive response for participants in this study
Total fat and the TCF7L2 gene (rs7903146)	We might expect participants in our investigation with the TCF7L2 gene variant TT to benefit from consuming a low-fat diet (LFD) (15% to 25% fat energy). A LFD and reducing saturated and trans-fats will help with weight loss and body-composition maintenance, especially if they are obese or overweight. A recommendation for obese or overweight participants with genotype CC or CT is to consume between 20% to 35% fat energy per day to help maintain lean fat as they do not respond to LFD (Foster & Wilson, 2013; Grau et al., 2010; Mattei et al., 2012).	With the TCF7L2 typical gene variant CC or CT, we might expect a normal BMI if following a healthy diet and active lifestyle.
Saturated fat and the APOA2 gene (rs5082)	We might expect participants in our investigation with the APOA2 gene variant CC compared to genotype TT or TC, most at risk of obesity, mainly if saturated-fat intake is high. All genotypes should reduce this risk by consuming a minimum of 8% to 10% /day of saturated fats and trans-fats. To maintain a healthy weight for genotype CC or TC, consume 20% to 35% of dietary fat and genotype CC 15% to 25% dietary fat.	With the TT or TC typical variant, we might expect baseline plasma levels of LDL cholesterol to be less than 3.4 mmol/L.
Saturated and unsaturated fat and the FTO gene (rs9939609)	We might expect participants in our investigation with the FTO gene variant AA or AT compared to genotype TT to be more responsive to reducing dietary intake of saturated fat. If these participants are overweight or obese increasing PUFA, especially n-3 PUFA will help with the lowering of BMI. Consuming less saturated fat and trans-fat and keeping to a minimum of 8% to 10% /day would benefit all genotypes. AT or AA genotypes would also benefit from consuming 5% PUFA / day and keeping to total-fat RDI.	With the TT typical variant, we might expect a normal BMI if following a healthy diet and active lifestyle.
Monounsaturated fatty acid (MUFA) and the PPARγ2 gene (rs1801282)	We might expect participants in our investigation with the PPARγ2 polymorphism Pro12Ala (rs1801282) genotype GG or GC to have a more significant weight and body fat loss if they consume a higher dietary intake of MUFA than genotype CC participants. Therefore, nutritional benefits for weight management for participants with genotype GG or GC would be consuming ≥ 50% of total fat intake from MUFA (Garaulet et al., 2011). Even though they are not responsive to a high-MUFA diet, participants with genotype CC would benefit from balancing their intake of total fat with a combination of fats. Mainly, saturated, MUFA, and PUFA would lower their risk of a multifactorial disease, especially if their BMI > 25 kg/m^2.	With the CC typical variant, we might expect a normal BMI if following a healthy diet and active lifestyle.
Protein and the FTO gene (rs9939609)	We might expect participants in our investigation with the FTO gene variant AA (rs9939609) along with a BMI of 25 to 30+ kg/m^2 to benefit from consuming a moderate- to high-protein diet (HPD) (25% to 35% protein energy) which may result in a more significant weight loss and reduced fat mass, compared to individuals on a low-protein diet (LPD) with genotype AA or carriers of the FTO gene variant TT or AT (Zhang et al., 2012). Participants with genotype TT or AT along with BMI of 25 to 30+ kg/m^2 may benefit from consuming a moderate to LPD (15% to 25% protein energy) to reduce fat mass (Zhang et al., 2012). For all other participants with a normal BMI, keeping to the RDI of protein of 52 g/day is advisable.	With the TT or AT typical variant, we might expect a normal BMI if following a healthy diet and active lifestyle.
Total cholesterol (total LDL-C and HDL-C) and the APOA5 gene (rs662799)	We might expect participants in our investigation with the APOA5 SNP rs662799 TT genotype to have a typical risk of high total cholesterol levels. They should ensure their levels do not exceed 5.0 mmol/L. Participants with the genotype CC or TC have an elevated risk of high total cholesterol. They should monitor their levels making sure to keep below 5.0 mmol/L and, if necessary, reduce all high cholesterol foods and adjust their diet and lifestyle according. Therefore, to control cholesterol levels, they should consume low quantities of saturated and trans-fats and increase PUFA, exercise, and keep stress low. Keeping LDL-C levels low and HDL-C levels high will reduce the risk of CAD and CVD especially, if overweight or obese.	With the typical variant genotype TT, we might expect fasting concentrations of total cholesterol levels to be < 5.0mmol/L and Total/HDL ratio < 4.5mmol/L.
LDL cholesterol (LDL-C) and the ABCG8 gene (rs6544713)	We might expect participants in our investigation with the ABCG8 gene variant SNP rs6544713 genotype CC to have a typical risk of high levels of LDL-C. CC genotype carriers should ensure their levels do not exceed 3.4 mmol/L. Participants with the genotype TT or TC have an elevated risk of high LDL-C levels and should monitor their levels ensuring levels are kept below 3.4 mmol/L. If necessary, they should adjust diet and lifestyle according, especially if overweight or obese, as these are the major modifiable risk factors of high LDL-C.	With the typical variant genotype CC, we might expect fasting concentrations of LDL levels to be < 3.4mmol/L.
HDL cholesterol (HDL-C) and the ABCA1 gene (rs1883025)	We might expect participants in our investigation with the ABCA1 gene variant SNP rs1883025 CC genotype to have a typical risk of low levels of HDL-C. CC genotype carriers should keep their levels > 1.0 mmol/L. Participants with the genotype TT or TC have an elevated risk of low HDL-C levels. They should monitor their levels, ensuring they keep them > 1.0 mmol/L, if necessary, adjusting diet and lifestyle according, especially if overweight or obese.	With the typical variant genotype CC, we might expect fasting concentrations of HDL levels to be > 1.0 mmol/L.

Physiological measure & related gene variant	Analysis of genetic response based on published associations for participants in this study	Analysis of digestive response for participants in this study
Triglycerides (TG) and the ANGPTL3 gene (rs10889353)	We might expect participants in our investigation with the ANGPTL3 gene variant SNP rs10889353 CC genotype to have a typical risk of high levels of TG, and they should make sure their levels keep < 2.0 mmol/L. Participants with the genotype AA or CA have an elevated risk of high TG and should monitor their levels, ensuring to keep them > 2.0 mmol/L. If necessary, they should adjust diet and lifestyle accordingly, especially if overweight or obese, as these are the major modifiable risk factors of high TG.	With the typical variant genotype CC, we might expect fasting concentrations of TG levels to be > 2.0 mmol/L.
Fat-taste perception and the CD36 gene (rs1761667)	We might expect participants in our investigation with the CD36 gene variant SNP rs1761667 AA genotype, termed typical tasters of fat, to have a typical ability to taste fat. Participants with the genotype GG or GA (supertasters) have an enhanced ability to taste fat (Bajit et al., 2020; Lopez-Ramos et al., 2005; Melis et al., 2015).	With the typical variant genotype AA, we might expect their fat-craving questionnaire answers to be higher than genotype GG or GA. Consequently, genotype AA carriers may be more at risk of being overweight or obese (Bajit et al., 2020; Lopez-Ramos et al., 2005; Melis et al., 2015).
Sugar preference and the GLUT2 gene (rs5400)	We might expect participants in our investigation with the GLUT2 gene variant SNP rs5400 CC genotype to have a typical preference for sugar intake. Participants with the genotype TT or CT have an elevated high preference for sugar intake and, if necessary, adjust diet and lifestyle according, especially if overweight or obese (Eny et al., 2008; Pénicaud et al., 2002; Routh et al., 2014).	With the elevated variant genotype TT or CT, we might expect sugar-preference questionnaire answers to be higher than for genotype CC. Therefore, CT or TT genotype carriers are more at risk of being overweight or obese, especially if BMI is 25+ kg/m2 due to higher intake of sugary foods (Eny et al., 2008; Pénicaud et al., 2002; Routh et al., 2014).
Susceptibility to hunger and the NMB gene (rs1051168)	We might expect participants in our investigation with the NMB gene variant SNP rs1051168 GG or GT genotype to have a typical susceptibility to hunger. Participants with the genotype TT have a psychologically driven elevated susceptibility to hunger. Therefore, adjust diet and lifestyle, especially if overweight or obese (Pénicaud et al., 2002; Rashid et al., 2015).	With the elevated variant genotype TT, we might expect their susceptibility to hunger to be higher than genotype GG or GT. Therefore, the TT genotype may be more at risk of being overweight or obese due to higher appetites, especially if BMI is 25+ kg/m2 (Eny et al., 2008; Pénicaud et al., 2002; Routh et al., 2014).
Fasting glucose and the ADCY5 gene (rs11708067)	We might expect participants in our investigation with the ADCY5 gene variant SNP rs11708067 GG genotype to have a typical fasting glucose level. Participants with the genotype GA or AA are more likely to have an elevated fasting glucose level. They may have to adjust diet and lifestyle, especially if overweight or obese (Dupuis et al., 2010).	With the elevated variant genotype GA and AA, we might expect their fasting glucose level to be higher than the typical variant genotype GG. GA or AA genotype may be more at risk of T2DM or being overweight or obese, especially if BMI is 25+ kg/m2 due to a higher concentration of fasting blood glucose (Dupuis et al., 2010).
Fasting insulin and the IRS1 gene (rs2943641)	We might expect participants in our investigation with the IRS1 gene variant SNP rs2943641 TT genotype to have a typical risk of high insulin levels. Participants with the genotype CT or CC are more likely to have an increased risk of elevated insulin levels. They may have to adjust diet and lifestyle, especially if overweight or obese (Almgren et al., 2017).	With the variant genotype CT or CC, we might expect them to be more at risk of high levels of insulin concentrations than the typical variant genotype TT. Therefore, CT or CC carriers may be more at risk of T2DM, especially if BMI is 25+ kg/m2 (Almgren et al., 2017; Kovacs et al., 2003).

Note. Nutrient reference values for Australia and New Zealand recommended by Australian Government, National Health and Medical Research Council, New Zealand Ministry of Health (2006) (see Appendix G). Plasma levels retrieved from Auckland District Health Board (2022). Published risk variants see Appendix A. Analysis of Genetic response based on published associations for participants in this study retrieved from Nutrigenomix (2020).

3.14 Funding

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Chapter 4: Results

4.1 Participant Characteristics

The collection of baseline data was conducted before food consumption (Table 4; see also Appendix B).

Table 4

Participant Characteristics

Characteristics at baseline	Mean (n = 30)	Standard deviation
Anthropometry		
Age (years)	28.0	3.8
Body weight (kg)	76.6	10.0
Body height (cm)	176.6	5.8
BMI (kg/m^2)	24.5	2.7
Systolic pressure (mm Hg)	117.3	11.7
Diastolic pressure (mm Hg)	75.7	9.0
Heart rate (bpm)	67.4	10.0

4.2 Nutritional Evaluation of Meal

The nutrition composition of participants' dietary intake the day prior to the clinic visit, based on 24-hour dietary recall, is presented in Table 5. The breakfast-meal nutrient composition was balanced for energy, carbohydrate, and fibre according to the food database analysis. A sample test meal was saved for later nutritional analysis; the nutritional composition of the actual meal sample is presented in Tables 6 and 7.

Table 5

Nutrient Composition of Participants' Dietary Intake the Day Prior to the Clinic Visit Based on 24-Hour Dietary Recall

Nutrient	Nutrient composition	
	Mean (n = 30)	Standard deviation
Energy (kJ)	9,956	3,910
Protein (g)	117	44
Total fat (g)	102	73
Carbohydrates (g)	238	95
Fibre (g)	24	12

Table 6

Nutritional Value of the Standardised Breakfast Meal Based on the Standardised Recipe

Nutrient	Nutrient value
Energy (kJ)	3,419
Protein (g)	61
Fat (g)	30
Saturated Fat (g)	12
Carbohydrates (g)	71
Fibre (g)	29
Sodium (mg)	983

Table 7

Nutrient Composition per 100 g of the Standardised Breakfast Meal Based on Actual Nutrition Composition of the Test Meal

Nutrient (unit per 100g)	Nutrient composition per 100g
Crude protein (%)	11.2
Fat (%)	6.7
Carbohydrates (%)	18.4
Total dietary fibre (%)	1.1
Sugars (g)	4.0
Sodium (g)	0.3
Iron (mg)	< 2.0
Zinc (mg)	1.4
Cholesterol (mg)	26.0

4.3 Digestive Responses Analysed Using Participant Data for Physiological Outcome Measures and the Corresponding Genetic-Risk Variant

The analysis of participants' data from plasma measures at baseline and physiological parameters for vitamin D, iron (iron overload and low iron), zinc, total cholesterol, LDL, HDL, triglycerides (TG), glucose and insulin and the corresponding qualitative genetic-risk statement was completed (Tables 8, 15 & 16).

Table 8

Combined Participant Data Including Plasma Measures at Baseline, the Physiological Parameters and the Corresponding Count Score of the Genetic Variant

Physiological marker – plasma measure	Mean value using n = 30 plasma measures	Standard deviation	Ref. marker & Gene variant	Median[a] value using n = 30 plasma measures	Total number of participants with[b]				Chi-square	*P* value
					Normal response ≤ median	Normal response > median	Increased response ≤ median	Increased response > median		
Vit D ng/mL	19.70	8.76	Vitamin D rs10741657 rs2282679	17.26	0	3	15	12	3.333	0.07
Iron µmol/L	17.22	6.09	Iron overload rs17342717 rs1800562 rs1799945	16.73	15	13	0	2	2.143	0.14
Iron µmol/L	17.22	6.09	Low iron rs4820268 rs7385804 rs3811647	16.73	7	10	8	5	1.222	0.27
Zinc mg/L		0.10	Zinc rs11126936	0.85	9	8	7	6	0.002	0.96
Total cholesterol mmol/L	4.60	0.73	Total cholesterol rs662799	4.41	9	11	7	3	1.674	0.20
LDL mmol/L	2.91	0.69	LDL rs6544713	2.84	11	9	4	6	0.600	0.44
HDL mmol/L	1.47	0.41	HDL rs1883025	1.41	9	7	6	8	0.536	0.46
TG mmol/L	1.14	0.65	TG rs10889353	1.00	1	0	14	15	1.034	0.31
LDL mmol/L	2.91	0.69	Sat Fat rs5082	2.84	13	15	2	0	2.143	0.14
Glucose mmol/L	4.96	0.41	Glucose rs11708067	5.04	1	2	13	14	0.238	0.63
Insulin µU/mL	7.72	4.13	Insulin rs2943641	6.89	7	0	8	15	9.130	2.50×10^{-3}

[a]Median values were calculated from the participants' physiological parameters (n = 30) using their baseline/ premeal recorded data. Each participant's physiological measures were categorised as either greater than or less than/equal to the median value.

[b]The qualitative genetic risk results were low, typical, elevated, enhanced, or diminished genetic risk. Low or typical risk indicates a low or normal response to a genetic variant, elevated, enhanced, or diminished genetic risk indicates an increased response to a genetic variant.

The analysis of participants' data for postprandial changes in LCPUFA (18:2 n-6, 18:3 n-6, 18:3 n-3,) concentrations in the CMRF for dietary intake of omega-6 and -3 and the corresponding qualitative genetic-risk statement was completed (Tables 9 & 17).

Table 9

Combined Participant Data From Postprandial Changes in Long-Chain Polyunsaturated Fatty Acid (LCPUFA) Concentrations in the Chylomicron-Rich Fraction of Omega-6 and -3 and the Corresponding Qualitative Genetic Risk

Physiological marker - plasma measure AUC[a] & ref. marker	Mean using n = 30 plasma measures	Standard deviation	Ref. marker & gene variant	Median[b] using n = 30 plasma measures	Number of participants with[c] Normal response ≤ median	Normal response > median	Increased response ≤ median	Increased response > median	Chi-square	*P* value
LCPUFA C18:2n-6	222.09	125.18	Omega -6 & -3 rs 174547	184.10	7	8	8	7	0.133	0.72
LCPUFA C18:3n-6	2.41	2.22	Omega -6 & -3 rs 174547	1.72	6	9	8	7	0.536	0.46
LCPUFA C18:3n-3	12.78	7.74	Omega -6 & -3 rs 174547	9.59	7	8	8	7	0.133	0.72

[a]AUC, area under the curve.

[b]Median values were calculated from the participants' physiological parameters (n = 30) using AUC data. The median AUC values were calculated using all-times recorded data (t-pre, t60, t120, t180, t240) of each participant's physiological measure, then categorised as either greater than or equal to/less than the median baseline values before performing chi-square.

[c]The qualitative genetic risk results were low, typical, elevated, enhanced, or diminished genetic risk. Low or typical risk indicates a low or normal response to a genetic variant, elevated, enhanced, or diminished genetic risk indicates an increased response to a genetic variant.

Participants' BMI was used to assess physiological parameters related to body composition and energy balance and the corresponding qualitative genetic-risk statement (Tables 10 & 18).

Table 10

Participants' BMI Measures to Assess Physiological Parameters and the Corresponding Qualitative Genetic Risk

BMI[a] kg/m^2	Mean (n=30)	Standard deviation	Ref.marker & Gene variant	Healthy BMI[b] (maximum)	Number of participants with[c] Normal response ≤ 25	Normal response > 25	Increased response ≤ 25	Increased response > 25	Chi-square	*P* value
BMI	24.5	2.69	Energy balance rs1800592	24.9	3	17	6	4	6.429	1.12×10^{-2}
BMI	24.5	2.69	Protein rs9939609	24.9	16	5	8	1	0.635	0.43
BMI	24.5	2.69	Total Fat rs7903146	24.9	19	10	1	0	0.517	0.47
BMI	24.5	2.69	Saturated & unsaturated fat rs9939609	24.9	9	9	5	7	0.201	0.65
BMI	24.5	2.69	Mono-unsaturated fat rs1801282	24.9	16	10	1	3	1.885	0.17

[a]BMI Categories (kg/m^2): Underweight < 18.5, Healthy/Normal 18.5–24.9, Overweight 25–29.9, Obese > 30 (National Heart Foundation of New Zealand, 2022).

[b]BMI healthy maximum value of 25 kg/m^2 was used to categorise the participants' (n = 30) BMI measures as either greater than 25 kg/m^2 or equal to/less than 25 kg/m^2, before performing chi-square.
[c]The qualitative genetic risk results were low, typical, elevated, enhanced, or diminished genetic risk. Low or typical risk indicates a low or normal response to a genetic variant, elevated, enhanced, or diminished genetic risk indicates an increased response to a genetic variant.

Participants' postprandial changes in an online visual analogue 100-point scale was used to assess fat-taste perception, sugar preference and hunger (using hunger, satisfaction, fullness, desire to consume sweet, or fatty-food appetite scores) and the corresponding qualitative genetic-risk statement (Tables 11 & 19).

Table 11

Participants' Postprandial Changes in Online Analogue Measures and the Corresponding Qualitative Genetic Risk

Appetite scores AUC[a]	Mean (n=30)	Standard deviation	Ref. marker & Gene variant	Median[b]	Number of participants with[c]				Chi-square	*P* value
					Normal response ≤ median	Normal response > median	Increased response ≤ median	Increased response > median		
Fat craving	134.92	92.68	Fat-taste perception rs1761667	117.40	3	1	11	15	1.489	0.22
Sugar craving	116.49	104.07	Sugar preference rs5400	76.88	14	10	1	5	3.333	6.79×10^{-2}
Hunger	191.26	94.03	Hunger rs1051168	184.15	12	15	1	2	0.136	0.71

[a]AUC, area under the curve.
[b]Median values were calculated from the participants' online analogue 100-point scale measures (n = 30) using AUC data. The median AUC values were calculated using all-times recorded data (t-pre, t0, t30, t60, t120, t180, t240) of each participant's online analogue 100-point scale measures. Then categorised as either greater than or equal to/less than the median values before performing chi-square.
[c]The qualitative genetic risk results were low, typical, elevated, enhanced, or diminished genetic risk. Low or typical risk indicates a low or normal response to a genetic variant, elevated, enhanced, or diminished genetic risk indicates an increased response to a genetic variant.

Recorded gastrointestinal symptoms experienced by participants during each time point of the study and over the entire study duration were collated (Table 12 & 13). Gastrointestinal symptoms could have affected the participants' appetite scores.

Table 12

Recorded Headache and Gastrointestinal Symptoms of Postprandial Meal Response in all Participants (n = 30) at Each Time Point

Time point	Headache	Bloating	Wind	Abdominal Pain	Nausea	Fatigue	Rumbling	Belching	Indigestion	Diarrhoea	Constipation
t-pre	1	2	4	1	2	10	5	0	0	0	0
t0	2	6	2	1	2	6	0	8	1	0	0
t30	4	8	8	2	3	7	5	8	2	0	0
t60	2	5	8	2	3	7	5	7	2	0	0
t120	4	3	8	2	1	9	4	6	1	0	1
t180	4	3	5	2	2	11	2	2	2	1	2
t240	4	5	6	3	2	10	3	2	1	1	2
Mean (n = 30)	3.0	4.6	5.9	1.9	2.1	8.6	3.4	4.7	1.3	0.3	0.7
Standard Deviation	1.3	2.1	2.3	0.7	0.7	1.9	1.9	3.3	0.8	0.5	1.0

Table 13

Recorded Headache and Gastrointestinal Symptoms of the Postprandial Meal Response in all Participants (n = 30) Over the Study Duration

Participant	Headache	Bloating	Wind	Abdominal Pain	Nausea	Fatigue	Rumbling	Belching	Indigestion	Diarrhoea	Constipation
1						x					
2	x					x					
3		x			x	x	x	x			
4											
5	x					x	x	x			
6	x		x			x					
7		x	x	x		x	x	x			
8	x	x	x	x	x	x	x	x	x	x	x
9	x	x	x	x	x	x	x	x	x		x
10			x				x				
11											
12											
13	x					x	x				
14		x	x			x	x	x			
15											
16			x								
17											
18					x						
19								x	x		
20		x	x				x	x	x		
21			x			x	x	x			
22	x	x	x			x					
23											
24											

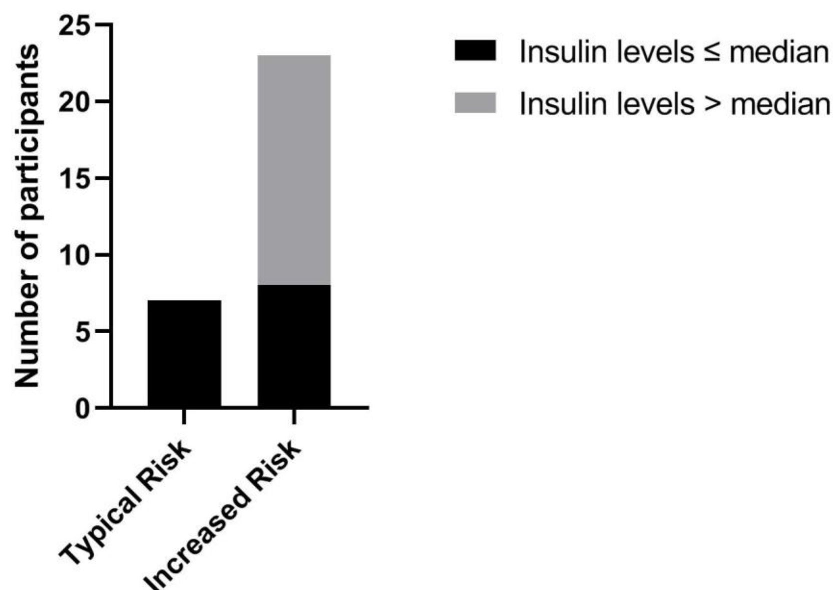
Participant	Headache	Bloating	Wind	Abdominal Pain	Nausea	Fatigue	Rumbling	Belching	Indigestion	Diarrhoea	Constipation
25											
26		x				x		x			
27						x					
28		x						x			
29											
30		x	x			x				x	

4.4 Results for Physiological-Outcome Measures and the Corresponding Genetic Variants (SNP)

There was a positive association ($P < 0.05$) between the insulin-signalling IRS1 gene variant rs2943641, the typical risk (TT) compared to the increased risk (CT or CC), in relation to postprandial insulin levels, $\chi^2 = (1, N = 30) = 9.130$, $P = 0.0025$, 95% CI [1.61, 4.93] (Figure 1, Table 8).

Figure 1

The Insulin-Signalling IRS1 Gene Variant SNP rs2943641

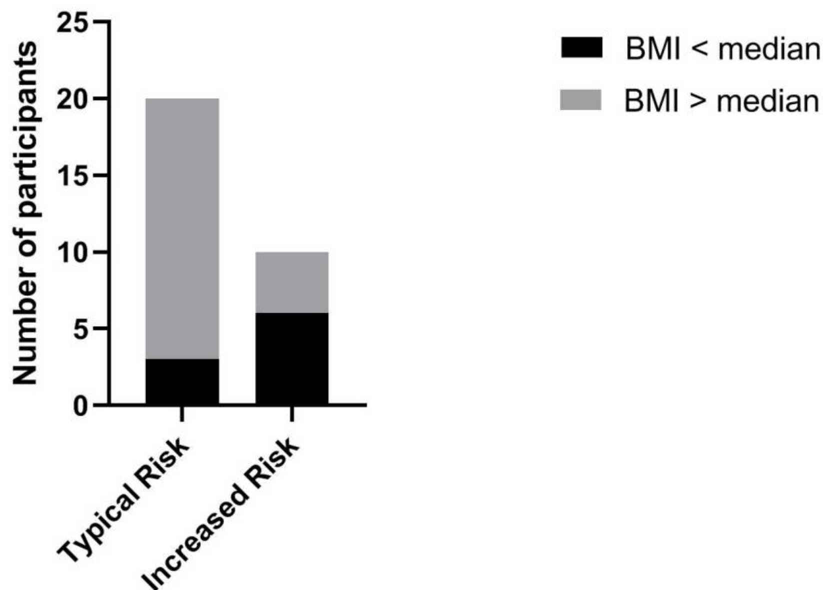


Note. The results compared participants with insulin levels ≤ median value of 6.89 μU/mL with insulin levels > median value (> 6.89 μU/mL) alongside their typical or increased risk.

The UCP1 gene variant -3826 rs1800592, the typical risk (AA) compared to the increased risk (GG or GA), was positively associated ($P = < 0.05$) with participants' BMI, $\chi^2 = (1, N = 30) = 6.429$, $P = 0.011$, 95% CI [0.081, 0.757] (Figure 2, Table 10).

Figure 2

The Energy-Balance UCP1 Gene Variant -3826 SNP rs1800592



Note. The results compared participants with BMI of < 25 kg/m^2 with BMI > 25 kg/m^2 alongside their typical or increased risk.

The "sugar preference" GLUT2 gene variant rs5400 was insignificant towards a difference between the typical risk (CC) compared to the increased risk (CT or TT) in relation to an elevated preference for sugar intake, χ^2 = (1, N = 30) = 3.333, *P* = 0.07, 95% CI [0.94, 19.81] (Table 11; Appendix H). However, only six participants had genotype CT or TT, indicating an elevated sugar preference. Therefore, a larger sample size may reveal differences as significant.

The remaining measures (vitamin D, iron, zinc, omega-6 and -3, protein, total fat, saturated, unsaturated and monounsaturated fat, cholesterol, LDL, HDL, triglycerides, fat taste, hunger and glucose) did not appear to associate with the genetic variants.

4.5 Variation in Data

Participants with values greater than the physiological-parameter median values did not necessarily associate with an increased genetic risk. However, there was a higher chance of an increased genetic risk if participants had a median value greater than the physiological-parameter value for: vitamin D levels (n = 15); TG levels (n = 15); glucose levels (n = 14); insulin levels (n = 15) and fat-taste perception (n = 15) (Table 14).

Table 14

Variation in Data

Physiological measure	Number of participants (n = 30) with physiological parameter < median	Number of participants (n = 30) with typical genetic risk and physiological parameter > median	Number of participants (n = 30) with increased risk and physiological parameter > median
Vit D	15	0	15
Iron overload	15	13	2
Low iron	15	10	5
Zinc	16	8	6
Total cholesterol	16	11	3
LDL	15	9	6
HDL	15	7	8
TG	15	0	15
LDL (For Sat Fat SNP APOA2)	15	15	0
Glucose	14	2	14
Insulin	15	0	15
LCPUFA C18:2n-6	15	8	7
LCPUFA C18:3n-6	14	9	7
LCPUFA C18:3n-3	15	8	7
Energy balance	9	17	4
Protein	24	5	1
Total Fat	20	10	0
Saturated & unsaturated fat	14	9	7
Monounsaturated fat	17	10	3
Fat-taste perception	14	1	15
Sugar preference	15	10	5
Hunger	13	15	2

Note. The table gives a generalised account of whether there is a relationship between an increased genetic risk and having a physiological-parameter measure higher than the median score calculated in Tables 8, 9, 10 and 11.

Chapter 5: Discussion

5.1 Major Findings

The study examined an individual's unique genetic background and considered the association with related physiological outcomes following a meal. This study is a step towards understanding the consequences of the long-term repeated consumption of a mixed meal during the postprandial state. Previous postmeal investigations have focused on the nutrient composition of a meal, single dietary nutrient-related genetic markers, and individual disease-risk factors. The literature reports associations between particular SNPs and a change in the physiological outcome, but many of these studies need further investigation and validation. Therefore, determining if genetic variation influences postprandial digestive responses by conducting research that maintains rigorous protocols on large populations will help confirm other associations.

The finding of this study was a positive association ($P < 0.05$) between the insulin-signalling IRS1 gene variant rs2943641 and postprandial insulin levels and the UCP1 gene variant -3826 rs1800592, was positively associated ($P = < 0.05$) with participants' BMI (Figures 1 & 2; Appendix H).

This investigation found that there was a positive association between the insulin-signalling IRS1 gene variant rs2943641, the typical risk (TT) compared to the increased risk (CT or CC), in relation to postprandial insulin levels ($P = 0.0025$). In the literature review, Almgren et al.'s (2017) population-based cohort study used 3,344 Swedish participants born between 1923 and 1950 and 4,905 Finnish participants. This study found a link between nondiabetic participants and fasting insulin levels. The IRS1 gene variant rs2943641 showed a significant association ($P = 2.4 \times 10^{-7}$) with postprandial insulin levels. The results of a RCT performed on 376 participants who had T2DM, and 380 healthy control participants, all from Saudi Arabia, concluded there was a positive relationship between subjects with T2DM and the IRS1 gene variant rs2943641 (OR = 1.482, 95% CI [1.176, 1.867], P = 0.001) (Alharbi et al., 2014). Therefore, based on Almgren et al. (2017) and Alharbi et al.'s (2014) published association and this study's findings on 30 young males (20–34 years) (P = 0.0025, 95% CI [1.61, 4.93]), the evidence suggests a positive relationship between the IRS1 gene variant rs2943641 and elevated insulin response. At-risk participants may have to adjust their diet and lifestyle, especially if they are overweight or obese (Almgren et al., 2017). Other factors, such as the diet and lifestyle of the participants (10 of the participants

in this study were overweight), could be responsible for the cause-and-effect relationship and may not be solely due to the rs2943641 SNP. The frequency of the high-risk C allele in the global population is 0.66. This frequency may account for participants with the risk-variant genotype CT and CC in this study, and is a similar frequency to the literature (gnomAD, n.d.).

The energy-balance UCP1 gene variant -3826 rs1800592 studied by Nagai et al. (2011) using 82 Japanese females aged 20–22 years from the same university campus in Japan were genotyped for the UCP1 gene polymorphism -3826 GA (rs1800592). The study explored the UCP1 gene variant rs1800592 linked to REE affecting energy balance (intake, expenditure and storage). The conclusion was that carriers of genotype GG and GA had lower rates of REE, and their energy needs were lower than the A allele carriers. This evidence suggests a positive relationship between the UCP1 gene variant polymorphism -3826 GA (rs1800592) and GG and GA genotype carriers, suggesting that the high-risk allele carriers may be at risk of excess body weight or a higher BMI. The literature reports that the UCP1 gene variant rs1800592 is involved in the oxidation of fatty acids and increases energy expenditure (used to assess energy balance; intake, expenditure and storage). BMI is the physiological measure linked to the UCP1 gene variant in this thesis. Despite the crude fit using BMI, this study reported a positive association. Ten participants in this investigation are carriers of the UCP1 gene variant -3826 GG or GA (rs1800592), and of these, four had a BMI greater than 25 kg/m^2. The genotype GG and GA participants could have a lower REE and a lower energy need than the AA genotype carriers ($P = 0.011$, 95% CI [0.081, 0.757]. Therefore, participants with GG or GA genotype may need to reduce their intake of calories if their BMI is greater than 25 kg/m^2 whilst increasing their energy expenditure (Brondani et al., 2012; Nagai et al., 2011; Pfannenberg et al., 2010). Further investigation is required with higher power using larger cohorts and a wider range of participants. The frequency of the high-risk allele in the population is unknown.

For the sugar-preference GLUT2 gene variant rs5400, this study found the results nonsignificant ($P = 0.2$). Only six participants had genotype CT or TT, indicating an elevated sugar preference. However, a larger sample size may reveal differences as significant. The published study identified that individuals with the GLUT2 gene variant rs5400 genotype CT or TT had a higher consumption of sugar over 1 month analysed from a FFQ (131 ± 5 vs 115 ± 3 g/day, $P = 0.007$) compared to genotype CC. However, FFQs can be unreliable (Routh et al., 2014). The study concluded that GLUT2 has been associated with increased sugar consumption and could account for sugar craving in specific

individuals (Eny et al., 2008). The regulation of glucose intake needs further investigation as elevated sugar craving has links to other factors, including mood, environment, culture, lifestyle, and pleasure-generated response (Routh et al., 2014).

The remaining psychological measures examined did not appear to associate with the genetic-risk variants despite scientific literature indicating associations between the other genetic-risk variants studied and outcomes.

5.2 Limitations and Future Studies

The strength of the association between the physiological parameters and the genetic variant is reliant on the GWA studies researched in the literature. These studies have shown a positively associated region of the genome where genetic variants located in genes have disease-causing effects. However, the studies varied in allele frequency, effect size, and the population they studied and only contributed to the increased likelihood of disease risk. This thesis has only focused on one SNP, not a region, associated with a physiological measure linked to the causal effect of a disease or health condition. Therefore, one major limitation is that perhaps there was a mismatch or crude match of some of the physiological measures to the genetic variant, which would account for the lack of association. For example, hunger was associated with the NMB gene; a better association might have been between hunger and the FTO gene variant rs9939609, linked to postprandial hunger responses (den Hoed et al., 2009). Diseases are complex, and some of the associated SNPs are linked with other risk variants (linkage disequilibrium) and dependent on factors such as lifestyle and environment. However, this research and other studies can help with the understanding and maybe the early detection of diseases and prevention and treatment intervention (Tam et al., 2019).

It is unclear whether performing the bivariate chi-square analysis was the correct analysis to test the associations or whether regression analysis would have been more appropriate. Chi-square for independence analysis seemed the most appropriate test to perform on the two categorical variables. The outcome was a description of the strength of the association, which was the intention. If this study used regression analysis, this would have predicted the likelihood of the outcome, which would not have indicated the strength of association of the two variables. It is questionable whether regression analysis would have improved the positive associations but could be a future improvement to this study.

This study was not powered because it had a relatively small sample size and involved a complex genetic assessment. However, this research will provide information to make predictions for developing hypotheses for future testing. If this experiment was performed

again, it would need to carry out a power analysis calculation and maintain a large-enough sample size throughout the trial to enable sufficient statistical power to maximise effect size (Hansen & Collins, 1994).

Pretest conditions can influence food study measurements; thus, standardising the requirements before the study is essential. These include standardising the premeals eaten 2 days before the study, controlling the amount of physical exercise performed before the study, the time of day the analysis is performed and rejecting participants with BMI kg/m^2 over 25 as they could have metabolic disorders (Lairon et al., 2007).

When measuring appetite responses, this study only used an online visual analogue 100-point scale, a subjective scale for appetite. It might have been more relevant to include objective measures of appetite in terms of appetite-related hormones (Thom et al., 2020).

Only gene variants were measured, not gene expression, which would have quantified the amount of a specific gene expressed within a cell. Variation of gene expression could affect tissues and consequently cause pathological phenotypes (Ackermann et al., 2013).

The physiological outcome measures did not necessarily reflect the kinetics and bioavailability of the dietary component and warrant caution when making an inference to digestion and absorption. The outcome measures can only be reported as the availability is higher or lower after consuming the standardised breakfast meal.

Other confounding factors could have influenced the results, e.g., BMI or eating patterns of participants; therefore, caution must be taken when concluding a causal inference between physiological outcome and genetic variant. But more research and evidence will confirm any causal inference.

Multinational RCTs focused on gene–nutrient interaction and postprandial digestive response must continue, ensuring enough statistical power so the results can be clinically significant. Therefore, conducting more extensive studies must continue with the aid of grant support as RCT postprandial research is costly and time consuming (Lairon et al., 2007).

5.3 Clinical Significance

The clinical significance of this trial could help clinicians, dietitians and nutritionists currently using genetic-test results to tailor nutrition advice. However, all registered practitioners need to be mindful of the strength of the relationships between SNPs and the physiological parameters.

5.4 Overview

Nutrigenomics is helping explain the gene–nutrient interaction to maintain health and reduce disease through early detection intervention plans. By using GWA studies and identifying the risk-gene variants, the challenge now is interpreting results and implementing correct dietary recommendations. However, advancement has been slow due to insufficient evidence and low replication of studies (Corella et al., 2009).

Biotechnology companies analysing an individual's SNPs could help with dietary intervention to improve the health and well-being of individuals (M. N. Mead, 2007). But the interpretation of research data can prove onerous and expensive, and is accompanied by the ethical constraints of handling, management, storage, and data privacy. Therefore, more cooperation and sharing of findings amongst nutrigenomic research groups from several disciplines, including medicine, molecular biology, nutrition and diet, genomics and bioinformatics, needs to be established worldwide to reduce the workload. This sharing will be invaluable for the findings accumulated on different ethnic and population groups by using their nutritional and genetic variation data (Sterling, 2008).

This study used a direct-to-practitioner biotechnology company Nutrigenomix (2020), to conduct the genetic-test analysis due to their secure data transfer, privacy, and security procedures when handling customers' DNA samples. Nutrigenomix provides buccal swab kits for collecting samples for registered practitioners and analyses variations in 70 genes related to nutrient metabolism, eating habits, and food intolerances. Once analysed, a customised nutrition and lifestyle report becomes available to identify any genetic-risk variants. The reports can only be interpreted by a healthcare professional, ensuring the patient understands and can utilise the information in their best interest.

Therefore, the increasing belief that nutrigenomics will be the "elixir" for health by introducing or reducing certain foods in one's diet based on a person's genotype is undoubtedly growing. Individualised personalised diets are "trendy," and growth is exponential in the biotechnology industries such as Illumina. These companies can easily report on health problems and risk of disease based on an individual's genotype. But caution is needed as not all biotechnology companies specifically detail their confidentiality procedures. Testing standards vary between companies meaning good judgement must be applied when interpreting results. Results from home-test kits could be of no value or harmful to an individual's health. Therefore, interpreting results should require a nutrition expert who can explain them safely to the client (Sterling, 2008).

People have unique characteristics such as gender, age, ethnicity, physical activity, microbiome, dietary intake, metabolism, and genetic genome. Foods and supplements are highly diverse structures and have complex biological properties. Estimating the total bioavailability of foods or supplements or knowing their exact action mechanism in each individual is impossible (Manach & Donovan, 2004). Therefore, assessing the bioavailability of food in a person's body differs depending on a person's characteristics and genetic variation (Manach & Donovan, 2004). This makes the field of nutrigenomics intricate and complicated where the investigation of dietary metabolism is concerned and, ultimately, it faces several challenges. Challenges for the future include discovering how nutrients affect the early stages of a disease or health condition and how dietary intake affects an individual's unique microbiome. Thus, more epidemiological research is needed to answer these questions (Pressman et al., 2017).

Gene–nutrient interaction is currently a new field of food studies research encompassing precision nutrition. Therefore, it is important to carry out more population nutrient-based studies depending on genotypes. However, the problem with food studies that use questionnaires is that they are prone to memory mistakes, making them inaccurate accounts that may not reflect typical dietary intake (da Rocha et al., 2014).

Several nutrigenomic epidemiological studies have demonstrated an association or statistical relationship between dietary nutrition, SNP genotyping and health (den Hoed, 2008; den Hoed et al., 2009; Ellis et al., 2021; Grimm & Steinle, 2011). Many studies have demonstrated a robust statistical relationship, but this does not establish cause and effect and only suggests causality. There is a need to know more about digestive metabolism and responses to foods consumed by individuals, and this is what this thesis examined (Pressman et al., 2017).

5.5 Conclusion

The results of this study provide evidence to suggest a positive association for the insulin-signalling IRS1 gene variant rs2943641, the typical risk (TT) compared to the increased risk (CT or CC) in relation to postprandial insulin levels. The UCP1 gene variant -3826 rs1800592, the typical risk (AA) compared to the increased risk (GG or GA), was positively associated ($P = < 0.05$) with the participants' BMI. This study did not confirm statistically significant results of the other psychological measures despite scientific literature indicating associations exist between the risk variants and physiological outcomes. However, as a pilot study, the statistical power is insufficient to determine the strength of the

relationship between all the outcomes measures and the related SNP during the postprandial digestive response to a standardised breakfast meal. This limitation is due to the limited sample size and the complexity of the genetic assessment. However, it is a secondary outcome, and this study will provide information to help make predictions for developing hypotheses for future testing. This study has highlighted many known associations between a particular SNP and a change in a physiological outcome, whilst providing information on how a genetic-risk variant could increase health risks and implement treatment or behaviour modification proposals. In the future, there is a need for more nutrigenomic research to establish the robustness and positive associations between genetic variants and related psychological outcomes. Whether the science of nutrigenomics is the key to producing the "perfect diet," the efficacy and utility of nutrient-related genetic markers are still under investigation.

References

Acalovschi, M., Ciocan, A., Mostean, O., Tirziu, S., Chiorean, E., Keppeler, H., & Lammert, F. (2006). Are plasma lipid levels related to ABCG5/ABCG8 polymorphisms? A preliminary study in siblings with gallstones. *European Journal of Internal Medicine*, *17*(7), 490–494. https://doi.org/10.1016/j.ejim.2006.04.012

Ackermann, M., Sikora-Wohlfeld, W., & Beyer, A. (2013). Impact of natural genetic variation on gene expression dynamics. *PLoS Genetics*, *9*(6), e1003514. https://doi.org/10.1371/journal.pgen.1003514

Afman, L., & Müller, M. (2006). Nutrigenomics: From molecular nutrition to prevention of disease. *Journal of the American Dietetic Association*, *106*(4), 569–576. https://doi.org/10.1016/j.jada.2006.01.001

Alathari, B. E., Sabta, A. A., Kalpana, C. A., & Vimaleswaran, K. S. (2020). Vitamin D pathway-related gene polymorphisms and their association with metabolic diseases: A literature review. *Journal of Diabetes & Metabolic Disorders*, *19*(2), 1701–1729. https://doi.org/10.1007/s40200-020-00561-w

Al-Goblan, A. S., Al-Alfi, M. A., & Khan, M. Z. (2014). Mechanism linking diabetes mellitus and obesity. *Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy*, *7*, 587. https://doi.org/10.2147/dmso.s67400

Alharbi, K. K., Khan, I. A., Munshi, A., Alharbi, F. K., Al-Sheikh, Y., & Alnbaheen, M. S. (2014). Association of the genetic variants of insulin receptor substrate 1 (IRS-1) with type 2 diabetes mellitus in a Saudi population. *Endocrine*, *47*(2), 472–477. https://doi.org/10.1007/s12020-014-0177-2

Al-Khalidi, B., Kimball, S. M., Rotondi, M. A., & Ardern, C. I. (2017). Standardized serum 25-hydroxyvitamin D concentrations are inversely associated with cardiometabolic disease in US adults: A cross-sectional analysis of NHANES, 2001–2010. *Nutrition Journal*, *16*(1), 1–12. https://doi.org/10.1186/s12937-017-0237-6

Allen, K. J., Gurrin, L. C., Constantine, C. C., Osborne, N. J., Delatycki, M. B., Nicoll, A. J., & Gertig, D. M. (2008). Iron-overload–related disease in HFE hereditary hemochromatosis. *New England Journal of Medicine*, *358*(3), 221–230. https://doi.org/10.1056/nejmoa073286

Almgren, P., Lindqvist, A., Krus, U., Hakaste, L., Ottosson-Laakso, E., Asplund, O., & Ahlqvist, E. (2017). Genetic determinants of circulating GIP and GLP-1 concentrations. *JCI Insight*, *2*(21), e93306. https://doi.org/10.1172/jci.insight.93306

Auckland District Health Board. (2022). *Test guide mobile*. https://testguide.adhb.govt.nz/eguidemob/?gm=939&gs=3

Australian Government, National Health and Medical Research Council, New Zealand Ministry of Health. (2006). *Nutrient reference values for Australia and New Zealand including recommended dietary intakes*. https://www.nrv.gov.au/nutrients

Azpiroz, F., Feinle-Bisset, C., Grundy, D., & Tack, J. (2014). Gastric sensitivity and reflexes: Basic mechanisms underlying clinical problems. *Journal of Gastroenterology*, *49*(2), 206–218. https://doi.org/10.1007/s00535-013-0917-8

Bäck, M. (2017). Omega-3 fatty acids in atherosclerosis and coronary artery disease. *Future Science OA, 3*(4), FSO236. https://doi.org/10.4155/fsoa-2017-0067

Baghurst, K. I., & Record, S. J. (2002). *Re-analysis of the 1995 National Nutrition Survey.* CSIRO.

Bajit, H., Mohammed, O. A., Guennoun, Y., Benaich, S., Bouaiti, E., Belghiti, H., & Aguenaou, H. (2020). Single-nucleotide polymorphism rs1761667 in the CD36 gene is associated with orosensory perception of a fatty acid in obese and normal-weight Moroccan subjects. *Journal of Nutritional Science*, *9*. https://doi.org/10.1017/jns.2020.18

Bandeali, S., & Farmer, J. (2012). High-density lipoprotein and atherosclerosis: The role of antioxidant activity. *Current Atherosclerosis Reports*, *14*(2), 101–107. https://doi.org/10.1007/s11883-012-0235-2

Barnes, S. (2008). Nutritional genomics, polyphenols, diets, and their impact on dietetics. *Journal of the American Dietetic Association, 108*(11), 1888–1895. https://doi.org/10.1016/j.jada.2008.08.014

Bayly, G. R. (2014). Lipids and disorders of lipoprotein metabolism. *Clinical Biochemistry: Metabolic and Clinical Aspects. Elsevier*, 702–36. https://doi.org/10.1016/b978-0-7020-5140-1.00037-7

Benyamin, B., Ferreira, M. A., Willemsen, G., Gordon, S., Middelberg, R. P., McEvoy, B. P., & Whitfield, J. B. (2009). Common variants in TMPRSS6 are associated with iron status and erythrocyte volume. *Nature Genetics*, *41*(11), 1173–1175. https://doi.org/10.1038/ng.456

Berry, S., Drew, D., Linenberg, I., Wolf, J., Hadjigeorgiou, G., Davies, R., Khatib, H. A., Hart, D., Surdulescu, G., Yarand, D., Nessa, A., Sheedy, A., Vijay, A., Asnicar, F., Segata, N., Chan, A., Franks, P., Valdes, A., & Spector, T. (2020). Personalised REsponses to DIetary Composition Trial (PREDICT): An intervention study to determine inter-individual differences in postprandial response to foods. *Research Square*. https://doi.org/10.21203/rs.2.20798/v1

Binia, A., Contreras, A. V., Canizales-Quinteros, S., Alonzo, V. A., Tejero, M. E., & Silva-Zolezzi, I. (2014). Geographical and ethnic distribution of single nucleotide polymorphisms within genes of the folate/homocysteine pathway metabolism. *Genes & Nutrition*, *9*(5), 421. https://doi.org/10.1007/s12263-014-0421-7

Bolhuis, D. P., Lakemond, C. M., de Wijk, R. A., Luning, P. A., & de Graaf, C. (2012). Effect of salt intensity in soup on ad libitum intake and on subsequent food choice. *Appetite*, *58*(1), 48–55. https://doi.org/10.1016/j.appet.2011.09.001

Bouchard, L., Drapeau, V., Provencher, V., Lemieux, S., Chagnon, Y., Rice, T., & Pérusse, L. (2004). Neuromedin β: A strong candidate gene linking eating behaviors and susceptibility to obesity. *The American Journal of Clinical Nutrition*, *80*(6), 1478–1486. https://doi.org/10.1093/ajcn/80.6.1478

Braakhuis, A., Monnard, C. R., Ellis, A., & Rozga, M. (2021). Consensus report of the Academy of Nutrition and Dietetics: Incorporating genetic testing into nutrition care. *Journal of the Academy of Nutrition and Dietetics*, *121*(3), 545–552. https://doi.org/10.1016/j.jand.2020.04.002

Brassard, P., Frisch, F., Lavoie, F., Cyr, D., Bourbonnais, A., Cunnane, S. C., & Carpentier, A. C. (2008). Impaired plasma nonesterified fatty acid tolerance is an early defect in the natural history of type 2 diabetes. *The Journal of Clinical Endocrinology & Metabolism*, *93*(3), 837–844. https://doi.org/10.1210/jc.2007-1670

Brondani, L. D. A., Assmann, T. S., Duarte, G. C. K., Gross, J. L., Canani, L. H., & Crispim, D. (2012). The role of the uncoupling protein 1 (UCP1) on the development of obesity and type 2 diabetes mellitus. *Arquivos Brasileiros de Endocrinologia & Metabologia*, *56*(4), 215–225. https://doi.org/10.1590/s0004-27302012000400001

Burdge, G. C., & Calder, P. C. (2005). Conversion of alpha-linolenic acid to longer-chain polyunsaturated fatty acids in human adults. *Reproduction Nutrition Development*, *45*(5), 581–597. https://doi.org/10.1051/rnd:2005047

Camp, K. M., & Trujillo, E. (2014). Position of the Academy of Nutrition and Dietetics: Nutritional genomics. *Journal of the Academy of Nutrition and Dietetics*, *114*(2), 299–312. https://doi.org/10.1016/j.jand.2013.12.001

Cannon, B., & Nedergaard, J. A. (2004). Brown adipose tissue: Function and physiological significance. *Physiological Reviews*, *84*(1), 277–359. https://doi.org/10.1152/physrev.00015.2003

Cerqueira, N. M., Oliveira, E. F., Gesto, D. S., Santos-Martins, D., Moreira, C., Moorthy, H. N., & Fernandes, P. A. (2016). Cholesterol biosynthesis: A mechanistic overview. *Biochemistry*, *55*(39), 5483–5506. https://doi.org/10.1021/acs.biochem.6b00342

Chan, A. W., Tetzlaff, J. M., Altman, D. G., Laupacis, A., Gøtzsche, P. C., Krleža-Jerić, K., Hróbjartsson, A., Mann, H., Dickersin, K., Berlin, J. A., & Doré, C. J. (2013). SPIRIT 2013 statement: Defining standard protocol items for clinical trials. *Annals of Internal Medicine*, *158*(3), 200–207. https://doi.org/10.7326/0003-4819-158-3-201302050-00583

Chang, C. L., & Deckelbaum, R. J. (2013). Omega-3 fatty acids: Mechanisms underlying "protective effects" in atherosclerosis. *Current Opinion in Lipidology, 24*(4), 345.

Chen, P. Y., Gao, W. Y., Liou, J. W., Lin, C. Y., Wu, M. J., & Yen, J. H. (2021). Angiopoietin-like protein 3 (ANGPTL3) modulates lipoprotein metabolism and dyslipidemia. *International Journal of Molecular Sciences*, *22*(14), 7310. https://doi.org/10.3390/ijms22147310

Chial, H. (2008). DNA sequencing technologies key to the Human Genome Project. *Nature Education*, *1*(1).

Christakos, S., Dhawan, P., Porta, A., Mady, L. J., & Seth, T. (2011). Vitamin D and intestinal calcium absorption. *Molecular and Cellular Endocrinology*, *347*(1–2), 25–29. https://doi.org/10.1016/j.mce.2011.05.038

Collins, F. S., Morgan, M., & Patrinos, A. (2003). The Human Genome Project: Lessons from large-scale biology. *Science*, *300*(5617), 286–290. https://doi.org/10.1126/science.1084564

Corella, D., Peloso, G., Arnett, D. K., Demissie, S., Cupples, L. A., Tucker, K., & Ordovas, J. M. (2009). APOA2, dietary fat, and body mass index: Replication of a gene–diet interaction in 3 independent populations. *Archives of Internal Medicine*, *169*(20), 1897–1906. https://doi.org/10.1001/archinternmed.2009.343

Dalgaard, L. T., & Pedersen, O. (2001). Uncoupling proteins: Functional characteristics and role in the pathogenesis of obesity and Type II diabetes. *Diabetologia*, *44*(8), 946–965. https://doi.org/10.1007/s001250100596

da Rocha, T. J., Korb, C., Schuch, J. B., Bamberg, D. P., de Andrade, F. M., & Fiegenbaum, M. (2014). SLC30A3 and SEP15 gene polymorphisms influence the serum concentrations of zinc and selenium in mature adults. *Nutrition Research*, *34*(9), 742–748. https://doi.org/10.1016/j.nutres.2014.08.009

del Campo-Albendea, L., & Muriel-García, A. (2021). Ten common statistical mistakes to watch out for when writing or reviewing a manuscript. *Enfermería Intensiva (English Edition), 32*(1), 42–44. https://doi.org/10.1016/j.enfie.2020.09.001

den Hoed, M., Smeets, A. J., Veldhorst, M. A., Nieuwenhuizen, A. G., Bouwman, F. G., Heidema, A. G., Mariman, E. C., Westerterp-Plantenga, M. S., & Westerterp, K. R. (2008). SNP analyses of postprandial responses in (an) orexigenic hormones and feelings of hunger reveal long-term physiological adaptations to facilitate homeostasis. *International Journal of Obesity*, *32*(12), 1790–1798. https://doi.org/10.1038/ijo.2008.195

den Hoed, M., Westerterp-Plantenga, M. S., Bouwman, F. G., Mariman, E. C., & Westerterp, K. R. (2009). Postprandial responses in hunger and satiety are associated with the rs9939609 single nucleotide polymorphism in FTO. *The American Journal of Clinical Nutrition*, *90*(5), 1426–1432. https://doi.org/10.3945/ajcn.2009.28053

Desmarchelier, C., Martin, J. C., Planells, R., Gastaldi, M., Nowicki, M., Goncalves, A., & Borel, P. (2013, October). A combination of SNPS is associated with the postprandial chylomicron triacylglycerol response in healthy male adults. 10. *International Congress on Coronary Artery Disease, 126*.

Desmarchelier, C., Martin, J. C., Planells, R., Gastaldi, M., Nowicki, M., Goncalves, A., & Borel, P. (2014). The postprandial chylomicron triacylglycerol response to dietary fat in healthy male adults is significantly explained by a combination of single nucleotide polymorphisms in genes involved in triacylglycerol metabolism. *The Journal of Clinical Endocrinology & Metabolism*, *99*(3), E484–E488. https://doi.org/10.1210/jc.2013-3962

Dib, M. J., Elliott, R., & Ahmadi, K. R. (2019). A critical evaluation of results from genome-wide association studies of micronutrient status and their utility in the practice of precision nutrition. *British Journal of Nutrition*, *122*(2), 121–130. https://doi.org/10.1017/s0007114519001119

Dumont, J., Goumidi, L., Grenier-Boley, B., Cottel, D., Marécaux, N., Montaye, M., & Meirhaeghe, A. (2018). Dietary linoleic acid interacts with FADS1 genetic variability to modulate HDL-cholesterol and obesity-related traits. *Clinical Nutrition*, *37*(5), 1683–1689. https://doi.org/10.1016/j.clnu.2017.07.012

Dupuis, J., Langenberg, C., Prokopenko, I., Saxena, R., Soranzo, N., Jackson, A. U., & Orrù, M. (2010). New genetic loci implicated in fasting glucose homeostasis and their impact on type 2 diabetes risk. *Nature Genetics*, *42*(2), 105–116.

Ellis, A., Rozga, M., Braakhuis, A., Monnard, C. R., Robinson, K., Sinley, R., & Vargas, A. J. (2021). Effect of incorporating genetic testing results into nutrition counseling and care on health outcomes: An evidence analysis center systematic review—Part II. *Journal of the Academy of Nutrition and Dietetics*, *121*(3), 582–605. https://doi.org/10.1016/j.jand.2020.02.009

Emanuele, D., Tuason, I., & Edwards, Q. T. (2014). HFE-associated hereditary hemochromatosis: Overview of genetics and clinical implications for nurse practitioners in primary care settings. *Journal of the American Association of Nurse Practitioners*, *26*(3), 113–122. https://doi.org/10.1002/2327-6924.12106

Eny, K. M., Wolever, T. M., Fontaine-Bisson, B., & El-Sohemy, A. (2008). Genetic variant in the glucose transporter type 2 is associated with higher intakes of sugars in two distinct populations. *Physiological Genomics*, *33*(3), 355–360. https://doi.org/10.1152/physiolgenomics.00148.2007

Farhud, D. D., Yeganeh, M. Z., & Yeganeh, M. Z. (2010). Nutrigenomics and nutrigenetics. *Iranian Journal of Public Health*, *39*(4), 1.

Feig, J. E., Hewing, B., Smith, J. D., Hazen, S. L., & Fisher, E. A. (2014). High-density lipoprotein and atherosclerosis regression: evidence from preclinical and clinical studies. *Circulation Research*, *114*(1), 205–213. https://doi.org/10.1161/circresaha.114.300760

Feingold, K. R., & Grunfeld, C. (2015). *Introduction to lipids and lipoproteins*. https://www.semanticscholar.org/paper/Introduction-to-Lipids-and-Lipoproteins-Feingold-Grunfeld/39273be6b60dce1fdb40a341fd7328862e3be722

Fenech, M. (2005). The genome health clinic and genome health nutrigenomics concepts: Diagnosis and nutritional treatment of genome and epigenome damage on an individual basis. *Mutagenesis*, *20*(4), 255–269. https://doi.org/10.1093/mutage/gei040

Flint, A., Raben, A., Blundell, J. E., & Astrup, A. (2000). Reproducibility, power and validity of visual analogue scales in assessment of appetite sensations in single test meal studies. *International Journal of Obesity*, *24*(1), 38–48. https://doi.org/10.1038/sj.ijo.0801083

Foster, R. H., & Wilson, N. (2013). Review of the evidence for the potential impact and feasibility of substituting saturated fat in the New Zealand diet. *Australian and New Zealand Journal of Public Health*, *37*(4), 329–336. https://doi.org/10.1111/1753-6405.12080

Fujihara, J., Yasuda, T., Kimura-Kataoka, K., Takinami, Y., Nagao, M., & Takeshita, H. (2018). Association of SNPs in genes encoding zinc transporters on blood zinc levels in humans. *Legal Medicine*, *30*, 28–33. https://doi.org/10.1016/j.legalmed.2017.10.009

Ganesh, S. K., Zakai, N. A., Van Rooij, F. J., Soranzo, N., Smith, A. V., Nalls, M. A., & Lin, J. P. (2009). Multiple loci influence erythrocyte phenotypes in the CHARGE Consortium. *Nature Genetics*, *41*(11), 1191–1198. https://doi.org/10.1038/ng.466

Garaulet, M., Smith, C. E., Hernández-González, T., Lee, Y. C., & Ordovás, J. M. (2011). PPARγ Pro12Ala interacts with fat intake for obesity and weight loss in a behavioural treatment based on the Mediterranean diet. *Molecular Nutrition & Food Research*, *55*(12), 1771–1779. https://doi.org/10.1002/mnfr.201100437

Garcia-Tsao, G., Sanyal, A. J., Grace, N. D., & Carey, W. (2007). AASLD Practice guidelines. *Hepatology*, *46*(3), 922–938.

Ghebreyesus, T. A., & Frieden, T. R. (2018). REPLACE: a roadmap to make the world trans-fat free by 2023. *The Lancet*, *391*(10134), 1978–1980. https://doi.org/10.1016/s0140-6736(18)31083-3

gnomAD. (n.d.). Genome aggregation database. https://gnomad.broadinstitute.org/

Grau, K., Cauchi, S., Holst, C., Astrup, A., Martinez, J. A., Saris, W. H., & Sørensen, T. I. (2010). TCF7L2 rs7903146–macronutrient interaction in obese individuals' responses to a 10-wk randomized hypoenergetic diet. *The American Journal of Clinical Nutrition*, *91*(2), 472–479. https://doi.org/10.3945/ajcn.2009.27947

Green, T. J., Skeaff, C. M., & Rockell, J. E. (2004). *Serum 25-hydroxyvitamin D Status of New Zealand Adolescents and Adults 15 years or older: Results of the 1997 National Nutrition Survey*. Ministry of Health.

Grimm, E. R., & Steinle, N. I. (2011). Genetics of eating behavior: established and emerging concepts. *Nutrition Reviews*, *69*(1), 52–60. https://doi.org/10.1111/j.1753-4887.2010.00361.x

Guyader, D., Jacquelinet, C., Moirand, R., Turlin, B., Mendler, M. H., Chaperon, J., & Deugnier, Y. (1998). Noninvasive prediction of fibrosis in C282Y homozygous hemochromatosis. *Gastroenterology*, *115*(4), 929–936. https://doi.org/10.1016/s0016-5085(98)70265-3

Hansen, W. B., & Collins, L. M. (1994). Seven ways to increase power without increasing N. *NIDA Research Monograph*, *142*, 184–184. https://doi.org/10.1037/e495862006-008

Haupt, A., Thamer, C., Machann, J., Kirchhoff, K., Stefan, N., Tschritter, O., & Fritsche, A. (2008). Impact of variation in the FTO gene on whole body fat distribution, ectopic fat, and weight loss. *Obesity*, *16*(8), 1969–1972. https://doi.org/10.1038/oby.2008.283

Hellstrand, S., Sonestedt, E., Ericson, U., Gullberg, B., Wirfält, E., Hedblad, B., & Orho-Melander, M. (2012). Intake levels of dietary long-chain PUFAs modify the association between genetic variation in FADS and LDL-C [S]. *Journal of Lipid Research*, *53*(6), 1183–1189. https://doi.org/10.1194/jlr.p023721

Hill, J. O., Wyatt, H. R., & Peters, J. C. (2012). Energy balance and obesity. *Circulation, 126*(1), 126–132. https://doi.org/10.1161/circulationaha.111.087213

Hinds, D. A., Stuve, L. L., Nilsen, G. B., Halperin, E., Eskin, E., Ballinger, D. G., & Cox, D. R. (2005). Whole-genome patterns of common DNA variation in three human populations. *Science*, *307*(5712), 1072–1079. https://doi.org/10.1126/science.1105436

Holick, M. (2001). Sunlight “D”ilemma: Risk of skin cancer or bone disease and muscle weakness. *Lancet, 357*, 4–6. https://doi.org/10.1016/s0140-6736(00)03560-1

Holmström, P., Marmur, J., Eggertsen, G., Gåfvels, M., & Stål, P. (2002). Mild iron overload in patients carrying the HFE S65C gene mutation: A retrospective study in patients with suspected iron overload and healthy controls. *Gut*, *51*(5), 723–730.

Holst, J. J. (2007). The physiology of glucagon-like peptide 1. *Physiological Reviews*, *87*(4), 1409–1439. https://doi.org/10.1152/physrev.00034.2006

Hoppenbrouwers, T., Cvejić Hogervorst, J. H., Garssen, J., Wichers, H. J., & Willemsen, L. E. (2019). Long chain polyunsaturated fatty acids (LCPUFAs) in the prevention of food allergy. *Frontiers in Immunology*, *10*, 1118. https://doi.org/10.3389/fimmu.2019.01118

Horne, J. R., Nielsen, D. E., Madill, J., Robitaille, J., Vohl, M. C., & Mutch, D. M. (2021). Guiding global best practice in personalized nutrition based on genetics: The development of a nutrigenomics care map. *Journal of the Academy of Nutrition and Dietetics*. https://doi.org/10.1016/j.jand.2021.02.008

Hosseini-Esfahani, F., Mirmiran, P., Daneshpour, M. S., Mehrabi, Y., Hedayati, M., Soheilian-Khorzoghi, M., & Azizi, F. (2015). Dietary patterns interact with APOA1/APOC3 polymorphisms to alter the risk of the metabolic syndrome: The Tehran Lipid and Glucose Study. *British Journal of Nutrition*, *113*(4), 644–653. https://doi.org/10.1017/s0007114514003687

Huang, L., & Tepaamorndech, S. (2013). The SLC30 family of zinc transporters–A review of current understanding of their biological and pathophysiological roles. *Molecular Aspects of Medicine*, *34*(2–3), 548–560. https://doi.org/10.1016/j.mam.2012.05.008

Innis, S. M. (2008). Dietary omega 3 fatty acids and the developing brain. *Brain Research*, *1237*, 35–43. https://doi.org/10.1016/j.brainres.2008.08.078

Jacobs, E. M., Hendriks, J. C., van Deursen, C. T., Kreeftenberg, H. G., de Vries, R. A., Marx, J. J., & Swinkels, D. W. (2009). Severity of iron overload of proband determines serum ferritin levels in families with HFE-related hemochromatosis: The HEmochromatosis FAmily Study. *Journal of Hepatology*, *50*(1), 174–183. https://doi.org/10.1016/j.jhep.2008.08.014

Jansen, P. R., Watanabe, K., Stringer, S., Skene, N., Bryois, J., Hammerschlag, A. R., & Posthuma, D. (2019). Genome-wide analysis of insomnia in 1,331,010 individuals identifies new risk loci and functional pathways. *Nature Genetics*, *51*(3), 394–403. https://doi.org/10.1038/s41588-018-0333-3

Jiang-Hua, Q., De-Chuang, J., Zhen-Duo, L., Shu-de, C., & Zhenzhen, L. (2014). Association of methylenetetrahydrofolate reductase and methionine synthase polymorphisms with breast cancer risk and interaction with folate, vitamin B 6, and vitamin B 12 intakes. *Tumor Biology*, *35*(12), 11895–11901. https://doi.org/10.1007/s13277-014-2456-1

Jump, D. B., Tripathy, S., & Depner, C. M. (2013). Fatty acid–regulated transcription factors in the liver. *Annual Review of Nutrition*, *33*, 249–269. https://doi.org/10.1146/annurev-nutr-071812-161139

Karczewski, K. J., Francioli, L. C., Tiao, G., Cummings, B. B., Alföldi, J., Wang, Q., Collins, R. L., Laricchia, K. M., Ganna, A., Birnbaum, D. P., Gauthier, L. D., Brand, H., Solomonson, M., Watts, N. A., Rhodes, D., Singer-Berk, M., England, E. M., Seaby, E. G., Kosmicki, J. A., ... MacArthur, D. G. (2020). The mutational constraint

spectrum quantified from variation in 141,456 humans. *Nature*, *581*(7809), 434–443. https://doi.org/10.1101/531210

Kathiresan, S., Willer, C. J., Peloso, G. M., Demissie, S., Musunuru, K., Schadt, E. E., & Cupples, L. A. (2009). Common variants at 30 loci contribute to polygenic dyslipidemia. *Nature Genetics*, *41*(1), 56–65. https://doi.org/10.1038/ng.291

Katsarou, M. S., Papasavva, M., Latsi, R., & Drakoulis, N. (2019). Hemochromatosis: hereditary hemochromatosis and HFE gene. *Vitamins and Hormones*, *110*, 201–222. https://doi.org/10.1016/bs.vh.2019.01.010

Kaur, G., Cameron-Smith, D., Garg, M., & Sinclair, A. J. (2011). Docosapentaenoic acid (22: 5n-3): A review of its biological effects. *Progress in Lipid Research*, *50*(1), 28–34. https://doi.org/10.1016/j.plipres.2010.07.004

Keller, K. L. (2012). Genetic influences on oral fat perception and preference: Presented at the Symposium "The Taste for Fat: New Discoveries on the Role of Fat in Sensory Perception, Metabolism, Sensory Pleasure and Beyond." *Journal of Food Science*, *77*(3), S143–S147. https://doi.org/10.1111/j.1750-3841.2011.02585.x

Khaksari, M., Mazzoleni, L. R., Ruan, C., Kennedy, R. T., & Minerick, A. R. (2017). Determination of water-soluble and fat-soluble vitamins in tears and blood serum of infants and parents by liquid chromatography/mass spectrometry. *Experimental Eye Research*, *155*, 54–63. https://doi.org/10.1016/j.exer.2016.12.007

Khan, R., & Mittelman, D. (2018). Consumer genomics will change your life, whether you get tested or not. *Genome Biology*, *19*(1), 1–4. https://doi.org/10.1186/s13059-018-1506-1

Kirkpatrick, S. I., Subar, A. F., Douglass, D., Zimmerman, T. P., Thompson, F. E., Kahle, L. L., George, S. M., Dodd, K. W., & Potischman, N. (2014). Performance of the Automated Self-Administered 24-hour Recall relative to a measure of true intakes and to an interviewer-administered 24-h recall. *The American Journal of Clinical Nutrition, 100*(1), 233–240. https://doi.org/10.3945/ajcn.114.083238

Klementova, M., Thieme, L., Haluzik, M., Pavlovicova, R., Hill, M., Pelikanova, T., & Kahleova, H. (2019). A plant-based meal increases gastrointestinal hormones and satiety more than an energy-and macronutrient-matched processed-meat meal in t2d, obese, and healthy men: A three-group randomised crossover study. *Nutrients, 11*(1), 157. https://doi.org/10.3390/nu11010157

Kohlmeier, M. (2015). *Nutrient metabolism: structures, functions, and genes*. Academic Press.

Kovacs, P., Hanson, R. L., Lee, Y. H., Yang, X., Kobes, S., Permana, P. A., & Baier, L. J. (2003). The role of insulin receptor substrate-1 gene (IRS1) in type 2 diabetes in Pima Indians. *Diabetes*, *52*(12), 3005–3009. https://doi.org/10.2337/diabetes.52.12.3005

Lairon, D., Lopez-Miranda, J., & Williams, C. (2007). Methodology for studying postprandial lipid metabolism. *European Journal of Clinical Nutrition*, *61*(10), 1145–1161. https://doi.org/10.1038/sj.ejcn.1602749

Lappalainen, T. J., Tolppanen, A. M., Kolehmainen, M., Schwab, U., Lindström, J., & Tuomilehto, J. (2009). The common variant in the FTO gene did not modify the effect of lifestyle changes on body weight: the Finnish Diabetes Prevention Study. *Obesity*, *17*(4), 832–836. https://doi.org/10.1038/oby.2008.618

Lasschuijt, M. P., de Graaf, K., & Mars, M. (2021). Effects of oro-sensory exposure on satiation and underlying neurophysiological mechanisms—what do we know so far? *Nutrients*, *13*(5), 1391. https://doi.org/10.3390/nu13051391

Lee, J. S., Chang, P. Y., Zhang, Y., Kizer, J. R., Best, L. G., & Howard, B. V. (2017). Triglyceride and HDL-C dyslipidemia and risks of coronary heart disease and ischemic stroke by glycemic dysregulation status: the strong heart study. *Diabetes Care*, *40*(4), 529–537. https://doi.org/10.2337/dc16-1958

Li, Y., Hruby, A., Bernstein, A. M., Ley, S. H., Wang, D. D., Chiuve, S. E., & Hu, F. B. (2015). Saturated fats compared with unsaturated fats and sources of carbohydrates in relation to risk of coronary heart disease: a prospective cohort study. *Journal of the American College of Cardiology*, *66*(14), 1538–1548. https://doi.org/10.1016/j.jacc.2015.07.055

Linderborg, K. M., Kaur, G., Miller, E., Meikle, P. J., Larsen, A. E., Weir, J. M., Nuora, A., Barlow, C. K., Kallio, H. P., Cameron-Smith, D., & Sinclair, A. J. (2013). Postprandial metabolism of docosapentaenoic acid (DPA, 22: 5n− 3) and eicosapentaenoic acid (EPA, 20: 5n− 3) in humans. *Prostaglandins, Leukotrienes and Essential Fatty Acids*, *88*(4), 313–319. https://doi.org/10.1016/j.plefa.2013.01.010

Liu, Z. (2007). Single nucleotide polymorphism (SNP). In Z. Liu (Ed.), *Aquaculture genome technologies* (pp. 59–72). Wiley. https://doi.org/10.1002/9780470277560.ch6

Lopez-Miranda, J., & Marin, C. (2010). Dietary, physiological, and genetic impacts on postprandial lipid metabolism. In J.-P. Montmayeur & J. le Coutre (Eds.), *Fat detection: Taste, texture, and post ingestive effects* (pp. 417–461). CRC Press/Taylor & Francis. https://doi.org/10.1201/9781420067767-c17

Lopez-Ramos, O., Panduro, A., & Martinez-Lopez, E. (2005). Genetic variant in the CD36 gene (rs1761667) is associated with higher fat intake and high serum cholesterol among the population of West Mexico. *J Nutr Food Sci*, *5*, 1–5. https://doi.org/10.3390/nu8020101

Lu, Y., Feskens, E. J., Boer, J. M., Imholz, S., Verschuren, W. M., Wijmenga, C., & Dollé, M. E. (2010). Exploring genetic determinants of plasma total cholesterol levels and their predictive value in a longitudinal study. *Atherosclerosis*, *213*(1), 200–205. https://doi.org/10.1016/j.atherosclerosis.2010.08.053

Lu, Y., Feskens, E. J., Dollé, M. E., Imholz, S., Verschuren, W. M., Müller, M., & Boer, J. M. (2010). Dietary n− 3 and n− 6 polyunsaturated fatty acid intake interacts with FADS1 genetic variation to affect total and HDL-cholesterol concentrations in the Doetinchem Cohort Study. *The American Journal of Clinical Nutrition*, *92*(1), 258–265. https://doi.org/10.3945/ajcn.2009.29130

Macrotrends. (2022). *The premier research platform for long term investors.* https://www.macrotrends.net/

Manach, C., & Donovan, J. L. (2004). Pharmacokinetics and metabolism of dietary flavonoids in humans. *Free Radical Research*, *38*(8), 771–786.

Marchand, V. (2010). Trans fats: What physicians should know. *Paediatrics & Child Health*, *15*(6), 373–375. https://doi.org/10.1093/pch/15.6.373

Mattei, J., Qi, Q., Hu, F. B., Sacks, F. M., & Qi, L. (2012). TCF7L2 genetic variants modulate the effect of dietary fat intake on changes in body composition during a weight-loss intervention. *The American Journal of Clinical Nutrition*, *96*(5), 1129–1136. https://doi.org/10.3945/ajcn.112.038125

Mayer, J. (1955). Regulation of energy intake and the body weight: The glucostatic theory and the lipostatic hypothesis. *Annals of the New York Academy of Sciences*, *63*(1), 15–43. https://doi.org/10.1111/j.1749-6632.1955.tb36543.x

McDonald, J. H. (2014). *Handbook of biological statistics* (3rd ed.). Sparky House Publishing. http://www.biostathandbook.com/chiind.html

Mead, J. R., Irvine, S. A., & Ramji, D. P. (2002). Lipoprotein lipase: structure, function, regulation, and role in disease. *Journal of Molecular Medicine*, *80*(12), 753–769. https://doi.org/10.1007/s00109-002-0384-9

Mead, M. N. (2007). Nutrigenomics: The genome–food interface. *Environ Health Perspect*, *115*(12), A582–A589. https://doi.org/10.1289/ehp.115-a582

Melis, M., Sollai, G., Muroni, P., Crnjar, R., & Tomassini Barbarossa, I. (2015). Associations between orosensory perception of oleic acid, the common single nucleotide polymorphisms (rs1761667 and rs1527483) in the CD36 gene, and 6-n-propylthiouracil (PROP) tasting. *Nutrients*, *7*(3), 2068–2084. https://doi.org/10.3390/nu7032068

Milan, A. M., Nuora, A., Pundir, S., Pileggi, C. A., Markworth, J. F., Linderborg, K. M., & Cameron-Smith, D. (2016). Older adults have an altered chylomicron response to a high-fat meal. *British Journal of Nutrition*, *115*(5), 791–799. https://doi.org/10.1017/s000711451500505x

Monrroy, H., Borghi, G., Pribic, T., Galan, C., Nieto, A., Amigo, N., & Azpiroz, F. (2019). Biological response to meal ingestion: Gender differences. *Nutrients*, *11*(3), 702. https://doi.org/10.3390/nu11030702

Moon, J., & Koh, G. (2020). Clinical evidence and mechanisms of high-protein diet-induced weight loss. *Journal of Obesity & Metabolic Syndrome*, *29*(3), 166. https://doi.org/10.7570/jomes20028

Moore, J. B. (2020). From personalised nutrition to precision medicine: The rise of consumer genomics and digital health. *Proceedings of the Nutrition Society*, *79*(3), 300–310. https://doi.org/10.1017/s0029665120006977

Mora, S., Otvos, J. D., Rifai, N., Rosenson, R. S., Buring, J. E., & Ridker, P. M. (2009). Lipoprotein particle profiles by nuclear magnetic resonance compared with standard lipids and apolipoproteins in predicting incident cardiovascular disease in women. *Circulation, 119*(7), 931–939. https://doi.org/10.1161/circulationaha.108.816181

Mortensen, L. S., Holmer-Jensen, J., Hartvigsen, M. L., Jensen, V. K., Astrup, A., De Vrese, M., & Hermansen, K. (2012). Effects of different fractions of whey protein on postprandial lipid and hormone responses in type 2 diabetes. *European Journal of Clinical Nutrition*, *66*(7), 799–805. https://doi.org/10.1038/ejcn.2012.48

Müller, T. D., Hinney, A., Scherag, A., Nguyen, T. T., Schreiner, F., Schäfer, H., & Reinehr, T. (2008). ‘Fat mass and obesity associated’ gene (FTO): No significant association of variant rs9939609 with weight loss in a lifestyle intervention and lipid metabolism markers in German obese children and adolescents. *BMC Medical Genetics*, *9*(1), 1–6. https://doi.org/10.1186/1471-2350-9-85

Mullins, V. A., Bresette, W., Johnstone, L., Hallmark, B., & Chilton, F. H. (2020). Genomics in personalized nutrition: Can you “eat for your genes”? *Nutrients*, *12*(10), 3118. https://doi.org/10.3390/nu12103118

Nagai, N., Sakane, N., Tsuzaki, K., & Moritani, T. (2011). UCP1 genetic polymorphism (–3826 A/G) diminishes resting energy expenditure and thermoregulatory sympathetic nervous system activity in young females. *International Journal of Obesity*, *35*(8), 1050–1055. https://doi.org/10.1038/ijo.2010.261

Nakajima, K., Nagamine, T., Fujita, M. Q., Ai, M., Tanaka, A., & Schaefer, E. (2014). Apolipoprotein B-48: A unique marker of chylomicron metabolism. *Advances in Clinical Chemistry*, *64*, 117–177.

National Center for Biotechnology Information. (2005). Clustered refsnps (rs) and other data computed in house. *SNP FAQ Archive*. https://www.ncbi.nlm.nih.gov/snp/

National Heart Foundation of New Zealand. (2022). *What is my BMI?* https://www.heartfoundation.org.nz/wellbeing/bmi-calculator

Nielsen, D. E., & El-Sohemy, A. (2014). Disclosure of genetic information and change in dietary intake: A randomized controlled trial. *PloS One*, *9*(11), e112665. https://doi.org/10.1371/journal.pone.0112665

Nishida, Y., Hachiya, T., Hara, M., Shimanoe, C., Tanaka, K., Sutoh, Y., & Wakai, K. (2020). The interaction between ABCA1 polymorphism and physical activity on the HDL-cholesterol levels in a Japanese population. *Journal of Lipid Research*, *61*(1), 86–94. https://doi.org/10.1194/jlr.p091546

Nunan, D., Aronson, J., & Bankhead, C. (2018). Catalogue of bias: Attrition bias. *BMJ Evidence-Based Medicine*, *23*(1), 21–22. https://doi.org/10.1136/ebmed-2017-110883

Nutrigenomix. (2020). *Nutrigenomix: Eat according to your genes. Training guide for healthcare professionals*. https://nutrigenomix.com/

Oh, B. (2019). Direct-to-consumer genetic testing: advantages and pitfalls. *Genomics & Informatics*, *17*(3). https://doi.org/10.5808/gi.2019.17.3.e33

Ordovas, J. M. (2008). Genotype–phenotype associations: Modulation by diet and obesity. *Obesity*, *16*(S3), S40–S46. https://doi.org/10.1038/oby.2008.515

Page, M. J., McKenzie, J. E., Bossuyt, P. M., Boutron, I., Hoffmann, T., Mulrow, C. D., & Moher, D. (2020). Mapping of reporting guidance for systematic reviews and meta-analyses generated a comprehensive item bank for future reporting guidelines. *Journal of Clinical Epidemiology*, *118*, 60–68. https://doi.org/10.1016/j.jclinepi.2019.11.010

Paoloni-Giacobino, A., Grimble, R., & Pichard, C. (2003). Genetics and nutrition. *Clinical Nutrition*, *22*(5), 429–435. https://doi.org/10.1016/s0261-5614(03)00064-5

Pasco, J. A., Henry, M. J., Kotowicz, M. A., Sanders, K. M., Seeman, E., Pasco, J. R., & Nicholson, G. C. (2004). Seasonal periodicity of serum vitamin D and parathyroid hormone, bone resorption, and fractures: the Geelong Osteoporosis Study. *Journal of Bone and Mineral Research*, *19*(5), 752–758. https://doi.org/10.1359/jbmr.040125

Passarino, G., De Rango, F., & Montesanto, A. (2016). Human longevity: Genetics or lifestyle? It takes two to tango. *Immunity & Ageing*, *13*(1), 1–6. https://doi.org/10.1186/s12979-016-0066-z

Patron, J., Serra-Cayuela, A., Han, B., Li, C., & Wishart, D. S. (2019). Assessing the performance of genome-wide association studies for predicting disease risk. *PLoS One*, *14*(12), e0220215. https://doi.org/10.1371/journal.pone.0220215

Pausova, Z., Syme, C., Abrahamowicz, M., Xiao, Y., Leonard, G. T., Perron, M., & Paus, T. (2009). A common variant of the FTO gene is associated with not only increased adiposity but also elevated blood pressure in French Canadians. *Circulation: Cardiovascular Genetics*, *2*(3), 260–269. https://doi.org/10.1161/circgenetics.109.857359

Pedersen, P., & Milman, N. (2009). Genetic screening for HFE hemochromatosis in 6,020 Danish men: Penetrance of C282Y, H63D, and S65C variants. *Annals of Hematology*, *88*(8), 775–784. https://doi.org/10.1007/s00277-008-0679-1

Pekkarinen, T., Takala, I., & Mustajoki, P. (1996). Two year maintenance of weight loss after a VLCD and behavioural therapy for obesity: Correlation to the scores of questionnaires measuring eating behaviour. *International Journal of Obesity and Related Metabolic Disorders*, *20*(4), 332–337.

Pénicaud, L., Leloup, C., Lorsignol, A., Alquier, T., & Guillod, E. (2002). Brain glucose sensing mechanism and glucose homeostasis. *Current Opinion in Clinical Nutrition & Metabolic Care*, *5*(5), 539–543. https://doi.org/10.1097/00075197-200209000-00013

Pepino, M. Y., Kuda, O., Samovski, D., & Abumrad, N. A. (2014). Structure-function of CD36 and importance of fatty acid signal transduction in fat metabolism. *Annual Review of Nutrition*, *34*, 281–303. https://doi.org/10.1146/annurev-nutr-071812-161220

Peregrin, T. (2001). The new frontier of nutrition science: nutrigenomics. *Journal of the Academy of Nutrition and Dietetics*, *101*(11), 1306.

Pfannenberg, C., Werner, M. K., Ripkens, S., Stef, I., Deckert, A., Schmadl, M., & Stefan, N. (2010). Impact of age on the relationships of brown adipose tissue with sex and adiposity in humans. *Diabetes*, *59*(7), 1789–1793. https://doi.org/10.2337/db10-0004

Phillips, C. M., Kesse-Guyot, E., McManus, R., Hercberg, S., Lairon, D., Planells, R., & Roche, H. M. (2012). High dietary saturated fat intake accentuates obesity risk associated with the fat mass and obesity-associated gene in adults. *The Journal of Nutrition*, *142*(5), 824–831. https://doi.org/10.3945/jn.111.153460

Phillips, T. (2008). Regulation of transcription and gene expression in eukaryotes. *Nature Education*, *1*(1), 199.

Pichler, I., Minelli, C., Sanna, S., Tanaka, T., Schwienbacher, C., Naitza, S., & Pramstaller, P. P. (2011). Identification of a common variant in the TFR2 gene implicated in the physiological regulation of serum iron levels. *Human Molecular Genetics*, *20*(6), 1232–1240. https://doi.org/10.1093/hmg/ddq552

Piers, L. S., Walker, K. Z., Stoney, R. M., Soares, M. J., & O'Dea, K. (2003). Substitution of saturated with monounsaturated fat in a 4-week diet affects body weight and composition of overweight and obese men. *British Journal of Nutrition*, *90*(3), 717–727. https://doi.org/10.1079/bjn2003948

Pressman, P., Clemens, R. A., & Hayes, A. W. (2017). Bioavailability of micronutrients obtained from supplements and food: A survey and case study of the polyphenols. *Toxicology Research and Application*, *1*, 2397847317696366. https://doi.org/10.1177/2397847317696366

Rafieian-Kopaei, M., Setorki, M., Doudi, M., Baradaran, A., & Nasri, H. (2014). Atherosclerosis: process, indicators, risk factors and new hopes. *International Journal of Preventive Medicine*, *5*(8), 927.

Rahbar, E., Waits, C. M., Kirby, E. H., Miller, L. R., Ainsworth, H. C., Cui, T., & Chilton, F. H. (2018). Allele-specific methylation in the FADS genomic region in DNA from human saliva, CD4+ cells, and total leukocytes. *Clinical Epigenetics*, *10*(1), 1–10. https://doi.org/10.1186/s13148-018-0480-5

Ramos-Lopez, O., Milagro, F. I., Allayee, H., Chmurzynska, A., Choi, M. S., Curi, R., De Caterina, R., Ferguson, L. R., Goni, L., Kang, J. X., Kohlmeier, M., Marti, A., Moreno, L. A., Pérusse, L., Prasad, C., Qi, L., Reifen, R., Riezu-Boj, J. I.,·San-Cristobal, R., … Martínez, J. A. (2017). Guide for current nutrigenetic, nutrigenomic, and nutriepigenetic approaches for precision nutrition involving the prevention and management of chronic diseases associated with obesity. *Lifestyle Genomics*, *10*(1–2), 43–62. https://doi.org/10.1159/000477729

Rashid, M., Islam, M. S., Salahuddin, M., Sayfullah, M., & Hossain, D. (2015). The biochemistry of hunger stimulating hormone: Why understanding this cascade in

hypothalamus is beneficial. *Biochem Physiol*, *4*, e139. https://doi.org/10.4172/2168-9652.1000e139

Ravisankar, P., Reddy, A. A., Nagalakshmi, B., Koushik, O. S., Kumar, B. V., & Anvith, P. S. (2015). The comprehensive review on fat soluble vitamins. *IOSR Journal of Pharmacy*, *5*(11), 12–28.

Reddy, V. S., Palika, R., Ismail, A., Pullakhandam, R., & Reddy, G. B. (2018). Nutrigenomics: Opportunities & challenges for public health nutrition. *The Indian Journal of Medical Research*, *148*(5), 632. https://doi.org/10.4103/ijmr.ijmr_1738_18

Roberts, C. K., Hevener, A. L., & Barnard, R. J. (2013). Metabolic syndrome and insulin resistance: Underlying causes and modification by exercise training. *Comprehensive Physiology*, *3*(1), 1. https://doi.org/10.1002/cphy.c110062

Roberts, S. B. (2000). High–glycemic index foods, hunger, and obesity: Is there a connection?. *Nutrition Reviews*, *58*(6), 163–169. https://doi.org/10.1111/j.1753-4887.2000.tb01855.x

Robinson, E., Almiron-Roig, E., Rutters, F., de Graaf, C., Forde, C. G., Tudur Smith, C., & Jebb, S. A. (2014). A systematic review and meta-analysis examining the effect of eating rate on energy intake and hunger. *The American Journal of Clinical Nutrition*, *100*(1), 123–151. https://doi.org/10.3945/ajcn.113.081745

Rodrigues, G. K., Resende, C. M., Durso, D. F., Rodrigues, L. A., Silva, J. L., Reis, R. C., & Alvarez-Leite, J. (2015). A single FTO gene variant rs9939609 is associated with body weight evolution in a multiethnic extremely obese population that underwent bariatric surgery. *Nutrition*, *31*(11-12), 1344–1350. https://doi.org/10.1016/j.nut.2015.05.020

Röhrl, C., & Stangl, H. (2013). HDL endocytosis and resecretion. *Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids*, *1831*(11), 1626–1633. https://doi.org/10.1016/j.bbalip.2013.07.014

Rolls, B. J. (2000). The role of energy density in the overconsumption of fat. *The Journal of Nutrition*, *130*(2), 268S–271S. https://doi.org/10.1093/jn/130.2.268s

Routh, V. H., Hao, L., Santiago, A. M., Sheng, Z., & Zhou, C. (2014). Hypothalamic glucose sensing: Making ends meet. *Frontiers in Systems Neuroscience*, *8*, 236. https://doi.org/10.3389/fnsys.2014.00236

Sachidanandam, R., Weissman, D., Schmidt, S. C., Kakol, J. M., Stein, L. D., Marth, G., & Altshuler, D. (2001). A map of human genome sequence variation containing 1.42

million single nucleotide polymorphisms. *Nature*, *409*(6822), 928–934. https://doi.org/10.1038/35057149

SanGiovanni, J. P., & Chew, E. Y. (2005). The role of omega-3 long-chain polyunsaturated fatty acids in health and disease of the retina. *Progress in Retinal and Eye Research*, *24*(1), 87–138. https://doi.org/10.1016/j.preteyeres.2004.06.002

Schroor, M. M., Mokhtar, F., Plat, J., & Mensink, R. P. (2021). Associations between SNPs in intestinal cholesterol absorption and endogenous cholesterol synthesis genes with cholesterol metabolism. *Biomedicines*, *9*(10), 1475. https://doi.org/10.3390/biomedicines9101475

Sedgwick, P., & Greenwood, N. (2015). Understanding the Hawthorne effect. *BMJ*, *351*. https://doi.org/10.1136/bmj.h4672

Sharma, P., Gillies, N., Pundir, S., Pileggi, C. A., Markworth, J. F., Thorstensen, E. B., & Milan, A. M. (2019). Comparison of the acute postprandial circulating B-vitamin and vitamer responses to single breakfast meals in young and older individuals: Preliminary secondary outcomes of a randomized controlled trial. *Nutrients*, *11*(12), 2893. https://doi.org/10.3390/nu11122893

Shea, M. K., Benjamin, E. J., Dupuis, J., Massaro, J. M., Jacques, P. F., D'agostino, R. B., & Booth, S. L. (2009). Genetic and non-genetic correlates of vitamins K and D. *European Journal of Clinical Nutrition*, *63*(4), 458–464. https://doi.org/10.1038/sj.ejcn.1602959

Sherry, S. T., Ward, M. H., Kholodov, M., Baker, J., Phan, L., Smigielski, E. M., & Sirotkin, K. (2001). dbSNP: The NCBI database of genetic variation. *Nucleic Acids Research,* *29*(1), 308–311. https://doi.org/10.1093/nar/29.1.308

Slater, N. A., Rager, M. L., Havrda, D. E., & Harralson, A. F. (2017). Genetic variation in CYP2R1 and GC genes associated with vitamin D deficiency status. *Journal of Pharmacy Practice*, *30*(1), 31–36. https://doi.org/10.1177/0897190015585876

Sollai, G., Melis, M., Mastinu, M., Pani, D., Cosseddu, P., Bonfiglio, A., & Tomassini Barbarossa, I. (2019). Human tongue electrophysiological response to oleic acid and its associations with PROP taster status and the CD36 polymorphism (rs1761667). *Nutrients*, *11*(2), 315. https://doi.org/10.3390/nu11020315

Speeckaert, M., & Delanghe, J. (2021). Vitamin D binding protein: A key regulator of vitamin D deficiency among patients with pneumonia. *Clinical Nutrition*, *40*(5), 2491–2492. https://doi.org/10.1016/j.clnu.2021.03.024

Stathopoulou, M. G., Dedoussis, G. V., Trovas, G., Theodoraki, E. V., Katsalira, A., Dontas, I. A., & Lyritis, G. P. (2011). The role of vitamin D receptor gene polymorphisms in the bone mineral density of Greek postmenopausal women with low calcium intake. *The Journal of Nutritional Biochemistry*, *22*(8), 752–757. https://doi.org/10.1016/j.jnutbio.2010.06.007

Sterling, R. (2008). The on-line promotion and sale of nutrigenomic services. *Genetics in Medicine*, *10*(11), 784–796. https://doi.org/10.1097/gim.0b013e31818c0441

Stewart, R. A., Held, C., Hadziosmanovic, N., Armstrong, P. W., Cannon, C. P., Granger, C. B., Hagström, E., Hochman, J. S., Koenig, W., Lonn, E., & Nicolau, J. C. (2017). Physical activity and mortality in patients with stable coronary heart disease. *Journal of the American College of Cardiology;70*(14), 1689–1700. https://doi.org/10.1016/j.jacc.2017.08.017

Stunkard, A. J., & Messick, S. (1985). The three-factor eating questionnaire to measure dietary restraint, disinhibition and hunger. *Journal of Psychosomatic Research*, *29*(1), 71–83. https://doi.org/10.1016/0022-3999(85)90010-8

Su, M., Subbaraj, A. K., Fraser, K., Qi, X., Jia, H., Chen, W., & Young, W. (2019). Lipidomics of brain tissues in rats fed human milk from Chinese mothers or commercial infant formula. *Metabolites*, *9*(11), 253. https://doi.org/10.3390/metabo9110253

Su, X., Kong, Y., & Peng, D. Q. (2018). New insights into apolipoprotein A5 in controlling lipoprotein metabolism in obesity and the metabolic syndrome patients. *Lipids in Health and Disease*, *17*(1), 1–10. https://doi.org/10.1186/s12944-018-0833-2

Tam, V., Patel, N., Turcotte, M., Bossé, Y., Paré, G., & Meyre, D. (2019). Benefits and limitations of genome-wide association studies. *Nature Reviews Genetics*, *20*(8), 467–484. https://doi.org/10.1038/s41576-019-0127-1

Thom, G., Dombrowski, S. U., Brosnahan, N., Algindan, Y. Y., Rosario Lopez-Gonzalez, M., Roditi, G., Lean, M. E., & Malkova, D. (2020). The role of appetite-related hormones, adaptive thermogenesis, perceived hunger and stress in long-term weight-loss maintenance: A mixed-methods study. *European Journal of Clinical Nutrition*, *74*(4), 622–632. https://doi.org/10.1038/s41430-020-0568-9

Timoshnikov, V. A., Kobzeva, T. V., Polyakov, N. E., & Kontoghiorghes, G. J. (2020). Redox interactions of vitamin C and iron: Inhibition of the pro-oxidant activity by deferiprone. *International Journal of Molecular Sciences*, *21*(11), 3967. https://doi.org/10.3390/ijms21113967

Tomei, S., Singh, P., Mathew, R., Mattei, V., Garand, M., Alwakeel, M., & Al Khodor, S. (2020). The role of polymorphisms in vitamin D-related genes in response to vitamin D supplementation. *Nutrients*, *12*(9), 2608. https://doi.org/10.3390/nu12092608

Tosi, F., Sartori, F., Guarini, P., Olivieri, O., & Martinelli, N. (2014). Delta-5 and delta-6 desaturases: crucial enzymes in polyunsaturated fatty acid-related pathways with pleiotropic influences in health and disease. In J. Camps (Ed.), *Oxidative stress and inflammation in non-communicable diseases-molecular mechanisms and perspectives in therapeutics* (pp. 61–81). Springer. https://doi.org/10.1007/978-3-319-07320-0_7

Vanlint, S. J. (2005). Vitamin D and adult bone health in Australia and New Zealand: A position statement. *Med J Aust, 183*(1), 52. https://doi.org/10.5694/j.1326-5377.2005.tb06895.x

Vimaleswaran, K. S., & Loos, R. J. (2010). Progress in the genetics of common obesity and type 2 diabetes. *Expert Reviews in Molecular Medicine*, *12*. https://doi.org/10.1017/s1462399410001389

Vincent, S., Planells, R., Defoort, C., Bernard, M. C., Gerber, M., Prudhomme, J., & Lairon, D. (2002). Genetic polymorphisms and lipoprotein responses to diets. *Proceedings of the Nutrition Society*, *61*(4), 427–434. https://doi.org/10.1079/pns2002177

Virtanen, K. A., & Nuutila, P. (2011). Brown adipose tissue in humans. *Current Opinion in Lipidology*, *22*(1), 49–54. https://doi.org/10.1097/mol.0b013e3283425243

Wang, Q., Afshin, A., Yakoob, M. Y., Singh, G. M., Rehm, C. D., Khatibzadeh, S., & Zajkás, G. (2016). Impact of nonoptimal intakes of saturated, polyunsaturated, and trans fat on global burdens of coronary heart disease. *Journal of the American Heart Association*, *5*(1), e002891. https://doi.org/10.1161/jaha.115.002891

Wang, T. J., Zhang, F., Richards, J. B., Kestenbaum, B., Van Meurs, J. B., Berry, D., & Spector, T. D. (2010). Common genetic determinants of vitamin D insufficiency: a genome-wide association study. *The Lancet*, *376*(9736), 180–188. https://doi.org/10.1016/s0140-6736(10)60588-0

Wolfe, R. R., Baum, J. I., Starck, C., & Moughan, P. J. (2018). Factors contributing to the selection of dietary protein food sources. *Clinical Nutrition*, *37*(1), 130–138. https://doi.org/10.1016/j.clnu.2017.11.017

World Cancer Research Fund International. (n.d.). Limit red and processed meat. https://www.wcrf.org/diet-activity-and-cancer/cancer-prevention-recommendations/limit-red-and-processed-meat/

Yang, Y. S., Song, H. D., Li, R. Y., Zhou, L. B., Zhu, Z. D., Hu, R. M., & Chen, J. L. (2003). The gene expression profiling of human visceral adipose tissue and its secretory functions. *Biochemical and Biophysical Research Communications*, *300*(4), 839–846. https://doi.org/10.1016/s0006-291x(02)02843-7

Yi, F., Brubaker, P. L., & Jin, T. (2005). TCF-4 mediates cell type-specific regulation of proglucagon gene expression by β-catenin and glycogen synthase kinase-3β. *Journal of Biological Chemistry*, *280*(2), 1457–1464. https://doi.org/10.1074/jbc.m411487200

Zaki, M. E., Amr, K. S., & Abdel-Hamid, M. (2013). APOA2 polymorphism in relation to obesity and lipid metabolism. *Cholesterol*, *2013*. https://doi.org/10.1155/2013/289481

Zeevi, D., Korem, T., Zmora, N., Israeli, D., Rothschild, D., Weinberger, A., & Segal, E. (2015). Personalized nutrition by prediction of glycemic responses. *Cell*, *163*(5), 1079–1094. https://doi.org/10.1016/j.cell.2015.11.001

Zeisel, S. H. (2007). Nutrigenomics and metabolomics will change clinical nutrition and public health practice: insights from studies on dietary requirements for choline. *The American Journal of Clinical Nutrition*, *86*(3), 542–548. https://doi.org/10.1093/ajcn/86.3.542

Zhang, X., Qi, Q., Zhang, C., Smith, S. R., Hu, F. B., Sacks, F. M., & Qi, L. (2012). FTO genotype and 2-year change in body composition and fat distribution in response to weight-loss diets: The POUNDS LOST Trial. *Diabetes*, *61*(11), 3005–3011. https://doi.org/10.2337/db13-er02a

Appendices

Appendix A: Main Studies Used in the Literature Review Examining the Association Between the Gene and Risk Variant, Outcomes and Limitations.

Study details	Sample(n) & design	Gene & variant and risk ref marker	Risk variant genotype	Measurement objectives	Outcome
Vitamin D Slater et al., 2017 – USA cross-sectional study on low vit D and associated gene variants: VDR (rs2228570), CYP2R1 (rs10741657), DHCR7 (rs12785878), and GC (rs2282679)	180 (n) mainly Caucasian 98 men and 82 women with varying ages between 21 and 80	1. CYP2R1 gene GG/GA rs10741657 2. GC gene GG rs2282679	MAF rs10741657 (OR 3.67; 95% CI: 1.35, 9.99) *P*= 0.011 rs2282679 (OR 0.42; 95% CI: 0.18, 0.93) *P* = 0.037	Binary logistic regression analysis was used to evaluate whether the variants and other potentially confounding factors were associated with the patient's Vit D status. Adjusted confounding factors included age, gender, height, weight, BMI, eGFR, disease states, concomitant medications, and vit D supplements.	For comparison, subjects were separated into two groups based on their Vit D level obtained at the time of enrollment and defined as either sufficient, total 25(OH)D ≤ 30 ng/mL, or insufficient, 25(OH)D < 30 ng/mL. Two genes, CYP2R1 (rs10741657) and GC (rs2282679), demonstrated a significant association related to vitamin D status. Subjects with one or more variant alleles at rs10741657 loci were almost 3.7 times more likely to have low Vit D. Subjects with one or more variant alleles at rs2282679 loci were 0.42 times as likely to have low Vit D. This study found a significant association with low Vit D for patients with variant alleles on genes CYP2R1 (rs10741657) and GC (rs2282679). **Limitations:** There were several potentially confounding variables as Vit D levels are dependent on biological non-gentic factors and environmental condition.
Vitamin D Wang et al., 2010 GWAS,15 cohorts from SUNLIGHT Consortium inc. Europe, Canada & USA to identify genetic variant that influence vit D	33,996 (n) European descent. COHORTS = 5 discovery (n=16,125) , 5 in-silico replication (n = 9,367) & 5 de-novo replication (n = 8,504)	1. CYP2R1 GG/GA rs10741657 2. GC GG rs2282679	4p12-$P = 2{\cdot}9{\times}10^{-109}$ overall for rs2282679 near GC 11q12-$P = 2{\cdot}1{\times}10^{-27}$ for rs12785878, near DHCR7 11p15-$P = 3{\cdot}3{\times}10^{-20}$ for rs10741657, near CYP2R1	25(OH)D conc. was measured and adjusted p values for effects of mild population stratification and to prevent inflation of type I error. Combined results of GWAS across cohorts using Z-score-weighted meta-analysis. *P* value of $< 5 \times 10^{-8}$ statistically significant.	Three of the variant alleles in GC (rs2282679), DHCR7 (rs12785878), and CYP2R1 (rs10741657) SNPs at three loci were SS to 25(OH)D concentration and explain 1-4% variation in 25(OH)D. The findings showed that all SNPs were associated with Vit D levels and linked to deficiency. Subjects with multiple variant alleles in the highest quartile were at a greater risk of

					deficiency. Vit D deficiency was defined as < 75 nmol/L (OR 2·47, 95% CI 2·20, 2·78, $P = 2·3×10^{-48}$) or < 50 nmol/L (1·92, 1·70, 2·16, $P = 1·0×10^{-26}$) in highest quartile than the lowest quartile. Risk of severe Vit D deficiency (25-OH D < 20 nmol/L), with an adjusted OR in the top quartile of 1.43 (95% CI, 1.13, 1.79; *P* = 0.02) compared to the lower quantile. The presence of risk alleles at the three loci more than doubled the odds of Vit D insufficiency. **Limitations:** There were several potentially confounding variables as Vit D levels are dependent on skin complexions, sunlight exposure, obesity suggesting the genetic variant may not be the only causal effect of low Vit D .
Iron overload Allen et al., 2008, 12 yrs Melbourne Collaborative Cohort Study Assessed for mutation HFE gene.	31,192 (n) 27–75 yrs (99% 40-69) European descent. Stratified random sampling 1,438(n) & 203(n) C282Y Homozygous (19 died)	C282Y AA rs1800562 **see Low Fe for: research on** SLC17A1 rs17342717 (**iron overload**) HFE AA rs1800562 & rs1799945 (**iron overload**)	Subjects with C282Y AA homozygous gene – 21 men had iron overload 28.4% (95% CI, 18.8, 40.2) & 1 female had iron overload 1.2% (95% CI, 0.03, 6.5)	Genotype groups compared by analysis of variance for continuous measures, serum ferritin analysed on natural log. scale, or by chi-square test for proportions. The hazard ratio (HR) for death from any cause among C282Y homozygotes, as compared with subjects who had no C282Y mutation, was 1.04 (95% CI, 0.67, 1.62; *P* = 0.87).	Genotyping of 29,676 of 31,192 samples (95.1%) was successful. The stratified random sampling of 1,438 included 203, C282Y homozygous carriers. Of the 74 men who completed the study and were C282Y homozygotes, 21 had iron overload related diseases. Of the women, 84 completed the study, and one had an iron overload. Therefore, homozygous C282Y carriers, especially men with serum ferritin levels of ≥ 1000 μg/L, are at greater risk of disease caused by the HFE gene. **Limitations:** 95% CI range in both males (18.8, 40.2) and females were too wide (0.03, 6.5) to give meaningful results. The study has high atrition rate and low power due to the small number of subjects with AA genotype who competed the study.
Low Fe Pichler et al., 2011 meta-analysis of two (GWAS)	5 population-based studies using	1. TMPRSS6 GG/GA rs4820268, 2. TRF2	GG genotype compared to the AA associated with a decrease in iron ($P = 1.3 \times 10^{-9}$) and an inc.	SNPs based on estimated power to replicate **Fe,** association TFR2 rs7385804, (*P* =	This meta-analysis GWAS 5 population-based studies identify gene loci associated with variation in serum

European & USA, replicated top variants in 3 cohorts	European and USA GWAS	CC rs7385804, A/C allele 3. TF AA rs3811647 4. SLC17A1 rs17342717 (**iron overload**) 5. HFE AA rs1800562 & rs1799945 (**iron overload**)	in transferrin ($P =$ 0.007) sTfR ($P =$ $1x10^{-4}$) and sTfR-ferritin index (P +0.005).	$5.0×10^{-4}$) confirmed previous association in TMPRSS6 rs4820268, ($P = 5.5×10^{-7}$) and HFE rs1799945, ($P = 0.00$). Direction and magnitude of effect sizes confirmed in all studies. **Ferritin**, association SLC17A1 rs17342717, ($P = 8.0×10^{-6}$) and this signal likely reflects association with HFE. In fact, SLC17A1 rs17342717 correlates with the HFE rs1800562, associated with HH. **Transferrin**, association of TS rs3811647 ($P =$ $1.2×10^{-10}$).	levels of iron markers. Including iron, transferrin, ferritin, soluble transferrin receptor (sTfR) and sTfR–ferritin index. The study used the subject's human liver samples data and analyses of hepcidin levels to find common variants in the TMPRSS6 gene. Forest plots estimated the genetic effects and were highly consistent across studies, with low I^2 values (% of the observed heterogeneity in excess of what can be explained by chance alone) of 8 and 0%, respectively. TFR2, TMPRSS6 and HFE were all shown to be involved in hepcidin regulation in iron. The TMPRSS6 SNP rs4820268 showed the strongest association with Fe levels (effect size for each copy of G allele from the combined analysis: 24.2 ug/ dl; 95% CI: -5.5, -3.0). **Limitations:** To prove results study needs a large sample to improve overall power.
Zinc da Rocha et al., 2014, cross-sectional GWAS Brazil study	56 women and 14 men 64.86 ± 8.75 years	SLC30A3 CC rs11126936	Results showed that the serum zinc levels in patients with the CC genotype of the rs11126936 polymorphism was lower than non-CC carriers.	Serum zinc measured by flame atomic absorption spectrophotometry, ref values 0.70 to 1.50 mg/L. Age and sex adjustments made. Correlation between age and serum zinc was estimated by Pearson correlation coefficient.	The study found age was negatively correlated with serum zinc levels ($r =$ −0.25, $P = 0.008$). In addition, 29 subjects in the sample (26.4%) had a serum zinc level below the reference value. The *SLC30A3* SNP rs11126936, after adjusting for age and sex, serum zinc mg/L were lower in the CC genotype (0.74 ± 0.30) than in the A carriers (0.89 ± 0.28, $P = 0.014$). When the subjects were grouped according to low or normal/high zinc levels, the low zinc levels (34.5%) were observed more frequently in the CC genotype carriers than the A allele carriers (18.2%, $P = 0.024$). **Limitations:** Small sample size, low power, no food-frequency questionnaire, diet

					recall, or anthropometric data. Therefore, this study needs to be read with caution.
Omegas-6 PUFA & 3 PUFA Lu, Feskens, Dollé et al., 2010 Doetinchem (GWAS) Cohort study, Netherlands	3575 (n), 48% men. Mean age 46.7 yrs	FADS1 CC/CT rs174547	Total cholesterol differed significantly with the rs174546 (complete linkage with the rs174547 variant) in genotype CC (*P* = 0.02). C allele was associated with a statistically significant lower HDL-cholesterol concentrations in subjects with a high n−6 PUFA intake (*P* = 0.004)	The relations between variants and plasma total, HDL-, and non-HDL cholesterol were explored with General Linear Models and adjusted for potential confounding effects.	Three variants were measured in the FADS1 gene variant and analysed n-6 & n-3 PUFA intake on plasma cholesterol. Subjects with high n-3 PUFA intakes had significantly higher HDL cholesterol than those with low n-3 PUFA intakes (*P* = 0.02). No significant associations between n-6 PUFA intake and any lipid variables were observed. The C allele was associated with higher total cholesterol and lower HDL cholesterol. However, this association was more pronounced and only statistically significant (*P* = 0.006) in subjects with a high n−3 PUFA intake. **Limitations:** The *P* values for interaction between rs174546 genotypes (in linkage with rs174547) and n−3 PUFA intake on total and low HDL-cholesterol concentrations were not statistically significant. In comparison, no difference was observed in subjects with a low n−6 PUFA intake. PUFA intake did not modify the associations between the other two variants (rs174570 and rs482548).
Energy balance Nagai et al., 2011 Japan GWAS Cohort study using noninvasive genotyping method	82 lean females. 20-22 yrs from same university campus genotyped for the -3826 GA of the UCP1 gene	UCP1 GG/GA rs 1800592	Diminished REE in G allele and reduced thermoregulatory SNS activity for GG genotype, suggest that attenuated UCP1-linked thermogenesis has adverse effect on the regulation of energy balance	Thermoregulatory SNS activity was assessed by heart rate variability power spectral analysis.	To investigate whether UCP1 polymorphism was associated with resting energy expenditure (REE) and thermoregulatory sympathetic nervous system (SNS) activity in humans. The frequencies of AA, GA and GG genotypes were 0.27, 0.45 and 0.28, respectively. No significant difference was found in anthropometric indexes among the three groups. However, in the GG group, the percentage

					of fat energy consumed lower (AA: 30.7 [+ or -] 1.1%, GA: 31.3 [+ or -] 1.0%, GG: 26.0 [+ or -] 1.2%, *P* < 0.01), and energy intake tended to be lower (AA: 7209 [+ or -] 310 kJ [d.sup.-1], GA: 7075 [+ or -] 280 kJ [d.sup.-1], GG: 6414 [+ or -] 264 kJ [d.sup.-1], *P* = 0.16). **Limitations:** The study had low power and only performed on young healthy women therefore the study's results are not reliable on their own.
Total fat Grau et al., 2010 Europe RCT parallel, 2-arm, open-label, 10-wk dietary intervention	771(n) & 739 completed European obese 75% women Aged 20–50 yrs	TCF7L2 TT rs7903146	Found significant interactions between genotype TT compared with CC and CT and diet in relation to weight. *P* for interaction: 0.023; n = 622)	Genotyping of the rs7903146 variant was performed by using the TaqMan SNP Genotyping Assays. Assessments of main effects was conducted by including the genotypes as covariates and as a separate covariate in the diet group.	Participants consumed a high-fat diet (HFD) 40-45 % fat energy (LFD) 20-25% fat energy. Both diets had 15% protein and 60-65 % carbohydrates and provided 600 kcal/d (2510 kJ/d). Anthropometric measures and body composition were assessed. The mean weight was 6.81 kg. There was no difference in weight loss for subjects with the CC/CT genotype between the HFD and LFD diet (*P* = 0.35). In individuals with the TT genotype, weight loss was 2.57 kg smaller (*P* = 0.0088) with the HFD than with the LFD diet. With the HFD diet, weight loss was 2.08 kg smaller (*P* = 0.010) for the TT genotype than for the CC/CT genotype. The effects of TCF7L2 rs7903146 on weight loss were larger than any effect found of 43 SNPs in 27 genes. **Limitations:** When analysing diet-related studies results participants change their behaviour ("Hawthorne Effect") and maintaining strict adherence to the diets throughout the study period is difficult. Thus, this research used post hoc analysis, and interpretation can be skewed.
Saturated fat Corella et al., 2009 USA Meta analysis of 3	FOS – 1454 non-Hispanic white	APOA2 CC rs5082	The genotype CC recorded an OR 1.84 (CI 95%, 1.38, 2.47, P < 0.001)	All populations, logistic regression models, included main effects and interaction terms,	Meta-analysis of study-specific estimates of ORs for the two strata of saturated-fat intake

populations, FOS, GOLDN, BPR. Cohort study	GOLDN 1078 European BPR 930 Puerto Rican All 26 – 80 yrs.		compared to genotype TT and TC when consuming (> 22g/day) high-saturated-fat diet.	fitted to test APOA2–saturated fat interaction in determine the OR of obesity. Study-specific ORs and 95% CI were estimated for 2 strata of < or > 22g/day of saturated fat. Multivariate adjustments performed.	< and > 20g of saturated-fat. The study's power was 80% and found that the APOA2 concentrati ons were significantly lower in individuals with the CC genotype. When low-saturated-fat intake, the APOA2 −265T>C SNP does not affect BMI. However, this SNP is significantly associated with BMI and obesity when high-saturated-fat intake, with a cut-off point of 22 g/d defining the two saturated fat strata. Different responses to saturated fat, depending on the individual genotype. The effect of saturated fat on BMI and obesity is highly dependent on the APOA2 −265T>C genotype. **Limitations:** Results only infer a gene–nutrient interaction between BMI and obesity in individuals who carry the APOA2 CC gene variant when consuming a saturated fat diet of > 22 g/day.
Saturated and unsaturated fat Phillips et al., 2012 France Case-control LIPIGENE	1754 (n) men & women aged 51 - 61yrs.	FTO AT/AA rs9939609	MS measurements are more elevated when on a high-saturated-fat diet and participants are more at risk of obesity. Genotype AA or AT were more receptive to weight loss compared to genotype TT.	To determine modulation by dietary-fat consumption, logistic analyses were repeated using the median of control individuals to dichotomise intakes and to examine associations in low and high consumers. Generalised estimating equation linear regression. Potential confounding factors used in the adjusted multivariate analysis.	This study investigated the FTO gene SNP rs9939609 links to obesity and metabolic syndrome (MS). MS was measured as having ≥ three of the following indicators of obesity, high-saturated-fat intake, elevated waist circumference, TG, fasting blood sugar and BP and low HDL. Measurements were recorded over 2 months and followed up at 7.5 years. The study concluded that the carriers of the A allele had higher MS measurements (OR = 1.66; 95% CI: 1.07, 2.57) *P* = 0.02 and carried a greater risk of obesity than the TT genotype. Waist circumference (*P* = 0.04) and BMI of ≥ 25 kg/m (*P* = 0.02) was higher in the AA and AT genotype participants whose saturated fat intake was ≥ 15.5 % and PUFA: the saturated fat ratio was < 0.38

					compared to the TT genotype. Gender-specific results were observed in the male risk allele carriers, but females were not significant (*P* = 0.09 and *P* = 0.08, respectively). **Limitations:** Due to the study using self-reporting questionnaires, it was prone to memory loss and inaccurate dietary accounts.
Monounsaturated fat Garaulet et al., 2011 Spanish Treatment Programme cohort Study	1456 (n) 20 -65 yrs attended five out-patient obesity clinics during 2009–2010 in the city of Murcia	PPARγ2 polymorphism Pro12Ala GG/GC rs1801282	Minor allele G associated with the PPARγ2 rs1801282 Pro12Ala SNP polymorphism and MUFA	Logistic regression models were used to estimate the OR and 95% CI of obesity and specific MetS components. Confounding controlled for and *P* = 0.039 for BMI and 0.02 for body fat	The study found a gene–diet interaction between the PPARγ2 Pro12Ala polymorphism and MUFA (% total fat) for BMI and body fat (%). Carriers of the Ala12 variant minor allele G were significantly less obese than homozygous major subjects (CC) when the MUFA intake was high (≥ 56% of total fat) (*P* = 0.02). **Limitations:** The study used overweight and obese subjects and no significant differences between carriers and noncarriers were found in the low-MUFA intake group (*P* = 0.75). Based on the results, it is still unclear whether PPARγ2 influences weight reduction in response to a dietary treatment.
Protein Zhang et al., 2012 USA. 2 years GWA POUNDS LOST study identified SNP & RCT 2x2 factorial design	742 (n) & 642 (86.5%) completed trial 61% female, 80% white, 15% black, 3% hispnic, 2% asian Aged 30-70 years	FTO AA rs1558902 genotype. has strong linkage disequilibrium with other obesity-associated FTO variants such as the rs9939609 genotype.	Measures of total fat, FFM, FM%, and % of trunk fat and the risk allele (A) was significantly associated with a 1.51kg greater weight loss in the high-protein group (HPD) (*P* = 0.010), but not in the low-protein group (LPD) at the end of 2 years.	A dual-energy X-ray absorptiometry (DEXA) scan was performed on 50% of a random sample. The allele frequency in two major ethnic groups (white and black) was compatible with Hardy-Weinberg equilibrium (*P* > 0.05). The Hardy-Weinberg equilibrium and comparison of categorical variables at baseline were assessed with chi-square test and adjusted for age, sex, and ethnicity.	Random assignment of the subject's to one of four diets. The primary outcomes were changes in body weight and weight circumference. Secondary outcomes were changes in body composition, including total fat mass, FFM, FM% and percentage of trunk fat, and fat distribution (TAT, VAT, SAT, and DSAT). Low protein versus high protein and low fat versus high fat were compared. Tests for genotype-diet protein interaction were significant on changes in FFM and FM% (for interactions, *P* = 0.034 and 0.049, respectively). There was no significant genetic effect or

					interaction between the FTO variant and dietary fat. **Limitations:** The lack of association with baseline BMI is probably largely because the participants were overweight or obese. The groups had relatively smaller variances in BMI than the general population. The study found that the FTO gene and a HPD were linked but not to the HFD. The study had 80% white participants, whilst overadjusted statistical comparisons increased the type II error.
Total cholesterol Lu, Feskens,Boer, et al., 2010 Netherlands 11 years Longitudinal GWAS study	1,668 (n) European descendant 20–59 years	APOA5 CC/TC rs662799	Adjusted for potential confounding the APOA5 SNP rs 662799 genotype CC and TC had total average cholesterol reading of 0.18 mmol/L higher compared to genotype TT participants.	The intake of total fat and cholesterol was averaged over the second and third surveys and the medians (34.9% energy and 224.9 mg/day, respectively) were used to categorise the subjects into low or high intake of fat or cholesterol.	1,668 European participants were followed over 11 years, and the study took three nonfasting cholesterol blood readings. The APOA5 variant rs662799 was statistically significant and associated with total blood cholesterol or LDL levels. When analysing only genotype CC carriers, the magnitude of their reading doubled (*P* = 0.0066) compared to genotype TC and TT. **Limitations:** The study did not prove clear evidence for the relationship between the APOA5 SNP rs662799 and its association to total cholesterol levels as environmental exposures of the participants could have influenced the results. Therefore, future studies performed on different ethnic groups and controlled for environmental exposures are needed.
LDL-C & HDL-C & TG Kathiresan et al., 2009 European Ancestry Meta-analysis using 7 GWA (The Framingham Heart, LOLIPOP, SUV1MAZ, inCHIANTI, DGI, FUSION and SardiNIA)	GWAS screened 19,840 (n) and replication in up to 20,623 (n)	*LDL-C* ABCG8 TT/TC rs6544713 HDL-C ABCA1 TT/TC rs1883025 TG ANGPTL3 AA/AC Rs10889353	it is hypothesised that; **ABCG8** - T allele leads to lower ABCG8 function resulting in higher levels of LDL-C, uptake of cholesterol and lipids, and a reduced secretion of lipids into the liver and bile. **ABCA1** - T allele leads to lower ABCG1 function resulting in lower levels of HDL-C uptake of cholesterol and lipids.	Variance-weighted meta-analysis and additional analysis applied a uniform analysis strategy to all sample sets to estimate regression coefficients (measuring association between each SNP and lipid levels). Test for association in each replication study using linear regression and	This study identified 30 distinct loci associated with lipoprotein levels (each with P < 5 x [10.sup. -8]), including 11 loci that reached GW significance for the first time. This meta-analysis used GWAS and a large-scale replication approach, mapping 30 loci that contribute to variation in lipoprotein concentrations in humans. The 11 newly

and large-scale replication study			**ANGPTL3 –** A allele leads to lower ANGPTL3 function resulting in higher levels of TG in the blood plasma.	adjusting for covariates.	defined loci included common variants associated with LDL-C near ABCG8, HDL-C near ABCA1 and TG near ANGPTL3 gene. The research concluded that the T allele carriers (TT and TC) were more at risk of high LDL-C than CC carriers. That TT and TC carriers had a 0.08 standard deviation lower HDL-C levels (*P* = 0.001) than individuals with the CC genotype. That carriers of the A allele (AA and CA) had 0.05 standard deviation higher TG levels (*P* < 0.0001) than the CC genotype. **Limitations:** Further investigations involving different ethnicities and population groups with rigorous experiment procedures using RCT are needed
Fat-taste perception Melis et al., 2015 Italy GWAS RCT	64 (n) Caucasian subjects (23 males, 41 females, age 27.6 ± 0.85 years)	CD36 GG/GA rs1761667	Genotype AA had reduction in the CD36 gene expression and classed “typical tasters.” Genotype GG were supertasters to detecting oleic acid and GA were considered intermediate tasters.	Homozygous for the G allele rs1761667 exhibited a 5-fold lower threshold for oleic acid than AA subjects (*P* = 0.041, Fisher LSD test).	Three sessions separated by 1 month; subjects were assessed for PROP taster status in the first two sessions. In the third session, sensitivity to oleic acid flavour was assessed. The study used a randomised taste test to examine if there was an association between oro-sensory exposure to oleic acid and CD36 genetic variant rs1761667. The study concluded a reduction of CD36 gene expression in genotype AA carriers. **Limitation:** the study had low power due to the small number of participants and more research is needed to investigate if this is linked to supertasters reducing dietary-fat intake due to earlier satiation.
Sugar preference Eny et al., 2008 Canada GWAS Cohort Study	2 populations P1-50 men,50 women BMI 30.7 ± 4.2 42-75 years P2-182 men, 405 women,	GLUT2 CT/TT rs5400	In P1 and P2 individuals with the SNP rs5400 genotype CT or TT had a higher consumption of sugar compared to genotype CC.	Unpaired *t*-tests assuming unequal variances were used to compare characteristics and dietary intakes between genotypes, and Wilcoxon tests were used if variables were skewed. The χ^2-test was used for categorical variables. Multiple linear regression adjusted	P1 - individuals with the GLUT2 genetic variant rs5400 genotype CT or TT had a more significant daily sugar consumption. Sugar consumption was assessed over a three-day food diary collated during two visits 2 weeks apart (visit 1: 112 ± 9 vs 86 ± 4 g/day, *P* = 0.01; visit 2:

	young and healthy 20-29 years			used to test for differences in nutrients and food groups consumed.	111 ± 8 vs 82 ± 4 g/day, *P* = 0.003) compared to genotype CC. P2 - individuals with the SNP rs5400 genotype CT or TT had a higher sugar consumption over 1 month analysed from a food-frequency questionnaire (FFQ) (131 ± 5 vs. 115 ± 3 g/day, *P* = 0.007) compared to genotype CC. Carriers of the T allele consumed more sucrose (55 ± 3 vs. 47 ± 1 g/day, *P* = 0.01), fructose (28.0 ± 1.3 vs. 25.4 ± 0.7 g/day, *P* = 0.04), and glucose (26.0 ± 1.2 vs. 23.7 ± 0.6 g/day, *P* = 0.03). Limitations: sugar craving have links to other factors, including mood, environment, culture, lifestyle, and pleasure-generated response. Further RCT-type research is needed to control individual food intake as FFQ are sometimes unreliable.
Susceptibility for hunger Bouchard et al., 2004 Quebec Prospective GWA study	274 males and 386 females aged 27-58 years with BMI of 20.3 kg/m^2 to 37.9 kg/m^2	NMB TT rs1051168	Results show a significant relationship between the NMB genetic variant rs1051168 genotype TT and eating behaviour with predisposition to obesity	A χ^2 test was applied to compare genotypic frequencies between groups with low, intermediate, and high scores on the 3FEQ susceptibility to hunger scores, there were no difference between sex. Genetic associations were assessed by analysis of covariance comparing mean phenotypic values across NMB genotype s. If significant differences were detected, Tukey's test was used to determine differences among genotypes.	The study aimed to identify numerous trait loci involved in eating behaviours. They found that the most significant linkage between locus and susceptibility to hunger (*P* < 0.0001) was the NMB genetic variant rs1051168 genotype TT compared to genotype GG or GT. The rs1051168 revealed a missense mutation resulting in a genetic variation within the NMB gene and was statistically significant (OR: 1.9; 95% CI: 1.15, 3.06; *P* = 0.01). After 6-year genotype carriers, TT gained more than twice as much body fat than noncarriers GG or GT genotype (3.6 compared with 1.5kg; *P* < 0.05). **Limitations:** Food-frequency studies are prone to recording errors of participants. For complex behaviours such as eating, it is unlikely that a single SNP could account for the results fully. Therefore,

					more research is needed to identify other mutations in the NMB gene or in that location.
Fasting glucose Dupuis et al., 2010 Europe meta-analysis study of 21 GWAs	46,186 European nondiabetic subjects that included loci associated with fasting glucose near the ADCY5 gene	ADCY5 GA/AA rs11708067	The A allele carriers had elevated fasting glucose levels and increased risk of T2DM compared to those that carried the G allele	Measured expression of the genes mapping closest to lead SNPs in DGKB/TMEM195, ADCY5**,** MADD.	This study was part of the Glucose and Insulin-related traits Consortium (MAGIC), which conducts large-scale meta-analyses using GW data for continuous diabetes-related traits in nondiabetic participants. After adjustments for BMI, the results demonstrated that the ADCY5 gene was associated with elevated fasting glucose levels of 0.027 mmol/L in A allele carriers ($P = 0.0001$). Therefore, A allele carriers had an increased risk of T2DM compared to G allele carriers. **Limitations:** The study's findings could have been due to other factors influencing the results, such as diet or lifestyle of the patients, and a cause-and-effect relationship may not be solely due to the rs11708067 SNP.
Fasting insulin Almgren et al., 2017 Swedish, Finish population-based cohort	3,344 Swedish male & female subjects born 1923–1950. Results meta-analysed with 4,905 Finnish subjects	IRS1 CT/CC rs2943641	CT or CC genotypes had greater levels of fasting insulin concentrations compared to TT genotype carriers	Serum insulin was assayed with an ELISA kit (K6219, Dako) and had no significant cross reactivity to proinsulin (range 0.5–206 U/l). Serum insulin was measured by an AutoDelfia fluoroimmunometric assay (B080-101, PerkinElmer). Association of SNPs with insulin levels was analysed using linear regression models and, for prediction of risk of T2DM using Cox regression models and adjusted for sex and age.	The study searched for a link between nondiabetic participants and fasting insulin levels. They found that a location near the IRS1 gene variant rs2943641 showed a significant association ($P = 2.4 \times 10^{-7}$). Almgren et al. concluded that participants who carried the CT or CC genotypes had greater fasting insulin levels than TT genotype carriers. **Limitation:** The study's findings could have been due to other factors influencing the results, such as diet or lifestyle of the patients, and a cause-and-effect relationship may not be solely due to the rs2943641 SNP.

Note. Gene name and reference SNP, frequency of the minor allele (MAF), risk variant (RV) effect size using beta coefficients (estimated results from regression analysis) odds ratios (OR) or confidence intervals (CI) are used to describe the strength of an association, and a $P < 0.05$ is statistically significant (SS).

Appendix B: Baseline Data of Participants Before Food Consumption

ID	BMI	BMI kg/m²	Weight (kg)	Height (cm)	Systolic pressure (mm Hg)	Diastolic pressure (mm Hg)	HR (beat/min)
1	21.6	Healthy	61.00	168.0	116	66	71
2	22.7	Healthy	77.58	184.9	108	62	72
3	21.9	Healthy	69.18	177.7	115	71	90
4	22.3	Healthy	69.42	176.3	101	71	74
5	24.6	Healthy	71.32	170.1	108	72	69
6	26.8	Overweight	83.60	176.7	119	84	70
7	22.1	Healthy	70.98	179.2	122	76	61
8	27.6	Overweight	80.00	170.3	130	79	59
9	24.7	Healthy	74.80	173.9	113	68	65
10	22.5	Healthy	66.60	172.0	96	66	59
11	22.6	Healthy	72.04	178.5	117	71	68
12	24.7	Healthy	71.38	170.1	110	71	53
13	25.9	Overweight	88.38	184.7	120	74	64
14	24.9	Healthy	82.14	181.7	124	80	63
15	23.8	Healthy	78.32	181.5	106	72	58
16	25.2	Overweight	92.70	191.8	120	77	63
17	29.1	Overweight	91.18	177.0	114	78	63
18	22.6	Healthy	69.70	175.7	141	94	92
19	23.3	Healthy	75.64	180.0	140	88	61
20	31.3	Obese	100.76	179.4	122	82	74
21	23.3	Healthy	75.04	179.6	131	87	60
22	25.3	Overweight	82.76	181.0	130	90	71
23	22.3	Healthy	69.06	175.8	122	82	64
24	29.5	Overweight	83.52	168.2	138	89	71
25	25.6	Overweight	80.20	177.0	99	57	80
26	24.1	Healthy	76.52	178.3	111	62	56
27	20.3	Healthy	55.68	165.6	101	80	79
28	23.7	Healthy	66.62	167.6	112	72	63
29	21.6	Healthy	68.12	177.7	114	70	49
30	29.2	Overweight	92.62	178.2	120	79	80
Mean	24.5		76.6	176.6	117.3	75.7	67.4
SD	2.7		10.0	5.8	11.7	9.0	10.0

Note. BMI Categories (kg/m^2): Underweight < 18.5, Healthy/Normal 18.5–24.9, Overweight 25–29.9, Obese > 30

Appendix C: Ethics Approval

Health and Disability Ethics Committees
Ministry of Health
133 Molesworth Street
PO Box 5013
Wellington
6011

hdecs@health.govt.nz

30 November 2020

Dr Andrea Braakhuis
The University of Auckland
Private Bag 92019
Auckland Mail Centre
Auckland 1142

Dear Dr Braakhuis,

Re:	**Ethics ref:**	**19/STH/226/AM02**
	Study title:	Acute Clinical Evidence of Digestive, Metabolic and Nutritional Differences in Beef and Meat-Alternative Meals

I am pleased to advise that this amendment has been *approved* by the Southern Health and Disability Ethics Committee. This decision was made through the HDEC Expedited Review pathway.

Please don't hesitate to contact the HDEC secretariat for further information. We wish you all the best for your study.

Yours sincerely,

Mrs. Helen Walker
Acting Chairperson
Southern Health and Disability Ethics Committee

Encl: Appendix A: documents submitted
Appendix B: statement of compliance and list of members

Appendix A
Documents submitted and approved

Document	Version	Date
Declined letter for previous application in respect of the same (or substantially similar) study: Amendment decline letter	1	02 October 2020
Covering letter: Memo	1	02 November 2020
PIS/CF: Updated PISCF	6	02 November 2020
Protocol: Updated protocol document	6	02 November 2020
Post Approval Form	AM02	02 November 2020
PIS/CF: Updated PISCF	7	23 November 2020
Covering letter: Ethics Memo for amendment 2	1	23 November 2020
Response to Request for Further Information		

Appendix B Statement of compliance and list of members
Statement of compliance
The Southern Health and Disability Ethics Committee:

- is constituted in accordance with its Terms of Reference
- operates in accordance with the *Standard Operating Procedures for Health and Disability Ethics Committees*, and with the principles of international good clinical practice (GCP)
- is approved by the Health Research Council of New Zealand's Ethics Committee for the purposes of section 25(1)(c) of the Health Research Council Act 1990
- is registered (number 00008713) with the US Department of Health and Human Services' Office for Human Research Protection (OHRP).

List of members

Name	*Category*	*Appointed*	*Term Expires*
Mrs Helen Walker	Lay (consumer/community perspectives)	19/08/2020	19/08/2021
Dr Pauline Boyles	Lay (consumer/community perspectives)	05/07/2019	05/07/2022
Dr Paul Chin	Non-lay (intervention studies)	27/10/2018	27/10/2021
Mr Dominic Fitchett	Lay (the law)	05/07/2019	05/07/2022
Dr Sarah Gunningham	Lay (other)	05/07/2016	05/07/2022
Assc Prof Mira Harrison-Woolrych	Non-lay (intervention studies)	28/06/2019	28/06/2020

Professor Jean Hay-Smith	Non-lay (health/disability service provision)	31/10/2018	31/10/2021
Dr Devonie Waaka	Non-lay (intervention studies)	18/07/2016	18/07/2019

Unless members resign, vacate or are removed from their office, every member of HDEC shall continue in office until their successor comes into office (HDEC Terms of Reference)

Appendix D: Participant Information Sheet

MEDICAL AND HEALTH SCIENCES

PARTICIPANT INFORMATION SHEET

Project title: **Acute Evidence of Digestive, Metabolic and Nutritional Differences in Beef, Lamb and Meat-Alternative Meals**

Principal Investigator: Dr Andrea Braakhuis (The University of Auckland)
Research Team: Dr Matt Barnett (The Liggins Institute), Dr Toan Pham (The University of Auckland), Ms Julie Brown (The University of Auckland)

Research introduction and aim

There has been much discussion about the nutritional value of eating red meat, however very few scientific studies have been conducted on red meat arising from different feeding systems (grain versus grass) and on meat-alternatives. The aim of this study is to investigate what nutrients end up in the bloodstream after consuming a grass-, grain-fed beef, or grass-fed lamb, or plant-based meat alternative containing meal.

Project description and invitation

You have been invited to participate because you are a male between the age of 20 and 34 years who eat red meat. This study will involve you visiting the Research Facility at The University of Auckland on four separate occasions in the morning. Each visit you will be required to consume a red meat or meat alternative containing meal and bloods will be taken for 4 hours after the meal. Before any meal, your saliva sample will be also taken only in the first visit.

Project Procedures

There will be four study visits in total with at least one week between meals. Any questions or concerns about the study will be discussed via email or phone prior to commencing. You will be asked to complete pre-screening documentation prior to be invited to participate. If you are satisfied with everything and agree to take part, we will ask you to sign the consent form (below) prior to testing. The study includes four test clinical visits. The four test occasions will occur on mutually agreed upon days, with at least one week, but no more than one month apart. Each test day you will be joined with other participants.

All interested participants will be asked to complete a screening questionnaire which will ask for weight, height, physical activity, ethnicity, education, and eating habits. Participants will be excluded if they are smokers, have previously tested with high cholesterol or blood lipids or demonstrated disordered eating habits.

Procedures for Test Days

The procedures for all four visits will be the same. Before each clinical visit, you will be asked to avoid eating foods high in fat and fibre as well as alcohol and caffeine. You are not to eat after 10 pm as this will affect your digestion in the morning. Except for water, you will be required to fast overnight and then come to the Clinical Research Centre as scheduled in the morning. You will be asked to provide informed consent and will have your height and weight measured. During each visit, we will ask you to rest quietly for 20 minutes before we measure your blood pressure. You will be asked to provide your saliva sample into a test-kit tube before having a test meal and this sample will be used to measure your specific nutrition-related genetic markers (only in the first visit). A small needle will be also placed into your arm vein. This can be slightly painful and can cause discomfort. The needle has a plastic cannula (thin tube) that will be left in your arm vein. This too is a little uncomfortable and you will not be

able to fully bend your arm. The researcher will then take 30 mL of blood, and this will be used to measure your resting amino acids and lipids.
After the first blood sample, you will be given a meat-containing meal to consume within 15 minutes. You will also be asked to complete a visual analogue scale (VAS) questionnaire to score your appetite and digestive symptoms, before consuming the meal, and at regular intervals during the trial. We will also ask you to record digestive symptoms as they happen with a separate questionnaire. Blood samples will be repeated every hour for 4 hours after your meal (in total 150 mL per visit). These blood samples will assess your digestion, metabolism and inflammatory responses.
After the 4 hours, the cannula will be removed which may cause mild discomfort. You will be offered lunch and you are free to go. Before departing we will invite you to make an appointment for the next clinical visits. We appreciate that this takes four visits to our research centre and approximately 20 hours of your time and would like to offer you a $400 gift voucher in total to reimburse for your time and efforts.

Blood and Saliva

Your blood will be used in the analysis of proteins, lipids, and sugars and markers of inflammation. These will provide vital insights into whether there are differences in the digestive responses to the four meals. We will be measuring metabolites (digested products of the meat and markers of your body's metabolic process) including amino acids (the digested products of proteins), lipids (the digested products of fats), sugars such as glucose (digestion products of carbohydrates), and hormones involved in digestion and absorption such as insulin. Some analysis techniques will take place in the laboratories of the Liggins Institute (University of Auckland). Your samples will also be sent to AgResearch Limited (Palmerston North, New Zealand) for analysis of things that we are unable to do in Auckland. After these analyses have been performed, it will not be possible to return any unused samples to you. You can request the return of your saliva or blood prior to any analysis; this would mean we would not use your information in the study.
Different versions of a gene can make us response differently to certain components in food such as the gluten in bread, the lactose in milk, the caffeine in coffee along with various proteins, fats, minerals and vitamins found in various foods. The differences between individuals can be explained by gene variations within the population. Some individuals may benefit from limiting their consumption of nutrient component (e.g. caffeine, gluten…) or increasing their intake of other nutrient components (e.g. omega-3 fat, zinc…). Understanding our genetic profile and its complications on our unique response to the foods, supplements and beverages we consume will provide us with the tools needed to make the best dietary choices. We will take gene samples from your saliva. Your saliva sample will be used in analysis of various genetic markers related to nutrition and physical activity. The sample will be sent to the Nutrigenomix laboratory (Nutrgenomix Australia, Level 10 & 11, 20 Martin Place, Sydney, Australia, 2000) for the genetic test. Nutrigenomix testing is a safe and noninvasive saliva collection kit or buccal swab developed for use by healthcare professionals. The Nutrigenomix test kit involves saliva or buccal swab collection, testing of the client's DNA for specific nutrition-related markers, and generation of a personalised nutrition and fitness report. The test analyses variations in 70 genes that impact nutrient metabolism, eating habits, weight management and body composition, food intolerances and physical activity. The accuracy of the genetic-test results is between 99.7 – 100%.
The information collected in this study will be kept for a total of 10 years. Your samples will be kept until the end of the analysis. At the end of this time a medical waste contractor will dispose of your tissue. If you would like a karakia said at this time, please indicate so in the consent portion of this form. Cremation and karakia ceremonies take place through the Auckland District Health Board, and occur every 2 months during the year.

Many iwi, hapu, and whānau disagree with transport of blood samples due to issues with the loss of rights to your whakapapa. However, it is acknowledged that individuals have the right to choose. These concerns may also apply to non-Māori. We encourage you to consult with your family or whānau before agreeing to participate, if you think this might apply to you. As the saliva samples are sent overseas they will be destroyed upon the completion of the testing for this trial.

Detection of Abnormalities

Some blood markers analysed in this research can be early indicators of diseases such as diabetes and heart disease. Any blood results outside of the normal healthy range will be provided to you. We will also inform your usual doctor of any results that might be significant for your health, so follow-up can be arranged if appropriate. The genetic test (Nutrigenomix) does not assess genetic predisposition to certain diseases. The test only includes genes related to nutrition and physical activity. Nevertheless, if an individual has a Nutrigenomix test, he/she is required to answer 'yes' on any legal forms or questionnaires that ask whether they have had a genetic test.

What if Something Goes Wrong?

This clinical trial is to be conducted principally for the benefit of the manufacturer or distributor of the medicine or item being trialled. Section 32 of the Accident Compensation Act 2001 provides that participants injured as a result of treatment received as part of this trial will not be eligible for publicly-funded compensation through the Accident Compensation Corporation (ACC). However, compensation may be available from the study's sponsor, Auckland UniServices Ltd., in line with industry guidelines. We can give you a copy of these guidelines if you wish. You would be able to take action through the courts if you disagreed with the amount of compensation provided.

If you have private health or life insurance, you may wish to check with your insurer that taking part in this study won't affect your cover.

You may have your friend, family, or whānau support help you understand the risks and/or benefits of this study or any other explanation you require. You are also welcome to have a friend, family, or whānau support with you during every session.

Right to Withdraw from Participation

You have the right to withdraw from this study at any time. Your contribution is entirely voluntary and if you chose to withdraw any remaining samples and data will be destroyed at that point, but data or samples that have already been collected and processed will continue to be used.

Anonymity Confidentiality and Risks

All samples and the measurements will be coded and recorded against this code to keep your identity confidential. Coding will be numerical and you will not be identifiable by this code. Each saliva sample is anonymized using a barcode and this is entered into a password protected online system. Nutrigenomix uses a Secure Socket Layer (SSL) protocol to encrypt information that is transmitted over the Internet. This technology uses 256-bit encryption, which ensures that confidential information and transactions cannot be viewed, intercepted or altered. Nutrigenomix will never reveal client information or genetic data to a third party except as required to provide the services requested, or as required by law. The only person able to link the code with your name is Dr Andrea Braakhuis who will keep the coding list in a locked filing cabinet. When the analysis is completed the researchers will analyse the whole group's data and report on averages. This data will be used for scientific publication and presentations. No person will be identifiable from the analysis. Although efforts will be made to protect your privacy, absolute confidentiality of your information cannot be guaranteed. Even with coded and anonymised information, there is no guarantee that you cannot be identified. The risk of

people accessing and misusing your information is currently very small but may increase in the future as people find new ways of tracing information.

Contact Details

For more information please contact either:

Dr Andrea Braakhuis
Discipline of Nutrition,
Faculty of Medical and Health Science,
The University of Auckland, New Zealand
Telephone: 09-923 6251
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Dr Toan Pham
Discipline of Nutrition,
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APPROVED BY THE HEALTH AND DISABILITY ETHICS COMMITTEE ON Approved Amendment – 18th November 2020. Reference Number 19/STH/226 AM02

Appendix E: Consent Form

MEDICAL AND HEALTH SCIENCES

CONSENT FORM
THIS FORM WILL BE HELD FOR A PERIOD OF 10 YEARS

Project title: **Acute Evidence of Digestive, Metabolic and Nutritional Differences in Beef, Lamb, and Meat-Alternative Meals**

Principal Investigator: Dr Andrea Braakhuis (The University of Auckland)
Research Team: Dr Matt Barnett (The Liggins Institute), Dr Toan Pham (The University of Auckland)

- I have read the Participant Information Sheet, have understood the nature of the research and why I have been selected. I have had the opportunity to ask questions and have them answered to my satisfaction.
- I agree to take part in this research.
- I have had the opportunity to use support from a family (whānau) member or a fried to help me ask questions and understand the research.
- I understand that I am free to withdraw participation at any time
- I understand that blood and saliva samples will be collected and used for research.
- I understand that samples will be sent around New Zealand and Australia for analysis and disposed of at the end of the study
    - I wish for a karakia said at the time of my tissue disposal (*please circle*). *Yes* *No*
- I understand that any blood results found to be outside the normal healthy range will be conveyed to me and that if I do not wish to be informed, I cannot participate in this study.
- I wish to receive the summary of findings. I understand that there may be a delay between data collection and the publication and availability of the research results (*please circle as appropriate*). *Yes* *No*
- I wish to receive a copy of my genetic test results *Yes* *No*
- I understand that the results from this study will be used for scientific publication and presentations.
- I agree not to restrict the use of any data or results that arise from this research provided such a use is only for scientific purposes.

Name

Signature Date

Researcher's Signature Date

APPROVED BY THE HEALTH AND DISABILITY ETHICS COMMITTEE ON
Provisionally Approved Amendment – 01 October
Reference Number 19/STH/226

Appendix F: Raw Data for Individual Participant for Physiological Outcome Measures and the Corresponding Qualitative Genetic Risk

Individual plasma measures at baseline for vitamin D, iron (iron overload and low iron), zinc, total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), triglycerides (TG), glucose and insulin and the corresponding qualitative genetic-risk statement.

Table F.1

Individual Participant Plasma Measures for Nutrients at Baseline and Corresponding Qualitative Genetic-Risk Statement

	SNP ref. marker	Plasma measure	SNP ref. marker	Plasma measure	SNP ref. marker	Plasma measure	SNP ref. marker	Plasma measure
ID	**Vit D** rs10741657 rs 2282679	**t-pre Vit D**	**Iron Overload** rs17342717 rs1800562 rs1799945	**t-pre Fe umol/L**	**Low Iron** rs4820268 rs7385804 rs3811647	**t-pre Fe umol/L**	**Zinc** rs11126936	**t-pre Zinc mg/L**
1	Elevated	12.35	low	14.25	typical	14.25	elevated	0.82
2	elevated	19.81	low	20.89	typical	20.89	typical	0.81
3	elevated	9.69	low	16.05	typical	16.05	elevated	0.91
4	typical	20.85	low	19.62	elevated	19.62	elevated	0.79
5	elevated	13.16	low	17.44	elevated	17.44	typical	0.80
6	elevated	12.72	low	16.62	elevated	16.62	elevated	0.74
7	elevated	20.53	low	9.36	elevated	9.36	elevated	0.85
8	elevated	24.11	low	16.84	typical	16.84	typical	0.74
9	elevated	16.99	low	15.83	typical	15.83	typical	0.80
10	elevated	8.40	low	14.37	typical	14.37	typical	0.93
11	elevated	21.54	low	12.61	typical	12.61	elevated	0.79
12	elevated	17.02	low	12.53	elevated	12.53	elevated	0.92
13	elevated	20.57	low	19.26	elevated	19.26	typical	1.10
14	elevated	25.73	low	13.72	elevated	13.72	typical	0.82
15	elevated	17.22	low	30.95	typical	30.95	typical	0.85
16	elevated	34.43	low	24.04	typical	24.04	typical	1.00
17	elevated	13.87	low	13.45	elevated	13.45	typical	0.69
18	elevated	13.27	low	25.37	typical	25.37	elevated	1.10
19	elevated	17.30	low	19.37	typical	19.37	elevated	0.92
20	elevated	28.65	low	17.26	typical	17.26	elevated	0.93
21	elevated	15.13	medium	18.11	elevated	18.11	typical	0.95
22	typical	19.72	medium	29.03	typical	29.03	typical	0.79
23	elevated	35.79	low	29.91	elevated	29.91	typical	1.00
24	elevated	11.81	low	9.06	typical	9.06	elevated	0.89
25	elevated	13.62	low	9.94	elevated	9.94	elevated	0.83
26	typical	48.86	low	21.18	typical	21.18	typical	0.86
27	elevated	10.54	low	7.52	elevated	7.52	typical	0.69
28	elevated	27.41	low	14.49	elevated	14.49	typical	0.94
29	elevated	17.12	low	17.64	typical	17.64	typical	0.97
30	elevated	22.77	low	9.87	typical	9.87	elevated	0.80

Median		17.26		16.73		16.73		0.85
SD[a]		8.76		6.09		6.09		0.10
Mean		19.70		17.22		17.22		0.87

Note. [a]SD, standard deviation

Table F.2

Individual Participant Plasma Measures at Baseline for Cholesterols, Triglycerides, Fats, Glucose and Insulin and Corresponding Qualitative Genetic-Risk Statement

	SNP ref. marker	Plasma conc	SNP ref. marker	Plasma conc	SNP ref. marker	Plasma conc	SNP ref. marker	Plasma conc	SNP ref. marker	Plasma conc	SNP ref. marker	Plasma conc	SNP ref. marker	Plasm conc
ID	Tot. Chol rs662799	t-pre Tot. chol mmol/L	LDL rs6544713	t-pre LDL mmol/L	HDL rs1883025	t-pre HDL mmol/L	TG rs10889353	t-pre TG mmol/L	Saturated Fat rs5082	t-pre LDL mmol/L	Glucose rs11708067	t-pre glucose mmol/L	Insulin rs2943641	t-pre Insulin μU/mL
1	typical	5.13	typical	3.47	typical	1.43	elevated	0.85	typical	3.47	elevated	4.89	elevated	8.63
2	elevated	4.06	typical	2.81	typical	1.31	elevated	1.14	typical	2.81	elevated	3.64	elevated	3.94
3	typical	3.21	elevated	1.58	elevated	1.52	typical	0.66	typical	1.58	elevated	4.89	elevated	10.23
4	typical	4.74	elevated	3.46	elevated	1.43	elevated	0.80	typical	3.46	elevated	5.22	elevated	8.69
5	elevated	4.55	elevated	3.30	elevated	1.23	elevated	0.61	typical	3.30	elevated	4.42	elevated	9.63
6	typical	5.26	typical	3.71	elevated	1.29	elevated	1.02	typical	3.71	elevated	5.59	elevated	17.25
7	typical	3.77	typical	2.49	elevated	1.21	elevated	0.72	typical	2.49	elevated	4.83	elevated	4.89
8	typical	5.43	typical	3.62	typical	1.25	elevated	1.67	typical	3.62	elevated	5.11	typical	5.76
9	typical	4.4	typical	2.87	elevated	1.45	elevated	0.60	typical	2.87	elevated	4.67	elevated	8.1
10	typical	4.03	typical	2.41	elevated	2.41	elevated	1.06	typical	2.41	elevated	5.23	elevated	6.32
11	typical	5.24	typical	2.90	typical	2.47	elevated	0.57	typical	2.90	elevated	4.56	elevated	4.76
12	typical	6.17	typical	4.49	elevated	1.48	elevated	1.05	typical	4.49	elevated	5.10	elevated	10.8
13	typical	3.87	typical	2.12	elevated	1.69	elevated	0.81	typical	2.12	elevated	4.83	elevated	13.15
14	typical	4.3	elevated	2.20	typical	2.06	elevated	0.59	elevated	2.20	elevated	4.81	typical	6.22
15	elevated	4.39	typical	2.47	typical	1.39	elevated	1.90	typical	2.47	elevated	5.46	typical	1.52
16	typical	4.67	elevated	3.00	typical	1.68	elevated	0.57	typical	3.00	typical	5.03	elevated	7.46
17	elevated	5.05	elevated	3.48	elevated	1.08	elevated	1.79	typical	3.48	elevated	5.05	elevated	16.43
18	typical	5.59	elevated	4.01	typical	1.06	elevated	1.68	typical	4.01	elevated	4.69	typical	3.28
19	elevated	3.96	typical	2.38	elevated	1.59	elevated	0.50	typical	2.38	elevated	4.57	elevated	5.01
20	elevated	6.06	elevated	4.42	typical	1.21	elevated	1.25	typical	4.42	elevated	5.13	typical	6.19
21	typical	3.85	elevated	2.40	typical	1.19	elevated	1.16	typical	2.40	elevated	4.86	elevated	7.59
22	typical	5.05	typical	3.27	typical	1.30	elevated	1.95	typical	3.27	typical	5.04	elevated	11.5
23	elevated	5.78	typical	2.88	elevated	2.14	elevated	1.43	typical	2.88	elevated	5.28	elevated	5.92
24	elevated	4.42	typical	2.94	elevated	0.97	elevated	1.80	typical	2.94	elevated	5.32	elevated	12.83
25	elevated	4.08	typical	2.62	typical	1.11	elevated	1.33	typical	2.62	elevated	5.06	elevated	6.22
26	elevated	4.22	typical	2.49	typical	1.65	elevated	0.54	elevated	2.49	elevated	4.41	typical	1.29
27	typical	4.25	typical	2.35	typical	1.73	elevated	0.98	typical	2.35	elevated	5.45	elevated	9.06
28	elevated	4.25	typical	2.15	typical	1.85	elevated	0.73	typical	2.15	elevated	5.17	typical	1.32

29	typical	3.8	typical	2.41	typical	1.17	elevated	0.83	typical	2.41	typical	4.84	elevated	4.49
30	typical	4.55	elevated	2.59	elevated	0.66	elevated	3.59	typical	2.59	elevated	5.65	elevated	13.12
Median		4.41		2.84		1.41		1.00		2.84		5.04		6.89
SD		0.73		0.69		0.41		0.65		0.69		0.41		4.13
Mean		4.60		2.91		1.47		1.14		2.91		4.96		7.72

Postprandial changes in long-chain polyunsaturated fatty acid (LCPUFA) (18:2 n-6, 18:3 n-6, 18:3 n-3,) concentrations in the CMRF for dietary intake of omega-6 and -3 and the corresponding quantitative genetic-risk statement.

Table F.3

Individual Participant Postprandial Changes in Long-Chain Polyunsaturated Fatty Acid and the Corresponding Qualitative Genetic-Risk Statement

	SNP ref. marker	**LCPUFA conc**	**LCPUFA conc**	**LCPUFA conc**
ID	**Omega-6&-3 (rs 174547)**	**AUC C18:2n-6**	**AUC C18:3n-6**	**AUC C18:3n-3**
1	elevated	123.00	0.57	5.53
2	typical	253.10	1.9	16.44
3	typical	153.90	0.97	7.83
4	typical	323.80	2.05	13.71
5	elevated	174.30	1.29	19.51
6	elevated	439.70	2.80	10.20
7	typical	162.70	0.65	7.47
8	elevated	278.70	3.28	18.12
9	elevated	135.40	1.57	7.95
10	typical	176.10	1.54	8.87
11	elevated	158.10	0.67	8.47
12	typical	192.10	0.73	22.24
13	typical	117.20	2.74	8.54
14	elevated	235.70	3.39	8.98
15	typical	26.18	0.76	2.80
16	typical	167.40	1.88	7.38
17	elevated	475.70	8.53	23.93
18	typical	349.10	4.99	15.32
19	elevated	27.10	0.33	2.57
20	typical	534.80	7.61	27.43
21	typical	398.80	7.24	25.90
22	typical	200.00	5.41	14.42
23	elevated	319.40	2.47	16.94
24	typical	234.40	2.09	19.59
25	elevated	172.70	1.34	10.21
26	elevated	113.10	0.46	2.94
27	elevated	75.27	0.59	4.65

28	elevated	221.00	0.74	7.00
29	typical	136.20	1.03	8.03
30	elevated	287.60	2.83	30.50
Median		184.10	1.72	9.59
SD		125.18	2.22	7.74
Mean		222.09	2.41	12.78

BMI to assess energy balance, protein, total fat, saturated and unsaturated fat, monounsaturated fat and the corresponding qualitative genetic risk statement. The categories of BMI were: Underweight < 18.5 kg/m^2, Healthy/Normal 18.5–24.9 kg/m^2, Overweight 25–29.9 kg/m^2, Obese > 30 kg/m^2 (BMI retrieved from National Heart Foundation of New Zealand, 2022).

Table F.4

Individual Participant BMI and the Corresponding Qualitative Genetic Risk Statement

	BMI	BMI	SNP ref. marker	SNP ref. marker	SNP ref. marker	SNP ref. marker	SNP ref. marker
ID	**BMI kg/m²**	**BMI**	**Energy Balance rs1800592**	**Protein rs9939609**	**Total Fat rs7903146**	**Saturated & Unsaturated Fat rs9939609**	**Monounsaturated Fat rs1801282**
1	21.6	Healthy	diminished	enhanced	typical	enhanced	typical
2	22.7	Healthy	diminished	typical	typical	typical	typical
3	21.9	Healthy	diminished	typical	typical	typical	typical
4	22.3	Healthy	diminished	enhanced	typical	enhanced	typical
5	24.6	Overweight	typical	typical	typical	enhanced	typical
6	26.8	Overweight	diminished	typical	typical	typical	typical
7	22.1	Healthy	diminished	typical	typical	enhanced	typical
8	27.6	Overweight	diminished	typical	typical	enhanced	typical
9	24.7	Healthy	diminished	typical	typical	typical	typical
10	22.5	Healthy	diminished	typical	typical	typical	typical
11	22.6	Healthy	diminished	typical	typical	typical	typical
12	24.7	Overweight	diminished	typical	enhanced	enhanced	typical
13	25.9	Overweight	typical	typical	typical	typical	typical
14	24.9	Overweight	diminished	typical	typical	enhanced	typical
15	23.8	Healthy	typical	enhanced	typical	enhanced	enhanced
16	25.2	Overweight	typical	typical	typical	typical	typical
17	29.1	Overweight	typical	typical	typical	enhanced	typical
18	22.6	Healthy	diminished	enhanced	typical	enhanced	typical
19	23.3	Healthy	diminished	typical	typical	enhanced	typical
20	31.3	Obese	typical	typical	typical	enhanced	enhanced
21	23.3	Healthy	typical	typical	typical	typical	typical
22	25.3	Overweight	typical	typical	typical	typical	enhanced
23	22.3	Healthy	diminished	typical	typical	typical	typical
24	29.5	Overweight	diminished	typical	typical	typical	enhanced
25	25.6	Overweight	diminished	typical	typical	typical	typical
26	24.1	Healthy	diminished	typical	typical	enhanced	typical

27	20.3	Healthy	diminished	typical	typical	typical	typical
28	23.7	Healthy	diminished	typical	typical	typical	typical
29	21.6	Healthy	diminished	typical	typical	enhanced	typical
30	29.2	Overweight	typical	enhanced	typical	enhanced	typical
Med	24.0						
SD	2.69						
Mean	24.5						

Postprandial changes in an online visual analogue 100-point scale to assess fat-taste perception, sugar preference and hunger (using hunger, satisfaction, fullness, desire to consume sweet, or fatty-food appetite scores) and the corresponding qualitative genetic risk statement.

Table F.5

Individual Participant Postprandial Changes in an Online Visual Analogue 100-Point Scale and the Corresponding Qualitative Genetic Risk Statement

	SNP ref. marker	Appetite score	SNP ref. marker	Appetite score	SNP ref. marker	Appetite score
ID	**Fat-Taste Perception rs1761667**	**AUC Fat Taste - yes 0 no 100**	**Sugar Preference rs5400**	**AUC Sweet craving - yes 0 no 100**	**Hunger rs1051168**	**AUC Hungry - no 0 yes very 100**
1	enhanced	199.80	typical	66.09	typical	368.00
2	enhanced	112.50	elevated	87.00	typical	58.92
3	enhanced	0.00	typical	0.00	typical	207.00
4	typical	80.25	typical	36.75	typical	347.50
5	enhanced	152.80	typical	13.50	typical	185.30
6	enhanced	273.00	typical	40.10	typical	96.67
7	enhanced	76.08	typical	180.30	typical	55.26
8	enhanced	122.30	elevated	150.50	typical	182.00
9	enhanced	94.25	typical	314.00	typical	183.00
10	enhanced	355.00	typical	248.50	typical	224.30
11	enhanced	65.00	typical	321.80	typical	346.50
12	enhanced	75.39	elevated	154.30	typical	251.80
13	enhanced	234.30	typical	225.00	typical	265.00
14	enhanced	229.00	typical	23.75	typical	114.20
15	enhanced	262.50	typical	136.80	elevated	98.47
16	enhanced	176.30	typical	61.76	elevated	229.30
17	enhanced	44.25	typical	32.25	typical	126.70
18	enhanced	39.00	elevated	260.00	typical	46.85
19	enhanced	250.50	typical	28.34	typical	95.50
20	typical	124.70	typical	101.50	typical	143.50
21	enhanced	35.00	typical	373.50	typical	265.50
22	typical	87.50	typical	55.00	typical	205.80
23	enhanced	182.30	typical	167.80	typical	273.50
24	enhanced	126.00	typical	123.50	typical	124.00

25	enhanced	218.50	typical	20.88	typical	261.80
26	enhanced	58.29	elevated	130.50	elevated	281.50
27	enhanced	265.50	elevated	66.75	typical	337.50
28	enhanced	37.50	typical	29.69	typical	158.80
29	enhanced	44.25	typical	23.02	typical	97.75
30	typical	25.97	typical	21.77	typical	106.00
Median		117.40		76.88		184.15
SD		92.68		104.07		94.03
Mean		134.92		116.49		191.26

Appendix G: Nutrient Reference Values for Dietary Components by Australian Government, National Health and Medical Research Council, New Zealand Ministry of Health (2006). Including Recommended Dietary Intakes (RDI) and Upper Limit (UL).

Dietary component	Men / years	RDI /day	Women / years	RDI /day	Upper Limit (UL)/day men & women 19+
Vitamin D	19-50 51-70	5.0 µg 10.0 µg	19-50 51-70	5.0 µg 10.0 µg	80 µg
Iron	19-70	8 mg	19-50 50-70	18 mg 8 mg (assumed over 50 postmenopausal)	45 mg
Protein	19-70 70+	52 g 65 g	19–70 70+	46 g 57 g	25% protein energy
PUFA - Omega 3 - Alpha-linolenic acid (ALA)	ALA - 19+	1.3 g (Total Omega 3 – 160 mg)	ALA - 19+	0.8 g (Total Omega 3 – 90 mg)	Omega 3 - 3000 mg
PUFA - Omega 6 - Linoleic Acid (LA)	19+	13 g	19+	8 g	No upper limit effects known
Saturated & trans-fat	19+	8-10% (2000 calories = 22g of fat)	19+	18-10%	10%
Total Fat (aim for balance saturated, MUFA, PUFA)	19+	20-35%	19+ 20-35%	20-35%	35%
Zinc	19-70	14 mg	19-70	8 mg	40 mg

Appendix H: Results of Findings

Energy balance and the UCP1 gene variant

Contingency	A	B
1 Table Analyzed	Energy balance & UCP1 gene variant	
2		
3 P value and statistical significance		
4 Test	Chi-square	
5 Chi-square, df	6.429, 1	
6 z	2.535	
7 P value	0.0112	
8 P value summary	*	
9 One- or two-sided	Two-sided	
10 Statistically significant (P < 0.05)?	Yes	
11		
12 Effect size	Value	95% CI
13 Relative Risk	0.2500	0.08059 to 0.7470
14 Reciprocal of relative risk	4.000	1.339 to 12.41
15		
16 Odds ratio	0.1176	0.02590 to 0.7174

Elevated insulin levels and the IRS1 gene variant

Contingency	A	B
1 Table Analyzed	Insulin levels & IRS1 gene variant	
2		
3 P value and statistical significance		
4 Test	Chi-square	
5 Chi-square, df	9.130, 1	
6 z	3.022	
7 P value	0.0025	
8 P value summary	**	
9 One- or two-sided	Two-sided	
10 Statistically significant (P < 0.05)?	Yes	
11		
12 Effect size	Value	95% CI
13 Relative Risk	2.875	1.607 to 4.930
14 Reciprocal of relative risk	0.3478	0.2028 to 0.6222
15		
16 Odds ratio	+infinity	3.134 to +infinity

Sugar preference and the GLUT2 gene variant

Contingency	A	B
1 Table Analyzed	Sugar preference GLUT2 & gene variant	
2		
3 P value and statistical significance		
4 Test	Chi-square	
5 Chi-square, df	3.333, 1	
6 z	1.826	
7 P value	0.0679	
8 P value summary	ns	
9 One- or two-sided	Two-sided	
10 Statistically significant (P < 0.05)?	No	
11		
12 Effect size	Value	95% CI
13 Relative Risk	3.500	0.9419 to 19.81
14 Reciprocal of relative risk	0.2857	0.05049 to 1.062
15		
16 Odds ratio	7.000	0.9315 to 87.33