UNIVERSITY OF LIVERPOOL

Applications of functional nutrition and nutrigenomics to improve public health through dietary interventions.

Thesis submitted in accordance with the requirements of the University of Liverpool for the degree of Doctor in Philosophy

by

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March 2018

Authors Declaration

Apart from the collaboration on health & genomic pathway analysis with Dr Alix Warbuton and Jamie Headington MSc on consumer research, this thesis represents the unaided work of the author.

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ACKNOWLEDGEMENTS

I would like to thank the individuals at Liverpool University who have supported me through the many years of PhD research. Most especially my supervisor, Professor James Stewart for his guidance, patience and challenge. I would like to thank the many exceptional scientists who have helped and collaborated with me especially Dr Alix Warburton, Dr Jill Bubb and Professor John Quinn. My appreciation also goes to my business colleagues Heather Reed, Charles Dalby and Jamie Headington for all their assistance. I would like to especially thank my wife Sarah for her enduring love and support.

Abstract:

Diet and lifestyle factors are well documented for their impact on health and wellbeing. Epigenetics provides the added connection between personal genetics and environmental factors, including food, interacting with health and disease.

Functional foods is a growth area of food development, augmentation and supplementation. However, more scientific validation of the claims made for functional foods and functional components is required to properly inform consumers and healthcare practitioners.

There is also growing consumer acceptance of personalised genetic analysis for health and wellbeing. The commerciality of providing cost-effective genetic diet and lifestyle advice is a new and developing area in the health industry.

All diseases have a genetic link, and genome-wide association studies (GWAS) are discovering genetic variations linked to complex diseases. However, nutrient information is absent for the development of dietary advice and the management and prevention of disease. The development of nutrigenomics provides information on the gene/nutrient interaction essential for the design of personalised nutrition.

Functional nutrition from a combination of personalised genetic analysis and nutrigenomic interventions, represent an important new nexus for improving public health and the future of disease prevention.

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Abbreviations

5-HTP	5 Hydroxytryptophan
ACE	Angiotensin-converting Enzyme
ADP	Adenosine diphosphate
AMP	Adenosine Monophosphate
ANOVA	Analysis of Variance
APOE	Apolipoprotein E.
ARE	Avenanthramides-rich extract
BHF	British Heart Foundation
BMI	Body Mass Index
bn	Billion
BP	Blood Pressure
Ca	Calcium
cAMP	Cyclic adenosine monophosphate
CCKR	Cholecystokinin Receptor
CEO	Chief Executive Officer
CH3	Methyl Group
CHD	Coronary Heart Disease
CI	Confidence Interval
CLA	Conjugated Linoleic Acid
CpG	Cytosine Phosphate guanosine Dinucleotide
CREB1	cAMP Responsive Element 1
CVD	Cardiovascular Disease
DHA	Docosahexaenoic acid
DMBA	Dimethoxybenzaldehyde
DNA	Deoxyribonucleic Acid
DNMT	DNA Methyltransferase
DP/CP	Dispersed Phase to Continuous Phase
DRI	Deietary Reference Intake
EBI	European Bioinformatics Institute

EFA	Essential Fatty Acid
EFSA	European Food Safety Authority
EGF	Epidermal Growth Factor
EPA	Eicosapentaenoic acid
EVOO	Extra virgin Olive Oil
F/V	Fruit/Vegetable
FARE	Fatal Allergic Reaction
FCE	Finished Consultant Episodes
FDF	Food and Drink Federation
FIR	Food Information for Consumers
FKHR	Forkhead in human rhabdomyosarcoma
FMCG	Fast Moving Consumer Goods
GDA	Guideline Daily Amounts
GDP	Gross Domestic Product
GEO	Gene Expression Omnibus
GI	Glycaemic Index
GI Tract	Gastro intestinal Tract
GMO	Genetically Modified Organism
GMP	Guanosine Monophosphate
GnRHR	Gonadotropin-releasing Hormone Receptor
GP	General Practioner
GRAS	Generally recognised as safe
GWAS	Genome Wide Association Studies
HAT	Histone acetyltranferase
HCC	Hepatocellular Carcinoma
HDAC	Histone deacetylase
HDL	High Density Lipoproteins
HIF1A	Hypoxia Inducible Factor 1
HPMCC	Hydroxypropylmethyl Cellulose
IFIC	International Food Information Council
IFT	Institure of Food Technologists

IGF-1	Insulin-like Growth Factor
IHD	Ishemic Heart Disease
IMP	Inosine Monophosphate
IOM	Institute of Medicines
IPA	Ingenuity Pathway Analysis
KCI	Potassium Chloride
LA	Linoleic Acid
LCT	Long Chain Triglycerides
LDL	Low Density Lipoproteins
LGA	Local government Association
LI	Lithium
LIAS	lipoic acid synthetase
MAPK	Mitogen-activated protein kinase
MCT	Medium Chain Triglycerides
MD	Mediterranean Diet
MDA	Malondialdehyde
MI	Myocardial Infarction
miRNA	MicroRNA
mRNA	Messenger RNA
MSG	Monosodium Glutamate
mtDNA	Mitochondrial DNA
MTHFR	Methylenetetrahydrofolate
MUFA	Monounsaturated fatty acids
n-3	Omega 3 Fatty Acid
NCBI	National Centre for Biotechnology Information
NCDs	Non Communicable Diseases
NCMP	National Child Measurement Program
NDNS	National Diet and Nutrition Survey
NHGRI	National Human Genome Research Institute
NHS	National Health Service
NK	Nattokinase

NPD	New Product Development
OA	Oats avenanthramides
OBG	Oat Beta-glucan
ODI	Overseas Development Institute
OECD	Organisation for Economic Co-operation and Development
PAI-1	Plasminogen activator inhibitor 1
PANTHER	Protein Analysis Through Evolutionary Pathways
PBMC	Peripheral blood mononuclear cells
PKB	Protein Kinase B
PN	Personal Genomics
PPAR	Peroxisome proliferator-activated receptors
PTEN	Phosphatase and Tensin Homolog
PUFA	Polyunsaturated Fatty acid
R	Correlation Coefficient R
RCT	Randomised Control Trials
RDA	Recommended Daily / Dietary Allowance
RDI	Recommended Daily Intake
RE	Retinal Equivalent
RIF	Random Intermittent Fasting
RNA	Ribonucleic Acid
SAM	S-adenosyl Methionine
SCT	Social Cognitive Theory
SFA	Saturated Fatty Acids
SNP	Single Nucleotide Polymorphism
SWOT	Strengths, Weaknesses, Opportunities and Threats
T2DM	Type 2 diabetes mellitus
TFA	Trans Fatty Acid
tPA	Tissue Plasminogen activator
uPA	Urokinase
WCRF	World Cancer Research Fund
WHO	World Health Organisation

YSTRs	Short Tandem Repeats on the Y Chromosome

Chapter 1

Introduction

1.1 The effects of diet on health

Diet is an important factor in promoting health and preventing chronic diseases such as cardiovascular disease (CVD), diabetes, obesity and cancer. Food products may be defined as functional if together with the basic nutritional impact it has beneficial effects on one or more functions of the human organism, thus either improving general health and physical conditions and/or decreasing the risk of the evolution of diseases.

The rapid growth of the global ageing population and the limited efficacy of available pharmacological therapies for age-related cognitive decline and neurodegenerative diseases means that there is an increasing interest and public demand for 'functional foods' that promote cognitive wellbeing and longevity (Cannella *et al*., 2009, Alles *et al*., 2012, Feart *et al*., 2015). Conversely, evidence supports the hypothosis that early nutrition during prenatal development, infancy and childhood affects both cognitive development and cognitive function and behaviour in later life (Anjos *et al*., 2013, Prado & Dewey, 2014, Barker *et al*., 2013, Jacka *et al*., 2013). Understanding the biological effects of nutrition throughout development and in later life has major implications for physical and mental health and is therefore of great importance for the public health sector, food product development, economic progress and consumer interest.

The beneficial effects of certain food groups, including plant foods and oily fish which provide high sources of plant bioactive compounds (*e.g*. polyphenols, glucosinolates and antioxidant vitamins) and omega-3 fatty acids, are well documented (Pallauf *et al*., 2013, Vauzour *et al*., 2010, Feart *et al*., 2013, Feart *et al*., 2015). Many modern diets and food supplements enriched with these bioactive compounds, claim to be beneficial to health by

boosting the immune system, supporting cardiovascular function, protecting cells against oxidative stress and promoting healthy skin, teeth and bones (Roll *et al*., 2011, Lamprecht *et al*., 2007, Chapple *et al*., 2012, Lamprecht *et al*., 2013, Cui *et al*., 2012, De Spirt *et al*., 2012, Esfahani *et al*., 2011). Addressing the synergistic effects of certain functional foods and supplements consumed in combination for health and wellbeing is of growing interest in the field of nutrition (Alles *et al*., 2012).

The purpose of this study is to provide an updated review surrounding the effects of functional dietary compounds on phenotypic outcomes relating to health. Further, this study addressed the potential biological effects of diet and functional foods using a pathway analysis approach to better our understanding of how nutrition can influence different regulatory mechanisms including development, immune responses, metabolic processing, hormonal control and Deoxyribonucleic Acid (DNA) repair. The findings of this analysis will have implications for public health, disease intervention, therapeutic management, nutrition-related behaviours, marketing strategy, economic growth and food development.

1.2 The role of functional food & nutriceuticals in the diet.

The Functional Foods Industry is an important and evolving area of food development intended to have a positive impact on health. The implications for the future of the food industry and the public health sector are extensive. The market for functional foods is growing. Despite initial scepticism about functional foods, many existing brands and products perform well in this competitive market. Customers choose functional foods over basic alternatives as a simple way to improve their health, with 82% of adults now consuming a functional food on a weekly basis (Mintel 2013). The global functional food

market is estimated to reach $149bn by 2018. (Business Communications Company (BCC) Research, 2008)

The top twelve key trends in food, nutrition and health comprise naturally functional foods, dairy, protein, energy, weight management, snacking, slow energy release, sugar alternatives, indulgent, free-form, seniors and kids-nutrition (New Nutrition Business, 2013). Similarly, functional food trends highlighted in 2014 were; speciality nutrition, real food, high protein, kid-specific, nutraceuticals, meat alternatives, performance nutrition, weight reduction and millennials (Institute of Food Technologists (IFT), 2014). The future landscape of functional foods will continue to focus on key public health concerns: weight management, cognitive health and cardiovascular health.

In the last decade, more consumers believe that foods contribute directly to their health. The growing trend for the purpose of food is not only to satisfy hunger and provide necessary nutrients, but also to improve health and reduce the risk of nutritional-related diseases. Many studies exist on different functional food components and their beneficial factors for improving health.

Food manufacturers are utilising these scientific backgrounds to develop new products which claim to have functional food health benefits. The functional food market is growing worldwide, and functional products exist in virtually all food categories. As new products are launched into the market every year, competition is becoming more intense. Health claims have become an important piece of product marketing, providing information to attract consumers to purchase. Health claims on food products are often used to highlight scientifically proven health benefits associated with consuming those products. It is also a way to promote awareness to the public about the benefits they will gain after

consumption. It is critical that the validity of claims is regulated to avoid misrepresentation of scientific fact.

Consumer acceptance is important for the successful development of functional foods in alignment with consumer needs. Therefore, consumer research is crucial to the product development process. Consumers understanding, perception and acceptance, is critical to the propensity of purchase and the development of the industry

Americans are accepting of functional foods and are prepared to include them in their diets. However, consumer studies reported lower frequency of consumption of functional food based upon lower perceptions of taste and enjoyment from functional foods. In Europe, food choice of functional food is growing continuously. Europeans are more critical of new products and technologies. They are suspicious of the safety of novel foods and their production processes. Therefore, it is fair to hypothesise that Europeans acceptance of functional foods is less unconditional compared to Americans (Siro *et al.* 2008),

Surveys confirm that consumer opinion on the healthiness of a product greatly depended on the carrier product itself. The healthiness of the carrier products might override the effect of enrichment with functional components (Ares & Gambaro, 2007). Surveys from different countries demonstrate different consumer attitudes and acceptance towards functional foods. Different socio-demographic parameters, gender, education, income levels *etc*., also have an effect on the customer acceptance level of functional foods (Siro *et al*., 2008)

Consumer knowledge and awareness of the health effects of newly developed functional ingredients seems to be rather limited (Siro *et al*., 2008). Willingness to try

functional food is driven by its attractiveness, credibility and uniqueness, which suggests that the healthy image of a functional food determines positive consumer acceptance (van Kleef *et al*., 2005).

Nutraceuticals are an important development in functional foods. Pharmaceutical products have traditionally been the established intervention for disease prevention and treatments. However, there is increasing awareness of the importance of a balanced diet and of foods that could potentially reduce the risk and assist in the treatment of disease. The term nutraceutical is used where there is overlap between pharmaceutical and food relative to health.

Cardiovascular disease (CVD) and diabetes are on the list of top 10 causes of death in the world (World Health Organisation (WHO), 2011). Obesity is believed to be a significant contributor to these diseases; according to WHO, obesity is the fifth leading risk for global deaths. Every year, there are around 2.8 million fatalities caused by obesity. By 2020, obesity and obesity-associated complications are estimated to attribute two-thirds of global diseases. The Global Functional Food Survey (2014) has acknowledged the role of nutraceuticals in the areas of weight management, cognitive health and cardiovascular health.

There is a growing awareness of cognitive decline with ageing in society. There are also links to cognitive decline and other dementias with diet. The most common cause of dementia is Alzheimer's disease which affects approximately half a million people in the UK (Alzheimer's Society, 2012). There is evidence to suggest that these diseases could potentially be prevented or reduced by functional food interventions. New technologies in nutraceuticals can also improve the bioavailability of functional foods in diets.

1.3 Nutritional importance of epigenetics & nutrigentetics.

Epigenetics and its association with functional foods, nutrigenetics and nutrigenomics, is a new and interesting area for next stage of development of functional foods. The human genome is made up of approximately three billion nucleotide pairs containing bioinformatic codes to control gene expression. A consensus definition of the concept of epigentic trait as "stably heritable phenotype resulting from changes in a chromosome without alteration in the DNA sequence" was formulated at the Cold Spring Harbor meeting in 2008. The changes in chromatin structures include DNA methylation, histone modifications and chromatin remodelling resulting in a change in somatically heritable states of gene expression.

Figure 1.1 shows a summary of the epigenetic mechanism of activating and repressing gene expression. Throughout our life cycle, development, environmental chemicals, drugs or pharmaceuticals, diet and maturation are factors that can influence the dynamic epigenetic phenotype. Epigenetics appears to be an important mechanism in many diseases for example, Tokunaga *et al*. (2013) reported that food and nutraceutical components are key determinants of enzyme function, which can suppress the expression of harmful genes. Therefore, epigenetics is an important mechanism in many diseases such as obesity, diabetes, cardiovascular diseases, degenerative diseases and immune diseases. Also, including the unknown aetiology of many others; of which may lead to the development of personalised disease prevention and treatment strategies (Choi and Friso, 2010).

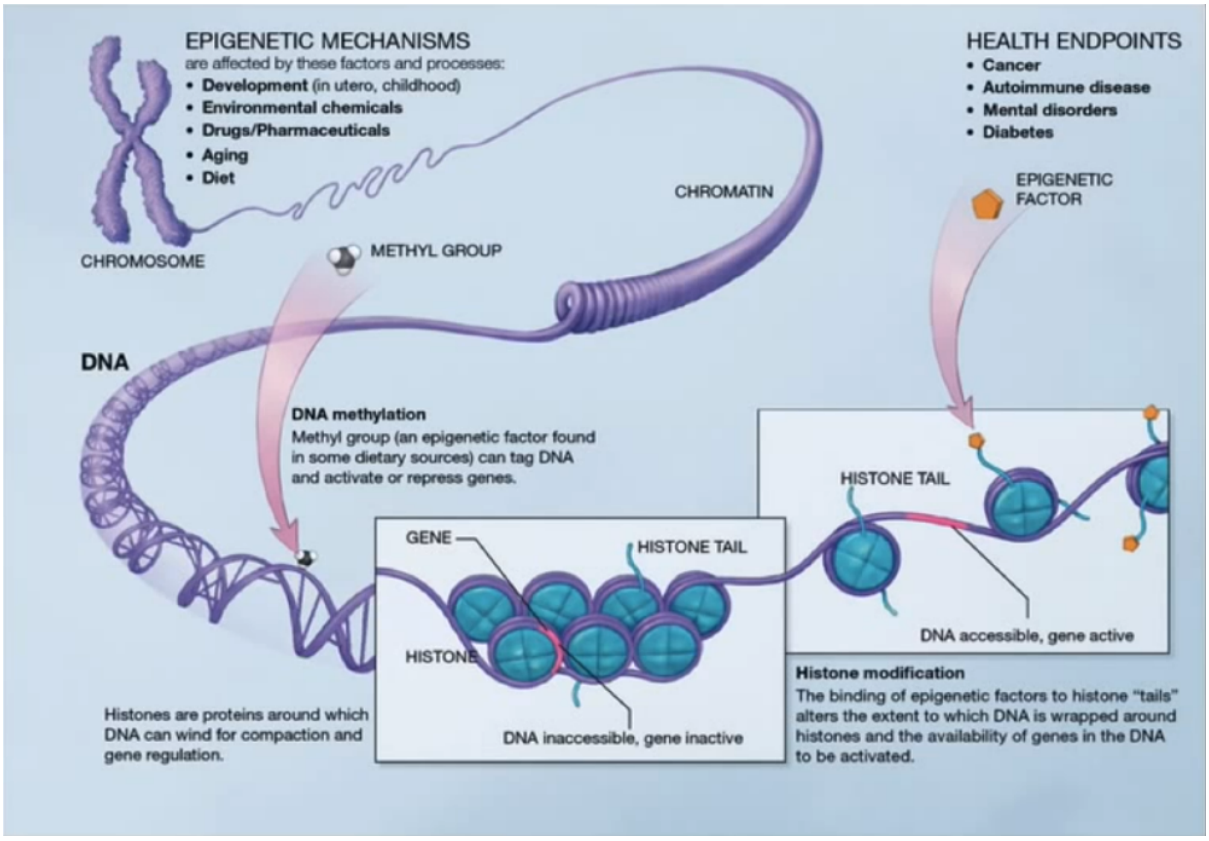


Figure 1.1. Epigenetic Mechanisms (Andersen, 2013)

Over recent generations, human diet has experienced some profound changes, and it is believed this will continue in the 21st century (Haggarty, 2013). Diabetes, obesity, cardiovascular diseases and cancer can all be categorised as non-communicable diseases (NCDs) because they are multifactorial and polygenic (Tokunaga *et al*., 2013). Epigenetic alterations change gene expression without altering DNA sequences, through DNA methylation, histone modifications and chromatin remodelling, which can mediate the pathogenesis of NCDs.

Recent research findings show that several nutrients and nutraceuticals can modulate gene expression, despite whether it is adversely or beneficially affected. Epigenetic changes by external or internal environmental factors can be inherited during cell

division, which will remain permanently in the acquired phenotype. Thus, environmental associated disease, as well as embryonic development and ageing, could be impacted by epigenetics. The discovery of epigenetic phenomena, which can be modified by nutrients and bioactive components, and the associated altering of gene expression at the transcriptional level is exceptionally important to nutritional science.

Nutrigenetics consists of three stages: nutrigenomics, proteomics and metabolomics. The main concept of nutrigenetics is to personalise nutrition such that dietary interventions can be made at an individual level. This technology might be able to provide tailored nutrition for individuals or population groups with the same health issue.

Reducing the risk of diet-related diseases and the associated healthcare costs will be of significant benefit to society (Wang and Bohn, 2012). Nutrigenomics describes how dietary nutrients affect the protein profile of an individual. Proteomics focuses on how altered protein profiles affects the biological system, and metabolomics is a cellular response to those changes. Nutraceutical companies are utilising nutrigenomic technology to develop new food ingredients according to an individual's genetics. This personalised diet could help the prevention or treatment of diseases such as diabetes, obesity and cardiovascular disease. It is clear that the genomic impact of diet is an important factor in promoting health and preventing chronic diseases such as cardiovascular disease (CVD), diabetes, obesity, cognitive decline and cancer.

There is rapid growth of the global ageing population. There is also limited efficacy of pharmacological therapies for age-related cognitive decline and neurodegenerative diseases. This is leading to increasing interest and public demand for functional foods that promote cognitive wellbeing and longevity (Cannella *et al*., 2009, Alles *et al*., 2012, Feart *et al*., 2015).

Understanding the biological effects of nutrition throughout development and in later life has major implications for physical and mental health and is therefore of great importance for the public health sector, food product development, economic progress and consumer interest. There also is a growing body of evidence that supports that early nutrition during prenatal development, infancy and childhood affects both cognitive development and cognitive function and behaviour in later life (Anjos *et al*., 2013, Prado & Dewey, 2014, Barker *et al*., 2013, Jacka *et al*., 2013).

1.4 Nutrient impact on genomics assessed through pathway analysis.

The beneficial effects of certain food groups, including plant foods and oily fish which provide sources of bioactive nutrients such as polyphenols, antioxidants, vitamins and omega-3 fatty acids, are well documented (Pallauf *et al*., 2013, Vauzour *et al*., 2010, Feart *et al*., 2013, Feart *et al*., 2015). Many modern diets and food supplements are enriched with these bioactive compounds and claim to be beneficial to health through boosting the immune system, supporting cardiovascular function and/or protecting cells against oxidative stress. (Roll *et al*., 2011, Lamprecht *et al*., 2007, Chapple *et al*., 2012, Lamprecht *et al*., 2013, Cui *et al*., 2012, De Spirt *et al*., 2012, Esfahani *et al*., 2011).

Addressing the synergistic effects of certain functional foods and supplements consumed in combination with health and wellbeing is of growing interest in the field of nutrition (Alles *et al*., 2012). The purpose of conducting pathway analysis was to provide an updated review surrounding the effects of dietary compounds on phenotypic outcomes such as longevity, mental health, body composition and cardiovascular function to inform on the health potential of different food groups. Further, we addressed the potential biological effects of diet and functional foods using a pathway analysis approach to better our

understanding of how nutrition can influence different regulatory mechanisms including development, immune responses, metabolic processing, hormonal control and DNA repair. The findings of this analysis will have implications for public health, disease intervention, therapeutic management, nutrition-related behaviours, marketing strategy, economic growth and food development.

Correctly channelled, functional foods and nutrigenomic applications could have a hugely positive impact on public health. Consumers are increasingly aware that food can contribute directly to their health. Today, the purpose of food is not only to satisfy hunger and to provide necessary nutrients, but also to improve health and reduce the risk of nutrition-related diseases. The current increase in NCDs and the associated rising cost of funding the NHS is an economic imperative that must be addressed.

The aim of this study was to investigate the effects of nutrition on phenotypic outcomes relevant to health and wellbeing through a systematic review. Also, an overlay of UK and global trends in eating patterns with morbidity/mortality rates of NCDs to address the correlation between diet and disease risk was reviewed. This study investigated consumer attitude to functional foods and genomic interventions to improve health and wellbeing. The predominant focus of this study is how the interconnection between functional foods, consumer acceptance and genomic technologies can have a positive and lasting impact on public health.

1.5 Consumer acceptance of diet based on personal genetics.

The application of genotype-based personalised nutrition could potentially revolutionise health promotion, healthcare and the food industry. If accepted, genomic-based personalised

nutrition could directly affect consumer food choice when health becomes the driving factor in food selection (German & Watkins, 2004). Conceptually, nutritional genomics is still in its infancy, and it is unclear how this rapidly developing science will be accepted by society (Castle & Ries, 2007; Ronteltap, van Trijp & Renes, 2007; Moskowitz, German & Saguy, 2005). A considerable number of studies have investigated the attitudes, knowledge and the interest of people in genetic testing within the US and European countries. However, this study has focused on the concept of genomic-based personalised nutrition within the UK population.

1.6 Implications for the future of public health, the NHS and the food industry.

The deterioration in public health has a clear impact on the associated escalation in the running cost of running the NHS and social care systems in the UK. While government addresses how to elicit a population effect to improve the nation's health, the reality is that the NHS is in continued financial distress.

Diet and its effect on health is a multifaceted area of research. This study aims to review the links by which the food industry, nutrigenomics technologies and the public sector could overlap to bring about a population effect improvement in health by focusing on individual nutrition and wellbeing.

The process flow of following chapters aims to assess the current and future situation in both science & business of nutrigenomics and how the benefits can be translated in to direct benefit to public health. This study will address:

1. The current situation of the UK and Global health trends with an associated review of the current functional food market and neutraceuticals.

2. An appraisal of the epigenetic importance and impact of food on health. A review of the epigenetic mechanism and nutrition, followed by a novel investigation of pathways analysis linked to nutritional interventions for health promotion and disease prevention.
3. An assessment and translation of this new nutrigenomic science combined with consumer acceptance to new and future genomic applications in the private and public health sectors with recommendations for improvements in public health.

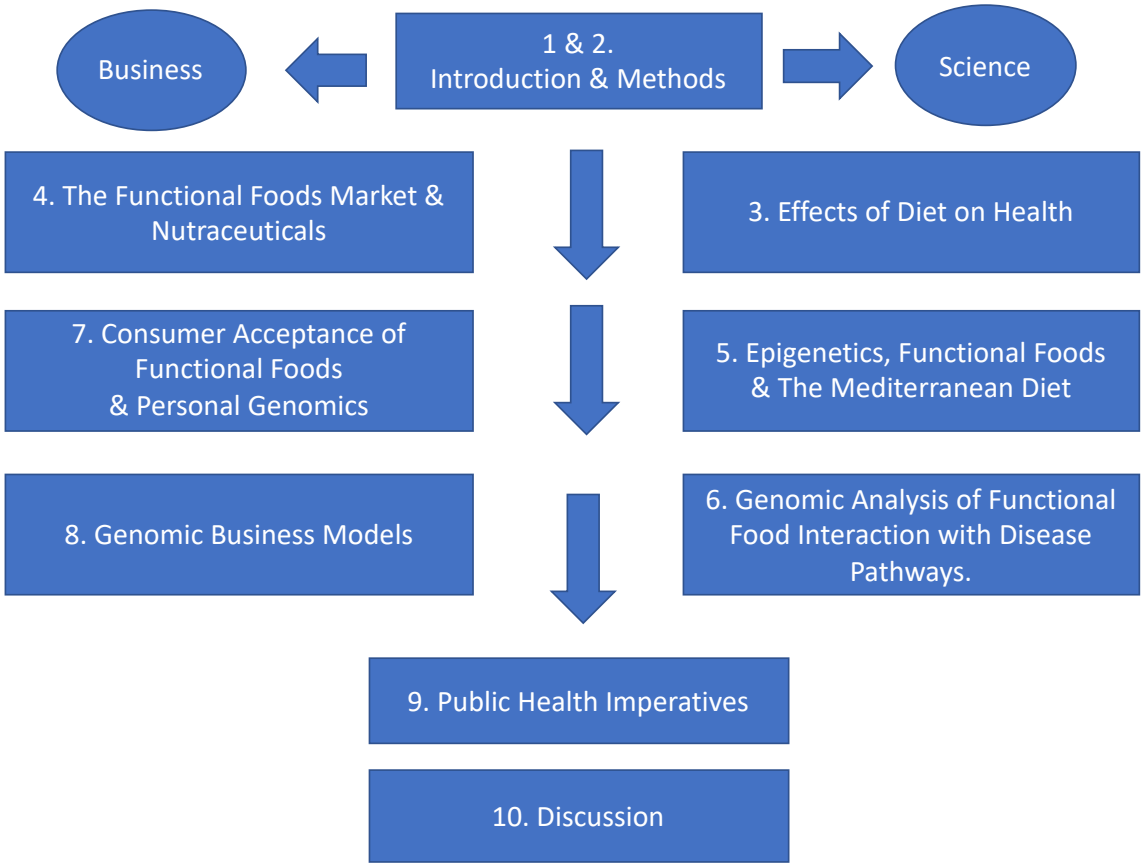


Figure1.2 Chapter Process Flow Diagram

Chapter 2

MATERIALS AND METHODS SECTION

2.1 Consumer Research

Consumer research was conducted using VYPR, a novel smart phone technology. The platform is designed to collect real time consumer feedback for new product development for FMCG applications. The resulting data collection can be used for demographic interpretation of consumer acceptance of new product concepts.

2.1.1 Sampling

The questionnaire was distributed to the general population of the UK between November 2017 and December 2017. Random sampling was used throughout. Participants were able to access the questionnaire through the VYPR application (see: https://www.vypr.it/#opinions-rewarded) on a 'first come first served' basis.

The questionnaire was designed to assess consumer's attitudes and preferences towards nutritional genomic products, services and activities. The questionnaire was developed using questions developed by existing studies (Roy *et al*., 2016; Stewart-Knox *et al*., 2008; Moran 2009; Cherkas *et al*., 2010; Henneman and Timmermans 2006; Castle, 2007; Petersen, 2005; Skirton *et al*., 2005; Rose *et al*., 2005; Catz *et al*., 2005). A theoretical framework from Berezowska *et al*., (2015) was adapted into the survey to assess consumer's intention to adopt nutritional genomics considering privacy risk, personalisation benefit, and the ability of service provider (Xu *et al*., 2009; Mayer and Davis, 1999).

Before responding to the questionnaire participants input their demographic data when downloading the app. In more detail, demographic data consists of gender and age. The questionnaire itself consisted of 15 questions on nutritional genomic products, services and activities. The questions were presented on a Likert scale whereby participants had the option of selecting 'agree', 'neutral' or 'disagree'. Due to the restrictions of the VYPR app, each

question was sent out separately therefore individual responses could not be tracked between questions. All respondents were anonymised and participated voluntarily.

2.1.2 Data Analysis

Respondents were categorised by age range (18-24, 25-34, 35-44, 45-54,55-64 and 65+) and gender (male and female) for comparison purposes. Responses by demographic were converted into percentages.

2.2 Systematic review

A reference search for the term 'diet' was performed using the PubMed search tool available through the *National Centre for Biotechnology Information* (NCBI) database (http://www.ncbi.nlm.nih.gov/pubmed/, accessed April 2015). The PubMed Advanced Search Builder was used and the following filters selected: species, human; article type, meta-analysis; publication dates, Jan 2012 to April 2015; journal categories, core clinical journals. Appropriate journals for inclusion in the systematic review were manually selected.

2.3 Gene expression arrays and databases

2.3.1 ArrayExpress

Human microarray data for dietary interventions with omega-3, flavonoids and resveratrol was downloaded from the ArrayExpress database (https://www.ebi. ac.uk/arrayexpress/). Datasets were analysed using the GEO2R function, available at http://www.ncbi.nlm.nih.gov/geo/info/geo2r.html, to determine gene expression changes between baseline measurements (control group) and following treatment intervention (treatment group) within the same individuals. Datasets were analysed using default parameters which generated a table of the top 250 genes ranked by significance (p-value).

Only genes that were significantly regulated ($p<0.05$) were included in the pathway analysis. Unadjusted p-values were used in this study in order to address extended biological networks associated with the dietary interventions. Datasets were filtered to include genes with a fold change in expression of more than 1.6 between the control and treatment groups. For treatment groups with large numbers of gene expression changes, only genes that were common across two or more studies for the same treatment condition were considered for analysis. For gene replicates within individual studies or duplicates across different studies for the same treatment group, data points with the least significant p-value were excluded. If replicates within individual studies had fold changes that were opposing (*i.e*. positive and negative values for the same gene) both data points were excluded from the analysis.

2.3.2 Bar Harbor BioTechnology

Candidate genes involved in obesity, diabesity and CVD were downloaded from *Bar Harbor* gene expression array systems available at https://www.bhbio.com/BHB/GUI/SP/Search.aspx, accessed May 2015. The panel of genes included on these gene expression arrays are based on experimental evidence from the literature. The gene list for CVD was compiled using the following plate descriptions: *Blood Coagulation*, *Hypertension* and *Inflammatory Cytokines & Receptors*. Duplicate gene entries were removed.

2.3.3 NHGRI-EBI GWAS Catalogue

The National Human Genome Research Institute (NHGRI)-European Bioinformatics Institute (EBI) catalogue of published GWAS was downloaded from http://www.ebi.ac.uk/gwas/docs/downloads (accessed August 2015). Reported genome-wide associated genes for ageing, breast cancer, cognitive function, CVD, diabesity,

neurodegeneration and psychiatric disorders were compiled and duplicates removed. GWAS included in this analysis are detailed in Tables A1-A7). Gene lists were compared against those associated with omega-3, flavonoids and resveratrol dietary interventions using hypergeometric distribution testing.

2.4 Significance testing of overlapping gene sets

2.4.1 Phyper test in R

The hypergeometric distribution test *phyper* available through the R package (https://www.r-project.org/) was used to compute statistical significance between the overlap of two gene sets using the command:

> phyper(q, m, n, k, lower.tail=FALSE)

where, q = overlap between list 1 and list 2, m = size of list 1, n = population size – list 1, and k = size of list 2. If 'phyper(q, m, n, k)' gives the probability of *x* overlap or fewer, '*lower.tail=FALSE'* gives the probability of *x* + 1 or more.

The p-value represents the probability of obtaining the observed number of overlap or larger by random chance. A p-value of <0.05 was considered significant. The reference gene set in the PANTHER database (20,814 genes) was selected as the population size based on subsequent pathway analysis methods using this reference list.

2.4.2 GeneOverlap in R

To address significant overlap of multiple gene sets, the GeneOverlap package in R which allows for testing and visualisation of gene overlaps was downloaded from https://www.bioconductor.org/packages/release/bioc/html/ GeneOverlap.html (accessed September 2015). Gene lists were saved as text files and uploaded into R. The GeneOverlap

function tests whether two variables are independent, which can be represented as a contingency table and then uses Fisher's exact test to find the statistical significance. The strength of the association is determined by the odds ratio; an odds ratio of ≤ 1 indicates no association between the two gene lists, an odds ratio of > 1 indicates a positive association.

2.5 Pathway analysis

2.5.1 Ingenuity Pathway Analysis (IPA)

Gene expression data obtained from ArrayExpress was analysed using the *Core Analysis* function of the IPA software (QIAGEN) under default parameters, detailed below:

1. General settings: a) Reference set, ingenuity knowledge base of genes and endogenous chemicals and b) Direct and indirect relationships
2. Networks: Interaction networks, include endogenous chemicals (default settings, 35 molecules per network; 25 networks per analysis)
3. Data sources: default settings (select all)
4. Confidence: experimentally observed
5. Species: default settings (select all)
6. Tissues and cell lines: default settings (unselected)
7. Mutation: default settings (select all)

Significance testing of the association between the uploaded dataset and the canonical/ well-established pathways available in the IPA library was measured using the following methods: 1) a ratio of the number of differentially expressed genes from the dataset that map to the pathway divided by the total number of genes that exist in the canonical pathway. The ratio is useful for determining which pathways overlap the most with the differentially expressed genes in a given dataset. 2) Fischer's exact test used to calculate a p-value determining the

probability that the association between the genes in the uploaded dataset and the canonical pathway is explained by chance alone. P-values of <0.05 were considered significant. 3) z-score analysis, used as a statistical measure of the match between expected relationship direction and observed gene expression of the uploaded dataset. It is a statistical measure of a score's relationship to the mean, *i.e*. the number of standard deviations from the mean of a normal distribution curve. Positive and negative z-scores indicate upregulated and downregulated pathways, respectively. In line with IPA cut-off values, z-scores of >2.0 or < -2.0 were considered significant.

2.5.2 PANTHER Classification System

Gene sets relating to CVD, obesity/diabesity and neurogenesis were subjected to pathway analysis using the freely available PANTHER (Protein ANalysis THrough Evolutionary Relationships) platform (Myi *et al*., 2009), available at http://www.pantherdb.org/ (accessed May 2015). This platform allows for analysis of gene function on a genome-wide scale. Gene lists were parsed into the *Gene List Analysis* tool and analysed using the *Functional Classification* method and *Statistical overrepresentation* test (Mi *et al*., 2013). The former returns the uploaded dataset as a gene list detailing the protein class, molecular functions, biological processes, cellular components or pathways that each gene in the user-defined list is associated with. Statistical overrepresentation testing compares the user-defined gene list to a reference gene list within the PANTHER database and determines whether a particular class of genes is over- or under-represented concerning a particular biological process or pathway. The significance is determined by the 'expected' number of genes within the test list to fall within a particular biological process or pathway based on the number of genes within the reference list for that process against the total number of genes within the

database (Mi *et al*., 2013). If more genes (from the test list) than expected (relative to the reference list) associate with a particular pathway, there is an overrepresentation; if less, there is an underrepresentation. P-values of <0.05 were considered significant.

Chapter 3

The effect of diet on health.

3.1 A systematic review of the effects of diet on different phenotypic outcomes relevant to health.

Certain dietary compounds such as plant foods (cereals, fruits, vegetables, legumes, tree nuts, seeds and olives) and oily fish have been linked to general health and wellbeing and the prevention of chronic diseases (Pallauf *et al*., 2013, Vauzour *et al*., 2010, Feart *et al*., 2013, Feart *et al*., 2015). A systematic review undertook to provide an up-to-date overview of the beneficial and adverse effects of dietary components on some phenotypic outcomes relevant to cardiovascular, cellular, cognitive, metabolic and immune system functioning. A reference search for the search term 'diet' was performed using the PubMed search engine within the NCBI database. Journals were filtered to include human-based meta-analyses performed between January 2012 and April 2015. To identify articles addressing phenotypic outcomes relating to dietary patterns, core clinical journals were selected and manually reviewed. Figure 3.1 illustrates the selection criteria for articles included in the systematic review. A total of 41 articles were eligible for inclusion and are detailed in Table 3.1.

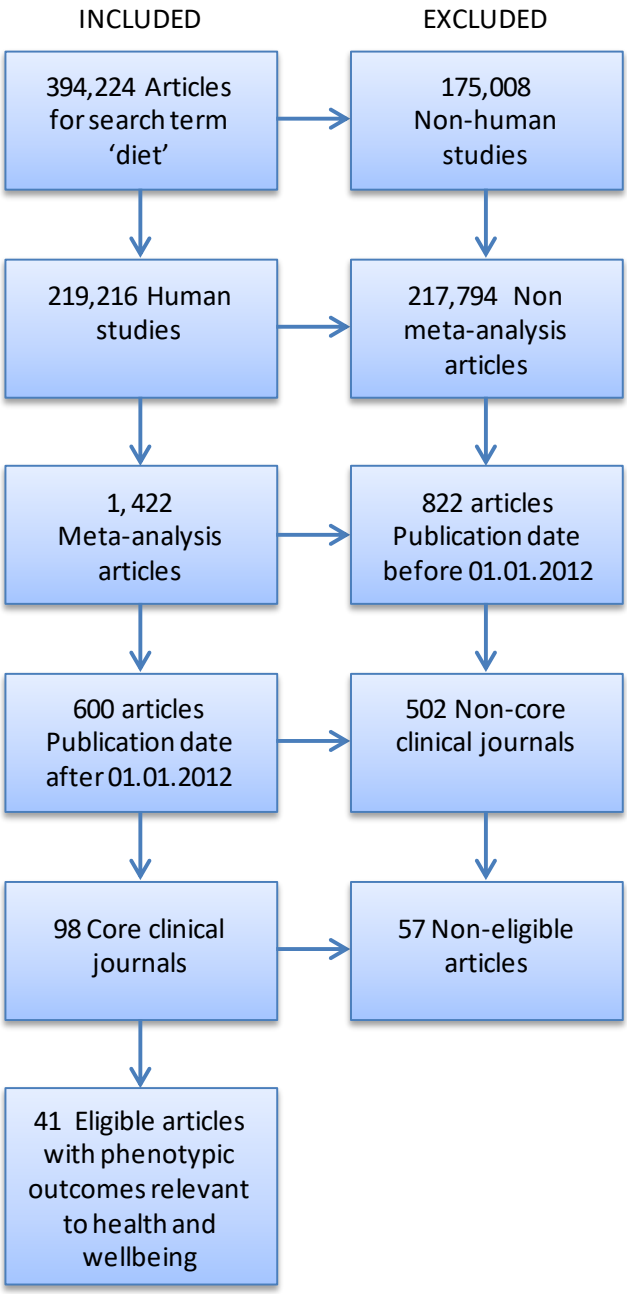


Figure 3.1 Selection of eligible articles for inclusion in the systematic review addressing the effects of diet on health and wellbeing. A flow diagram depicting reasons for exclusion of articles identified under the search term 'diet' in NCBI PubMed, specifically the CCJ subset (Accessed May 2015). Articles were filtered by *species*, *article type*, *publication date* and *journal categories*. Non-eligible are articles not addressing the effects of diet on phenotypic outcomes associated with health and wellbeing were manually excluded. A total of 41 articles were selected for inclusion.

Table 3.1. Effects of diet on phenotypic outcomes relevant to health and well-being

Food group	Number of individuals	Phenotypic outcome	Direction of outcome	Relative risk [95% CI]	Reference
Whole-grain:					
Whole-grain vs. non-whole grain control diet	400,492 participants including 14,427 cases	CHD	Higher whole-grain intake has a protective effect against CHD.	CHD risk (highest vs. lowest whole-grain intake): -0.79 [-0.74, -0.83]	(Tang *et al*., 2015)
	2,060 participants	Weight and body composition	Whole-grain consumption has a small beneficial effect on total body fat but not body weight compared with controls.	Fat (%): −0.48 [−0.95, −0.01] Weight (kg): 0.06 [−0.09, 0.20]	(Pol *et al*., 2013)
OBG content: oat bran; oatmeal; oat flour	2,506 individuals (healthy, diabetic or high cholesterol)	Cholesterol	Adding ≥3 g OBG/day to the diet reduces LDL and total cholesterol without changing HDL cholesterol or triglyceride concentration (mmol/L). The effect is significantly greater for individuals with type-2 diabetes (P=0.004).	LDL: -0.25 [-0.20, -0.30] HDL: -0.03 [-0.08, 0.01] Total: -0.30 [-0.24, -0.35] Triglyceride: -0.02 [-0.06, 0.01]	(Whitehead *et al*., 2014)
Fruit and vegetables:					
Fruit and vegetables	833,234 participants	Mortality from all causes, CVD and cancer	Higher consumption of fruit and vegetables was significantly associated with a lower risk of all-cause mortality, particularly CVD but not cancer.	All-cause mortality (increment of one serving per day of F/V): *F/V:* -0.95 [-0.92,-0.98] *Fruit:* -0.94 [-0.90, -0.98] *Veg:* -0.95 [-0.92, -0.99] Disease mortality (for each additional serving of F/V per day): *CVD:* -0.96 [-0.92, -0.99] *Cancer:* -0.97 [-0.90, 1.03]	(Wang *et al*., 2014)
	275 individuals (healthy or cardio-metabolic disorders)	Weight and body composition	Increased fruit and vegetable intake do not affect body weight.	Weight: 1.04 [−1.10, 1.17]	(Kaiser *et al*., 2014)

Food group	Number of individuals	Phenotypic outcome	Direction of outcome	Relative risk [95% CI]	Reference
	1,290,045 participants including 3,912 cases	Liver cancer (HCC)	Increased vegetable but not fruit intake is associated with reduced risk of HCC.	Vegetable intake: *High vs. low:* -0.72 [-0.63, -0.83] *Daily increase (100 g/d):* -0.92 [-0.88, -0.95] Fruit intake: *High vs. low:* -0.93 [-0.80, 1.09] *Daily increase (100 g/d):* -0.99 [-0.94, 1.05]	(Yang *et al*., 2014)
Whole fruit and fruit juices	187,382 healthy participants (at baseline)	Type-2 diabetes	Greater consumption of specific whole fruits, particularly blueberries, grapes and apples, is significantly associated with a lower risk of type-2 diabetes, whereas greater consumption of fruit juice is associated with a higher risk.	Diabetes risk (for every three servings/week of fruit consumed): *Whole fruit:* -0.98 [-0.96, -0.99] *Blueberries:* -0.74 [-0.66, -0.83] *Grapes/raisins:* -0.88 [-0.83, -0.93] *Prunes:* -0.89 [-0.79, 1.01] *Apples/pears:* -0.93 [-0.90, -0.96] *Bananas:* -0.95 [-0.91, -0.98] *Grapefruit:* -0.95 [-0.91, -0.99] *Peaches/plums/apricots:* -0.97 [-0.92, 1.02] *Oranges:* -0.99 [-0.95, 1.03] *Strawberries:* 1.03 [-0.96, 1.10] *Cantaloupe:* 1.10 [1.02, 1.18] *Fruit juice:* 1.08 [1.05, 1.11]	(Muraki *et al*., 2013)
Vegetarian diet	21,915 participants	Blood pressure (systolic and diastolic)	Consumption of vegetarian diets may lower BP relative to omnivorous diets.	Observational studies of BP (mm Hg): *Systolic:* −6.9 [−9.1 to −4.7] *Diastolic:* −4.7 [−6.3 to −3.1] For RCTs of BP (mm Hg): *Systolic:* −4.8 [−7.6 to −3.1] *Diastolic:* −2.2 [−3.5 to −1.0]	(Yokoyama *et al*., 2014)

Food group	Number of individuals	Phenotypic outcome	Direction of outcome	Relative risk [95% CI]	Reference
Carotenoids	924,969 participants including 21,218 cases	Breast cancer	Dietary β-carotene and blood concentrations of carotenoids (α- and β-carotene and lutein) significantly associated with reduced risk of breast cancer.	Dietary β-carotene (increment of 5 mg/day): -0.95 [-0.91, -0.99] Blood concentrations of carotenoids: *Total (per 100 µg/dL):* -0.78 [-0.61, -0.99] *β-carotene (per 50 µg/dL):* -0.74 [-0.57, -0.97] *α-carotene (per 10 µg/dL):* -0.82 [-0.73, -0.92] *Lutein (per 25 µg/dL):* -0.68 [-0.52, -0.89]	(Aune *et al*., 2012)
Plant sterols/ stanols (dietary and supplement form)	263 participants (normal to high baseline cholesterol)	Cholesterol	Plant sterol/stanol intake from dietary sources and supplements effectively lower 'bad' LDL-cholesterol (mmol/L).	*Supplements:* -0.31 [−0.39, −0.23] *Dietary:* −0.31 [−0.35, −0.27]	(Amir Shaghaghi *et al*., 2013)
Resveratrol	388 participants including 79 cases	Glucose control and insulin sensitivity	Resveratrol supplements significantly improve glucose control and insulin sensitivity in diabetic individuals but do not affect glycaemic measures in non-diabetics.	Fasting glucose concentration (mg/dL): *All participants:* -9.67 [-21.09, 1.74] *Non-diabetics:* -0.58 [-3.41, 2.26] *Diabetics:* -35.22 [-52.13, -18.30] Fasting insulin concentration (µl U/ml): *All participants:* -1.51 [-3.53, 0.51] *Non-diabetics:* -0.47 [-1.82, 0.87] *Diabetics:* -4.55 [-6.54, -2.56]	(Liu *et al*., 2014)
Fat:					
Saturated fat (MCT vs. LCT)	239 individuals on MCT diet; 250 individuals on control diet (LCT)	Weight and body composition	Replacement of LCTs with MCTs in the diet can reduce body weight and composition without adversely affecting blood lipid levels.	Weight (kg): –0.51 [–0.80, –0.23] Waist (cm): –1.46 [–2.04, –0.87] Hip (cm): –0.79 [–1.27, –0.30] Body fat (d): *Total:* –0.39 [–0.57, –0.22] *Subcutaneous:* –0.46 [–0.64, –0.27] *Visceral:* –0.55 [–0.75, –0.34]	(Mumme & Stonehouse, 2015)

Food group	Number of individuals	Phenotypic outcome	Direction of outcome	Relative risk [95% CI]	Reference
Total fat	73,589 participants	Weight and body composition	Lowering total fat intake can reduce body weight and composition without adversely affecting lipid levels or BP. Correlation between high total fat intake and weight gain also observed in children and young people.	Weight (kg): -1.60 [−2.00, −1.20] BMI (kg/m2): −0.51 [−0.76, −0.26] Waist (cm, only tested in women): -0.30 [−0.58, −0.02]	(Hooper *et al*., 2012)
TFA (natural and industrially produced)	208 participants (healthy, overweight/ obese or hyperlipidaemic)	Glucose homeostasis and triglyceride and cholesterol (total, LDL and HDL) concentrations	TFA intake (2.59% to 7.8% total energy intake) does not significantly change circulating glucose and insulin concentrations. High TFA intake increases circulating total and LDL-cholesterol and decreases HDL-cholesterol concentrations. TFAs from natural sources (up to 4.19 % of daily energy intake) have no adverse effects on risk markers for CVD in healthy adults.	Glucose levels: 1.08 [−1.14, 1.29] Insulin levels: −1.02 [−1.23, 1.19] Cholesterol levels: *Total:* 1.28 [1.04, 1.51] *LDL:* 1.36 [1.13, 1.60] *HDL:* −1.25 [−1.48, −1.01]	(Aronis *et al*., 2012, Gayet-Boyer *et al*., 2014)
Dairy products	426,055 participants including 26,976 cases	Type-2 diabetes	The inverse relationship between risk of type-2 diabetes and consumption of dairy products (400 g total daily intake), low-fat dairy products (200 g daily intake) and cheese (50 g daily intake).	Total dairy (400 g/day): -0.93 [-0.87, -0.99] High fat dairy (200 g/day): -0.98 [-0.94, 1.03] Low fat dairy (200 g/day): -0.91 (-0.86, -0.96] Milk (200 g/day): -0.87 [-0.72, 1.04] Cheese (50 g/day) -0.92 [-0.86, -0.99] Yoghurt (200 g/day): -0.78 [-0.60, 1.02]	(Aune *et al*., 2013)
	RCTs for 2,101 participants	Weight and body composition	Overall, increasing dairy consumption does not significantly reduce body weight and fat loss in long-term studies or studies without energy restriction. However, dairy products have modest effects on reducing body fat and may be beneficial in facilitating weight loss in short-term interventions or energy-restricted diets.	Weight (kg): -0.14 [-0.66, 0.38] Fat (kg) −0.45 [−0.79, −0.11]	(Chen *et al*., 2012)

Food group	Number of individuals	Phenotypic outcome	Direction of outcome	Relative risk [95% CI]	Reference
Calcium intake	9,095 cases	Stroke	Dietary calcium intake was shown to be inversely associated with stroke in populations with low to moderate calcium intakes and in Asian populations but positively correlated (weak association) with stroke in populations with high dietary calcium intake.	<700 mg/day: -0.82 [-0.76, -0.88] ≥700 mg/day: 1.03 [1.01, 1.06]	(Larsson *et al.*, 2013)
Omega-fatty acids:					
Linoleic acid (omega-6 fatty acid)	310,602 participants; 12,479 total CHD events including 5,882 CHD deaths	CHD	Dietary LA intake inversely associated with CHD risk in a dose-response manner; 15% lower risk of CHD events and 21% lower risk of CHD deaths. The beneficial effect of LA on lowering CHD risk may only be effective in combination with high n-3 in the diet.	CHD events: -0.85 [-0.78, -0.92] CHD mortality: -0.79 [-0.71, -0.89]	(Farvid *et al.*, 2014, Ramsden *et al.*, 2013, Fattore *et al.*, 2014)
Fish consumption	408,305 participants	CHD	The inverse association between fish consumption and risk of the acute coronary syndrome. Fish consumption appears beneficial in the primary prevention of acute coronary syndrome and a higher consumption (≥4 times per week) is associated with greater protection.	≥4 times per week: -0.79 [-0.70, -0.89] 100g serving per week: -0.95 [-0.92, -0.97]	(Leung Yinko *et al.*, 2014)
Omega-3, omega-6 or combined supplements	723 and 294 cases (ages 3-18 years) for fatty-acid and artificial colour exclusion diets, respectively.	ADHD	Fatty acid supplementation and exclusion of artificial food colour may reduce symptom severity in children with ADHD.	Fatty acid supplementation: -1.21 [-1.05, -1.36] Artificial colour exclusion: -1.32 [-1.06, -1.58]	(Sonuga-Barke *et al.*, 2013)

Food group	Number of individuals	Phenotypic outcome	Direction of outcome	Relative risk [95% CI]	Reference
Simple sugars:					
Fructose †	637 participants for isocaloric trials and 119 participants for hypercaloric trials	Weight and body composition in diabetic, overweight/obese and normal weight individuals	Fructose intake does not affect body weight relative to isocaloric diets for other carbohydrate comparators, except in overweight or obese individuals. Excess energy in the diet from fructose intake increases body weight, although confounding from excess energy cannot be excluded.	Fructose vs. any carbohydrate comparator on body weight (kg): Diabetics 0.12 [-0.32, 0.56] Overweight/obese: -0.55 [-1.09, -0.02] Normal weight: -0.13 [-0.37, 0.10] Normal diet with excess energy from fructose on body weight (kg): Obese/overweight: 1.26 [0.70, 1.81] Normal weight: 0.37 [0.15, 0.58]	(Sievenpiper *et al*., 2012)
Dietary sugars and sugar-sweetened beverages	Not stated	Weight and body composition	Intake of free sugars or sugar-sweetened beverages is a determinant of body weight. Change in body fatness that occurs with modifying intakes seems to be mediated via changes in energy intakes since the isoenergetic exchange of sugars with other carbohydrates was not associated with weight change.	Sugary beverages and overweight/obesity risk in children: 1.55 [1.32, 1.82] Dietary sugar intake and weight (kg) in adults: Reduced intake: −0.80 [−0.39, −1.21] Increased intake: 0.75 [0.30, 1.19]	(Te Morenga *et al*., 2013)
Protein:					
Meat	1,932,391 participants including 13,313 cases	Meat intake and risk of oesophageal cancer	Meat consumption is associated with oesophageal cancer risk. High red meat and low poultry intake are associated with an increased risk of oesophageal squamous cell carcinoma. High meat intake, especially processed meat, increases the risk of oesophageal adenocarcinoma.	Highest vs. lowest consumption categories: *Total meat:* 1.19 [-0.98, 1.46] *Red meat:* 1.55 [1.22, 1.96] *Processed meat:* 1.33 [1.04, 1.69] *White meat:* -0.72 [-0.60, -0.86] *Poultry:* -0.83 [-0.72, -0.96] *Fish:* -0.95 [-0.76, 1.19]	(Zhu *et al*., 2014)

Food group	Number of individuals	Phenotypic outcome	Direction of outcome	Relative risk [95% CI]	Reference
Meat; low-fat diets with high-protein vs standard-protein intake	1,063 participants	Weight and body composition, resting energy expenditure, satiety and appetite and cardio-metabolic risk factors	High protein diets relative to calorie-matched standard protein diets provide modest benefits for reductions in body weight, fat mass and triglycerides concentration.	Weight (kg): −0.79 [−1.50, −0.08] Fat mass (kg): −0.87 [−1.26, −0.48] Triglycerides (mmol/L): −0.23 [−0.33, −0.12]	(Wycherley *et al.*, 2012)
Nuts	1) 83,445 participants including 12,655 type-2 diabetes; 8,862 CVD; 6,623 IHD; 6,487 stroke and 48,818 mortality cases 2) 40,102 participants and 12,814 hypertension cases 3) RCTs for 1,006 participants	Mortality from all causes, type-2 diabetes, stroke, IHD and hypertension	1) Nut intake is inversely associated with all-cause mortality, overall CVD, IHD and hypertension but not significantly associated with diabetes and stroke. 2) Higher consumption of nuts was associated with reduced risk of CHD and hypertension but not stroke or type-2 diabetes. 3) Nuts do not affect body weight or waist circumference.	1) Type-2 diabetes (accounting for BMI): 1.03 [-0.91, 1.16] IHD: -0.72 [-0.64, -0.81] CVD: -0.71 [-0.59, -0.85] Stroke: -0.91 [-0.81, 1.02] All-cause mortality: -0.83 [-0.76, -0.91] 2) Hypertension: -0.66 [-0.44, 1.00] 3) Weight (kg) –0.47 [–1.17, 0.22] BMI (kg/m^2): –0.40 [–0.97, 0.17] Waist (cm): –1.25 [–2.82, 0.31]	(Luo *et al.*, 2014, Flores-Mateo *et al.*, 2013, Zhou *et al.*, 2014, Afshin *et al.*, 2014)
Legumes; dietary pulse intake	1,037 participants	IHD, stroke, type-2 diabetes, cholesterol	1) Legume intake (4 weekly 100 g servings) inversely associated with IHD but not significantly associated with stroke or diabetes. 2) Dietary pulse intake (130 g per day) significantly reduces 'bad' LDL cholesterol levels.	1) IHD: -0.86 [-0.78, -0.94] Stroke: -0.98 [-0.84, 1.14] Diabetes: -0.78 [-0.50, 1.24] 2) LDL (mmol/L): −0.17 [−0.25, −0.09]	(Afshin *et al.*, 2014, Ha *et al.*, 2014)
Vitamins and minerals:					
Vitamin B	RCTs for 54,913 participants	Homocysteine levels and cerebrovascular disease	Vitamin B supplementation significantly reduced overall stroke events.	Stroke: -0.93 [-0.86, 1.00]	(Ji *et al.*, 2013)

Food group	Number of individuals	Phenotypic outcome	Direction of outcome	Relative risk [95% CI]	Reference
Vitamin D	5,292 participants and RCTs for 13,033 participants	CVD; cardiac failure, MI and stroke	Vitamin D supplementation offers protection against cardiac failure in older people (mean or median age ≥60) but does not appear to protect against MI or stroke.	Cardiac failure: -0.75 [-0.58, -0.97] MI: -0.97 [-0.75, 1.26] Stroke: 1.06 [-0.80, 1.32]	(Ford *et al*., 2014)
Iron-fortified foods *	11,750 iron-fortified subjects and 9,077 control subjects	Effects on haemoglobin and serum ferritin, iron deficiency and anaemia, zinc and iron status, infections and mental and motor development	Associated with significant increases in haemoglobin and serum ferritin, reduced risk of anaemia and iron deficiency and improvements in other indicators of iron nutrition. No observable effect on serum zinc concentrations, infections, physical growth and mental and motor development.	Haemoglobin (g/dL): 0.42 [0.28, 0.56] Serum ferritin (µg/L): 1.36 [1.23, 1.52] Anaemia: -0.59 [-0.48, -0.71] Iron deficiency: -0.48 [-0.38, -0.62]	(Gera *et al.*, 2012)
Magnesium	313,041 individuals and 11,995 CVD, 7,534 IHD, and 2,686 fatal IHD events	CVD	Circulating (per 0.2 mmol/L increment) and dietary (per 200 mg/day increment) magnesium are inversely associated with CVD risk.	Circulating magnesium and risk of: *CVD:* -0.70 [-0.56, -0.88] *IHD:* -0.83 [-0.75, 1.05] *Fatal IHD:* -0.61 [-0.37, 1.00] Dietary magnesium and risk of: *CVD:* -0.89 [-0.75, 1.05] *IHD:* -0.78 [-0.67, -0.92] *Fatal IHD:* -0.73 [-0.62, -0.86]	(Del Gobbo *et al*., 2013)
	1,703 cases and 2,253 control subjects	Colorectal tumours	Higher intakes of dietary magnesium (100 mg/day increase) are associated with lower a 12-13% lower risk of colorectal tumours.	Colorectal cancer: -0.88 [-0.81, -0.97] Colorectal adenomas: -0.87 [-0.75, 1.00]	(Wark *et al*., 2012)
Selenium	13,254 participants including 5,007 cases	Prostate cancer	Increased selenium concentrations are associated with lower risk of prostate cancer.	Prostate cancer risk: -0.29 [-0.14, -0.61]	(Hurst *et al*., 2012)

Food group	Number of individuals	Phenotypic outcome	Direction of outcome	Relative risk [95% CI]	Reference
Sodium	5,508 participants including 1,478 hypertension cases	Blood pressure, CVD and adverse biomarker levels, *e.g*. blood lipids, catecholamine levels and renal function	Decreased sodium intake significantly reduces resting systolic and diastolic BP in adults and children and has no adverse effects on blood lipids, catecholamine levels or renal function. Increased sodium intake is associated with an increased risk of stroke and fatal CHD in adults.	Reducing sodium intake in adults: Systolic BP (mm Hg): -3.39 [-2.46, -4.31] Diastolic BP (mm Hg): -1.54 [-0.98, -2.11] Increasing sodium intake in adults: Stroke risk: 1.24 [1.08, 1.43] Stroke mortality: 1.63 [1.27, 2.10] CHD mortality: 1.32 [1.13, 1.53] Reducing sodium intake in children Systolic BP (mm Hg): -0.84 [-0.25, -1.43] Diastolic BP (mm Hg): -0.87 [-0.14, -1.60]	(Aburto *et al*., 2013, He *et al*., 2013)
Healthy vs unhealthy diet	127,733 participants, excluding cases (n= ~14,846)	Depression	Higher intake of fruit, vegetables, fish and whole-grains has the potential to reduce the risk of depression.	Risk of depression: Healthy diet: -0.84 [-0.76, -0.92] Western diet: 1.17 [-0.97, 1.68]	(Lai *et al*., 2014)

Note: Data based on randomised controlled trials (RCT) or observational studies. Data presented as relative risk or change in the continuous variable being measured. *Common iron-fortificants used across studies were: ferrous sulfate (28%); NaFeEDTA (20%); electrolytic iron (13%); ferric pyrophosphate (8%); hydrogen-reduced iron, heme (3%) or ferric orthophosphate (3%); ferrous fumarate (7%); amino acid chelates (2%); iron gluconate (1%) and ammonium citrate (1%). † Comparators in isocaloric trials: starch, sucrose, glucose, high-fructose corn syrup, dextromaltose and galactose; diet alone was the comparator in hypercaloric trials. Abbreviations: *BMI, body mass index; BP, blood pressure; CHD, coronary heart disease; CI, confidence interval; CVD, cardiovascular disease; d, standard mean difference; F/V, fruit and vegetables; HDL, high-density lipoprotein; HCC, Hepatocellular carcinoma; IHD, ischemic heart disease; LA, Linoleic acid; LCT, long-chain triglycerides; LDL, low-density lipoprotein; MCT, medium-chain triglycerides; MI, myocardial infarction; OBG, oat beta-glucan; TFA, trans fatty acid*.

3.1.1 Effects of diet on CVD and diabetes

The main findings of the systematic review on the effects of diet and risk of CVD, diabetes and obesity are summarised below and in Figures 3.2 and 3.3. In general, increasing the daily consumption of whole-grains, fruit and vegetables, dairy (including low-fat dairy and cheese), oily fish, protein (including nuts, legumes and pulses) and certain vitamins and minerals can have a protective effect against CVD, hypertension, high cholesterol, type-2 diabetes and body weight and fat mass.

Foods that are high in salt, free sugar and trans-fatty acids (TFA) should be consumed in moderation and prepared from naturally sourced ingredients (as opposed to industrially processed) concerning fats and simple sugars. Lowering salt intake can reduce the risk of stroke, coronary heart disease (CHD) and hypertension; cutting-down on dietary sugar intake particularly sugary beverages can decrease obesity risk in adults and children; and reducing total fat and TFA intake can reduce body weight and composition in adults and children and lower cholesterol in healthy and overweight/obese individuals, respectively. These findings are dependent on multiple factors including age, sex, health status, genetics, environment and culture and should only be considered as a guideline.

3.1.1.1 Whole-grain

<u>Beneficial effects:</u>

- Increasing whole-grain intake has a protective effect against CVD and diabesity (Figure 3.2A and 3.3B):
  - Reduces ‘bad’ low-density lipoprotein (LDL) and total cholesterol levels without changing ‘good’ high-density lipoprotein (HDL) cholesterol levels.
  - This beneficial effect of whole-grain on cholesterol levels is significantly greater ($p<0.01$) for individuals with type-2 diabetes.

- Whole-grain consumption has a small beneficial effect on reducing total percentage body fat but not body weight, Figure 3.3B.

3.1.1.2 Fruit & vegetables

Beneficial effects:

- Higher consumption of fruit and vegetables significantly lowers the risk of all-cause mortality (Figure 3.2A):
  - Particularly CVD; the average reduction in mortality risk is 4% for each additional serving per day of fruit and vegetables (combined effect).
- Greater consumption of specific whole fruits, particularly blueberries, grapes and apples, significantly lowers risk of type-2 diabetes, Figure 3.3A.
- Resveratrol supplements significantly improve glucose control and insulin sensitivity in diabetic individuals, Figure 3.3A.
- Consumption of vegetarian diets may lower blood pressure (BP) relative to omnivorous diets, Figure 3.2B.

No or adverse effects:

- Fruit and vegetable intake does not affect weight and body composition in healthy adults and individuals with CVD, Figure 3.3A.
- Greater consumption of fruit juice significantly increases the risk of type-2 diabetes, Figure 3.3A.

3.1.1.3 Simple sugars

Beneficial effects: Consumption of fructose, found naturally in honey, fruits, berries and most root vegetables, relative to isocaloric diets for other carbohydrate comparators promotes weight loss in overweight/obese individuals (Figure 3.3B).

No or adverse effects:

- Fructose intake does not affect body weight relative to isocaloric diets for other carbohydrate comparators in healthy or diabetic individuals (Figure 3.3B).
- Dietary sugar intake is positively correlated with body weight in adults, including excess energy from fructose intake (Figure 3.3B).
- Sugary beverages increase obesity risk in children (Figure 3.3A).

3.1.1.4 Dietary fat

Beneficial effects:

- Replacement of long-chain triglycerides/fatty acids (LCTs, *e.g*. butter, rapeseed oil and soybean oil) with medium-chain triglycerides (MCTs, *e.g*. palm kernel oil and coconut oil) or lowering total fat intake can reduce body weight and composition in adults, Figure 3.3B
    - Does not adversely affect other cardiovascular risk factors such as BP or lipid levels.
    - A positive correlation between total fat intake and weight gain also observed in children and teenagers/young adults.

No or adverse effects:

- TFAs from natural sources (milk and body fat of ruminants, as opposed to industrially produced from vegetable fats) have no adverse effects on risk markers for CVD in healthy adults (Figure 3.3A):
    - Based on TFA consumption of up to 4.2 % of daily energy intake.
    - The current recommended daily allowance is 0·5 to 1.0 %.

- High TFA intake (natural and industrial sources) at a range of 2.59 to 7.8 % of total energy intake:
    - Does not affect circulating glucose and insulin concentrations in healthy or overweight/obese adults, Figure 3.3A.
    - Does increase total and 'bad' LDL-cholesterol concentrations and decreases 'good' HDL-cholesterol concentrations, Figure 3.3A.

3.1.1.5 Omega-fatty acids

Beneficial effects:

- Fish consumption is inversely associated with risk of the acute coronary syndrome, Figure 3.2A.
- Dietary omega-6 intake lowers CHD risk. However, this beneficial effect may be dependent on high levels of dietary omega-3, Figure 3.2A.

3.1.1.6 Dairy

Beneficial effects:

- Increasing total dairy intake including low-fat dairy products (300–400 g/day) or cheese (~50 g/day) may protect against type-2 diabetes (Figure 3.3A):
    - Possibly due to the high content of calcium, magnesium, vitamin D and whey proteins which may reduce body fat and insulin resistance.
- Dairy products have modest short-term effects on reducing body fat, Figure 3.3B.
- Calcium intake is inversely associated with risk of stroke in Asian populations which have low-to-moderate average calcium intakes (<700 mg/day), Figure 3.2A.

No or adverse effects:

- Increasing dairy consumption does not significantly reduce body weight and fat loss in long-term interventions or diets without energy restriction, Figure 3.3B.
- Consumption of high-fat dairy products, milk or yoghurt, has no effect on the risk of type-2 diabetes, Figure 3.3A.
- Calcium intake is weakly positively associated with increased risk of stroke in populations with high calcium intakes (≥700 mg/day), Figure 3.2A.

3.1.1.7 Protein

Beneficial effects:

- High protein diets provide modest benefits for reducing body weight, fat mass and circulating blood triglyceride concentrations, Figure 3.3B.
- Higher consumption of nuts is associated with reduced risk of ischaemic heart disease (IHD), overall CVD, hypertension and all-cause mortality, Figure 3.2A.
- Legume intake is inversely associated with IHD, Figure 3.2A.
- Consumption of dietary pulses significantly reduces 'bad' LDL cholesterol levels, Figure 3.3B.

No or adverse effects:

- Legume intake and higher nut consumption is not significantly associated with reduced risk of type-2 diabetes and stroke, Figure 3.2A and 3.3A.
- Nut consumption does not affect BMI, body weight or waist circumference, Figure 3.3B.

3.1.1.8 Vitamins & minerals

Beneficial effects:

- Vitamin B supplementation lowers stroke events; vitamin D supplementation protects against cardiac failure, Figure 3.2A.
- Iron-fortified foods reduce the risk of anaemia and iron deficiency, Figure 3.2.
- Decreased sodium intake significantly reduces resting BP in adults and children with no adverse effects on other blood or renal functions, Figure 3.2B.
- Higher intakes of dietary magnesium are associated with reduced risk of IHD (200 mg/day), Figure 3.2A.

No or adverse effects:

- Vitamin D supplementation does not appear to protect against myocardial infarction or stroke, Figure 3.2A.
- Increased sodium intake is associated with an increased risk of stroke, stroke mortality and fatal CHD in adults (Figure 3.2A).
    - Recommended 3 g/day should become the long-term target for population salt intake.

Figure 3.2 A

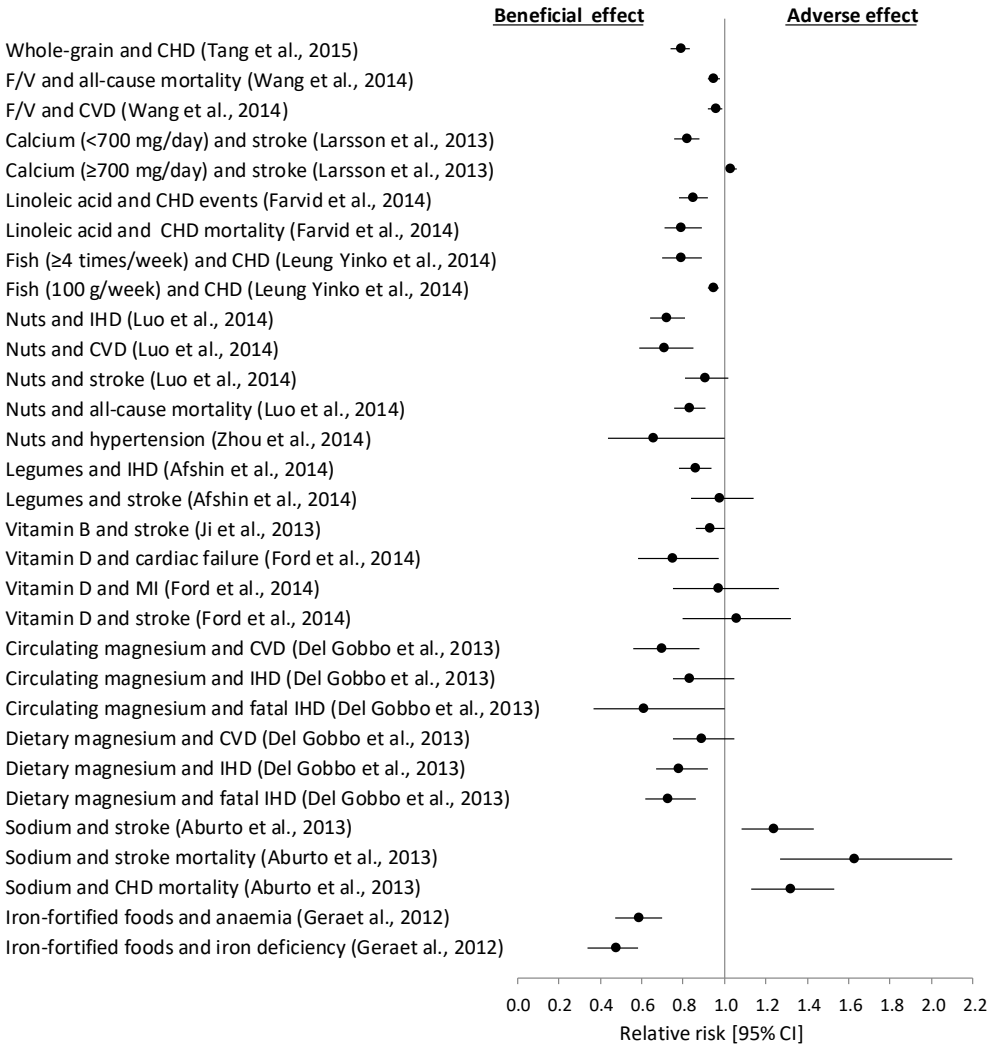

Beneficial effect
Adverse effect
Whole-grain and CHD (Tang et al., 2015)
F/V and all-cause mortality (Wang et al., 2014)
F/V and CVD (Wang et al., 2014)
Calcium (<700 mg/day) and stroke (Larsson et al., 2013)
Calcium (≥700 mg/day) and stroke (Larsson et al., 2013)
Linoleic acid and CHD events (Farvid et al., 2014)
Linoleic acid and CHD mortality (Farvid et al., 2014)
Fish (≥4 times/week) and CHD (Leung Yinko et al., 2014)
Fish (100 g/week) and CHD (Leung Yinko et al., 2014)
Nuts and IHD (Luo et al., 2014)
Nuts and CVD (Luo et al., 2014)
Nuts and stroke (Luo et al., 2014)
Nuts and all-cause mortality (Luo et al., 2014)
Nuts and hypertension (Zhou et al., 2014)
Legumes and IHD (Afshin et al., 2014)
Legumes and stroke (Afshin et al., 2014)
Vitamin B and stroke (Ji et al., 2013)
Vitamin D and cardiac failure (Ford et al., 2014)
Vitamin D and MI (Ford et al., 2014)
Vitamin D and stroke (Ford et al., 2014)
Circulating magnesium and CVD (Del Gobbo et al., 2013)
Circulating magnesium and IHD (Del Gobbo et al., 2013)
Circulating magnesium and fatal IHD (Del Gobbo et al., 2013)
Dietary magnesium and CVD (Del Gobbo et al., 2013)
Dietary magnesium and IHD (Del Gobbo et al., 2013)
Dietary magnesium and fatal IHD (Del Gobbo et al., 2013)
Sodium and stroke (Aburto et al., 2013)
Sodium and stroke mortality (Aburto et al., 2013)
Sodium and CHD mortality (Aburto et al., 2013)
Iron-fortified foods and anaemia (Geraet al., 2012)
Iron-fortified foods and iron deficiency (Geraet al., 2012)
0.0 0.2 0.4 0.6 0.8 1.0 1.2 1.4 1.6 1.8 2.0 2.2
Relative risk [95% CI]

Figure 3.2 B

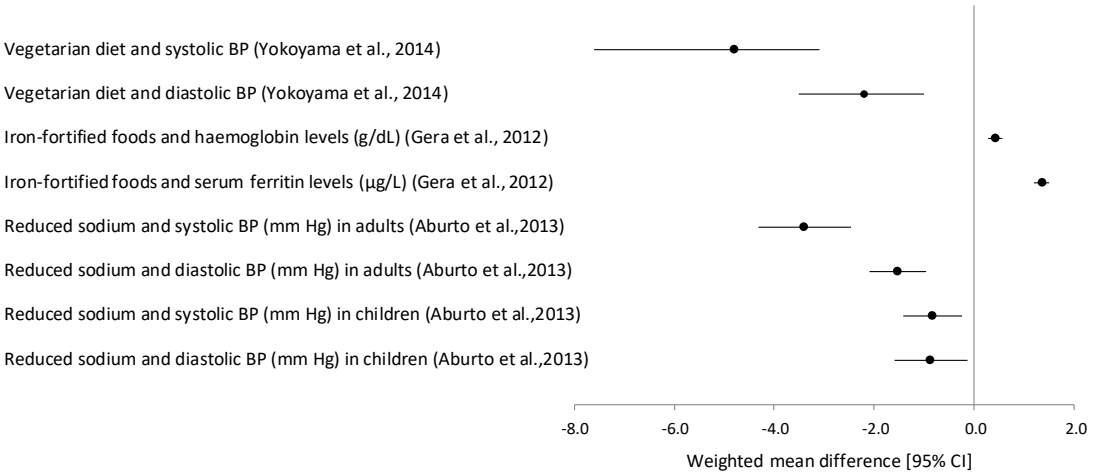


Figure 3.2. Dietary risk factors for cardiovascular disease. Data points represent pooled estimates of relative risk for categorical variables (A) or weighted mean differences for continuous variables (B) for different food groups and their association with CVD based on meta-analyses detailed in Table 1. Horizontal lines represent 95% confidence intervals. Values are based on the random-effects analysis. Abbreviations: *BP, blood pressure; CHD, coronary heart disease; CI, confidence intervals; CVD, cardiovascular disease; F/V, fruit and vegetables; IHD; ischaemic heart disease; MI, myocardial infarction BMI, body mass index; CI, confidence intervals; d, standard mean difference; F/V, fruit and vegetables; HDL, high-density lipoprotein; LDL, low-density lipoprotein; TFAs, trans-fatty acids*.

Figure 3.3 A

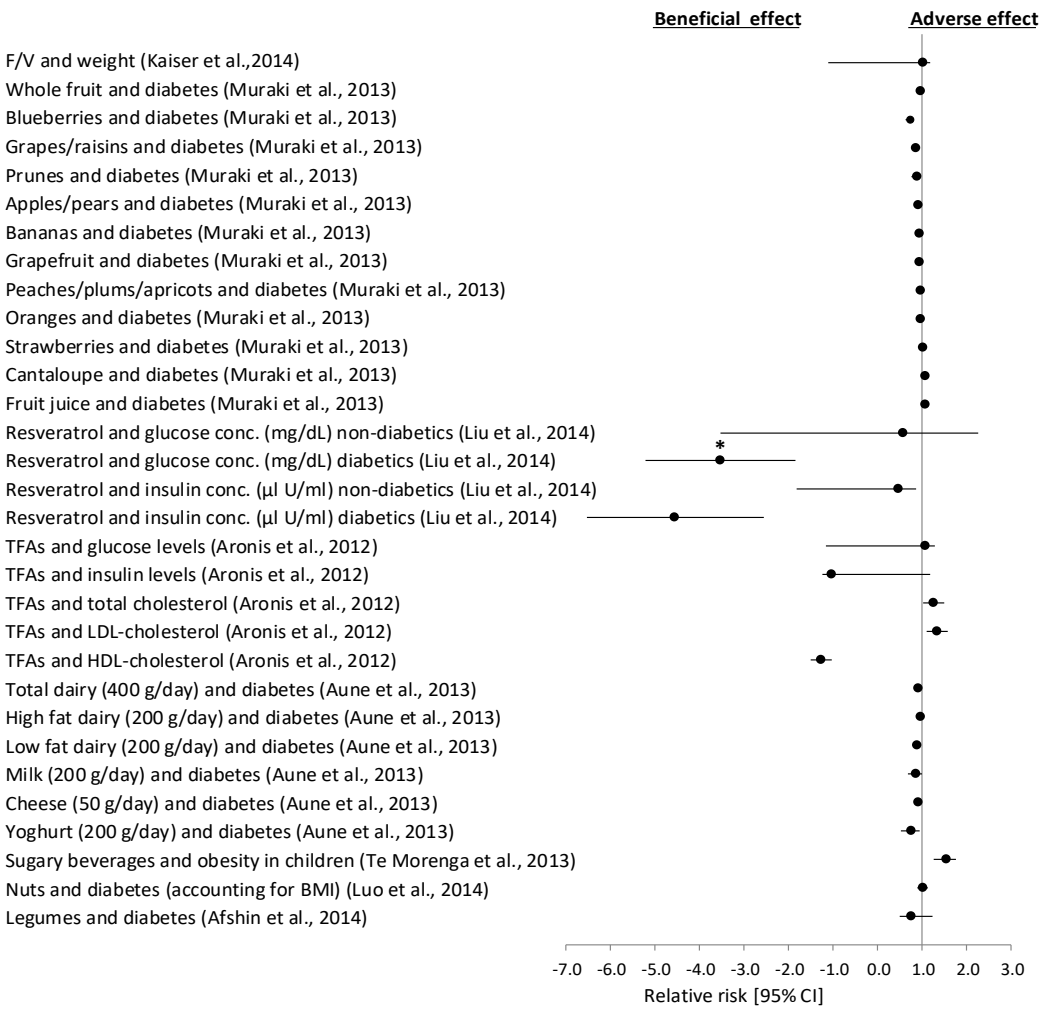

Beneficial effect
Adverse effect
F/V and weight (Kaiser et al.,2014)
Whole fruit and diabetes (Muraki et al., 2013)
Blueberries and diabetes (Muraki et al., 2013)
Grapes/raisins and diabetes (Muraki et al., 2013)
Prunes and diabetes (Muraki et al., 2013)
Apples/pears and diabetes (Muraki et al., 2013)
Bananas and diabetes (Muraki et al., 2013)
Grapefruit and diabetes (Muraki et al., 2013)
Peaches/plums/apricots and diabetes (Muraki et al., 2013)
Oranges and diabetes (Muraki et al., 2013)
Strawberries and diabetes (Muraki et al., 2013)
Cantaloupe and diabetes (Muraki et al., 2013)
Fruit juice and diabetes (Muraki et al., 2013)
Resveratrol and glucose conc. (mg/dL) non-diabetics (Liu et al., 2014)
Resveratrol and glucose conc. (mg/dL) diabetics (Liu et al., 2014)
*
Resveratrol and insulin conc. (µl U/ml) non-diabetics (Liu et al., 2014)
Resveratrol and insulin conc. (µl U/ml) diabetics (Liu et al., 2014)
TFAs and glucose levels (Aronis et al., 2012)
TFAs and insulin levels (Aronis et al., 2012)
TFAs and total cholesterol (Aronis et al., 2012)
TFAs and LDL-cholesterol (Aronis et al., 2012)
TFAs and HDL-cholesterol (Aronis et al., 2012)
Total dairy (400 g/day) and diabetes (Aune et al., 2013)
High fat dairy (200 g/day) and diabetes (Aune et al., 2013)
Low fat dairy (200 g/day) and diabetes (Aune et al., 2013)
Milk (200 g/day) and diabetes (Aune et al., 2013)
Cheese (50 g/day) and diabetes (Aune et al., 2013)
Yoghurt (200 g/day) and diabetes (Aune et al., 2013)
Sugary beverages and obesity in children (Te Morenga et al., 2013)
Nuts and diabetes (accounting for BMI) (Luo et al., 2014)
Legumes and diabetes (Afshin et al., 2014)
-7.0 -6.0 -5.0 -4.0 -3.0 -2.0 -1.0 0.0 1.0 2.0 3.0
Relative risk [95% CI]

Figure 3.3 B

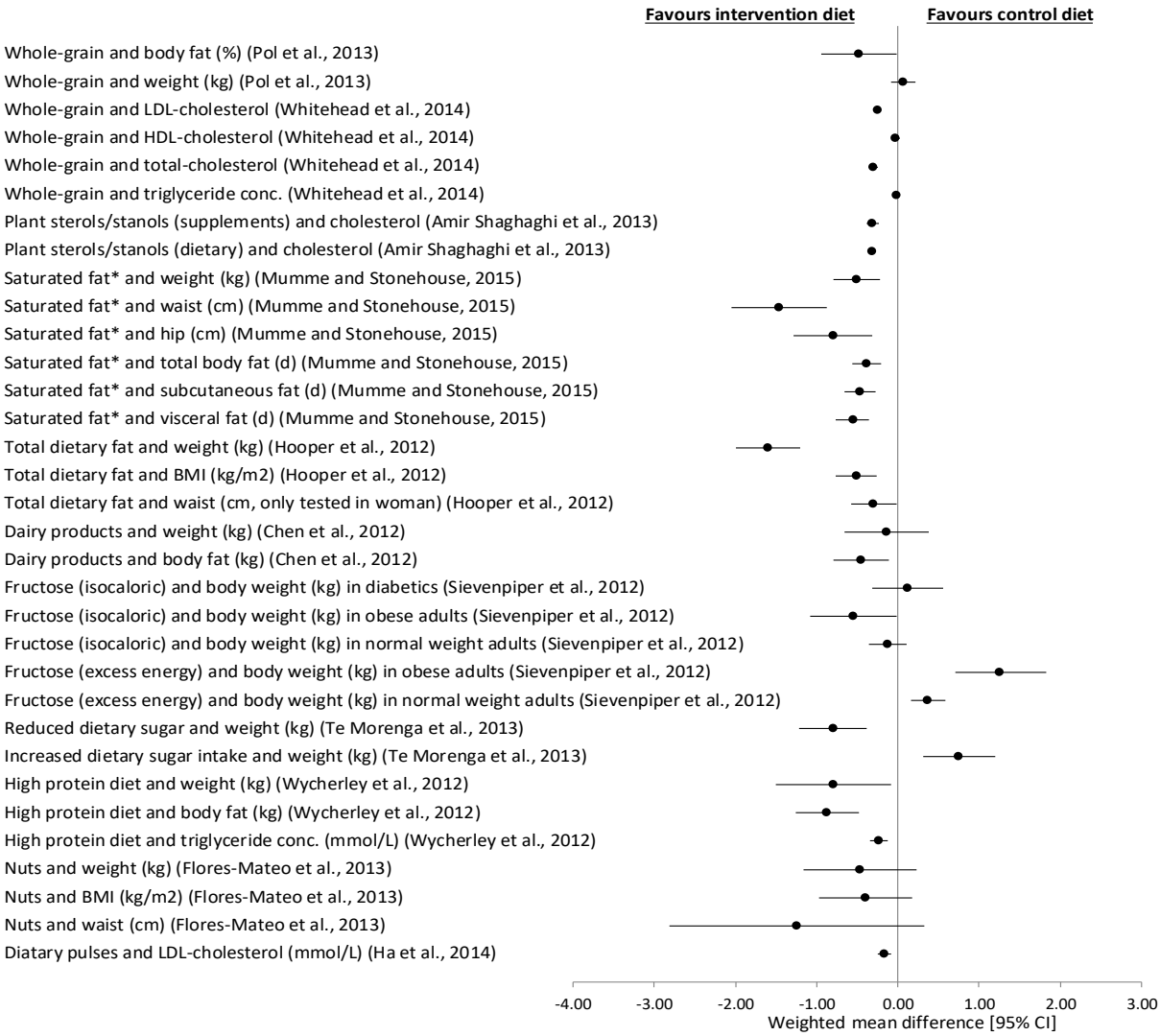


Figure 3.3. Dietary risk factors in diabetes. Data points represent pooled estimates of relative risk for categorical variables (A) or weighted mean differences for continuous variables (B) for different food groups and their association with body weight and composition, obesity and type-2 diabetes based on meta-analyses detailed in Table 1. Horizontal lines represent 95% confidence intervals. Values are based on the random-effects analysis. A, * Data represented as -10 fold; actual value [95% CI] -35.22 [-52.13, -18.30]. B, * Dietary intervention for saturated fat refers to the replacement of long-chain triglycerides with medium-chain triglycerides in the diet. Abbreviations: *BMI, body mass index; CI, confidence intervals; d, standard mean difference; F/V, fruit and vegetables; HDL, high-density lipoprotein; LDL, low-density lipoprotein; TFAs, trans-fatty acids*.

3.1.2 Effects of diet on cancer risk

The main findings of the systematic review on the effects of diet and risk of cancer are summarised below and in Figure 3.4. In general, diets high in fruit and vegetables and low in processed meats may have a protective effect against certain cancers including breast, colorectal, liver, oesophageal and prostate cancer.

3.1.2.1 Fruit & vegetables

- Higher consumption of fruit and vegetables:
  - Does not significantly lower the risk of cancer mortality
  - However, may reduce the risk of liver cancer
- Dietary intake and blood concentrations of carotenoids (α- and β-carotene and lutein), which are naturally occurring pigments synthesised by plants, significantly associated with reduced risk of breast cancer

3.1.2.2 Protein

- Meat consumption is associated with oesophageal cancer risk:
  - High intake of red meat and low intake of poultry are associated with an increased risk of oesophageal squamous cell carcinoma
  - High intake of processed meat supports an increased risk of oesophageal adenocarcinoma

3.1.2.3 Vitamins & minerals

- Higher intakes of dietary magnesium (100 mg/day) are associated with a 12-13% lower risk of colorectal tumours.
- Increased selenium concentrations associate with lower risk of prostate cancer

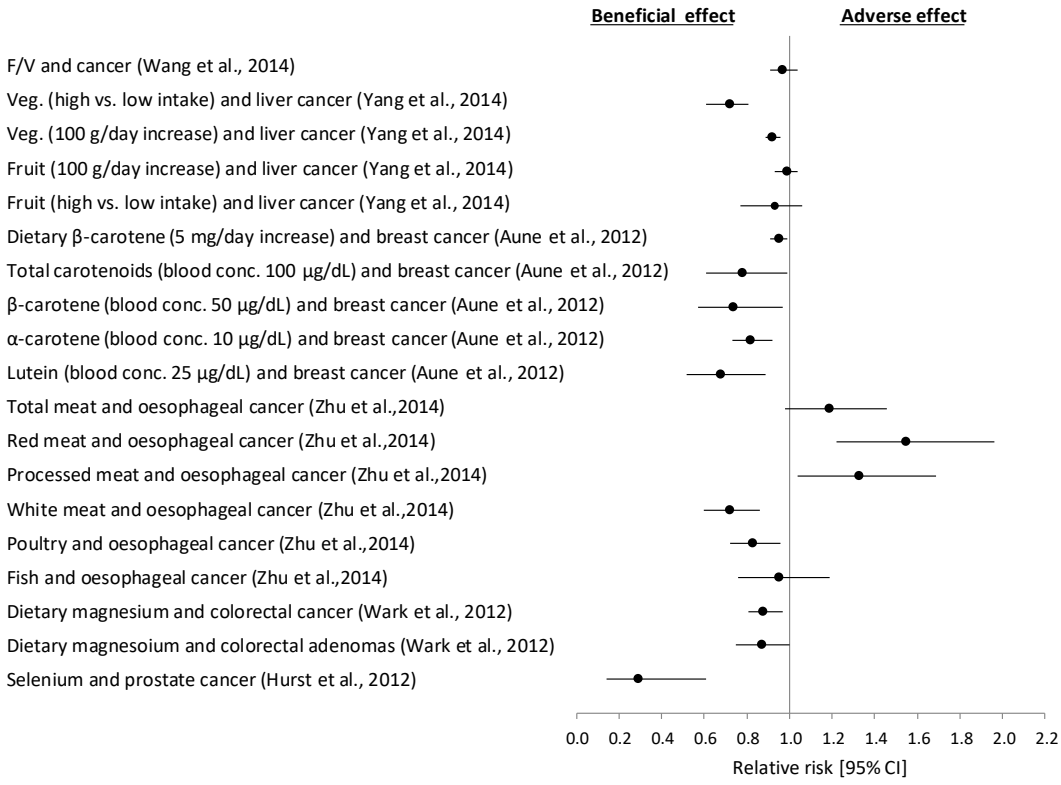


Figure 3.4. Dietary risk factors for cancer. Data represents pooled estimates of relative risk (dots) and 95% confidence intervals (horizontal lines) for different food groups and their association with cancer-based on meta-analyses detailed in Table 1. Values are based on random-effects analysis. Abbreviations: *CI, confidence intervals; F/V, fruit and vegetables*.

3.1.3 Effects of diet on neurological function

An emerging body of evidence indicates that diet plays an important role in mental health (Jacka and Berk, 2007, Jacka *et al*., 2013, Murakami and Sasaki, 2010). Our systematic review of the effect of diet on general health and wellbeing showed that a healthy dietary pattern high in fruit, vegetables, fish, whole-grains and low-fat dairy, and low-to-moderate in red or processed meats significantly reduced the risk of depression, Figure 3.5. In contrast, Western dietary patterns rich in processed meat, high-fat dairy, refined grains, sugary beverages and desserts showed a trend towards an increased risk of depression. However, this did not reach statistical significance (Figure 3.5). Free fatty-acid supplementation and exclusion of artificial food colours in the diet also had a beneficial effect on mental health; reducing the risk of attention deficit hyperactivity disorder (ADHD), Figure 3.5.

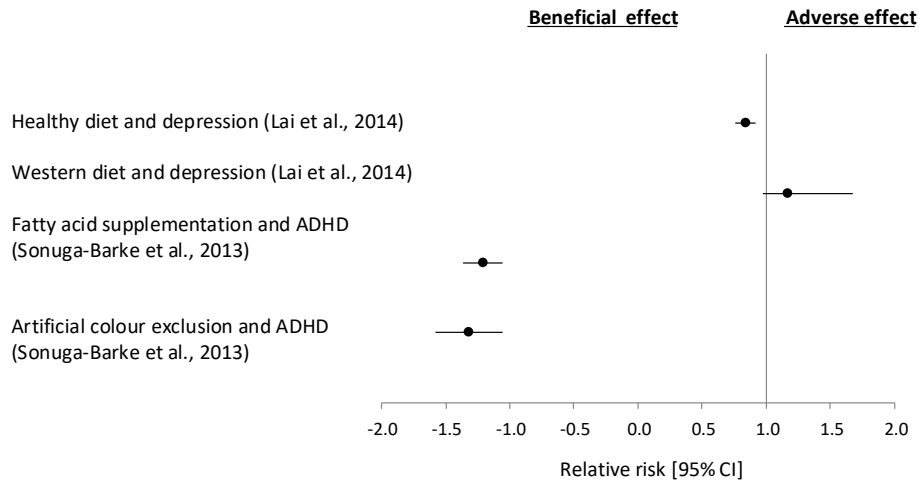


Figure 3.5. Dietary risk factors in neurological disease. Data represents pooled estimates of relative risk (dots) and 95% confidence intervals (horizontal lines) for different food groups and their association with depression and ADHD based on meta-analyses detailed in Table 1. Values are based on random-effects analysis. Abbreviations: *ADHD, attention deficit hyperactivity disorder; CI, confidence intervals*.

3.2 UK dietary patterns and their impact on general health and wellbeing

As indicated from our systematic review, poor quality diet can increase the risk of non-communicable diseases such as CVD, diabesity and cancer. It is therefore a global priority to promote healthy dietary patterns to reduce disease risk. Dietary patterns in the UK were addressed using findings from the most recent National Diet and Nutrition Survey (NDNS), which is based on a representative sample of 1,000 individuals per year living in private households in the UK (NDNS, 2015). The major findings of the NDNS report covering years 1 to 4 (2008/2009 to 2011/2012) of the rolling program, based on mean values for age and gender assigned groupings, are summarised in Figure 3.6 alongside key findings from our systematic review of the literature.

Overall, the population is consuming more than the recommended amounts of saturated fat, added sugars and salt, and not enough fruit, vegetables, oily fish and fibre (NDNS, 2015). Household income was shown to influence dietary patterns, with lower income quintiles associating with poorer quality diets. However, dietary intakes which failed to meet dietary recommendations (see Figure 3.6) were consistent across all income quintiles. In comparison to dietary patterns in 2008/2009, mean energy intake and consumption of total fat, saturated fat, total meat and red meat were generally lower in 2011/2012, with no differences in fruit and vegetable intake or percentage risk of vitamin and mineral deficiencies. Mean intakes of saturated fat, TFAs and non-milk extrinsic sugars were lower in the most recent NDNS relative to previous surveys, and total fat typically made smaller contributions to total energy intakes. Also, intake of fibre was higher, and protein consumption contributed greater to total energy intakes in the most recent survey (NDNS, 2015).

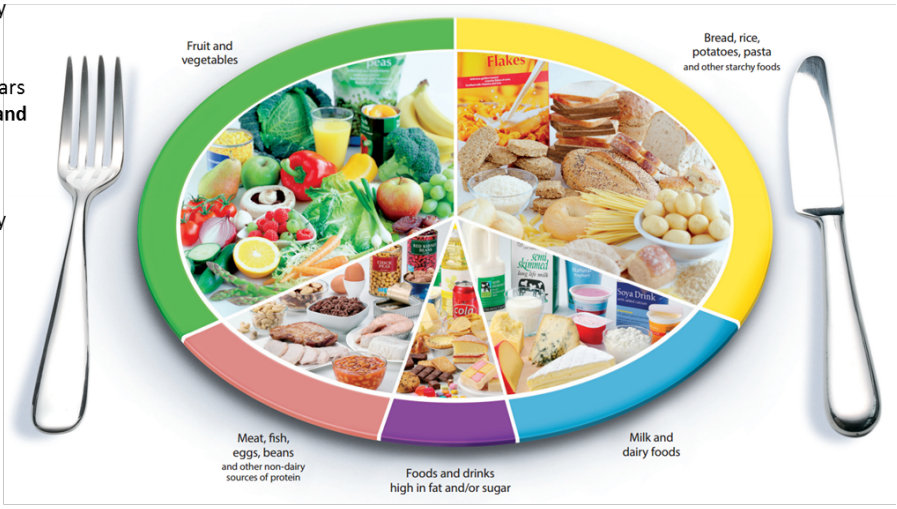
Food intake *in line with* dietary recommendations:
- Total fat and TFAs
- Vitamins (excluding vitamin D)

Fruit and vegetables (†):
- ↓ risk of all-cause mortality (particularly CVD), type-2 diabetes, hypertension and cancer
- **Fruit juice can ↑ risk of type-2 diabetes**

Whole-grain:
- ↓ levels of 'bad' LDL cholesterol
- ↓ % body fat

Food intake *above* dietary recommendations:
- Mean saturated fat
- Non-milk extrinsic sugars
- **Salt (↑ risk of stroke and CVD mortality)**

Food intake *below* dietary recommendations:
- Fruit and vegetables
- Fibre (non-starch polysaccharides)
- Oily fish
- Vitamin D
- Vitamin A, riboflavin and folate [a, b]
- Iron and minerals [c, d]



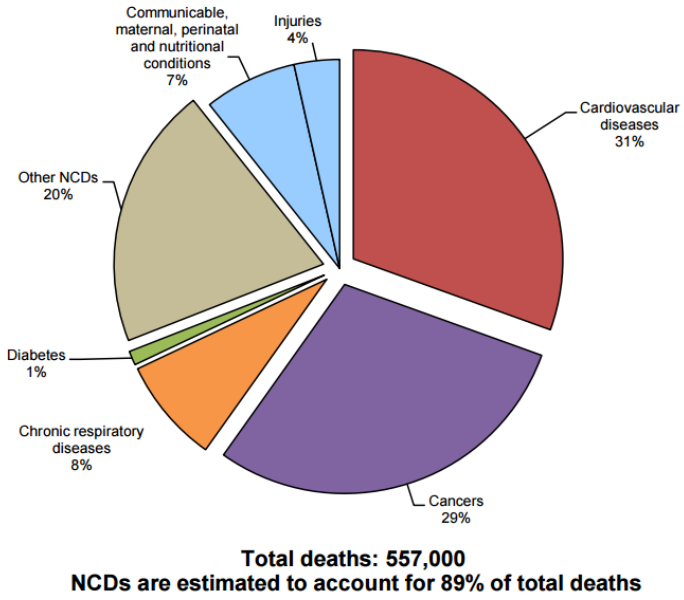
Protein:
- Oily fish reduces risk of CVD; Nuts reduce risk of CVD, hypertension and all-cause mortality; legumes reduces risk of IHD; pulses reduce 'bad' LDL cholesterol levels
- **High intake of red and processed meat increases risk of certain cancers**

Dietary fats:
- Replacement of LCTs* with MCTs** or lowering total fat intake can ↓ body weight and composition
- **TFAs ↑ total and 'bad' LDL cholesterol levels and ↓ 'good' HDL cholesterol concentrations**

Dairy:
- Low-fat dairy products and cheese reduces risk of type-2 diabetes
- Modest short-term effects on reducing body fat
- Low-to-moderate calcium intake reduces risk of stroke

Figure 3.6. UK dietary patterns based on the National Diet and Nutrition Survey (NDNS). A summary of the NDNS covering years 1 to 4 (2008/09 to 2011/12) of the rolling program is represented on the left, representative of 1,000 UK participants (500 children aged 1.5 and upwards and 500 adults), *NDNS* (2015). Beneficial and adverse effects of the different food groups are summarised from a systematic review of the literature. [a] Proportion of 11-18 year old girls only; no indication from biochemical status data that this age group was at risk of deficiency. [b] Riboflavin deficiency in women aged 19-64 years. [c] Iron deficiency risk in girls aged 11-18 years and women aged 19-64 years. [d] Mineral deficiency, particularly magnesium, potassium and selenium, in a substantial proportion of older children and adults. † Eaten individually or in combination. *LCTs, *e.g.* butter, rapeseed oil and soybean oil; **MCTs, *e.g.* palm kernel oil and coconut oil. Image modified from http://www.nhs.uk/Livewell/Goodfood/Pages/eatwell-plate.aspx, accessed November 2015.

A



B

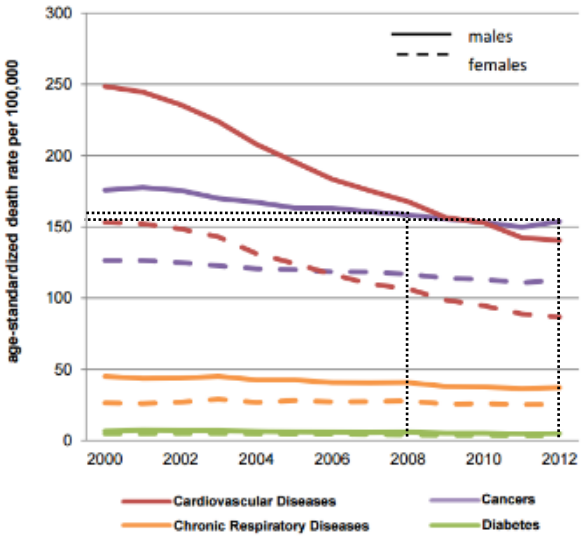


Figure 3.7. UK profile of non-communicable diseases (NCDs). Data based on 2014 World Health Organisation NCD country profiles. A, Percentage mortality rates based on all ages and both sexes. B, Age-standardised death rates per 100,000 of the population. Image modified from http://www.who.int/nmh/countries/gbr_en.pdf, accessed November, 2015.

Non-communicable diseases account for approximately 89% of total UK mortalities, with CVD (31%) and cancer (29%) accounting for the greatest proportions of deaths within this disease category (Figure 3.7A). Analysis of UK mortality rates for both CVD and cancer showed

reductions in the number of deaths per 100,000 of the population from 2008 to 2012, particularly concerning CVD (Figure 3.7B). This supports trends towards healthier dietary patterns in the UK in 2011/2012 relative to 2008/2009, including reduced intake of red meat, saturated fat and TFAs, and increased consumption of fibre (NDNS, 2015).

3.3 Global dietary patterns and disease trends

Our analysis of dietary patterns and non-communicable disease burden was extended globally. A recent study addressing global dietary patterns from 1990 to 2010 found that diets based on healthy products improved worldwide. However, diets based on unhealthy products worsened and at a rate that outpaced healthy dietary patterns for many countries (Imamura *et al*., 2015), Figure 3.8. High-income nations had better quality diets based on healthy products, but significantly poorer diets based on unhealthy products relative to low-income nations, with heterogeneity in dietary patterns observed both regionally and globally (Figure 3.8). In general, older adults and females were reported to have substantially higher quality diets relative to younger adults and males, respectively.

To correlate dietary patterns with disease burden on a worldwide scale, global maps representing morbidity and mortality rates for non-communicable diseases were downloaded from the World Health Organization (WHO) (http://www.who.int/gho/map_gallery/en/, accessed November 2015).

Fig 3.8 A

Fig 3.8 B

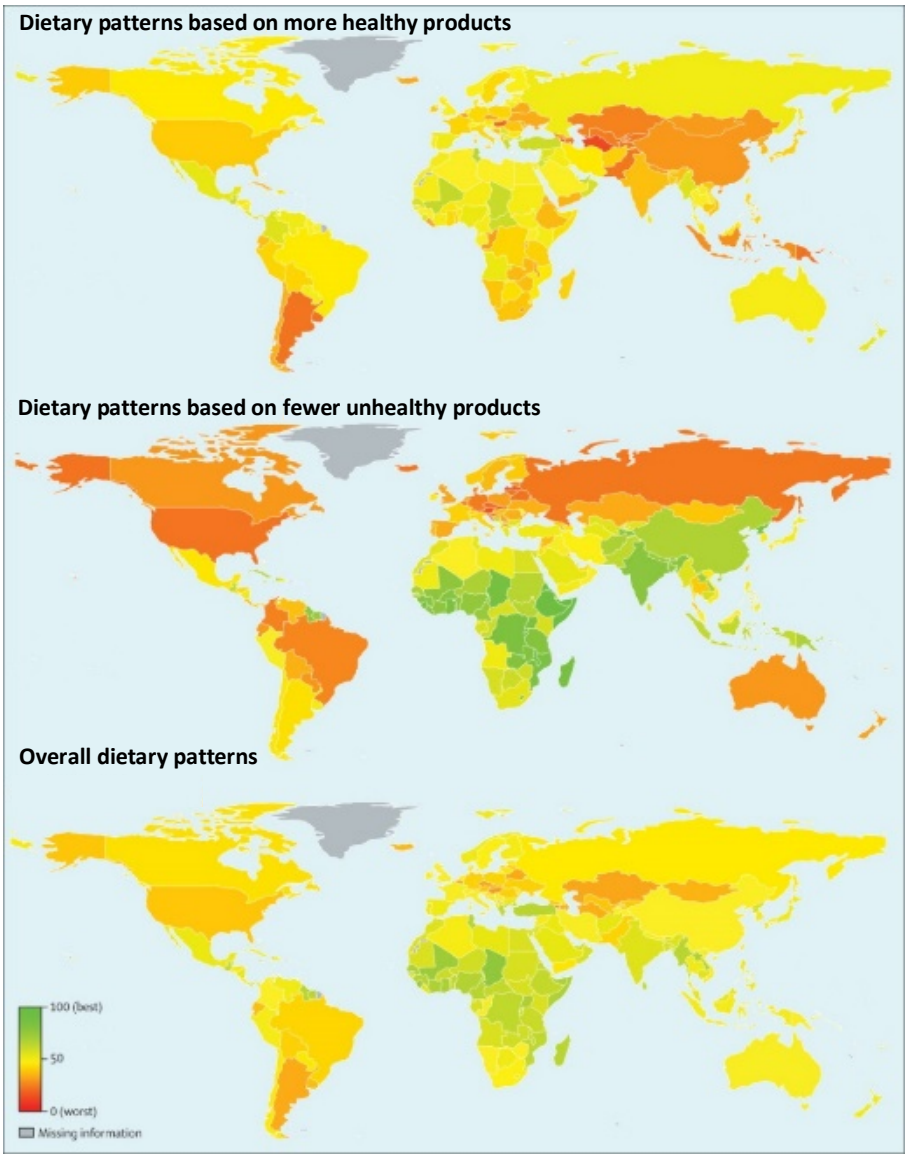


Figure 3.8. Global dietary patterns are relative to global income. A Global income based on gross national income (GNI) per capita for 2013. Low-, middle- and high-income economies are respectively defined as those with a GNI per capita of $1,045 or less, $1,045 to $12,746, and $12,746 or more. Lower-middle-income and upper-middle-income economies are separated at a GNI per capita of $4,125. Image modified from http://data.worldbank.org/maps2015, accessed November 2015. B, Global dietary patterns among men and women in 2010. Values represent degrees of adherence to healthy and unhealthy diet trends, ranging from 0 (least healthy) to 100 (most healthy). Global mean scores were 44·0 (SD, 10·5) and 52·1 (SD, 18·6) for healthy and unhealthy dietary patterns, respectively, with weak inter-correlation between countries. Image is taken from *Imamura et al.* (2015).

The overlap between dietary patterns based on consumption of fewer unhealthy products and the prevalence of raised cholesterol showed a strong positive correlation, *i.e*. the fewer unhealthy products consumed, the lower the prevalence of raised cholesterol; the more unhealthy products consumed, the higher the prevalence of raised cholesterol (Figure 3.9). This was particularly apparent for low- to middle-income countries including India, Pakistan, Afghanistan, China and African countries, where healthier dietary patterns (based on consumption of fewer unhealthy products) correlate with the lower prevalence of raised cholesterol. The opposite trend was observed in high-income countries, with the highest prevalence of raised cholesterol reported in several European countries, including the UK (Figure 3.9). In contrast to blood cholesterol levels the prevalence of raised BP was high in African countries, and also in parts of Europe (Northern and Eastern) and Asia (Central, Northern and Eastern; excluding China). Global mortality rates for CVD were largely comparable to trends observed for the global prevalence of raised BP, Figure 3.9. Global cancer mortality rates again identified Central, Northern and Eastern Asia (including China) and Northern and South-Eastern Europe as most at risk, in addition to parts of South America and Africa. The strong correlation between unhealthy eating patterns and the prevalence of overweight adult males based on body mass index (BMI) was also observed Figure 3.10. This was particularly apparent in upper-middle- to high-income nations, excluding China (see Figure 3.8A for a map of global income). In North America, South America and parts of Europe, there is also a positive correlation between unhealthy eating patterns, high BMI and morbidity rates for the major depressive disorders (Figure 3.10). High levels of depression are also observed in Southern Asia.

Fig 3.9

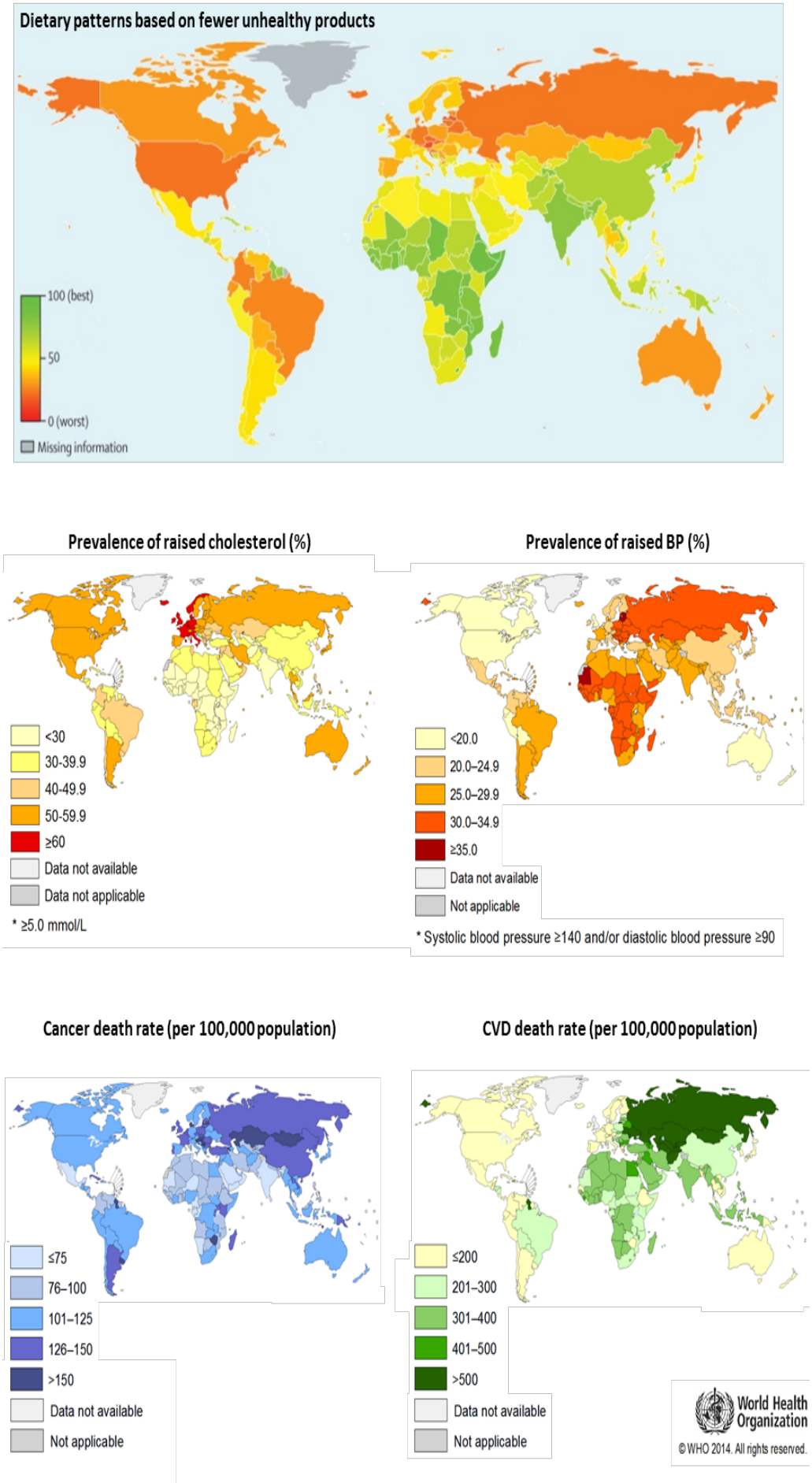

Dietary patterns based on fewer unhealthy products
100 (best)
50
0 (worst)
Missing information
Prevalence of raised cholesterol (%)
<30
30-39.9
40-49.9
50-59.9
≥60
Data not available
Data not applicable
* ≥5.0 mmol/L
Prevalence of raised BP (%)
<20.0
20.0–24.9
25.0–29.9
30.0–34.9
≥35.0
Data not available
Not applicable
* Systolic blood pressure ≥140 and/or diastolic blood pressure ≥90
Cancer death rate (per 100,000 population)
≤75
76–100
101–125
126–150
>150
Data not available
Not applicable
CVD death rate (per 100,000 population)
≤200
201–300
301–400
401–500
>500
Data not available
Not applicable
World Health Organization
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Figure 3.9. Global burden of non-communicable diseases about global dietary patterns. *Top panel*, Global dietary patterns among men and women in 2010. Values represent degrees of adherence to healthy and unhealthy dietary patterns, based on consumption of fewer unhealthy products, ranging from 0 (least healthy) to 100 (most healthy). Image modified from *Imamura et al.* (2015). *Middle panel*, Age-standardised prevalence of raised blood cholesterol (age 25 and over, both sexes) in 2008 and raised blood pressure, BP, (age 18 and over, males) in 2014. *Lower panel*, Age-standardised mortality rates per 100,000 of the population for cancer and cardiovascular disease, CVD, in 2012. Data is representative of both sexes. Images downloaded and modified from the World Health Organisation (http://gamapserver.who.int/mapLibrary/app/searchResults.aspx, accessed November 2015).

Figure 3.10

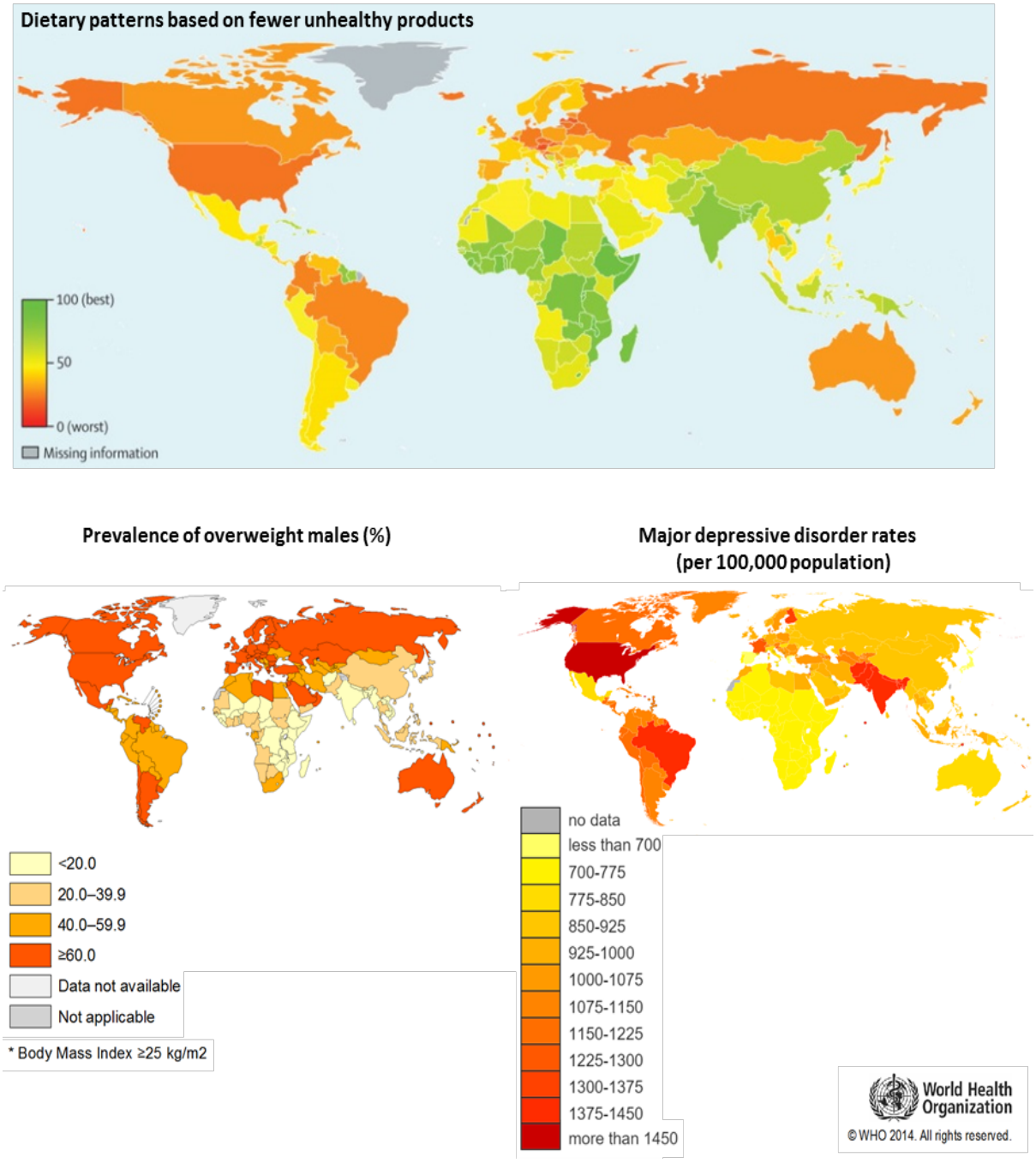


Figure 3.10. Global burden of weight gain and major depressive disorder about global dietary patterns. *Top panel*, Global dietary patterns among men and women in 2010. Values represent degrees of adherence to healthy and unhealthy dietary patterns, based on consumption of fewer unhealthy products, ranging from 0 (least healthy) to 100 (most healthy). Image modified from *Imamura et al.* (2015). *Bottom panel*, Age-standardised overweight males based on body mass index (age 18 and over) in 2014 and age-standardised disability-adjusted life year rates for the major depressive disorder (both sexes) in 2004. Images downloaded and modified from the World Health Organisation.

(http://gamapserver.who.int/mapLibrary/app/searchResults.aspx, (accessed November 2015).

https://en.wikipedia.org/wiki/Major_depressive_disorder#/media/File:Unipolar_depressive_disorders_world_map_-_DALY_-_WHO2004.svg, accessed November 2015.

3.4 Summary

Diet can influence the risk of chronic diseases, such as CVD and cancer, which account for an estimated 89% of total deaths in the UK. Although UK dietary patterns are becoming healthier concerning lower intakes of fat and red meat, and higher intakes of fibre, consumption of fruit, vegetables, saturated fat and salt do not meet national dietary recommendations.

Both in the UK and globally, dietary patterns are linked to the economic status which has implications for disease burden, particularly in low-income quintiles. There is an opportunity in the market to promote affordable, healthier food choices to reduce the disease burden of dietary-related non-communicable disease

Chapter 4

The Functional Food Market & Nutraceuticals

4.1 Natural Functional Food and Drinks

Natural functional foods are expected to be the greatest trend set to impact the food industry. Products that sit within this category are considered to have inherent characteristics that boast naturally healthy ingredients promoted for their natural occurrence; inherent nutrition benefit, simple formulation or nothing added declaration. For example, oatmeal contains beta-glucan, this has been clinically proven to reduce blood cholesterol levels. Additionally, blueberries are considered a natural functional food or informally a 'superfood' as they are rich in antioxidants, phytoflavinoids, potassium, vitamin C and act as an anti-inflammatory.

Another example is Goji berries which have an extensive and impressive list of nutritional benefits. These include 19 amino acids and 21 minerals, including germanium which is an anti-cancer fighting component. Goji berries have 13% protein which is more than whole-wheat and a beta-carotene content which is higher than carrots. They also provide vitamin C and Beta-Sisterol, an anti-inflammatory which aids in lowering cholesterol, Cyperone which reduces blood pressure, Solavetivone an anti-bacterial/fungal compound, Physalin a natural active compound against leukaemia, Betaine which reduces homocysteine and Lycium Barbarum Polysaccharide which is protective in Hepatoma Cancer cell lines.

Water consumption is essential for the body to function optimally, especially as an adult human body's weight is made up of between 50-70% water. Coconut water is an example of a natural functional food which has been re-discovered by the general public looking for water alternatives. Sales of this product in the US had a dramatic increase from

$0 in 2007 to an impressive $390m in 2013. Its success is believed to have been encouraged by the 'naturally healthy – nothing added image'.

4.2 Heart Health

According to The British Heart Foundation, Heart and Circulatory disease combined contribute to more than a quarter of all UK deaths, on average 161,000 annually. The cost of this is estimated to be £19bn each year which includes premature death, loss of productivity, hospital treatment and prescriptions. The main risk factors associated with CHD are having high blood pressure, high blood cholesterol levels, diabetes, being overweight/obese, lack of exercise, unhealthy diet and stress. Many of these factors are influenced by the environment, ethnicity or genetic predisposition. However, diet can have a major impact. Reducing the associated dietary risk factors could impact drastically on the mortality rate of CHD and related illnesses globally.

4.2.1 Cholesterol

Cholesterol is a lipid substance which is secreted by the liver and found in some foods. It is not a harmful substance. However, having excessively high amounts of lipids in the blood, known as hyperlipidaemia can cause detrimental health effects.

Cholesterol is transported around the body by proteins and when the two combine, they are known as lipoproteins; there are two types known as LDL and HDL. Low-Density Lipoproteins (LDL) carries cholesterol from the liver to cells which require it, if it transports more than the cells need, it can build up on artery walls which can cause atherosclerosis – narrowing of the arteries, which in turn can restrict blood flow and potentially cause heart-attack or stroke. Therefore, low levels of LDL are crucial to maintaining healthy bodily

function. High-Density Lipoproteins (HDL) are required in high levels as it is beneficial for bodily function, it removes unneeded cholesterol away from cells and back to the liver, where is it broken down or excreted as waste (NHS A, 2013).

Plant sterols and stanols (phytosterols) can aid in the reduction of blood cholesterol. These components are naturally occurring in plants and similarly structured to cholesterol; therefore, they can compete for absorption, which in turn can reduce the amount of cholesterol that is absorbed. Many manufacturers have included phytosterols in their products to create functional foods with the additional 'cholesterol reducing' benefit. Scientific evidence suggests that consuming between 1.5g and 2.4g of plant sterols daily can significantly reduce LDL by up to 10%. '

4.2.2 High Blood Pressure

Up to 30% of people in the UK suffer from hypertension. It is a measure of the strength of blood pressure against the artery walls and if left untreated, can increase the risk of heart attack/stroke. (NHS 2015). The factors which increase the likelihood of hypertension include age, high caffeine, salt or alcohol intake, poor exercise level, certain ethnicities or being overweight.

4.2.3 Salt

Salt consumption is considered a substantial factor which contributes to increased blood pressure. (Desmond 2006). Salt can be reduced in food subsequently reducing dietary intake in the following ways:

4.2.3.1 Reducing Salt Content

Possibly the easiest way to reduce the amount of salt in food would simply be to not add so much in the first place. The trend for convenience ready-meals are commonly associated with high salt contents to improve shelf-life and reduce wastage. In these pre-packed products, the consumer has limited control over the amount of salt ingested.

4.2.3.2 Salt Substitutes

Potassium Chloride (KCl) is the most common salt substitute for low or reduced sodium/salt food. (Desmond, 2006). Flavour enhancers such as yeast extracts, lactates, monosodium glutamate and nucleotides, used in addition to salt can enrich the overall flavour and result in less salt being added.

4.2.3.3 Optimising the physical form of salt

Salt is characterised by crystal size and shape (Desmond, 2006). The size of salt crystal can influence the delivery of the salty taste that is imparted when consumed. Finer salt crystals are believed to give a more rapid release of saltiness in comparison to large crystals. (Campden BRI, 2012). Optimised sodium chloride in a restructured format, has no bitter aftertaste often associated with potassium alternatives. Overall salt content can be reduced by 25-50%, using a re-engineered salt particle whose physical structure has been altered to facilitate a hollow centre in microscopic particles, therefore acting with the same functionality and properties as salt.

4.3 **Weight Management**

Weight management is a key trend for the functional food market (Canadean Ltd, 2013), (IFT, 2014), (Nutraceuticals, 2012). Estimates are that half the UK population will be obese by 2050. Being overweight is defined as having a BMI (Body Mass Index) of 25.0 – 29.9, obesity is defined as having a BMI of 30 or above. (BBC A, 2014). A survey published in 2012 found that just over a quarter of all adults (26%) in England are obese and a further 41% of men and 33% of women are overweight. Functional foods can help aid in weight loss in three areas:

4.3.1 Reducing energy intake

Developing food products which increase satiety is hypothesised to increase fullness when consumed and the individual will be encouraged to cease eating sooner (Choudhary and Grover, 2012). Therefore, total energy intake will be reduced. This can be accomplished by increasing the main non-caloric value, *e.g*. water, fibre and reducing the caloric value, *e.g*. fat and sugar. Insoluble fibre is considered non-caloric as is not digested by the body, carbohydrates and protein provide 4 calories per gram and fat contribute 9 per gram, as shown in table 4.1.

Table 4.1 Calories per Gram of Macronutrients

Food Group	Calories Per Gram
Fat	9
Alcohol	7
Carbohydrate	4
Fat	4

4.3.2 Increasing the main non-caloric value

4.3.2.1 Water

Water is an essential nutrient for the human body, and as it is not stored, it needs to be regularly consumed to keep the body replenished for optimum efficiency. Liquids can promote satiety in the short term, while long-term structured snacks help individuals lose weight. (Choudhary and Grover, 2012).

The COMMISSION DIRECTIVE 96/8/EC of 26th February 1996, has stringent regulations on the name, energy value, nutritional value, labelling and advertisement of any product presented as a replacement for one or more meals of the daily diet. It stipulates that the energy provided shall be no less than 200kcal and no more than 400kcal per meal. Protein content should be no less than 25% or any more than 50% of the total energy, and the total energy derived from fat should not exceed 30%. Finally, any replacement meal substitute should provide at least 30% of the vitamins and minerals as specified in table 4.2.

Table 4.2 COMMISSION DIRECTIVE 96/8/EC of 26th February 1996, Vitamin and Mineral Requirements

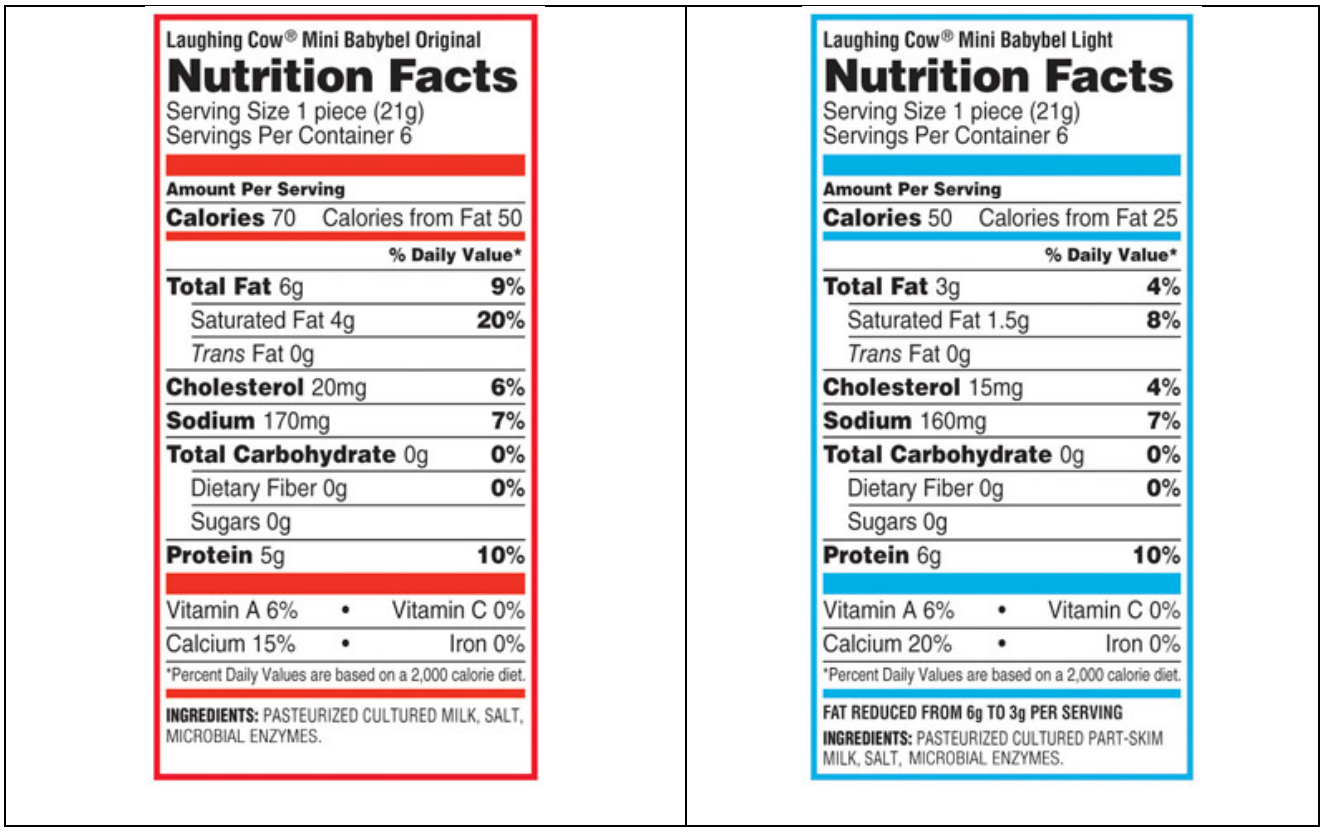
Vitamin/Mineral	Measure	Amount
Vitamin A	µg (RE)	700
Vitamin D	µg	5
Vitamin E	mg (TE)	10
Vitamin C	mg	45
Thiamin	mg	1.1
Riboflavin	mg	1.6
Niacin	mg (NE)	18
Vitamin B_6	mg	1.5
Folate	µg	200
Vitamin B_{12}	µg	1.4
Biotin	µg	15
Pantothenic acid	mg	3
Calcium	mg	700
Phosphorus	mg	550
Potassium	mg	3100
Iron	mg	16
Zinc	mg	9.5
Copper	mg	1.1
Iodine	µg	130
Selenium	µg	55
Sodium	mg	575
Magnesium	mg	150
Manganese	mg	1

4.3.2.2 Fibre

Fibre is another non-caloric component in food. Insoluble-fibre, as a dietary material containing substances such as cellulose, lignin, and pectin, which is resistant to the action of digestive enzymes. Fibre is often used in functional foods as it is believed to aid with weight loss for several reasons; firstly, fibre is a tough and chewy material, requiring maximal mastication to break it down, this stimulates the sensory glands which prepare the body for food intake. Secondly, fibre is not digested by the body which means that any fibre consumed will not contribute to the daily caloric intake. Additionally, fibre empties the gastric tract at a slower rate than other macronutrients meaning the nutrients derived from fibre are absorbed by the body slowly. These qualities mean that increasing fibre in the diet reduce caloric intake and leaves the consumer with a longer sensation of satiety and a slow constant supply of essential nutrients.

4.3.3 Decreasing the main caloric value

As shown in table 4.1, fat contains over double the calories per gram (9) than protein and carbohydrate (4). Food brands have developed ranges to reduce the fat, salt and sugar in their products, creating lower fat versions labelled: Low fat, lite, light, reduced fat, low sugar. Cheese is an example of a high fat and high calorie product that can be reformulated to deliver low fat versions as in figure 4.3



Laughing Cow® Mini Babybel Original

Nutrition Facts

Serving Size 1 piece (21g)
Servings Per Container 6

Amount Per Serving	
Calories 70	Calories from Fat 50
	% Daily Value*
Total Fat 6g	**9%**
Saturated Fat 4g	**20%**
Trans Fat 0g	
Cholesterol 20mg	**6%**
Sodium 170mg	**7%**
Total Carbohydrate 0g	**0%**
Dietary Fiber 0g	**0%**
Sugars 0g	
Protein 5g	**10%**

Vitamin A 6% • Vitamin C 0%
Calcium 15% • Iron 0%

*Percent Daily Values are based on a 2,000 calorie diet.

INGREDIENTS: PASTEURIZED CULTURED MILK, SALT, MICROBIAL ENZYMES.

Laughing Cow® Mini Babybel Light

Nutrition Facts

Serving Size 1 piece (21g)
Servings Per Container 6

Amount Per Serving	
Calories 50	Calories from Fat 25
	% Daily Value*
Total Fat 3g	**4%**
Saturated Fat 1.5g	**8%**
Trans Fat 0g	
Cholesterol 15mg	**4%**
Sodium 160mg	**7%**
Total Carbohydrate 0g	**0%**
Dietary Fiber 0g	**0%**
Sugars 0g	
Protein 6g	**10%**

Vitamin A 6% • Vitamin C 0%
Calcium 20% • Iron 0%

*Percent Daily Values are based on a 2,000 calorie diet.

FAT REDUCED FROM 6g TO 3g PER SERVING

INGREDIENTS: PASTEURIZED CULTURED PART-SKIM MILK, SALT, MICROBIAL ENZYMES.

Figure 4.3: Nutritional Comparison of Cheese.

4.3.4. Incorporating compounds

Many naturally occurring ingredients and compounds are believed to be helpful in weight management. Incorporating these ingredients into new products is considered an ideal way to build a substantial health claim into a new product. (Choudhary and Grover, 2012)

4.3.4.1 Caffeine and ephedrine

A combination of the ingredients caffeine and ephedrine are thought to be effective in long-term weight management. This is due to caffeine's ability to inhibit the phosphodiesterase-induced degradation of cAMP and ephedrine's capability of enhancing the sympathetic release of catecholamines. When operating synergistically, studies have shown that they are effective in long-term weight loss.

4.3.4.2 Green tea

Green tea contains both tea catechins and caffeine which operate synergistically to prevent the inhibition of enzymes which break down body mass. Furthermore, tea catechins have antiangiogenic properties that may prevent the development of obesity (Choudhary and Grover, 2012).

4.3.4.3 Calcium

A diet high in Calcium (Ca) is associated with lower BMI (Choudhary and Grover, 2012). Results from clinical trials suggest that calcium supplementation generates small, statistically significant weight loss in obese and overweight individuals (Daniels, 2014).

4.3.4.4 Conjugated linoleic acid

Conjugated linoleic acid (CLA) is a fatty acid which reduces body fat mass while preserving lean body mass. CLA affects two enzymes – one which breaks down fat in cells and another which breaks down fat in the blood. CLA prevents fat from being stored.

4.3.4.5 Glucomannan

A compound produced from the konjac root; the fibre can absorb a large amount of water which creates a feeling of satiety which in turn can suppress appetite and reduce energy intake. Glucomannan also reduces carbohydrate absorption which prevents insulin spiking and balances cholesterol and triglyceride levels.

4.3.4.6 Chitosan

Chitosan is a dietary fat inhibitor derived from shellfish, which cannot be digested by the body. If consumed in unison with a fat-laden meal it can allow dietary lipids to pass through the body unabsorbed and undigested.

4.4 Protein

The consumer trend continues for meat-free food alternatives to meat, minced beef, chicken and fish (Butler & Smithers, 2014). Meat and protein alternatives are the trends that will drive functional food purchases, with eggs being the most popular alternative followed by beans, lentils and legumes (IFT, 2014), (New Nutrition Business, 2013). The term 'flexitarians' is fast becoming a descriptive title for meat-eaters who are actively seeking non-meat alternatives. Some of the current trending meat alternatives include Seitan, mushroom and mycoproteins which are processed to mimic the texture of meat. The recommended GDA for protein is 45g (figure 4.4), and functional alternatives to meat protein play an important role in modern diets.

High protein functional foods are prevalent in sports nutrition. Products are consumed in the format of whey protein isolate powders, shakes, and supplements. Developments in this market have fulfilled the need for more convenient, on-the-go, ready-to-eat formats.

Other high protein products may also come from dairy sources such as 'Quark' a naturally fat-free soft cheese. Oat milk is a non-dairy product made from soaked and blended oats which are soy, dairy, milk and lactose-free. The milk is often enriched with calcium, vitamin D, riboflavin and vitamin B12. It is high in fibre and protein and low in unsaturated fat. It is rich in beta-glucans which are heart healthy, due to their blood

cholesterol reducing abilities. Similarly, sales of almond milk rose from $0 in 2009 to $407m in 2013 (Boothroyd, 2013).

Table 4.4: Recommended Daily Amount (Source: FDF)

Food Group	Recommended Daily Amount
Protein	45g
Carbohydrate	270g
Fibre	24g
Calories	2,000
Sugar	90
Fat	70
Sat Fat	20
Salt	6

4.5 Free-From

Whether it's free-from gluten, dairy, egg, nut, soya or even suitable for vegans/vegetarians, cutting out problematic ingredients or those which many are sensitive, allergic or intolerant is a growing market. A food allergy is an adverse immune response to a food protein. Whereas food intolerance or non-allergic food hypersensitivity where an individual has difficulty digesting a particular food or food composite.

Any food which contains a protein can cause an allergic reaction however only eight types of food account for 90% of all food allergies in the United States. These include peanuts, tree nuts, milk, eggs, wheat, soy, fish and shellfish (FARE, 2014). Food intolerances have fuelled a £260m sales boom expanding the 'free-from' market by 50% in just two years (Kantar Worldpanel, 2014).

4.5.1 'Gluten-free'

Gluten is a protein composite found in foods processed from wheat or related grains; many individuals react to gliadin, a protein found in gluten where parts of the digestive tract become inflamed, which can interfere with the absorption of nutrients. The number of Coeliac disease diagnoses increased 400% between 1990 and 2011 (BBC B, 2014). The 'Gluten-free' market has huge potential; the market was worth over £175m, a 25% growth since 2011 and had the most food launches of any category in 2013 (Carter, 2014).

4.5.2 Dairy-free

The dairy industry has an opportunity to grow the market by 273 million gallons by targeting the lactose intolerant consumer segment. There is continued demand for lactose-free milk, but also for easily digestible dairy products such as Mozzarella, Cheddar cheese and yoghurt.

4.5.3 Egg-free

Egg allergies are common allergic reactions to the proteins in the albumen (egg white). However, it is usually recommended that suffers refrain from consuming the entire egg as complete separation of the albumen and yolk is difficult. Functional egg replacers substitute the function of egg and can include products developed from potato starch, tapioca and calcium carbonate.

4.5.4 Nut-free

Peanuts (a legume) and tree nuts are the two most common food allergens. They can cause severe and potentially fatal allergic reactions known as anaphylaxis (FARE, 2014). To

prevent an anaphylactic reaction, strict avoidance of peanuts and tree nuts, which includes but is not limited to, walnuts, almonds, hazelnuts, cashews, pistachios and brazil nuts is recommended. Due to entire avoidance being the only known preventative measure, the food industry has responded with various functional nut-free products made from roasted soy.

4.6 Children

Functional food aimed at children has high potential growth rate due to current health issues and pressures. Health will be the main driver of the children's functional food and beverage market, bolstered by innovations in four key areas: packaging, fortification, taste and trust (TSG, 2008). Trends in oobesity, hyperactivity, brain function and gut health are key aspects of child health that could be addressed by diet.

4.6.1 Obesity

One in five children starting school is overweight and latest studies indicating that if a child is overweight by age five, they are unlikely to change. Significant interest in child health and nutrition has become paramount, and childhood obesity is considered one of the most serious global public health challenges. The latest results from the National Child Measurement Programme (NCMP) 2012/2013, suggests that 18.9% of children aged 10-11 are obese and a further 14.4% are overweight. Results from the National Health Survey also reflects this, finding that 28% of children in the UK aged 2 to 15 are classified as either overweight or obese (Public Health England, 2014). In response to the growing childhood obesity epidemic, many functional 'low fat' foods aimed at children are now available.

4.6.2 Hyperactivity & brain function

E numbers, additives, colours and flavourings are under scrutiny from parents. Healthier children's sweets made from natural grains and fruit juice instead of glucose, provide a natural functional alternative to artificial ingredients. Additionally, studies in children have shown that essential fatty acid supplements improve focus and concentration.

4.6.3 Gut health

Two major concerns in child health are digestion and immunity. Children's probiotics and omega 3 are commonly used to improve gut function and strengthen the immune system.

4.6.4 Formula milk

The first functional food that many will have their initial exposure to is infant formula. Infant formula is considered a complete food which is manufactured from purified cow's milk in a powdered format. Formula milk is the most basic of functional foods as despite being a powder when diluted with water it is considered a complete substance in providing all essential macro and micronutrients. Formula can be given to an infant exclusively for the first six months. Formula milk for infants and follow-on milk for toddlers added nearly $5bn to the functional food market in 2013. This market ranks first over energy drinks ($3.5bn), pre and pro-biotic yoghurts ($2.4bn) and water ($2bn) (Mercola, 2013). Functional Baby drinks remain the smallest segment of the baby food market, capturing only 2% of spend. There is potential to grow this market by launching non-sweet drinks for children, as 21% of parents are interested in this concept (Mintel B ,2014).

4.6.5 Baby food

Baby food is usually the next stage of formula or breast milk. It is usually in a smooth, soft, puree format as infants cannot chew, due to underdeveloped muscles and teeth. 82% of parents with a child aged 0-4 feed them homemade food on a daily basis. 36% feed their child pre-made food as often (Mintel B, 2014).

Figure 4.1, illustrates how the baby food market growth is forecasted from 2012 to 2017. Despite monetary growth year on year, from $11.4bn in 2012 to an estimated $14.1bn in 2017, the % annual market growth is set to decelerate over the same period. Innovation is essential for market growth in this area and functional foods continue to play an important role (Mintel B, 2014).

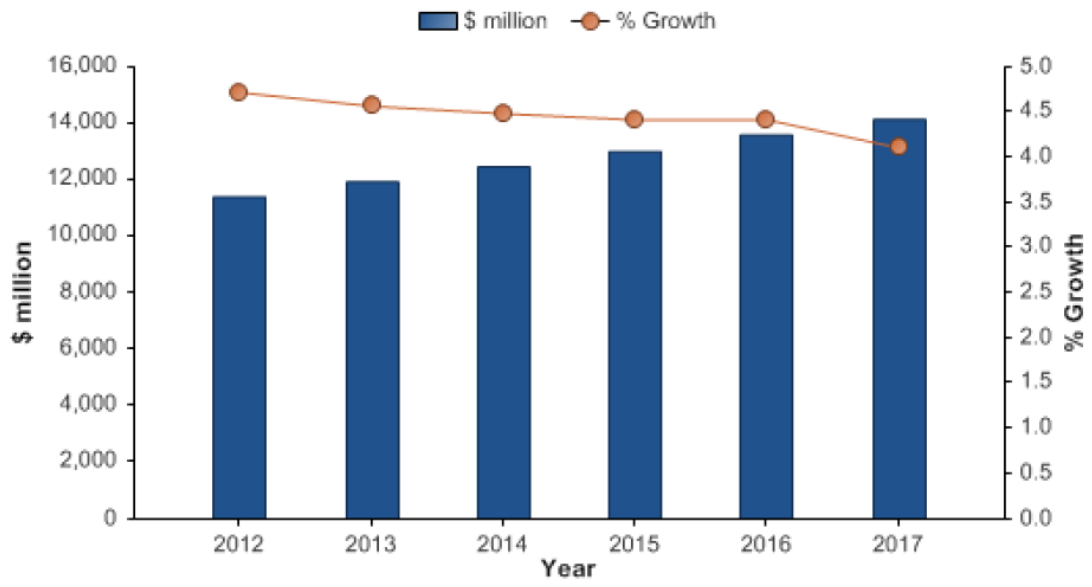


Figure 4.1: Global Baby Food Market Value Forecast: $ Million, 2012 - 2017

4.6.6 Ready meals

Convenient meals specifically designed for children is an ideal market for functional food. There are many aspects of child health which can be enhanced by a diet including dental and bone growth, brain development, limiting weight gain and many others. However, targeting children is a sensitive area as any contentious ingredients used, or controversial claims will be heavily scrutinised due to the innocence of the target sector and the imperative of good quality nutrition needed at this life stage.

4.7 Energy

Products providing 'healthy fuels' for energy will be a key trend over the coming years. The introduction of legislation in 2014 by the EU regarding caffeine in food and drinks, placed stringent regulations on the labelling of high caffeine products (Mintel, 2014). If 150mg of caffeine per litre is exceeded, products must have a 'high caffeine content' label.

4.7.1 Slow energy release & breakfast biscuits

Breakfast is often considered the most important meal of the day. The time constraints of modern living reduce the opportunity to prepare and eat at this meal occasion. Lack of time, has driven a desire for quick, convenient, food 'on-the-go' options. Products which have become popular as a breakfast offering are those which provide a nutritious and sustained energy source. The new breakfast biscuits category has grown 78.9% to £76m year-on-year in less than four years (Bamford, 2014), (Evans, 2012).

4.7.2 Bars & snacking

Many energy-rich products come in bar format which is easy to transport, portion controlled and quick to consume. Snacking is generally accepted as food and drink consumed between

main meals (Chaplin and Smith, 2011). The convenience food culture has encouraged the snacking market to grow 3.3% in 2013 (Conlumino, 2014). It is the least developed market for all eating occasions but the most promising regarding long-term growth with 98.8% of people eating snacks at some stage of the day; fruit and cereal bars are the most common snack in the morning.

4.7.3 Sugar

Sugar has several different functions; its broad properties include sweetening products, enhancing taste and aroma, affecting the weight and volume, improving texture and colour, increasing shelf-life, aiding in the fermentation process, assisting with water retention to prevent drying out and reducing the freezing point. Due to its multi-functioning role, substitutes which can replace each property are uncommon. Natural alternatives are known as 'sugar substitutes', and synthetic varieties are called 'artificial sweeteners'. The NHS recommends that an adult male should consume no more than 70g of sugar per day and adult females no more than 50g daily (NHS C, 2013).

Consumers are becoming increasingly sceptical about anything artificial. A recent study involving 2000 participants contends that 38% actively avoid foods or drinks which contain artificial sweeteners as they believe it can have a more adverse effect on health than natural sugar (Quinn, 2014). Examples include aspartame, lactitol, xylitol, saccharin and sorbitol. In contrast, consumer opinion of natural sugar alternatives are positive with 40% of respondents claiming they would purchase more low sugar variety products if they contained natural sweeteners such as stevia, agave nectar or honey instead of artificial variations (Quinn, 2014).

4.7.3.1 Plant sources for functional sugar replacements

4.7.3.1.1 *Sysepalum Dulficum*

A red fruit berry, commonly known as miracle fruit. This berry can affect the perception of taste due to its innate glycoprotein – miraculin. This protein binds to the taste receptors on the tongue and acts as a sweetness inducer changing the flavour of sour foods such as lemon, lime and vinegar to taste sweet when instantly consumed after miracle berries (Gayot, 2012).

4.7.3.1.2 Stevia

'Stevia' is a natural sweetener that is 250-300 times sweeter than normal sugar, without providing any calories, carbohydrates, or increased blood sugar level. The compound is made from leaves found on the *Stevia Rebaudiana Bertoni* plant. The food additive has been approved by the EU since 2011 but has been used in the US since 2008 and sold in Japan for over 40 years (BBC D, 2013). Stevia is approximately 2000% more expensive than sugar. Therefore, the price of stevia is a barrier to wide commercial use.

4.8 Manufacturers

The global functional food market is dominated by some of largest global food and drink manufacturers (table 4.5). Manufacturing products with different functional ingredients to provide a range of health benefits.

Table 4.5. Samples of functional food and drink products from Danone, Unilever, Kellogg, Kraft, Coca-Cola and Yakult.

	Company	Product	Functional ingredient or effect	Health benefits
Foods	Danone	Activia (yoghurt)	Bifidus ActiRegularis	improve gastrointestinal and immune function
		Actimel (yoghurt drink)	L. Casei cultures, calcium, Vit B6 and D	Strengthen immunity, bone health, heart health
	Unilever	Flora pro.activ	Plant sterols	Reduce LDL cholesterol
		Slim fast (meal replacement)	High fibre and protein content, low calorie	Weight management
	Kellogg's	Raisin Bran	Omega 3 from flaxseed	Reduce LDL cholesterol levels
		Smart Start	antioxidant	Reduce oxidative stress from developing a wide range of diseases.
		Fibre Plus	High fibre, Antioxidant (Vit E & Zinc)	Improve gut health; Reduce oxidative stress from developing a wide range of diseases.
	Kraft	Mayo	Fat-free; olive oil	Weight management, reduce LDL cholesterol level and blood pressure
	Yakult	Yakult	Probiotics: Lactobacillus casei and Bifidobacterium	Improve gastrointestinal and immune function
	Coca-cola	Powerade ION4	Mineral salts (sodium chloride, magnesium chloride,	Replenish energy level

Drinks			calciukm chloride, potassium phosphate)	
		Glaceau	Vitamins (B6, C, E, pantothenic acid (Vit B5) and folic acid)	Anti-inflammatory properties;
		5 Alive	Antioxidant; phenylalanine	Anti-infammatory properties; Cognitive function
		Relentless	Taurine; caffeine; Guarana	Enhance cognitive function;
	Kellogg's	To go-breakfast shake	High protein and fibre content	Weight management

4.9 Opportunities and market drivers

Functional food products and ingredients that emphasise health benefits with direct scientific evidence are more likely to be successful due to reduced scepticism among consumers. Important future developments will be in the area of personalised nutrition which will allow manufacturers to capitalise on the growing demand for targeted functional foods. Overall the functional food industry is a remarkably successful and diverse market which continues to grow.

4.10 Nutraceuticals

Functional foods and ingredients that have a role in the overlap between pharmaceuticals and food are commonly referred to as Nutraceuticals. There are a number of common conditions where nutraceuticals have a purpose and certain technologies can improve the bioavailability of ingredients.

4.10.1 Obesity

4.10.1.1 Epidemiology

Around 2.8 million people die annually from obesity. Related conditions lead to other undesirable metabolic effects on blood pressure, cholesterol and insulin resistance *etc*. Commonly, these conditions are attributed to coronary heart disease, stroke and type II diabetes *etc*. In England, there were approximately 24.7% of adults who were obese in 2012. Figure 4.2 shows the increasing trend from 1993 to 2012; the trend in obesity is increasing and estimated by 2050 to affect 60% of adult men, 50% of adult women and 25 % of children (Public Health England, 2007).

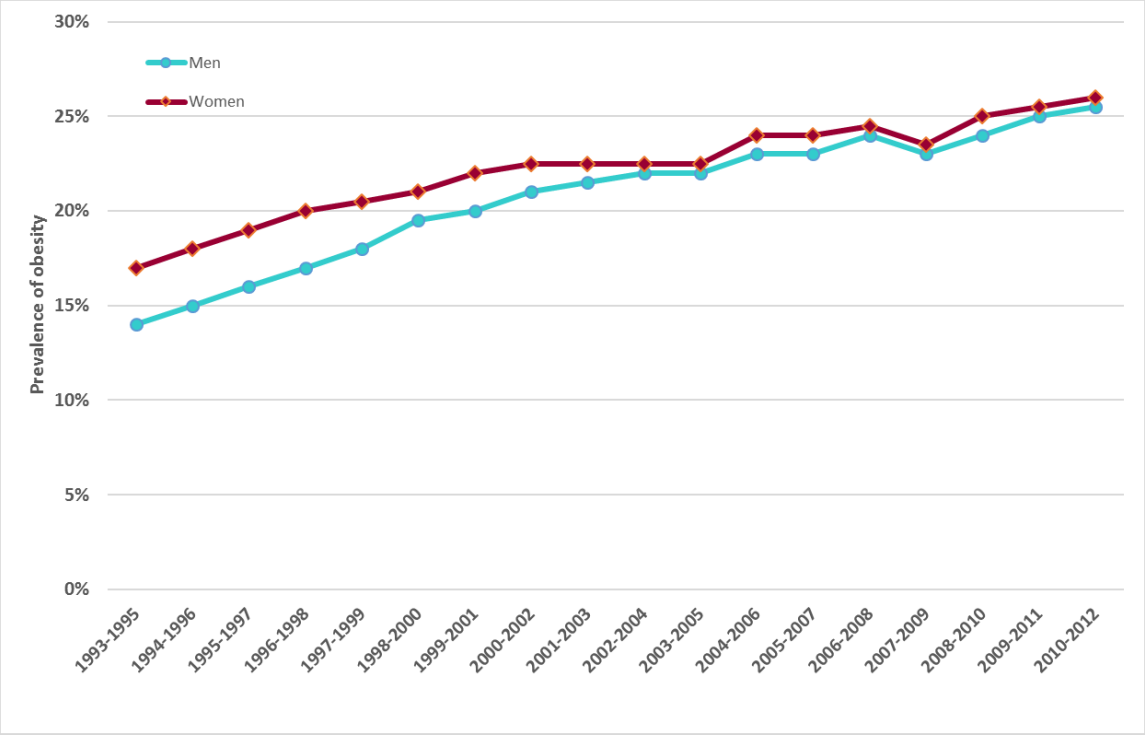


Figure 4.2 . Prevalence in obesity, Public Health England, 2012

4.10.1.2 Treatment

Although there are drugs which target the treatment of obesity. There are relatively few anti-obesity medications that have been approved to be safe and effective, obtainable without a prescription in the UK. Many of the currently available pharmaceutical drug interventions have deleterious side effects such as gastrointestinal and cardiovascular risk factors (NICE). The challenge for nutraceutical companies is to deliver non-pharmacological alternatives to obesity drugs without side effects. With the global increase in obesity, nutraceutical inventions have an important role for the prevention and treatment of obesity.

Evidence shows that consumption of dietary polyphenols may be beneficial for obesity management. Thousands of different types of polyphenols have been identified in a wide variety of fruits and vegetables. They can be categorised as flavonoids, phenolic acids, lignans and stilbenes (Baboota *et al*., 2013). Consumption of dietary polyphenols delivers multiple benefits, for example, antioxidant, inhibiting free radicals and reactive oxygen species and anti-inflammatory. Most importantly, dietary polyphenols may inhibit pre-adipocyte to adipocyte differentiation causing adipocyte apoptosis, decrease absorption of fat from the gut, promote catabolism in adipose and liver tissue and suppress lipid biosynthesis. Also, anti-inflammatory molecules may be promoted in adipose tissue to reduce oxidative stress under obesity.

The Polyphenols industry is growing rapidly due to increased public awareness of its benefits to maintain good health. In 2011, the global polyphenols market hit $580m; it is predicted to reach $873m in 2018. Natural polyphenols can be found in tea, particularly in

green tea. It is expected the global tea polyphenols market will hit $368m by 2020 (Academia, 2013).

Energy intake has a significant impact on developing obesity. Conventional functional foods such as fibre, protein or carbohydrate reduce energy density in food intake. Fibre substantially contributes to lower total caloric content. It also leads to slower gastric emptying and rate of nutrient absorption. Since protein (7kcal/g) and carbohydrate (4kcal/g) are less energy dense than fat (9kcal/g); altering macronutrient composition of the diet reduces fat proportion which also impacts on reducing the energy density of the diet.

4.10.2 Cardiovascular Disease (CVD)

4.10.2.1 Epidemiology

Cardiovascular disease remains the leading cause of death in the world. CVD represents a group of disorders of the heart and blood vessels, which includes: coronary heart disease, cerebrovascular disease, peripheral arterial disease, rheumatic heart disease, congenital heart disease, deep vein thrombosis and pulmonary embolism. According to WHO, in 2008 there were 17.3 million fatalities from cardiovascular disease, which represent 30% of global deaths. The increase in CVD mortality rate forecasts that 23 million people will die from CVD annually by 2030. Populations affected were predominately from low and middle-income countries.

Figure 4.3 shows the CVD-related cause of death in the UK in 2010 (BHF, 2012). Women show a higher risk of CVD than men across all causes of CVD-related deaths; CVD mortality rate mainly affects older age groups.

		All ages	Under 35	35-44	45-54	55-64	65-74	75+
All causes	Men	270,945	8,015	6,997	14,120	30,587	54,052	157,174
	Women	290,721	4,639	4,209	9,531	20,366	38,205	213,771
	Total	561,666	12,654	11,206	23,651	50,953	92,257	370,945
All diseases of the circulatory system (I00-I99)	Men	87,528	504	1,409	3,984	8,982	16,766	55,883
	Women	91,550	274	566	1,523	3,382	9,004	76,801
	Total	179,078	778	1,975	5,507	12,364	25,770	132,684
Coronary heart disease (I20-I25)	Men	46,591	102	681	2,539	5,899	9,952	27,418
	Women	33,977	36	166	586	1,495	4,084	27,610
	Total	80,568	138	847	3,125	7,394	14,036	55,028
Stroke (I60-I69)	Men	19,287	91	224	515	1,126	2,883	14,448
	Women	30,079	62	131	425	813	2,326	26,322
	Total	49,366	153	355	940	1,939	5,209	40,770
Other diseases of the circulatory system (I00-I19, I26-I59, I70-I99)	Men	21,650	311	504	930	1,957	3,931	14,017
	Women	27,494	176	269	512	1,074	2,594	22,869
	Total	49,144	487	773	1,442	3,031	6,525	36,886

Table 4.6 Death by cause, by sex and age, UK 2010 (BHF, 2012)

4.10.2.2 Treatment

CVD can be treated with medications when a patient is at high risk of developing the disease. Most common medications prescribed to treat CVD include angiotensin-converting enzyme (ACE) inhibitors, which are used to lower blood pressure; statins which are used to lower blood cholesterol levels and aspirin to prevent blood clots (NHS, 2012). By 2015, the global market for cardiovascular drugs is due to exceed US$111.8bn (GIA, 2010).

Nutraceutical products may help prevent cardiovascular disease. Plant sterols, a plant-based substance, has shown a positive effect on reducing CVD risk, by blocking cholesterol absorption in the small intestine. Consumption of functional food and beverages containing plant sterols can lower cholesterol, especially LDL cholesterol level, by at least 6% without lowering HDL cholesterol level. Functional foods with plant sterols include whole grain bread, fortified margarine and fortified fruit juices. Globally, sales of plant sterol products have reached approximately €2.5bn in 2013. Dietary supplements are another approach to obtain adequate amounts of plant sterols to reduce CHD risk (IFICF, 2007). Additionally, substances such as catechins in green tea, flavanols in coffee, polyphenols (resveratrol, procyanidins and quercetin) found in red wine have all been shown to reduce the risk of CVD in clinical trials (Tempest, 2012).

4.10.3 Diabetes

4.10.3.1 Epidemiology

Approximately 360 million people in the world have diabetes, which is approximately 8.5% of the global population. It is a significant impact on both society and economy. The top 5 countries with the highest number of diabetic patients are China, India, US, Russia and Brazil (Diabetes UK, 2011). In the UK, there are 2.9 million people diagnosed with diabetes, and it is expected to rise to 4 million by 2025. Diabetes is a lifelong condition where blood sugar remains at high levels. There are two types of diabetes: type 1 and type 2. In the UK, type 2 diabetes accounts for about 90% of all adults who are diagnosed with diabetes, leaving 10% for type 1 diabetes. Children who are affected, are commonly diagnosed with type 1 diabetes. However, there is an increasing number of children and young adults developing type 2 diabetes. Type 1 diabetes, where the pancreas does not produce insulin, is not curable. Type 2 diabetes is a condition where the pancreas does not produce enough insulin to reduce blood sugar level. It usually affects adults over the age of 40 (NHS, 2012).

4.10.3.2 Treatment

Diabetes treatment may include lifestyles changes such as regular physical activity and healthy diet. However, in some serious conditions, medication and insulin treatment will be needed. There are several types of medications for diabetes which include: Biguanide, Sulphonylureas, Alpha-glucosidase inhibitor, Prandial glucose regulators, Thiazolidinediones, Incretin mimetics, DPP-4 inhibitors, and SGLT2 inhibitors. Diabetes can be preventable by altering nutrition and dietary pattern. Figure 4.4 shows foods, nutrients and dietary patterns that can reduce the risk of diabetes development (Giacco *et al.*, 2013).

Table 4.7 Foods, nutrients and dietary patterns associated with risk of developing type 2 diabetes.

The Functional Food Market & Nutraceuticals

	Increased Risk	Degree of Evidence	Decreased Risk	Degree of Evidence
Foods	Soft Drinks	++	Whole Grains	++
	Red meat and processed meat	++	Tea and Coffee	++
	Oil and hydrogenated margarine	+	Milk and dairy products low in fat	++
	Eggs	+	Fruits, vegetables, legumes	+
			Moderate alcohol consumption	+
Nutrients	Saturated fatty acids	+	Fibres	++
	Trans fatty acids	+	Unsaturated fatty acids	++
			Antioxidants	+
			Magnesium	+
Dietary Pattern	High glycaemic index	++	Low glycaemic index	+
	Western Diet		Mediterranean Diet	+++
			Diet low in fat	+++

Degree of evidence from perspective epidemiological studies and clinical trials = +++ High, ++ Moderate, + Reasonable

Food with a low glycaemic index (GI) have a direct effect on reducing the risk of diabetes as it does not raise blood glucose to a high level. Cereals such as oats are rich in β-glucan which is a highly viscous soluble fibre. It plays a significant role in slowing gastric emptying and limits the rise of blood glucose level. Glucose has a GI of 84%, and fructose is 29%; therefore, replacing glucose with fructose is another way to reduce GI of the food without affecting the palatability (Giacco *et al.*, 2013). Slow-release carbohydrate food is another healthy option for diabetics.

There are also dietary supplements with specific types of functional ingredients that are beneficial for diabetes (Davi *et al.*, 2010).

- Vanadium: this mineral is valuable for both type 1 and types 2 diabetes because it transports glucose into the cells which is similar to the action of insulin. Reduction in fasting glucose level is seen after consumption.
- Biotin: a member of B vitamin complex, causes an increase in insulin production by stimulating live glucokinase activity.
- Coenzyme Q10: clinical trials show an effect of improved insulin resistance by lowering glucose and fasting insulin levels.

4.11 Nutrient interventions for disease (table 4.8– Appendix 1)

A number of nutrient interventions for diseases ranging from Metabolic Syndrome, Alzheimer's, CVD, Atherosclerosis and Diabetes, can be found in table 4.8.

4.12 Nutraceutical technologies

The bioavailability of certain functional ingredients is reduced or destroyed in food processing from heat or oxidation. Capsule technology protects the bioavailability of sensitive components, provides improved shelf life and timed delivery of nutrients.

4.12.1 Microencapsulation

Microencapsulation technologies have been developed to ensure the bioavailability of ingredients. Microencapsulation is a process of enclosing micron-sized particles of solids or droplets of liquids or gasses in within an inert shell to isolate and protect the substance from the external environment (Wang & Bohn, 2012). The end product of this process can be called microparticles, microcapsules or microspheres depending on the forms and internal structure. Particle size diameters which are less than 1µm are called nanoparticles, nanocapsules or nanospheres. Particle size diameters between 300 - 800 µm are known as microparticles, microcapsules or microspheres. Finally, particle size diameters that are larger than 1000µm are called macroparticles.

The main purpose of this technology is to (i) protect sensitive substances from external environment; (ii) mask the organoleptic properties like colour, taste, odour of the substance; (iii) improve stability and bioavailability of the food ingredient or nutrient; (iv) obtain controlled release of the drug substance and (v) target release of the drug.

Microparticles consist of two parts which are core material and coat material. The core material is usually an active ingredient or substance such as pharmaceutical ingredients, proteins, peptides, volatile oils, food material, *etc*. The core materials are protected by the coating material such as gelatine, ethyl cellulose or hydroxyl propyl methyl

cellulose (Jyothi *et al*., 2010). Since most nutrients are sensitive to heat, oxygen, water or another physical process, low moisture and high temperature are needed to control and kill harmful organisms to aid in extending shelf-life. Therefore, preserving the beneficial structures and nutrients of a functional ingredient is important for bioavailability (Onwulata, 2011).

Encapsulation also offers targeted delivery and controlled release of nutrients. For example, delivery of probiotic bacteria in dairy products has been difficult because of the high die-off rate during long-term storage. Types of preparation and protection methods must be carefully selected to protect the live bacteria and ensure successful delivery. Coating material must be maintained and well tolerated due to environmental stress such as acidity and gastric condition in the digestive process (Amin *et al*., 2013). A fundamental understanding of the physical and chemical factors of the core and coating material will facilitate the delivery system to the desired site (Augustin *et al*., 2013).

4.12.2 Capsule-in-capsule technology

Capsule-in-capsule technology is a biphasic delivery system suitable for combination or dual release products. It is achieved by placing a smaller pre-filled capsule into a larger filled capsule. The content of the inner capsule can be in the form of liquid, semi-solid, powder or pellets; whereas the outer capsule can only be either a liquid or semi-solid formulation.

Figure 4.3 . Examples of various fills of capsule-in-capsule technology: liquid/liquid; liquid/semi-solid; liquid/beads (Rao *et al*., 2013)

This single dosage can deliver 2 different types of substances, released at different times in different regions of the GI tract. It is easy to achieve multiple release profiles by using an appropriate coating and modifying the release formulation in the outer or inner capsule (Rao *et al*., 2013). For instance, with an enteric coating, the capsule is capable of surviving through the low pH in the stomach, upper or lower intestinal tract and delivery to the colon to meet target release profile optimising site delivery (Capsugel, 2013). This method is suitable for both pharmaceutical and nutraceutical industries.

Chapter 5

Epigenetics, Functional Foods and the Mediterranean Diet

5.1 Epigenetics

Epigenetics is the study of heritable changes in gene expression that does not involve changes to the underlying DNA sequence — a change in phenotype without a change in genotype.

5.1.1 The epigenetic mechanism

5.1.1.1 DNA methylation

DNA methylation is essential for cell development and stabilising cell function. It is a DNA modification which involves a biochemical addition of a methyl group (CH_3) to the 5^{th} carbon position of cytosine-phosphate-guanosine (CpG) dinucleotides via the DNA methyltransferase (DNMT) enzyme (Choi & Friso, 2010). The purpose of DNA methylation is to silence the gene and therefore reduce gene expression; this may be the default state of a gene (Zeisel, 2009).

5.1.1.2 Histone modification

In contrast to DNA which can only be modified by methylation, there are 6 types of histone modification include methylation, acetylation, phosphorylation, biotinylation, sumoylation and ADP-ribosylation (Choi and Friso, 2010). However, the functional interaction between DNA and histones are modulated mainly by methylation and acetylation. Histones are a family of basic proteins which are tightly wrapped around by DNA to prevent transcription. When histone is modified by methylation or acetylation, it changes the structure of histone protein by adding a methyl or acetyl group to it and exposes the DNA attached to it. This creates space through the DNA strands to facilitate transcription factors (Zeisel, 2009). The histone acetylation status is maintained by histone acetyltransferase (HAT) and histone

deacetylase (HDAC). Histone methylation status is balanced by histone methyltransferases and histone demethylase. A study has shown that inhibiting HDAC could repress silenced genes in cancer cells. Therefore certain bioactive food components have been investigated to determine whether they could act as HDAC inhibitors.

Sulforaphane from broccoli and sprouts, diallyl sulphide from garlic and butyrate from fibre have all shown association with HDAC inhibitory effects (Choi & Friso, 2010). Histone acetylation is also highly associated with inflammation; HDAC regulates proinflammatory and anti-inflammatory genes in disease such as cancer.

5.1.1.3 MicroRNA

MicroRNA (miRNA) are small non-coding RNA that regulate gene expression by translational repression. They play an important role in controlling DNA methylation and histone modification (Choi & Friso, 2010).

mRNA function via base-pairing with complementary sequences of mRNA; therefore, mRNA is silenced because they can no longer be translated into proteins by ribosomes as ribosomes are blocked. Aberrant expression of miRNA has shown a positive association with development or progression in numerous diseases including cancer by altering cell proliferation and apoptosis processes.

5.1.2 Nutrients in epigenetics

The epigenetic mechanism is exceptionally important in the nutritional field because food and nutrient components are not only able to affect enzymes directly in the system, they are also capable of modifying gene expression at the transcriptional level (Choi & Friso, 2010).

Maternal diets affect foetal development changing epigenetic marks. A maternal diet which is high in choline during pregnancy has been shown to result in changes in gene expression that control brain development in the foetus. Choline deficiency during pregnancy is associated with a significant and irreversible change in hippocampal function of the foetus; and long-term potentiation and altered memory in their adulthood. Therefore, it is critical to obtain an adequate amount of choline during pregnancy as it can cause lifelong changes in brain structure and function by altering epigenetic marking (Zeisel, 2009).

5.1.2.1 Nutritional epigenomics.

Alteration in the process of gene expression can result from epigenetic changes rather than a change in DNA sequence. Nutrients are involved in epigenetic changes that can alter and modify the intracellular signalling pathways (García-Segura, Pérez-Andrade & Miranda-Ríos, 2013).

5.1.2.2 Molecular targets for bioactive food ingredients

Bioactive food ingredients and components function to maintain normal cellular activity, affect the neoplastic transition of normal cells to cancerous cells, and alter the biological behaviour of the neoplasm. The responses to dietary compounds that have a role in preventing cancer may be related to the diversity of the enzymes being processed and modified (Elsamanoudy *et al*., 2016).

Garlic is associated with autoactivation of CYP2E1 but does not affect other CYP450 isozymes by the same mechanism. Genetic polymorphisms in the regulatory regions of the genes of metabolising enzymes and transporter proteins, such as AhR and PXR, may affect

the total response to the bioactive food constituents (Yang *et al*., 2007). Food components could modify the neoplastic progression as well as apoptosis (Meeran, 2002)

Key points in the cell cycle are regulated by different protein kinase complexes that are composed of cyclin and cyclin-dependent kinase molecules. These key cell cycle points are also affected by combined dietary components. It has been proved that dietary factors, either essential or non-essential, can adjust and modify the cell cycle checkpoints, and consequently have a role in reducing the progression and proliferation of a tumour (Meera, 2008).

Apigenin (celery, parsley), curcumin (turmeric), epigallocatechin-3-gallate (green tea), resveratrol (red grape and berries), genistein (soybean), and allyl sulfur (garlic) have been reported to affect the cell cycle by different mechanisms (Elsamanoudy *et al*., 2016). Some bioactive dietary components may also enhance apoptosis by stimulating the free radical formation of reactive oxygen/nitrogen species in the cell (Kim, Yim & Chung, 2008).

5.1.3 Epigenetics response to diet

DNA methylation patterns have been shown to be influenced by the intake of multiple and combined food ingredients such as vitamin A and zinc; even nonessential dietary components may have a role (Ross, 2003). Methyl-deficient diets could lead to an evident alteration in the methylation patterns observed in the process of transformation of normal cells to cancerous cells (Fang *et al*., 2007). It is reported that genistein and related soy isoflavones through their possible direct regulatory effect on DNA methyltransferase enzyme can reactivate methylation-silenced genes. Genistein is a phytoestrogen compound of the isoflavone class that is found in plants and has a structure similar to estrogen.

Dietary cholesterol performs an inhibitory effect on the transcription of hydroxymethyl-glutaryl-CoA reductase gene. Dietary polyunsaturated fatty acids repress mRNA production of fatty acid synthase in hepatocytes by decreasing mRNA for lipogenic enzymes. This process depends on the degree of unsaturation of fatty acids (Leu & Schmidt, 2008).

5.1.3.1 Resveratrol

Resveratrol, is a bioactive component in red grape skins and novel potent activator of Sirt1, has an anti-inflammatory effect against colitis and colitis-associated colon cancer (Cui *et al*., 2010; Sanchez-Fidalgo *et al*., 2010). The anti-inflammatory effect of resveratrol is conveyed through the inhibitory effects of iNOS, COX-2, and NF-kB (Youn *et al*., 2009). It has been suggested that histone acetylation by activated NF-kB can be repressed by resveratrol. Resveratrol has also been shown to inhibit HDAC. Altered enzyme activity by this compound may affect physiological and pathological processes during our lifetime by altering gene expression.

Other possible beneficial properties in various pathologies include CVD, type 2 diabetes mellitus (T2DM), neurodegenerative disorders and rheumatoid arthritis (Choo *et al*., 2014; Trotta *et al*., 2015, 2016; Banu *et al*., 2016). These benefits are largely ascribed to the polyphenol components of these products. Resveratrol exists as two isoforms, cis-resveratrol and trans-resveratrol. The latter isoform being more biologically active than its cis counterpart (Calabrese *et al*., 2010; Artero *et al*., 2015).

5.1.3.2 Nattokinase

Nattokinase (NK) is a serine protease purified and extracted from natto, a traditional Japanese food produced from the fermentation of soybeans with the bacterium, *Bacillus*

subtilis (natto). NK can break down blood clots by directly hydrolysing fibrin, and plasmin substrate converts endogenous prourokinase to urokinase (uPA), degrades PAI-1 (plasminogen activator inhibitor-1), and also increases tissue plasminogen activator (t-PA) which supports fibrinolytic activity (Yatagai *et al*., 2008).

Unlike common fibrinolytic proteases, such as t-PA and uPA, which can produce various side effects such as bleeding, NK exhibits little to no side effects. Studies also indicate that an oral administration of NK can be absorbed by the intestinal tract (Fujita *et al*., 2011).

Human trials have demonstrated that NK provides support to the circulatory system by thinning the blood and dissolving blood clots (Jensen *et al*., 2016).

The effects of NK are similar to the well-known blood thinner, aspirin (Jang *et al*., 2013). Studies also indicate that NK can ameliorate other diseases such as hypertension (Fujita *et al*., 2011), stroke (Chang, 2008), Alzheimer's disease (Fadl *et al*., 2013) and atherosclerosis (Dogné *et al*., 2006).

5.1.3.3 Serratiopeptidase

Serratiopeptidase or serrapeptase is an enzyme isolated from the non-pathogenic enterobacteria Serratia E15 found in silkworms. It is widely used to combat various kinds of inflammation and inflammatory disorders (Jadav *et al*., 2010). It also plays a vital role in the management of atherosclerosis as it possesses fibrinolytic and caseinolytic properties (Bhagat *et al*., 2013).

Serratiopeptidase was reported to have a direct effect on the movement of immune cells and recruitment of other lymphocytes at the site of inflammation (Chappi, Suresh & Patil, 2015). Serratiopeptidase reduces capillary permeability induced by histamine,

bradykinin, and serotonin; breaks down abnormal exudates and proteins; facilitates the absorption of decomposed products through blood and lymphatics (Nakamuera *et al*., 2003). The clinical use of serratiopeptidase during allergic conditions was shown to reduce the viscosity of mucous and improve the elimination of it through bronchopulmonary secretions (Klein & Kullich, 2000).

5.1.3.4 Oats

Oats (Avena sativa L.) are a commonly consumed wholegrain cereal and contains many kinds of phytochemicals that possess antioxidant properties, such as tocotrienols, phenolic acids, flavonoids, sterols, and phytic acid (Peterson, 2001). Oats avenanthramides (OA), which are unique to oats, are regarded as key phytoalexins in the defence mechanisms of oats against certain pathogens (Miyagawa *et al*. 1995). Recent research shows that OA exhibits excellent antioxidant activities both *in vitro* (Bratt *et al*., 2003) and *in vivo* (Ji *et al*., 2003).

5.1.4 Epigenetic Control in disease

5.1.4.1 Epigenetics in obesity

The information from exposure to environmental influences during embryogenesis and early development is thought to be stored in our epigenome as an archive, and may affect health and disease states in adulthood (Bramswig & Kaestner, 2012). Environmental influences such as food intake contribute towards obesity, which also plays a major role in several pathogenesis of diseases including diabetes.

5.1.4.2 Epigenetics in cardiovascular disease

DNA methylation patterns can result in permanent phenotype changes in blood pressure. These alterations of gene expression are reversible through dietary compounds to reduce risk without any harmful effects. Curcumin, a type of polyphenols found in the spice turmeric, has been shown to inhibit HAT activity. In a study using an animal model, consumption of 50mg/kg/day of curcumin inhibited p300HAT activity, which leads to ventricular hypertrophy prevention and preserved systolic function (Ordovas & Smith, 2010).

5.2 Epigenetics of the Mediterranean diet

Epidemiological and clinical studies have established the health benefits of the Mediterranean diet, which is associated with a lower incidence of atherosclerosis, cardiovascular diseases, some types of cancer, and overall mortality (Corella & Ordovas, 2014).

The Mediterranean diet is largely vegetarian; olives and olive oil derived from the olive tree are important components. Olive oil consumption has been associated with a decreased risk of cardiovascular disease and certain cancers (Toledo *et al*., 2015).

It is now well-established that, in olives and extra virgin olive oil (EVOO), not only the monounsaturated fatty acid constituents but also the minor phenolic components and other phytochemicals have important health benefits (Lou-Bonafonte, 2012). *In vivo* and *in vitro* studies have shown that EVOO components have positive effects on metabolic parameters, such as plasma lipoproteins, oxidative damage, inflammatory markers, pro-thrombotic markers, platelet function, and antimicrobial activity (Konstantinidou *et al*., 2013). The

Mediterranean Diet was able to reduce the levels of established biomarkers of inflammation and resulted in a trend toward normalising the microbiota in Crohn's disease patients (Marlow *et al*., 2013).

The replacement of saturated fatty acids (SFA) with monounsaturated fatty acids (MUFA) may improve health, by reducing metabolic stress and oxidative phosphorylation activity in peripheral blood mononuclear cells (PBMC). The MUFA diet also changed the expression of genes involved in B-cell receptor signalling and endocytosis signalling (Dijk *et al*., 2012). The Mediterranean diet may have additional antiatherogenic effects by lowering proinflammatory plasma proteins (Lou-Bonafonte *et al*., 2015).

A Mediterranean diet high in olive oil may reduce the risk of non-alcoholic fatty liver disease progression to non-alcoholic steatohepatitis (Moral *et al*., 2015). A diet with high amounts of olive and fish oils together with butter induced 72 genes and inhibited 180 genes related to lipolysis or lipogenesis (Eletto *et al*., 2005). The Mediterranean diet with virgin olive oil elicited changes in 241 genes. The most prevalent differentially expressed pathways were related to atherosclerosis and hypertension (*i.e*., ADRB2, IL7R, IFN-gamma, MCP1, TNFalpha) and were often associated with an improvement in systemic markers for oxidation and inflammation (Castaner *et al*., 2013).

5.2.1 Influence of monounsaturated fatty acids

The inclusion of refined olive oil leads to a more anti-inflammatory gene expression profile (Van Dijk *et al*., 2009). Thus, the replacement of dietary saturated fat with a

monounsaturated fatty acid diet could prevent adipose tissue inflammation and may reduce the risk of inflammation-related diseases (Van Dijk *et al*., 2009)

Fish oil consumption decreased gene expression in pathways related to mitochondrial physiology and insulin synthesis/secretion, slowed tumour growth and increased survival rates in mice with tumours (Lloyd *et al*., 2013). This suggests the type of dietary fat consumed may be important for controlling tumour progression. The fatty acid composition of diets modified gene expression patterns of adipocytes, PBMC, coronary artery smooth muscle, and human prostate cancer cells. The inclusion of MUFA from olive oil led to a lower expression of inflammatory genes and, consequently, to a less atherogenic profile, and tumour progression was controlled when compared to other sources of fat.

Supplementation with either olive oil, eicosapentaenoic acid (EPA), or docosahexaenoic acid (DHA) on gene expression significantly affected gene expression in the following pathways: (1) interferon signalling, (2) receptor recognition of bacteria and viruses, (3) G protein signalling, glycolysis, and glycolytic shunting, (4) S-adenosyl-l-methionine biosynthesis, and cyclic adenosine monophosphate (cAMP)-mediated signaling including cAMP responsive element protein 1 (CREB1), as well as many other individual genes, such as hypoxia inducible factor 1 alpha subunit(HIF1A) (Tsunoda *et al*., 2015). EPA supplementation was associated with significant effects on gene expression involving the interferon pathway, as well as downregulation of CREB1 and HIF1A, which may relate to its beneficial effect on cardiovascular risk reduction.

5.2.2 Influence of Olive Oil enriched in phenolic compounds

Olive oil phenolic compounds differentially expressed genes that seemed to be involved in inflammatory processes mediated by transcription factor NF-kappaB, activator protein-1

transcription factor complex AP-1, cytokines, mitogen-activated protein kinases (MAPK), or arachidonic acid pathways which were repressed by phenolic compounds, thereby switching the activity of PBMC to a less deleterious inflammatory profile (Camargo *et al*., 2010).

Phenolic compounds modulate levels of mRNA and miRNA which play a role in metabolism, inflammation, and cancer. These results indicate that changes in gene expression are part of the postprandial protective action of olive oil consumption and phenolic compounds exert an influence (D'Amore *et al*., 2016). These studies indicate that the consumption of virgin olive oil with phenolic compounds, either in a postprandial regimen or over a relatively short period, influences the expression of genes related to atherosclerosis development and progression, and that this effect is observed at the moderate doses consumed in this dietary pattern.

5.2.2.1. Influence of EVOO isolated phenolic compound extracts

In bone-marrow mesenchymal stem cells, oleuropein was found to upregulate the expression of 60% of adipogenesis-repressed genes (Casado-Diaz *et al*., 2017).

Hydroxytyrosol, the main phenolic compound in minor constituents of olive oil, has been shown to be a potent antioxidant and has anti-atherogenic and anti-cancer properties which result in the upregulation of numerous antioxidant proteins and enzymes, including heme oxygenase-1, glutaredoxin, and glutathione peroxidase. This may account for the reduction in oxidative stress and suggests a mechanism for the chemoprevention of cancer by hydroxytyrosol. Alteration in the expression of transcription factors such as STAT3, STAT6, SMAD7, and ETS-1 may also contribute to anti-cancer and anti-inflammatory effects. Furthermore, the downregulation of the telomerase reverse transcriptase subunit, observed in the erythroleukemic cell line K562 treated with this compound (Rafehi *et al*., 2012).

Together with changes in the complement system, the Warburg effect, and chromatin remodeling, are other candidate genes involved in the prevention of cancer by hydroxytyrosol (Nan *et al*., 2014). These studies provide evidence that tyrosol and hydroxytyrosol can control growth, oxidative stress, and differentiation in different experimental models.

5.3 Summary

The link between epigenetics and nutrition is an evolving area which has not been extensively researched or well understood. However, the epidemiological evidence of the importance of diet, nutrition and health is well accepted. Nutritionally complex diets such as the Mediterranean diet confers population-level health benefits and provided the inspiration and focus for the pathway analysis in this study.

Chapter 6

Genomic pathway analysis of functional foods and their interaction with immune response and disease pathways.

6.1 Introduction and Aims of the study

The purpose of this study was to assess the epigenetic potential of key nutitional elements of the Mediteranean diet; Resveratol, Flavanoids and Omega 3 fatty acids (EPA & DHA). An assessment of their impact on different regulatory mechanisms including development, immune response, metabolic processing and disease using a pathway analysis approach.

Aims:

- Identify genes associated with biological and disease processes identified from the systematic review to be important dietary responses to nutrition.
- Perform pathway analysis of candidate genes for diet-related diseases to determine major gene ontology processes associated with these conditions.
- Address biological pathways associated with gene expression changes linked to functional foods identified from the literature as being beneficial for cognitive health, immune function and preventing CVD to identify potential common regulatory mechanisms important for nutritional health.
- Compare gene sets associated with functional foods to those identified from genome-wide association studies (GWAS) for common diseases and biological functions to identify potential overlap which may have therapeutic implications.

6.2 Pathway analysis of genes implicated in diet-associated chronic diseases identifies immune response and developmental pathways

CVD, diabetes, body composition and weight were identified as major phenotypic outcomes associated with dietary composition from our systematic review of the literature. To address biological processes associated with these phenotypic outcomes pathway analysis was

performed using candidate genes for each of these conditions. Gene lists for CVD, obesity and diabesity were downloaded from Bar Harbor BioTechnology expression array systems. CVD genes were overlaid with those identified from association studies and/or animal studies to be involved in cardiovascular pathologies, accessed from:

http://tcm.zju.edu.cn/chd/download.

Pathway analysis of biological processes relating to the gene sets was performed using the publicly available PANTHER Classification System software. Gene lists were parsed into the *Gene List Analysis* tool and analysed using the *Functional Classification* method and *Statistical overrepresentation* test (Mi *et al*., 2013).

Candidate genes for CVD were enriched for developmental and immune response pathways relating to angiogenesis (the formation of new blood vessels from existing ones) and inflammation mediated by chemokine and cytokine signalling, respectively (Figure 6.1). Like CVD, analysis of pathways associated with genes implicated in diabesity also identified developmental and immune response pathways as well as G-protein signalling to be important (Figure 6.2). Enrichment analysis of gene sets for CVD and diabesity was therefore performed to identify overlapping pathways for these diet-related conditions. This identified signalling along the insulin/IGF-1 (insulin-like growth factor)-protein kinase B (PKB) Cascade as a significant pathway (Figure 6.3).

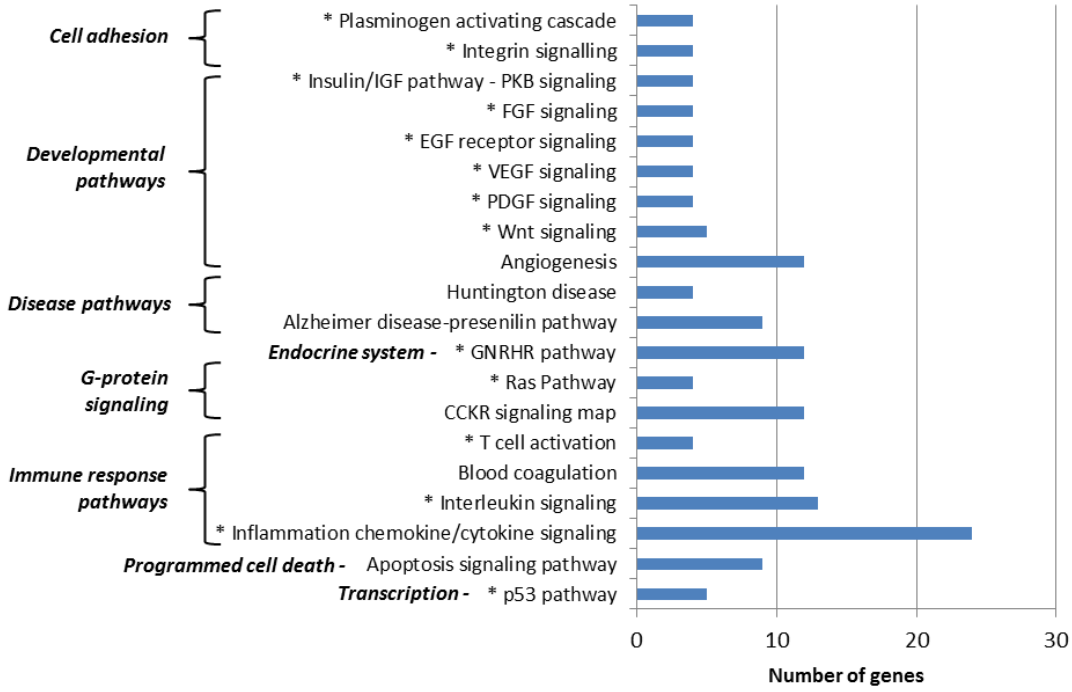


Figure 6.1 Gene ontology pathways associated with candidate genes for cardiovascular disease (CVD). Genes were analysed using the *functional classification* tool through the PANTHER pathway analysis software. A total of 53 pathways were identified using this method. Pathways with 4 or more target genes from the uploaded gene list are displayed. The gene list was enriched for factors implicated in immune response and developmental pathways. * Factors and signalling pathways were shown to be involved in the induction or modulation of angiogenesis. Abbreviations: *CCKR, cholecystokinin receptor; EGF, Epidermal growth factor; FGF, fibroblast growth factor; GNRHR, Gonadotropin-releasing hormone receptor; IGF, insulin-like growth factor; PDGF, Platelet-derived growth factor; PKB, protein kinase B; VEGF, Vascular endothelial growth factor. phosphatidylinositol-3; PKB, protein kinase B; TRHR, Thyrotropin-releasing hormone receptor; VEGF,*

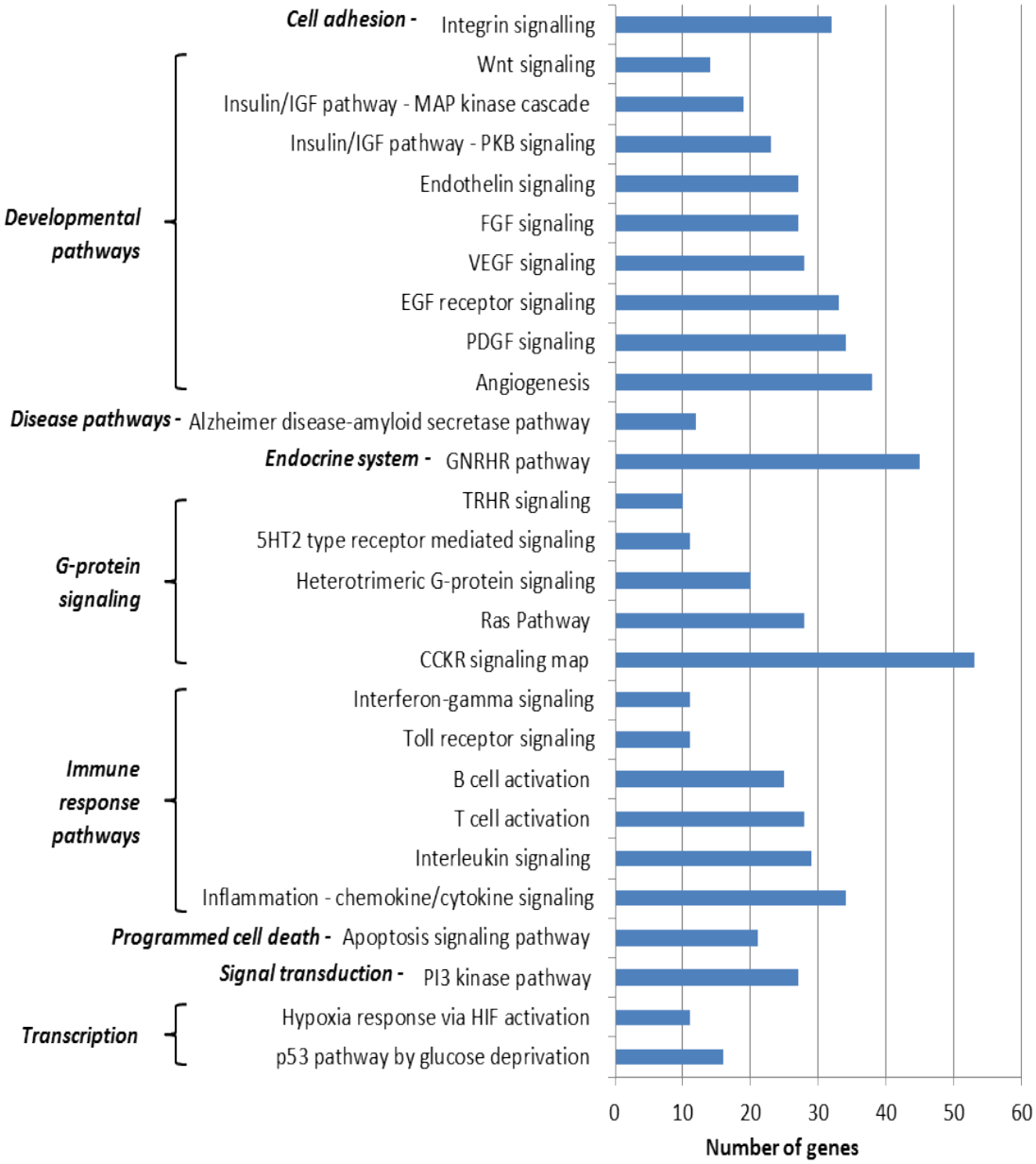


Figure 6.2 Gene ontology pathways associated with obesity and diabesity candidate genes. Genes were analysed using the *functional classification* tool through the PANTHER pathway analysis software. A total of 85 pathways were identified using this method. Pathways with 10 or more target genes from the uploaded gene list are displayed. The gene list was enriched for factors implicated in immune response, developmental and G-protein signalling pathways. Abbreviations: *CCKR, cholecystokinin receptor; EGF, Epidermal growth factor; FGF, fibroblast growth factor; GNRHR, Gonadotropin-releasing hormone receptor; HIF, Hypoxia-inducible Factor; IGF, insulin-like growth factor; MAP, mitogen-activated protein kinase; PDGF, Platelet-derived growth factor*; *PI3, Vascular endothelial growth factor; 5HT2, 5-hydroxytryptamine receptor 2*. *Figures are p-values*.

The peptide hormone, insulin, and growth factor, IGF-1, play important roles in several biological processes including regulation of metabolism and cell growth, survival and maintenance through activation of common signalling pathways within the cell (Taniguchi *et al*., 2006), see Figure 6.4. Certain functional foods, such as those rich in polyphenols (found in large quantities in fruit, vegetables, tea extracts, red wine, coffee and chocolate) have been shown to act upon the insulin/IGF-1 pathway and may protect against chronic diseases including CVD and cancer (Stoclet *et al*., 2004, Pandey and Rizvi, 2009, Manach *et al*., 2005). Extracts of strawberry, wild blueberry and cranberry have been shown to activate important downstream signalling cascades within this regulatory pathway (Figure 6.4), influencing both cell migration and angiogenesis (Tulio *et al*., 2012); important biological processes in age-related and disease-related vascular repair and wound healing (Kolluru *et al*., 2012). Polyphenols from black tea extracts have been shown to mimic the effects of insulin/IGF-1 signalling within the cell by activating the longevity-associated gene FKHR/FOXO1a (Cameron *et al*., 2008). Whereas soy and tomato products have been reported to counteract the tumour-promoting activity of IGF-1 in prostate cancer cells, inhibiting cell growth and proliferation and inducing apoptosis (Wang *et al*., 2003), Figure 6.4. Collectively these findings suggest that polyphenol-rich foods which target insulin-signalling within the cell may have therapeutic potential in the treatment of chronic diseases, supporting their role as 'functional foods'.

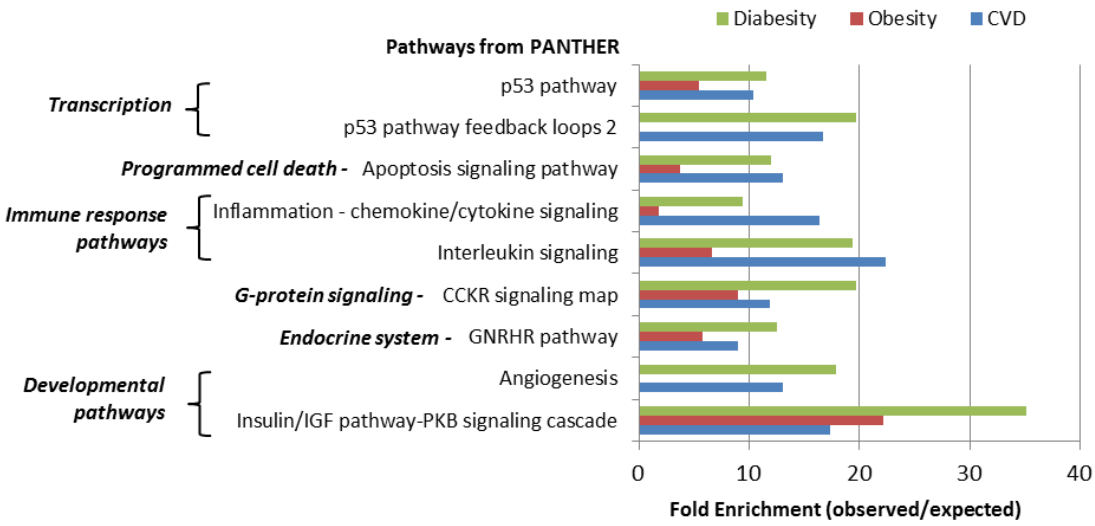


Figure 6.3 Enrichment analysis of statistically significant pathways associated with cardiovascular disease (CVD), obesity and diabesity. Statistical overrepresentation testing was performed using the binomial statistics tool available through PANTHER to compare classifications of gene lists for CVD, obesity and diabesity. Uploaded gene lists were compared to a reference list containing all human genes within the PANTHER database to statistically determine over- or under-representation of PANTHER classification categories using the binomial distribution test (Cho and Campbell, 2000). The analysis was performed using *the PANTHER GO-slim Pathway* annotation dataset, and Bonferroni correction was applied to correct for multiple testing. The gene list was enriched for factors implicated in developmental pathways involving insulin/IGF signalling, outlined in Figure 6.4.

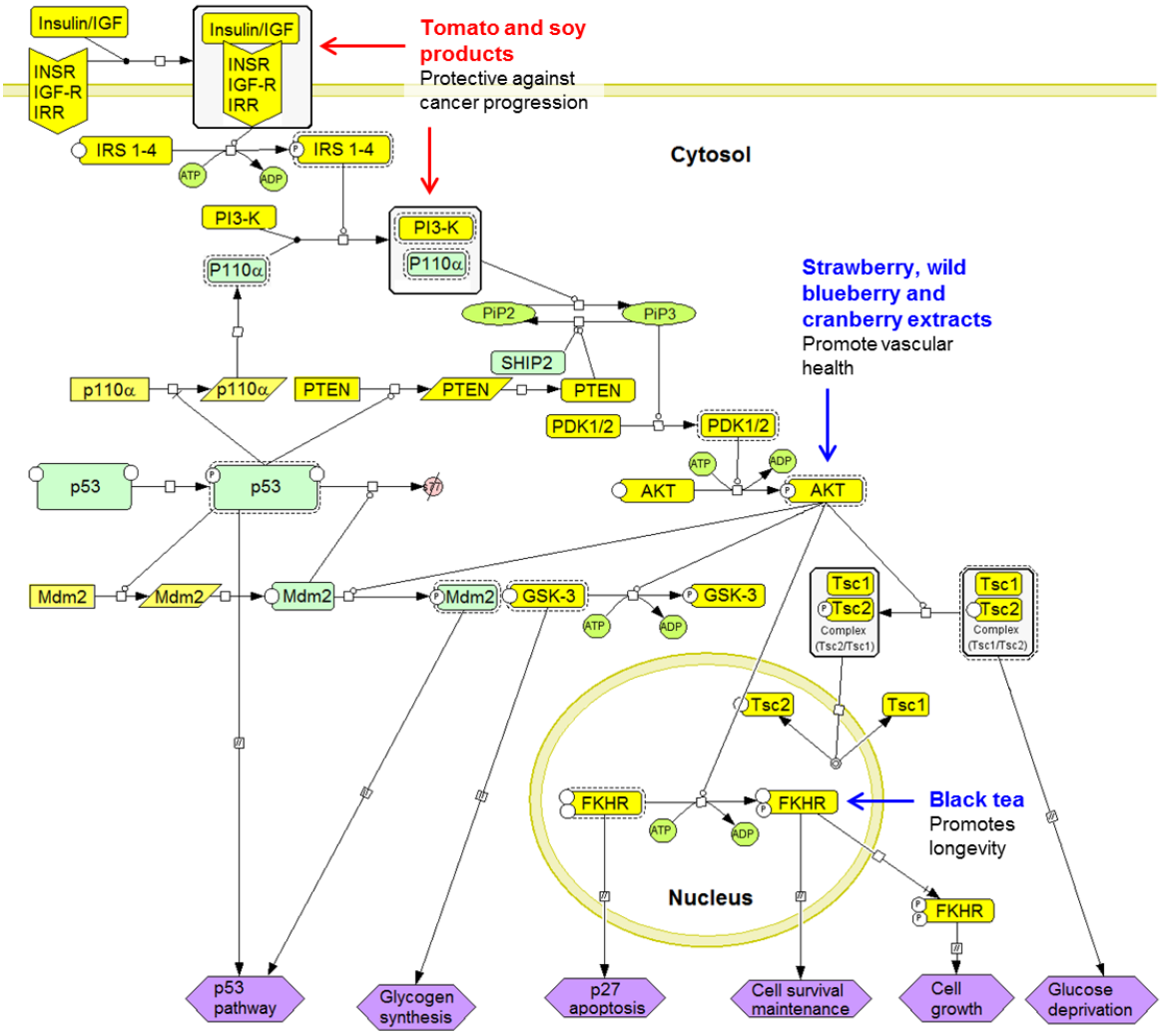


Figure 6.4 Insulin/IGF pathway-protein kinase B (PKB/AKT) signalling cascade pathway map for genes associated with cardiovascular disease, diabesity and obesity. The insulin receptor (INSR), insulin-like growth factor receptor (IGF-R) and insulin-receptor-related receptor (IRR) are a family of cell surface proteins which bind insulin and insulin-like growth factor (IGF). They are involved in regulating tissue homeostasis, metabolism and cellular processes including cell growth, proliferation and survival. All of these receptors are structurally similar and utilise common downstream signalling cascades, including the phosphatidylinositol-3-kinase (PI3-K)/PKB (Akt) pathway illustrated in the above schematic. Dysregulation of this pathway has been implicated in several pathologies including cancer, neurodegenerative diseases and metabolic disorders. Dietary compounds can regulate different components of the insulin/IGF pathway to promote health. Foods listed in red and blue respectively mark downregulation and upregulation of pathway components. Uploaded genes involved in insulin/IGF signalling are highlighted in bright yellow and include: AKT/PKB, FKHR/FOXO1a, GSK3B, IGF1R, INSR, IRS1-2-4, PDPK1, PIK3, PTEN, TSC1/2. Pathway map modified from PANTHER (http://pantherdb.org/).

6.3 Enrichment analysis of genes implicated in neurogenesis identified overlapping pathways with CVD and diabetes.

Diet has also been associated with cognitive function and neurogenesis (the generation of new neurons), an important process involved in learning and memory (Deng *et al*., 2010, Cameron & Glover, 2015). Calorie restriction and certain functional foods such as omega-3, flavonoids and resveratrol have been shown to promote neurogenesis (Gomez-Pinilla, 2008, Mattson, 2005, Mattson, 2008, Greenwood & Parasuraman, 2010, Stangl and Thuret, 2009, Beltz *et al*., 2007, Crupi *et al*., 2013). The antioxidant properties of calorie restriction and these bioactive compounds are believed to support neurogenesis by protecting new neurons from cellular damage which is associated with normal ageing and disease pathways (Prior, 2003).

Comparative enrichment analysis was performed using candidate genes for neurogenesis against those implicated in CVD and diabesity to identify common biological pathways which may be modulated in response to diet. Angiogenesis, important in cardiovascular health and disease, and hormonal signalling along the cholecystokinin receptor (CCKR) and gonadotropin-releasing hormone receptor (GnRHR) pathways were identified as significant pathways ($p<0.05$) implicated in CVD, diabesity and neurogenesis, Figure 6.5. The CCKR and GnRHR signalling pathways are associated with eating patterns and reproductive health and disease, respectively (Gibbs *et al*., 1973, Kissileff *et al*., 1981, Smith *et al*., 1981, Skorupskaite *et al*., 2014). Disruption of these hormonal pathways has been implicated in a number of common pathological conditions including obesity, cancer and neurodegenerative diseases (de Krom *et al*., 2007, DiVall *et al*., 2015, Cheung & Wong, 2008, Nuruddin *et al*., 2014, Mazurek & Beal, 1991, Hays & Paul, 1982, Geibel *et al*., 2014, Baldwin & Shulkes, 2007). Alzheimer's and Huntington's disease were identified as

common disease pathways enriched for genes associated with neurogenesis and CVD, whereas developmental EGF-receptor signalling was identified as a common pathway amongst genes implicated in neurogenesis and diabesity (Figure 6.5). Axon guidance mediated by Slit/Robo was identified as the most significant pathway enriched for genes involved in neurogenesis along with Notch signalling and TGF-signalling, Figure 6.5.

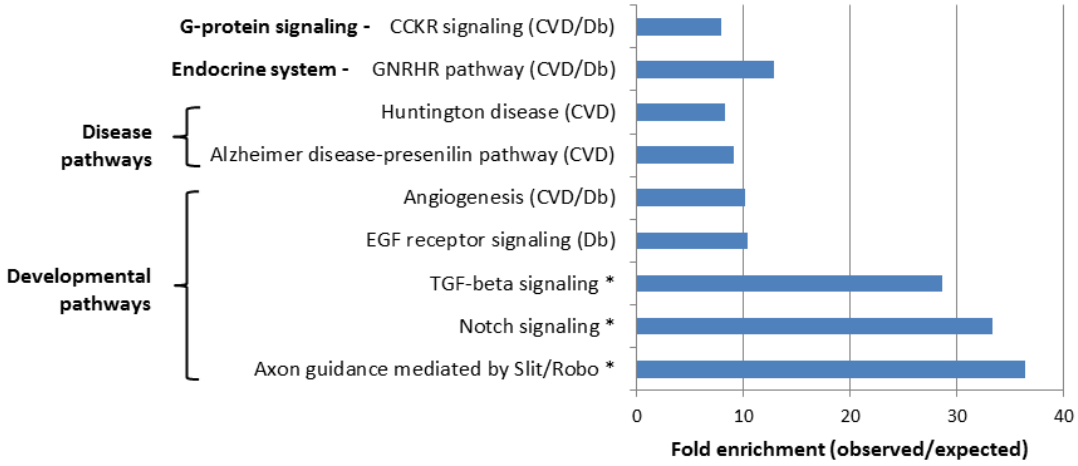


Figure 6.5 Enrichment analysis of statistically significant pathways associated with neurogenesis. Statistical overrepresentation testing was performed using the binomial statistics tool available through PANTHER to identify pathway classifications for genes associated with neurogenesis. Uploaded genes were compared to a reference list containing all human genes within the PANTHER database to statistically determine over- or under-representation of PANTHER classification categories using the binomial distribution test (Cho and Campbell, 2000). The analysis was performed using *the PANTHER Pathway* annotation dataset, and Bonferroni correction was applied to correct for multiple testing. The gene list was enriched for factors implicated in developmental pathways. CVD, Db and CVD/Db indicate that the corresponding pathways are also statistically enriched for genes implicated in cardiovascular disease, diabesity or both. * Fold enrichment only reached statistical significance for the neurogenesis gene set. Abbreviations: *CCKR, cholecystokinin receptor; CVD, cardiovascular disease; Db, diabesity; EGF, Epidermal growth factor; GNRHR, Gonadotropin-releasing hormone receptor; TGF, transforming growth factor*.

6.4 Determining regulatory pathways affected by the functional foods omega-3, flavonoids and resveratrol

Pathway analysis of genes associated with CVD, diabesity and neurogenesis, highlighted from the systematic review of the literature to be the major phenotypic outcomes of diet, identified processes relevant to development, immune response pathways and neurodegeneration to be important. Functional food components such as omega-3, flavonoids and resveratrol are well publicised for their beneficial effects on health and wellbeing. To explore the potential effects of these functional food components on modifying biological systems, human microarray data relating to gene expression changes following dietary intervention with these bioactive compounds were downloaded from the publicly available *ArrayExpress* database. Studies included in the pathway analysis are detailed in Table 6.1. Datasets for each study were analysed using the GEO2R function; an interactive web tool that allows users to compare two or more groups of samples that are publicly available in the Gene Expression Omnibus (GEO) repository of microarray data (Davis & Meltzer, 2007). It provides a simple interface that enables 'R' statistical analyses to be performed without command line expertise. This allowed for analysis of differential gene expression changes within the same individuals at baseline (control group) and following treatment intervention (treatment group).

The total number of significant gene expression changes across different studies for each of the treatment conditions addressed were 3,638 for omega-3 (mean: 1,213; range: 542 - 1,827), 2,960 for flavonoids (mean: 1,480; range: 915 – 2,045) and 3,704 for resveratrol (mean: 925; range: 555 – 1,424). Statistical significance of the overlap between the gene lists was determined using R.

Table 6.1

Treatment	ArrayExpress ID	Study description	Study cohort	Supplementation description	Array ID	Citation
Omega-3	E-GEOD-48368	Gene expression profile of PBMCs after intake of fish oil for seven weeks	n=17 healthy subjects	Fish oil capsules providing a daily intake of 1.6 g EPA/DHA (0.7 g EPA; 0.9 g DHA)	Illumina HumanHT-12 V4.0 expression beadchip array	-
	E-GEOD-12375	Gene expression profile of PBMCs after intake of fish oil for six months	n=23 healthy elderly subjects (≥ 65 years)	Fish oil capsules providing a daily intake of 1.8g EPA/DHA (1,093 ± 17 mg EPA; 847 ± 23 mg DHA)	NuGO human Hs1a520180 array	(Bouwens *et al*., 2009)
	E-GEOD-20114	Gene expression profile of PBMCs after DHA supplementation for 90 days	n=4 hypertriglyceridemic but otherwise healthy men	DHA oil capsules providing a daily intake of 3 g DHA and 0 g EPA	Affymetrix GeneChip Human Genome U133 Plus 2.0 array	(Dawson *et al*., 2012)
Flavonoids	E-GEOD-13899	Gene expression profile of monocytes after quercetin supplementation for 2 weeks	n=4 healthy subjects	Capsules providing a daily intake of 150 mg quercetin (naturally occurring polyphenol of the flavonoid subclass *flavonols*)	Affymetrix GeneChip Human Genome U133 Plus 2.0 array	(Boomgaarden *et al*., 2010)
	E-GEOD-34145	Gene expression profile of PBMCs following dietary intake of anthocyanins for 8 weeks	n=3 overweight female subjects	Daily consumption of bilberry puree and dried bilberries equivalent of 400 g fresh bilberries (contain the highest levels of polyphenols, particular anthocyanins of the flavonoid subclass *flavanols*, amongst the commonly consumed berries). Other sources of carbohydrates in the subjects' diet were substituted with the provided bilberry products.	Illumina Human-6 v2 Expression BeadChip	(Kolehmainen *et al*., 2012)
Resveratrol	E-GEOD-42432	Gene expression profile of adipose tissue following 30 days resveratrol supplementation	n=9 obese but otherwise healthy male subjects	Double-blind randomised crossover study; daily intake of placebo and 150 mg/day resveratrol [99% resVida] with a 4-week washout period in between	Affymetrix Human Gene 1.1 ST Array [HuGene-1_1-st]	(Konings *et al*., 2014)

Table 6.1

Treatment	ArrayExpress ID	Study description	Study cohort	Supplementation description	Array ID	Citation
Resveratrol	E-GEOD-41168	Gene expression profile of adipose tissue following 12 weeks resveratrol supplementation	n=13 healthy, postmenopausal female subjects	Daily supplementation with 75 mg/day resveratrol	Affymetrix GeneChip Human Genome U133 Plus 2.0 Array	-
	E-GEOD-41168	Gene expression profile of skeletal muscle (vastus lateralis) following 12 weeks resveratrol supplementation	n=9 healthy, postmenopausal female subjects	Daily supplementation with 75 mg/day resveratrol	Affymetrix GeneChip Human Genome U133 Plus 2.0 Array	-
	E-GEOD-32357	Gene expression profile of skeletal muscle (vastus lateralis) following 30 days of resveratrol supplementation	n=10 obese but otherwise healthy male subjects	Double-blind randomised crossover study; daily intake of placebo and 150 mg/day resveratrol [99% resVida] with a 4-week washout period in between	Affymetrix Human Gene 1.1 ST Array [HuGene-1_1-st]	(Timmers *et al*., 2011)

Table 6.1 Microarray study descriptions included in the pathway analysis

Note: Gene expression changes were analysed in the same individuals using baseline measurements verses treatment interventionunless stated otherwise. Abbreviations: *DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; PBMCs, peripheral blood mononuclear cells*.hypergeometric distribution test *phyper*. Significant overlap was observed between the gene sets for omega-3 and flavonoids ($p = 4.67 \times 10^{-25}$) and flavonoids and resveratrol ($p = 0.015$) but not for omega-3 and resveratrol ($p = 0.624$), Figure 6.6. A total of 141 genes were common across the three treatment groups. Analysis of this gene list using the overrepresentation function in PANTHER identified flavin biosynthesis ($p = 0.02$), p53 pathway by glucose deprivation ($p = 0.01$), Notch signaling ($p= 0.03$), interleukin signaling ($p= 0.03$), apoptosis signaling ($p = 0.04$), angiogenesis ($p = 0.02$) and Alzheimer's disease (presenilin pathway, $p= 0.05$) as the most enriched pathways, Figure 6.6. To establish a more manageable number of genes for pathway analysis, the datasets were filtered using the following criteria: 1) Select genes that are common across two or more studies for the same treatment condition and/or 2) Select genes that have a fold change of more than 1.6 between the control and treatment groups. This resulted in 505 genes for omega-3, 571 genes for flavonoids and 307 genes for resveratrol. To define potential regulatory mechanisms which may be operating in response to omega-3, flavonoids and resveratrol, we parsed the gene expression data into IPA, an integrated data-mining platform for biological pathway analysis. Enrichment analysis was performed using the Core Analysis function which provides an overview of biological relationships, mechanisms, functions and pathways relating to the uploaded dataset based on log fold changes in gene expression. The gene lists were also analysed using PANTHER. The top canonical pathways associated with the dietary interventions tested are listed in Figure 6.7. Intervention with omega-3 enriched for genes implicated in T cell signalling and hypoxia; flavonoids activated B cell signalling and resveratrol.

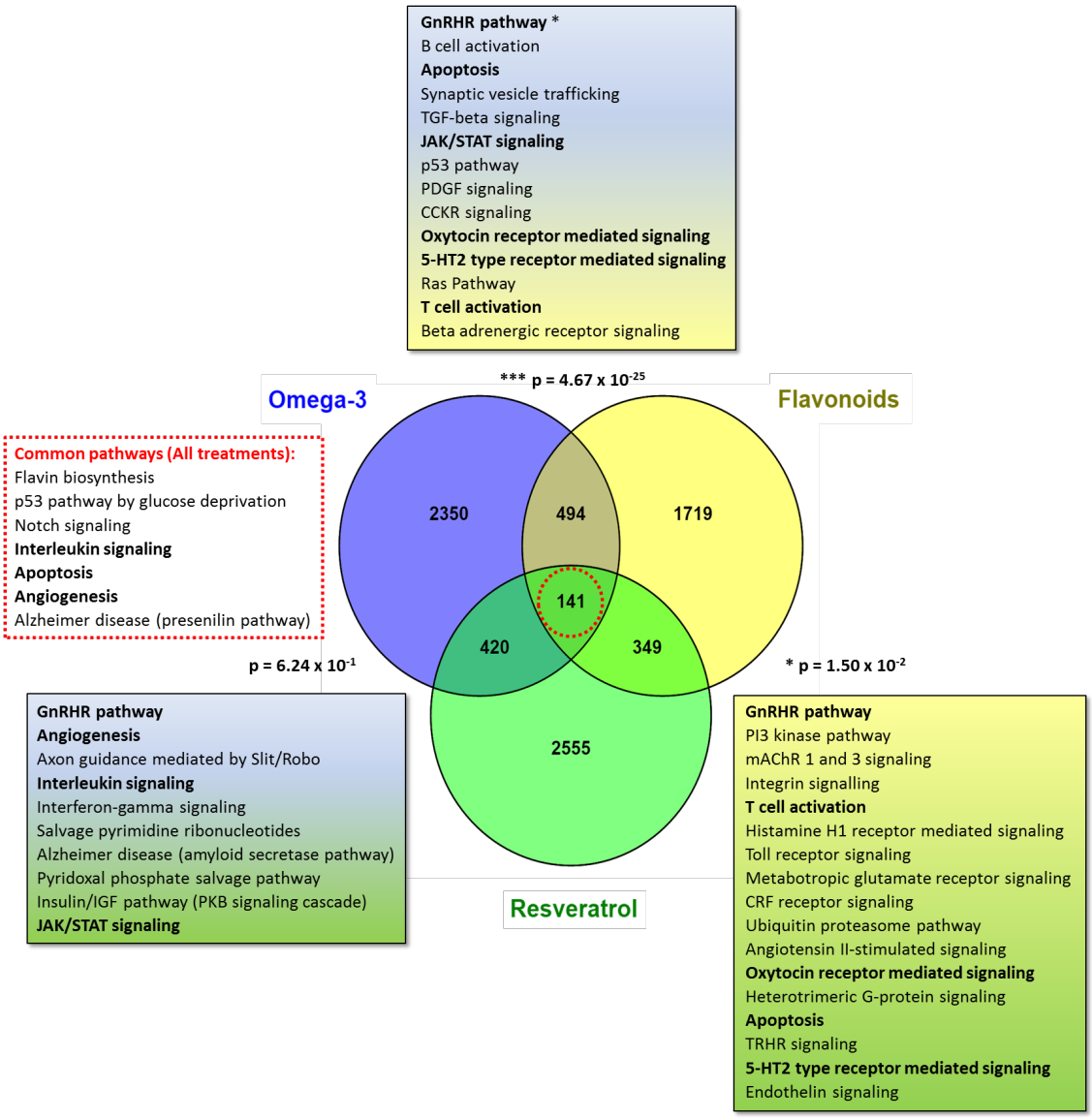


Figure 6.6 Enrichment analysis of overlapping gene sets associated with dietary interventions with omega-3, flavonoids and resveratrol. Gene expression data were downloaded from ArrayExpress (https://www.ebi.ac.uk/arrayexpress/). Studies included in the analysis are detailed in Table 6.1. Significant gene expression changes are represented as a Venn diagram showing treatment-specific and overlapping profiles across the three dietary interventions. Overlapping gene sets (omega-3 and flavonoids; omega-3 and resveratrol; flavonoids and resveratrol; all treatments) were subjected to statistical overrepresentation testing using PANTHER pathway analysis software. Over-represented pathways were determined using the binomial distribution test (Cho & Campbell, 2000). Bold font indicates common pathways across the different gene sets. See Figure 6.7 for pathways associated with the separate treatment conditions. *Withstood Bonferroni correction for multiple testing. Abbreviations: *CCKR, cholecystokinin receptor; CRF, Corticotropin releasing factor; JAK/STAT, Janus kinase/Signal transducer and activator of transcription; mAChR, Muscarinic acetylcholine receptor; PDGF, Platelet-derived growth factor; PKB, protein kinase B; TGF, Transforming growth factor; TRHR, Thyrotropin-releasing hormone receptor; 5-HT2, 5-hydroxytryptamine/serotonin*.

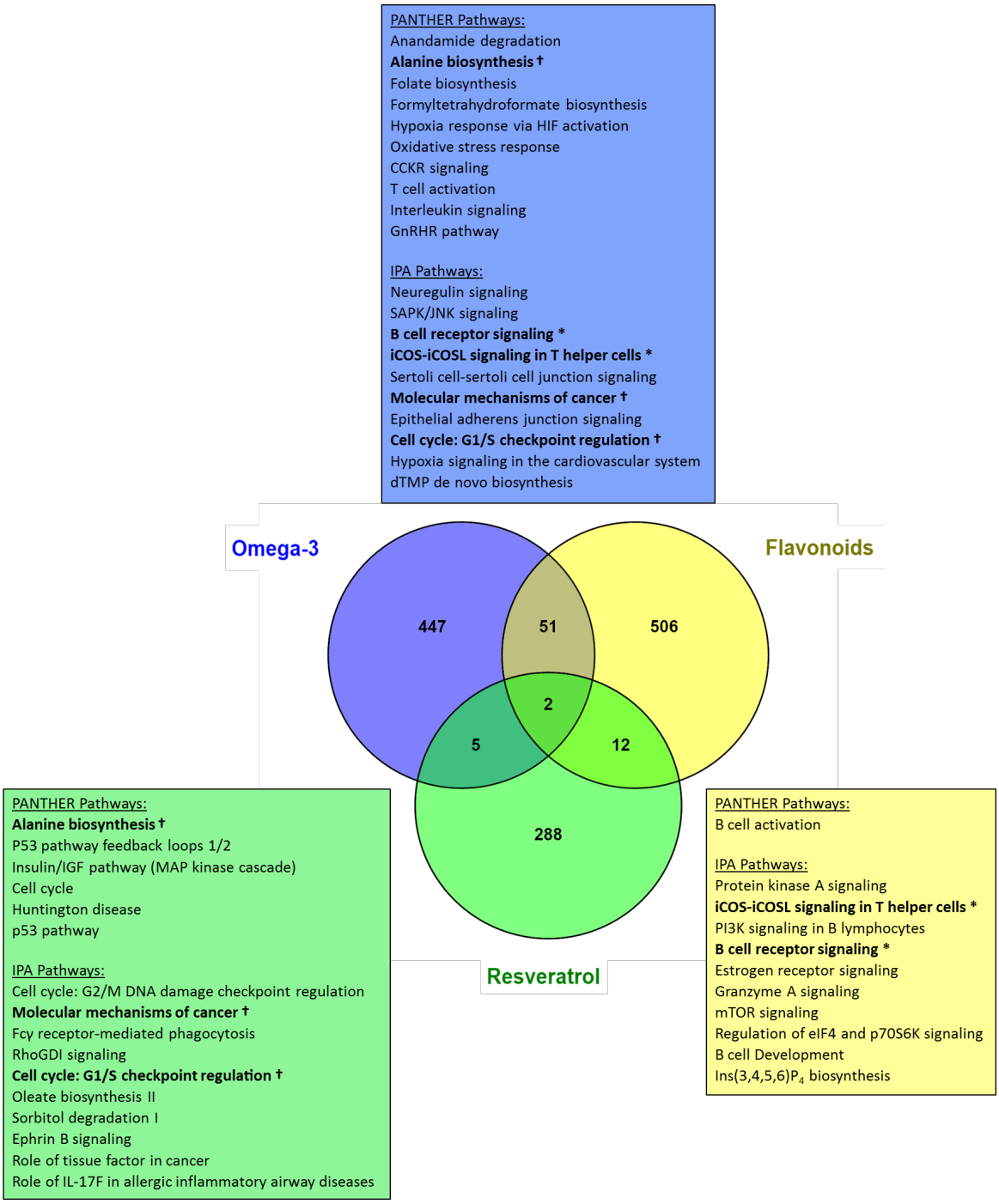


Figure 6.7 Top canonical pathways enriched for genes significantly regulated in response to dietary intervention with omega-3, flavonoids and resveratrol. Gene expression data were downloaded from ArrayExpress (https://www.ebi.ac.uk/arrayexpress/). Studies included in the analysis are detailed in Table 2. Gene sets were filtered to include significant gene expression changes observed in 2 or more studies for the same treatment condition and with a fold change of >1.6. These are represented as a Venn diagram showing treatment-specific and overlapping gene expression profiles. Each gene set was subjected to statistical overrepresentation testing using IPA and PANTHER pathway analysis platforms. Bold font indicates common pathways between different intervention groups; * omega-3 and flavonoids, † omega-3 and resveratrol. Pathways associated with overlapping gene sets (for all

significant gene expression changes) are represented in Figure 6.6. Abbreviations: *CCKR, cholecystokinin receptor; dTMP, deoxythymidine monophosphate; eIF4, Eukaryotic Initiation Factor-4; GnRHR, Gonadotropin-releasing hormone receptor; HIF, Hypoxia-inducible Factor; iCOS, Inducible costimulatory; iCOSL, iCOS ligand; IGF, insulin-like growth factor; Ins(3,4,5,6)P_4, D-myo-inositol (3,4,5,6)-tetrakisphosphate; MAP, mitogen-activated protein kinase; mTOR, mechanistic target of rapamycin; PI3K, phosphatidylinositol-3 kinase; RhoGDI, Rho GDP-dissociation inhibitor; SAPK/JNK, Stress-activated protein kinases/Jun amino-terminal kinases*.

Pathway analysis using IPA indicated overlap between the different treatment groups. These were B cell receptor signalling and iCOS-iCOSL signalling in T helper cells between the omega-3 and flavonoids treatment groups (Figure 6.8), and molecular mechanisms of cancer and G1/S checkpoint regulation between omega-3 and resveratrol (Figure 6.9). Alanine biosynthesis was also identified as a common pathway associated with omega-3 and resveratrol treatment based on statistical overrepresentation testing using the Gene List Analysis function in PANTHER.

Disease mechanisms implicated in immunological diseases and cancer, conditions which have been identified from the literature to be targeted by certain functional foods (Simopoulos, 2002, Dragan *et al*., 2007, Shukla & Singh, 2011, Petro, 2011, Ramos-Romero *et al*., 2012, Batra & Sharma, 2013, Laviano *et al*., 2013, Tanaka, 2014, Fabian *et al*., 2015), were overlaid onto the common pathways identified from IPA in order to identify potential therapeutic targets for the treatment of these pathological conditions (Figures 6.8 and 6.9). Comparative analysis was also performed using IPA to identify other common pathways associated with all three of the treatment conditions. The top 20 canonical pathways are shown in Table 6.2. Again, the overlap between omega-3 and flavonoids (8/20 pathways) and omega-3 and resveratrol (3/20 pathways) was observed, with no overlap between the flavonoids and resveratrol treatment groups. In addition to the overlapping pathways identified in Figure 6.7, there was an enrichment of pathways relating to immune response, disease signalling and signal transduction (Table 6.2).

Fig 6.8 A

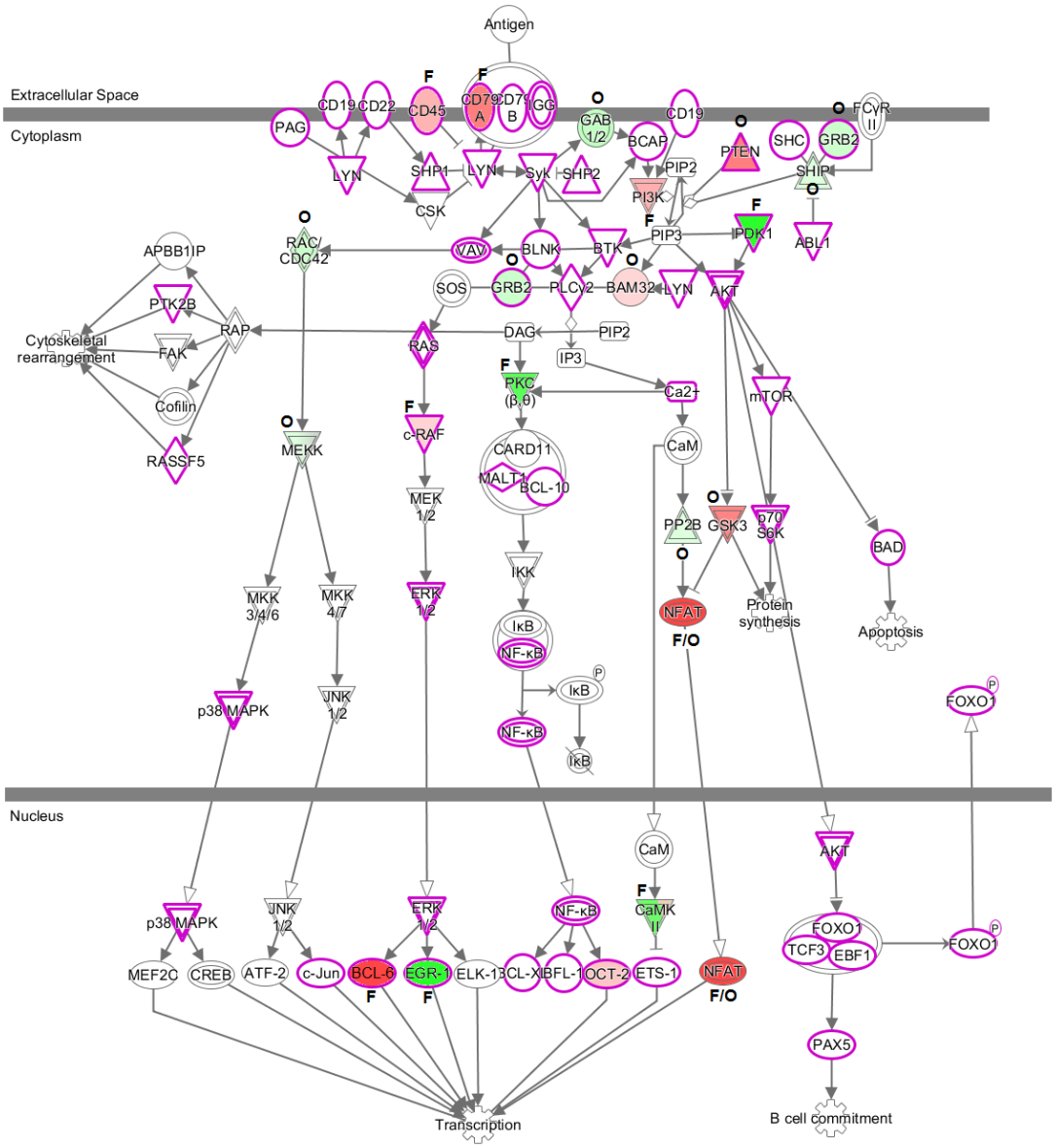

Antigen
Extracellular Space
Cytoplasm
Nucleus
CD19
CD22
CD45
CD79A
CD79B
IGG
PAG
LYN
SHP1
CSK
Syk
SHP2
GAB 1/2
BCAP
CD19
PIP2
PI3K
PTEN
SHC
GRB2
FCγR II
SHIP
ABL1
PIP3
PDK1
RAC/ CDC42
VAV
BLNK
BTK
SOS
GRB2
PLCγ2
BAM32
LYN
AKT
APBB1IP
PTK2B
RAP
FAK
Cytoskeletal rearrangement
Cofilin
RASSF5
RAS
DAG
PIP2
IP3
PKC (β,θ)
Ca2+
mTOR
c-RAF
MEKK
CARD11
MALT1
BCL-10
CaM
MEK 1/2
PP2B
GSK3
p70 S6K
BAD
IKK
MKK 3/4/6
MKK 4/7
ERK 1/2
IκB
NF-κB
NFAT
F/O
Protein synthesis
Apoptosis
p38 MAPK
JNK 1/2
IκB
NF-κB
FOXO1
CaM
AKT
p38 MAPK
JNK 1/2
ERK 1/2
NF-κB
CaMK II
FOXO1
TCF3
EBF1
FOXO1
MEF2C
CREB
ATF-2
c-Jun
BCL-6
EGR-1
ELK-1
CL-XL
BFL-1
OCT-2
ETS-1
NFAT
F/O
PAX5
Transcription
B cell commitment

Fig 6.8 B

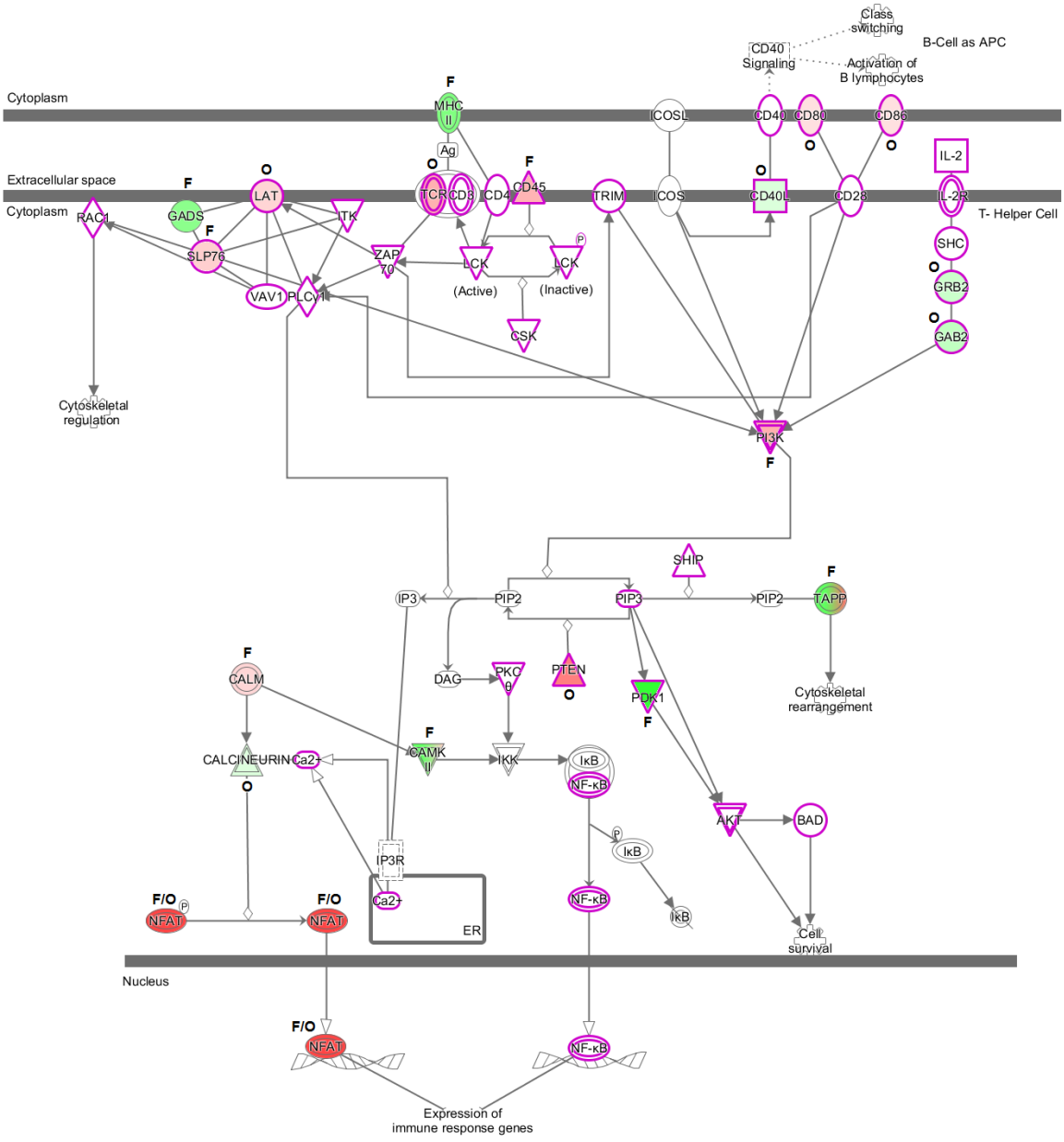
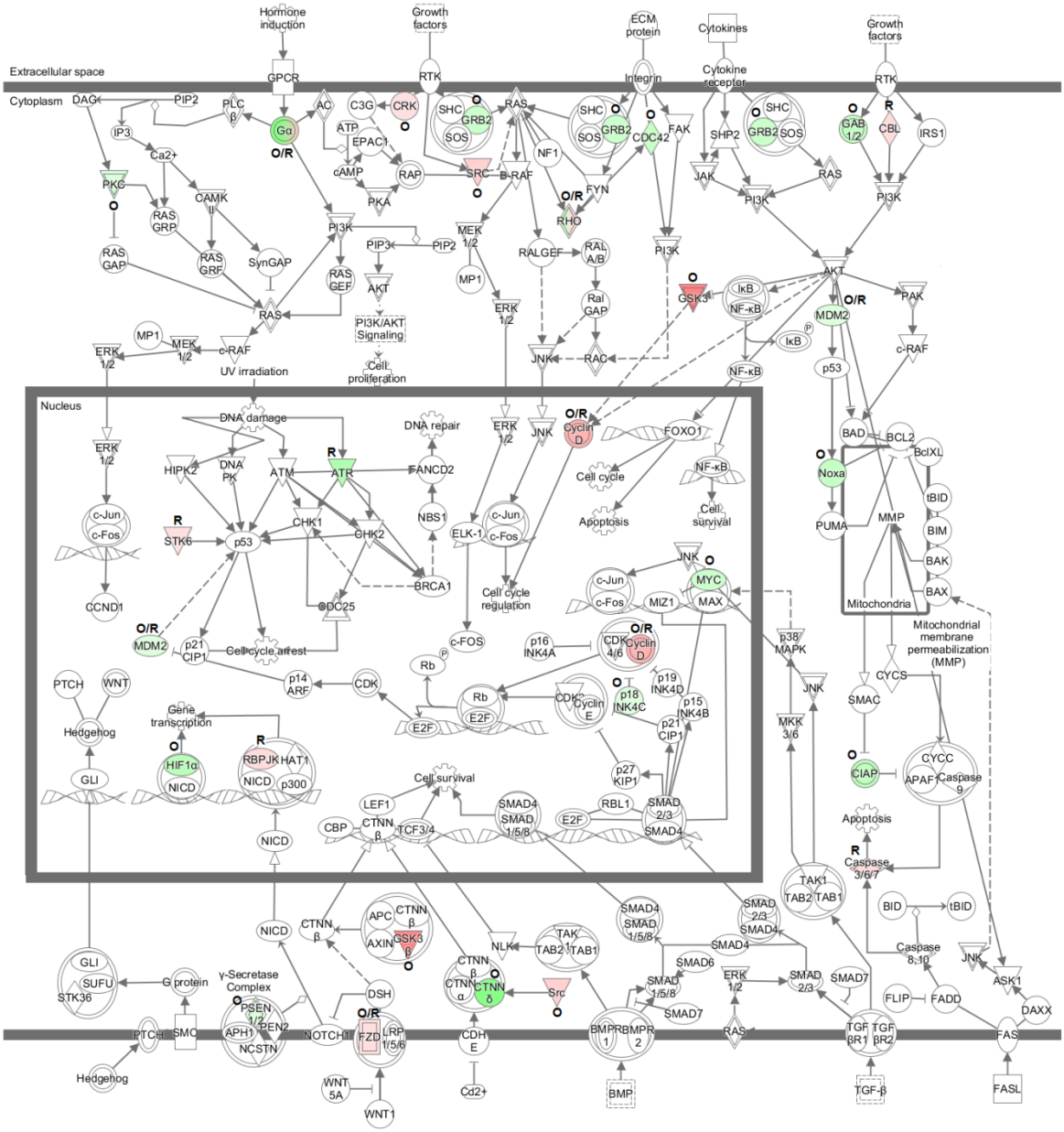


Figure 6.8 Common pathways associated with dietary interventions with omega-3 and flavonoids. Significant gene expression changes associated with diets rich in omega-3, flavonoids and resveratrol were uploaded into IPA and comparative pathway analysis performed. B cell receptor signalling (A) and iCOS-iCOSL signalling in T helper cells (B) were identified as significant pathways common to both the omega-3 and flavonoids treatment groups. Red and green nodes represent up- and downregulated genes from the uploaded dataset with colour intensity positively correlating with fold change. F, O and F/O respectively mark genes regulated in response to flavonoids, omega-3 or both. Nodes outlined in purple indicate those associated with immunological disease (A) and cancer (B). Pathway maps modified from IPA (http://www.ingenuity.com/). Abbreviations: *APC, antigen-presenting cells; ER, endoplasmic reticulum*.

Fig 6.9 A


Hormone induction
Growth factors
ECM protein
Cytokines
Growth factors
Extracellular space
Cytoplasm
GPCR
RTK
Integrin
Cytokine recepter
RTK
DAG
PIP2
PLC β
AC
C3G
CRK
SHC
GRB2
SOS
RAS
SHC
GRB2
SOS
CDC42
FAK
SHC
GRB2
SOS
GAB 1/2
CBL
IRS1
IP3
Gα
ATP
EPAC1
Ca2+
O/R
cAMP
RAP
SRC
B-RAF
NF1
SHP2
JAK
RAS
PI3K
PI3K
PKG
CAMK II
PKA
O/R
FYN
RHO
RAS GRP
PI3K
MEK 1/2
RALGEF
RAL A/B
PI3K
RAS GAP
RAS GRF
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IkB
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AKT
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PAK
RAS GEF
AKT
ERK 1/2
Ral GAP
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MP1
MEK 1/2
c-RAF
PI3K/AKT Signaling
IkB
c-RAF
ERK 1/2
UV irradiation
Cell proliferation
JNK
RAC
NF-kB
p53
Nucleus
DNA damage
DNA repair
ERK 1/2
JNK
O/R
Cyclin D
FOXO1
BAD
BCL2
BclXL
ERK 1/2
HIPK2
DNA PK
ATM
R
ATR
FANCD2
Cell cycle
NF-kB
Noxa
c-Jun
c-Fos
R
STK6
p53
CHK1
CHK2
NBS1
ELK-1
c-Jun
c-Fos
Apoptosis
Cell survival
PUMA
MMP
tBID
BIM
JNK
O
MYC
BAK
c-Jun
c-Fos
MIZ1
MAX
BRCA1
Cell cycle regulation
Mitochondria
BAX
CCND1
CDC25
O/R
MDM2
p21 CIP1
Cell cycle arrest
c-FOS
p16 INK4A
O/R
CDK 4/6
Cyclin D
Mitochondrial membrane permeabilization (MMP)
p38 MAPK
PTCH
WNT
Rb
P
p14 ARF
CDK
p19 INK4D
CYCS
Gene transcription
Rb
E2F
CDK 2
Cyclin E
p18 INK4C
p15 INK4B
JNK
SMAC
Hedgehog
E2F
p21 CIP1
MKK 3/6
R
HIF1α
RBPJK
HAT1
p27 KIP1
O
CIAP
CYCC
APAF
Caspase 9
GLI
NICD
NICD
p300
Cell survival
LEF1
SMAD4
SMAD 1/5/8
RBL1
SMAD 2/3
Apoptosis
CBP
CTNN β
TCF3/4
E2F
SMAD4
R
Caspase 3/6/7
NICD
TAK1
TAB2
TAB1
BID
tBID
CTNN β
APC
CTNN β
AXIN
GSK3 β
SMAD4
SMAD 1/5/8
SMAD 2/3
SMAD4
NLK
TAK
TAB2
TAB1
Caspase 8,10
JNK
GLI
SUFU
STK36
G protein
γ-Secretase Complex
CTNN β
CTNN α
CTNN δ
Src
SMAD6
ERK 1/2
SMAD 1/5/8
SMAD 2/3
SMAD7
FLIP
FADD
ASK1
DAXX
DSH
SMAD7
PTCH
SMO
PSEN 1/2
APH1
PEN2
NCSTN
NOTCH1
O/R
FZD
LRP 1/5/6
CDH E
BMPR 1
BMPR 2
RAS
TGF βR1
TGF βR2
FAS
Hedgehog
WNT 5A
WNT1
Cd2+
BMP
TGF-β
FASL

Fig 6.9 B

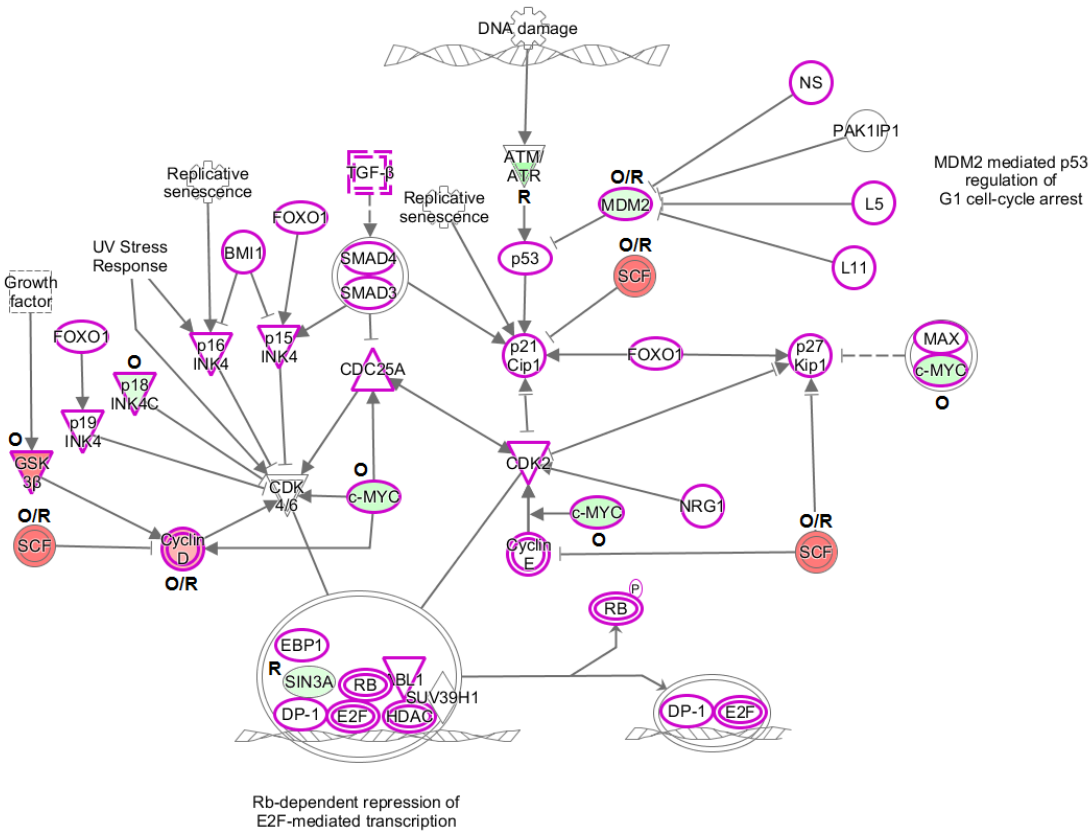

Figure 6.9 Common pathways associated with dietary interventions with omega-3 and resveratrol. Significant gene expression changes associated with diets rich in omega-3, flavonoids and resveratrol were uploaded into IPA and comparative analysis performed. Molecular mechanisms of cancer (A) and G1/S checkpoint regulation (B) were identified as significant pathways common to both the omega-3 and resveratrol treatment groups. Red and green nodes represent up- and downregulated genes from the uploaded dataset with colour intensity positively correlating with fold change. O, R and O/R respectively mark genes regulated in response to omega-3, resveratrol or both. Nodes outlined in purple indicate those associated with cancer (B). Pathway maps modified from IPA (http://www.ingenuity.com/).

Table 6.2 *P*-Values for the top 20 common canonical pathways significantly regulated following dietary intervention with omega-3, flavonoids and resveratrol

Functional Group	Canonical Pathway	Omega-3	Flavonoids	Resveratrol
Cell cycle	G1/S Checkpoint Regulation	**4.19×10^{-3}**	-	**1.31×10^{-2}**
	CHK Proteins in Cell Cycle Checkpoint Control	3.80×10^{-1}	5.02×10^{-2}	**4.35×10^{-2}**
	G2/M DNA Damage Checkpoint Regulation	3.27×10^{-1}	1.27×10^{-1}	**6.45×10^{-4}**
Developmental pathways	Wnt/β-catenin Signaling	**7.56×10^{-3}**	6.60×10^{-2} ↑	9.43×10^{-2}
	Cardiac β-adrenergic Signaling	9.96×10^{-2} ↑	**6.78×10^{-3}**	2.94×10^{-1}
	Insulin Receptor Signaling	2.17×10^{-1}	**2.19×10^{-3}**	2.98×10^{-1}
Disease pathways	Molecular Mechanisms of Cancer	**1.33×10^{-3}**	3.20×10^{-1}	**6.34×10^{-3}**
	Glioma Signaling	**2.63×10^{-2}** ↓	**1.04×10^{-2}**	3.93×10^{-1}
	Role of NFAT in Cardiac Hypertrophy	**2.84×10^{-2}**	**6.01×10^{-3}**	-
Immune response pathways	B Cell Receptor Signaling	**1.11×10^{-3}**	**4.34×10^{-5}**	4.59×10^{-1}
	iCOS-iCOSL Signaling in T Helper Cells	**1.14×10^{-3}**	**1.87×10^{-5}**	-
	CD28 Signaling in T Helper Cells	**2.32×10^{-2}**	**3.10×10^{-3}**	-
	Fcγ Receptor-mediated Phagocytosis	**2.40×10^{-2}**	2.09×10^{-1}	**1.06×10^{-2}**
	PI3K Signaling in B Lymphocytes	1.92×10^{-1}	**2.24×10^{-5}**	2.75×10^{-1}
	PKCθ Signaling in T Lymphocytes	**7.43×10^{-3}**	**1.03×10^{-2}**	-
Signal transduction	Protein Kinase A Signaling	**4.53×10^{-2}**	**6.52×10^{-6}**	-
	PI3K/AKT Signaling	**9.44×10^{-3}**	**1.30×10^{-2}**	-
	3-phosphoinositide Biosynthesis	1.78×10^{-1}	**2.33×10^{-3}**	3.91×10^{-1}
	mTOR Signaling	2.94×10^{-1}	**1.04×10^{-3}**	5.27×10^{-2}
Transcription	DNA Methylation and Transcriptional Repression	8.15×10^{-2}	9.00×10^{-2}	**3.24×10^{-2}**

Note: Functional enrichment analysis for the gene sets differentially regulated by dietary intervention with omega-3, flavonoids or resveratrol was performed using IPA software. Statistical significance was determined using Fisher's exact test. Bold font indicates significant pathways ($p<0.05$). Net effects of gene expression changes on pathway activation or repression were determined using activation z-scores (threshold, <-2.0; >2.0). ↓ Downregulated pathways; ↑ upregulated pathways.

6.5 Disease pathways targeted by the functional foods omega-3, flavonoids and resveratrol support a therapeutic role

To investigate the role of omega-3, flavonoids and resveratrol in the prevention and/or treatment of disease, IPA Core Analysis was used to identify the top disease pathways and toxicity functions associated with the filtered gene sets for these treatment conditions. The net effect of gene expression changes was addressed using z-scores which predict an increase (positive z-score) or decrease (negative z-score) in pathway activation. Omega-3 was suggested to have anti-viral (decreased viral infection and viral replication), anti-tumorigenic (decreased autophagy of colon cancer cell lines and differentiation of tumor cells; increased apoptosis of lymphoma cells) and pro-survival properties (decreased necrosis and/or cell death of kidney cells and epithelial tissue; increased cell viability of fibroblast cell lines), Table 6.4. Flavonoids were strongly associated with development (decreased growth failure; increased size of the embryo and cell survival/viability) and immune and inflammatory responses (decreased chronic inflammatory disorder; the increased immune response of cells and phagocytosis, Table 6.4. Resveratrol was enriched for processes involved in cell structure (decreased stabilisation of microtubules, microtubule dynamics and organisation of cytoplasm and cytoskeleton) and cell cycle (decreased cell cycle progression and mitosis), Table 6.5. Common toxicity functions associated with the different treatment groups was addressed through comparative enrichment analysis which identified processes relevant to cardiovascular, hepatic and renal function (Table 6.6). Activation z-scores suggested a beneficial role for omega-3 in reducing hypertrophy of the heart and liver cancer, while flavonoids associated with reduced inflammation of the liver.

Table 6.3.

Disease or Function	p-value	Activation state	z-score	Molecules
Necrosis of kidney	1.16×10^{-3}	Decreased	-2.5	ATF6, ATG7, BNIP3L, CD44, CDC42, GLS, GSK3B, HGF, HIF1A, IER3, IL18, IRF4, KEAP1, MYC, NDEL1, PMAIP1, PPARG, PPP3CA, PSEN1, PTGIS, RASSF4, RHOT1
Viral Infection	3.13×10^{-3}	Decreased	-2.4	ACVR2A, ALOX5, APOC1, AQR, ATG7, ATRX, CAMK1, CD40LG, CD44, CD80, CDC42, CHMP2B, COPB2, CRTC3, CTSB, CYP2U1, DLG1, FBXO44, GAB2, GAS6, GRB2, GSK3B, HGF, HNRNPM, IER3, IL10RA, IL18, IMPDH1, IRF4, ISG20L2, LAMP2, MAT2A, MDM2, MED14, MED27, MKNK1, MLH1, MSR1, MYC, NDUFAF2, OPN1SW, PDE4B, PDE4D, PDE5A, PIGK, PKD1L2, POU2F2, PPARG, PPP1R8, PPP3CA, PSMA1, PTPRJ, PVR, RNF10, RPS5, SF3A1, SFXN3, SMARCA2, SMU1, SMURF1, SNRPA1, SNX10, SPG7, SYNJ1, TMPRSS2, TMPRSS4, TNFSF14, TRIM25, TUBB4B, TUBG1, YY1, ZC3H12A, ZNF292
Cell death of kidney cells	1.46×10^{-3}	Decreased	-2.2	ATF6, ATG7, BNIP3L, CD44, CDC42, GLS, GSK3B, HGF, HIF1A, IER3, IRF4, KEAP1, MYC, NDEL1, PMAIP1, PPARG, PPP3CA, PSEN1, PTGIS, RASSF4, RHOT1
Contractility of cardiac muscle	4.26×10^{-4}	Decreased	-2.2	ARSB, ATE1, BTC, COX7A1, GAB2, HBEGF, IER3, IL18, LAMP2, MTMR14, PDLIM5, PLN
Hypertrophy	6.11×10^{-4}	Decreased	-2.2	CDK9, CTSB, CYP1B1, EHD4, GRB2, GSK3B, GUCY2C, HBEGF, HGF, HIF1A, IER3, IL18, IL6ST, MBNL1, MDM2, MYC, NFATC3, OSM, PDE5A, PPARG, PPP3CA, PTEN, PTGIS, RHEB, SKP2, SRC, STK3, TPPP2
Replication of virus	3.37×10^{-4}	Decreased	-2.1	ACVR2A, ATG7, ATRX, CD40LG, CDC42, COPB2, CYP2U1, DLG1, FBXO44, GAS6, GSK3B, HNRNPM, IL10RA, IL18, LAMP2, MDM2, MED14, MED27, MKNK1, MLH1, MSR1, MYC, OPN1SW, PPP1R8, PSMA1, RPS5, SF3A1, SMU1, SMURF1, SNX10, TMPRSS2, TRIM25, ZC3H12A
Necrosis of epithelial tissue	2.91×10^{-3}	Decreased	-2.1	AIFM1, ARHGAP18, ATG7, ATRX, BNIP3L, CD40LG, CD44, CDC42, CTSB, FAAH, GAS6, GSK3B, HGF, IER3, IL18, IRF4, KEAP1, KLF10, MDM2, MYC, NDEL1, OSM, PMAIP1, PPARG, PSEN1, PTEN, PTGIS, RASSF4, SRC, STK3, TNFSF14
Autophagy of colon cancer cell lines	2.34×10^{-3}	Decreased	-2.0	ATG7, BNIP3L, MDM2, MYC
Differentiation of tumour cells	3.01×10^{-3}	Decreased	-2.0	CD44, GAB2, MYC, OSM, PDE4D, PPARG, PTEN
Production of lactic acid	4.66×10^{-3}	Decreased	-2.0	AIFM1, EWSR1, HIF1A, MYC, PTEN
Apoptosis of lymphoma cells	2.73×10^{-3}	Increased	2.0	CD40LG, IL6ST, MS4A1, MYC
Cell viability of fibroblast cell lines	6.15×10^{-4}	Increased	2.1	CDC42, GAS6, GSK3B, HGF, MLH1, MYC, NARFL, OGG1, PDK4, SRC

Table 6.3. Top disease pathways and biological functions enriched for genes significantly regulated following dietary intervention with omega-3

Note: Functional enrichment analysis was performed using IPA software. Statistical significance was determined using Fisher's exact test. All pathways reached statistical significance ($p<0.05$). Net effects of gene expression changes on disease pathways and biological functions were determined using activation z-scores (threshold, <-2.0; >2.0). Negative values indicate downregulated pathways; positive values upregulated pathways. Molecules represent genes from the uploaded dataset.

Table 6.4

Disease or Function	p-value	Activation State	z-score	Molecules
Growth Failure	2.83×10^{-3}	Decreased	-2.2	ATM, B4GALT1, BCL6, CFLAR, CHM, EGR1, ETV6, H3F3A/H3F3B, HFE, HIST1H1C, HIST1H1D, HNRNPD, LTBP3, NFAT5, NUMB, PDS5A, PTGES3, PTPN12, RAF1, RBBP6, REV3L, SLC19A1, SMC1A, SNX27, SOD2, STK4, SYNJ1, TIA1, TRIM33, TRIP12, UBR2, VDAC3, ZNF148
Chronic inflammatory disorder	1.45×10^{-3}	Decreased	-2.2	ADM, ANXA1, ATM, B2M, BCL6, C17orf59, CAMK2D, CD79A, CELF2, CR1, CYBA, DUSP2, EEF1E1, FOXO3, FUBP1, GLUL, GPSM3, GZMA, H3F3A/H3F3B, HAVCR2, HLA-DOA, IKZF1, IL16, IL7R, JAK1, KIR3DL2, LRRK2, LTB, MYCBP2, NLRP3, NR3C1, NUMB, NXPE3, PDE4B, PDE4C, PDE5A, PSMD1, PTPRC, RGCC, RPS16, RSBN1, S1PR3, SELP, SNRNP200, SOCS3, STK17B, TAGAP, TMEM192, TNRC6B, VIM, XIAP
Immune response of cells	2.98×10^{-5}	Increased	2.1	ANXA1, ATM, ATXN3, BCL6, CBL, CD44, CD47, CD48, CD79A, CLEC7A, CLIP1, CR1, CYBA, CYP2S1, ERAP1, FPR1, HAVCR2, IL7R, JAK1, KLRB1, LCP2, LGALS3, MAP3K8, MAP4K1, MLST8, MYO1G, NLRX1, NR3C1, PFDN6, PRKCB, PTPRC, REL, RGCC, SERPINB9, SLC11A1, SOCS3, TRIM38, VIM
Size of embryo	4.32×10^{-3}	Increased	2.2	ACVR1, CENPU, HES1, HIST1H1C, HIST1H1D, LAMA5, MDM4, MORF4L2, NUMB, PDPK1, RAF1, RAPGEF2, RBBP6, REL, REV3L, SLC8A1, SOCS3, STK4, TGS1, TRIM33, UBR2
Cell survival	8.83×10^{-5}	Increased	2.4	ACVR2A, ATM, ATRX, B2M, BCL6, BCLAF1, CA2, CAMK2D, CAMK2G, CBL, CD44, CD47, CD48, CD79A, CFLAR, CR1, CTDSPL, CYBA, DDX3X, DHX9, EGR1, EIF4G1, EMP1, ETV6, EWSR1, FBXO9, FOXO3, HBB, HELB, HES1, IGF2BP3, IL7R, IQGAP1, JAK1, KDM4B, LAMA5, LGALS3, LRRK2, MAP3K8, MBP, MDM4, MICAL2, mir-21, MTMR6, NFAT5, NR3C1, NT5C3A, NUPL1, PDPK1, PFDN6, PPP2R2A, PPP2R5C, PRKCB, PTGDR2, PTPRC, PTPRE, RAF1, REL, REV3L, RSF1, S1PR3, SERPINB9, SLC11A1, SLC19A1, SLC22A3, SMC1A, SMC3, SOCS3, SOD2, SPN, TRIM33, VIM, VKORC1, XIAP
Cell viability	2.31×10^{-4}	Increased	2.5	ACVR2A, ATM, ATRX, B2M, BCL6, BCLAF1, CA2, CAMK2D, CAMK2G, CBL, CD44, CD47, CD48, CD79A, CFLAR, CR1, CTDSPL, CYBA, DHX9, EGR1, EIF4G1, ETV6, EWSR1, FBXO9, FOXO3, HBB, HELB, IGF2BP3, IL7R, IQGAP1, JAK1, KDM4B, LAMA5, LGALS3, LRRK2, MAP3K8, MBP, MDM4, MICAL2, mir-21, MTMR6, NFAT5, NR3C1, NT5C3A, NUPL1, PDPK1, PFDN6, PPP2R2A, PPP2R5C, PRKCB, PTGDR2, PTPRC, PTPRE, RAF1, REL, REV3L, RSF1, S1PR3, SERPINB9, SLC19A1, SLC22A3, SMC3, SOCS3, SOD2, SPN, TRIM33, VKORC1, XIAP
Phagocytosis	1.14×10^{-4}	Increased	3.1	ANXA1, CBL, CD163, CD44, CD47, CD48, CLEC7A, CLIP1, CR1, CYBA, CYP2S1, DLG1, EPG5, ERAP1, FPR1, IQGAP1, LGALS3, MAP1S, MYO1G, NR3C1, PRKCB, PTPRC, RGCC, SLC11A1, SYNJ1, SYNJ2BP, VIM, ZFYVE16

Table 6.4 Top disease pathways and biological functions enriched for genes significantly regulated following dietary intervention with flavonoids
Note: See Table 6.3 for the legend.

Table 6.5. Top disease pathways and biological functions enriched for genes significantly regulated following dietary intervention with resveratrol *Note:* See Table 6.3 for legend.

Disease or Function	p-value	Activation State	z-score	Molecules
Organization of cytoplasm	3.43×10^{-3}	Decreased	-2.5	AURKA, BCL6, BMP2K, CASP6, CD44, CIT, DRD1, EPHA5, EPHA8, GRK5, HTR6, IFT57, IQGAP1, KIF3B, METRN, MTFR2, NEU3, NEUROD1, ONECUT2, PI4K2B, PIP5K1A, PLK1, POU3F2, PRC1, RACGAP1, RHOJ, RYR2, TACC3, TESK2, TMEM135, TRPV2
Organization of cytoskeleton	5.98×10^{-3}	Decreased	-2.5	AURKA, BCL6, BMP2K, CASP6, CD44, CIT, DRD1, EPHA5, EPHA8, GRK5, HTR6, IFT57, IQGAP1, KIF3B, METRN, NEU3, NEUROD1, ONECUT2, PI4K2B, PIP5K1A, POU3F2, PRC1, RACGAP1, RHOJ, RYR2, TACC3, TESK2, TRPV2
Microtubule dynamics	4.66×10^{-3}	Decreased	-2.4	AURKA, BMP2K, CASP6, CD44, CIT, DRD1, EPHA8, GRK5, HTR6, IFT57, IQGAP1, KIF3B, METRN, NEU3, NEUROD1, ONECUT2, PI4K2B, PIP5K1A, POU3F2, PRC1, RACGAP1, RYR2, TACC3, TESK2, TRPV2
Cell cycle progression	8.42×10^{-6}	Decreased	-2.2	ADARB1, AURKA, BCL6, CALCB, CASC5, CASP6, CCND3, CD44, CDKN3, CIT, GRK5, GYPA, HECW2, HERC5, IL11, IRS2, KDM2B, MDM2, MXD1, NEUROD1, NUCKS1, PLK1, PPARA, RACGAP1, RLIM, SGOL1, TACC3, TP63, ZBTB16, ZNF274
Mitosis	1.06×10^{-2}	Decreased	-2.2	AURKA, CASC5, CDKN3, CIT, GYPA, IL11, IRS2, MDM2, PLK1, PPARA, RACGAP1, SGOL1
Stabilization of microtubules	1.20×10^{-2}	Decreased	-2.0	BMP2K, IQGAP1, PI4K2B, TESK2
Insulin sensitivity	1.08×10^{-2}	Increased	2.2	CALCB, CD44, GCGR, IRS2, PPARA

Table 6.6 Top 20 common toxicity functions associated with dietary intervention with omega-3, flavonoids and resveratrol

Biological System	Toxicity function	Omega-3	Flavonoids	Resveratrol
Cardiovascular	Cell death of cardiomyocytes	**3.41×10^{-2}**	**4.01×10^{-3}**	3.80×10^{-1}
	Failure of heart	1.53×10^{-1}	**6.88×10^{-3}**	**4.68×10^{-2}**
	Hypertrophy of cardiac muscle	**6.93×10^{-5}** ↓	1.47×10^{-1}	2.03×10^{-1}
	Hypertrophy of cardiomyocytes	**2.45×10^{-3}** ↓	1.59×10^{-1}	3.74×10^{-1}
	Hypertrophy of heart	**9.34×10^{-4}** ↓	4.65×10^{-1}	3.11×10^{-1}
Hepatic	Apoptosis of hepatocytes	**2.09×10^{-2}** ↓	6.95×10^{-2}	3.67×10^{-1}
	Cell death of liver cells	**2.64×10^{-3}**	**1.09×10^{-2}**	3.21×10^{-1}
	Hepatic steatosis (fatty liver)	**2.15×10^{-4}**	1.74×10^{-1}	6.56×10^{-2}
	Advanced stage hepatic steatosis	7.00×10^{-2}	7.40×10^{-2}	**2.68×10^{-2}**
	Hepatomegaly (enlargement of the liver)	**3.96×10^{-3}**	-	1.75×10^{-1}
	Steatohepatitis (fatty liver disease)	**3.13×10^{-3}**	**4.00×10^{-3}**	3.17×10^{-1}
	Inflammation of liver	6.00×10^{-2}	**5.39×10^{-3}** ↓	8.35×10^{-2}
	Liver cancer	**1.53×10^{-3}** ↓	**1.91×10^{-5}**	8.69×10^{-2}
	Hepatocellular carcinoma	**1.18×10^{-3}** ↓	**2.67×10^{-5}**	6.87×10^{-2}
	Tumorigenesis of hepatocellular carcinoma	**2.34×10^{-2}**	1.40×10^{-1}	2.10×10^{-1}
	Proliferation of hepatocytes	**6.99×10^{-4}**	4.16×10^{-1}	4.46×10^{-1}
Renal	Apoptosis of kidney cell lines	**1.54×10^{-2}**	**4.54×10^{-3}**	4.52×10^{-1}
	IgA nephropathy (inflammation of the glomeruli)	**3.41×10^{-2}**	1.75×10^{-1}	**2.98×10^{-2}**
	Increased quantity of creatinine	**3.01×10^{-4}**	6.32×10^{-1}	**4.82×10^{-2}**
Haematopoietic	Increased quantity of red blood cells	8.86×10^{-2}	**1.29×10^{-2}**	2.25×10^{-1}

Note: Functional enrichment analysis for the gene sets differentially regulated by dietary intervention with omega-3, flavonoids or resveratrol was performed using IPA software. Statistical significance was determined using Fisher's exact test. Bold font indicates significant toxicity functions ($p<0.05$). Net effects of gene expression changes on toxicity functions were determined using activation z-scores (threshold, <-2.0; >2.0). ↓ Downregulated processes; ↑ upregulated processes.

6.6 Functional foods target biological processes and disease pathways identified from GWAS

To further address how omega-3, flavonoids and resveratrol may converge onto major disease pathways, gene expression changes associated with these functional foods were compared against GWAS hits for different biological functions and diseases. Reported genome-wide associated genes for ageing, breast cancer, cognitive function, CVD, diabesity, neurodegeneration and psychiatric disorders were downloaded from the NHGRI-EBI GWAS catalog, a publicly available collection of published GWAS for more than 100,000 single-nucleotide polymorphisms (SNPs) with SNP-trait associations of $p<1 \times 10^{-5}$ (Welter *et al*., 2014). Hypergeometric distribution testing using the *GeneOverlap* package in R was used to determine the significant overlap between the GWAS datasets and genes associated with dietary interventions with omega-3, flavonoids and resveratrol. As illustrated in Figure 6.10A, significant overlap was observed between the two lists representing genes regulated in response to omega-3 and those implicated in breast cancer risk from GWAS ($p = 0.01$). A total of 32 genes were identified as being common between the two gene lists. These were uploaded into the PANTHER pathway analysis platform and overrepresentation testing performed. Enrichment of genes implicated in metabolic processes (Figure 6.10B) and developmental pathways (Figure 6.10C) was identified as being significantly associated with this gene set. No other significant associations were found between gene lists for the functional foods and diseases/biological functions tested.

The odds ratio analysis in Figure 6.10A, illustrates the significant benefit afforded by Omega 3 in breast cancer with a *p*-value of 0.01.

Fig 6.10 A

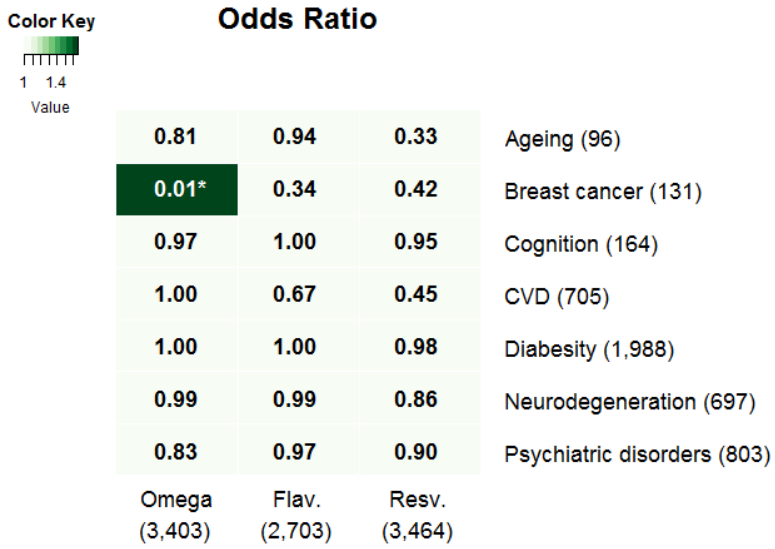

Fig 6.10 B

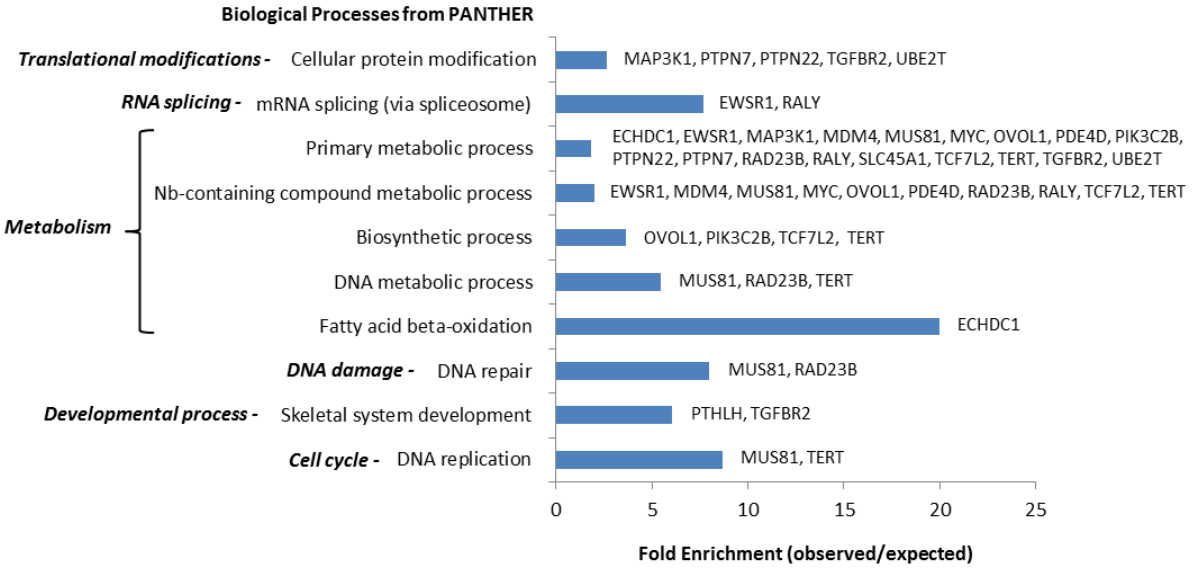

Fig 6.10 C

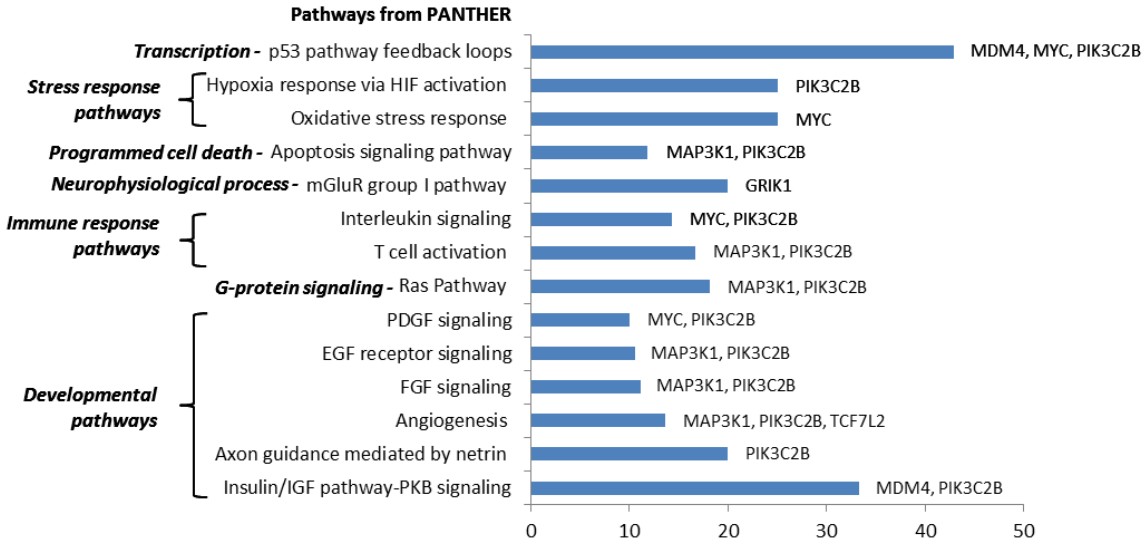

Figure 6.10 Significance testing of overlap between genes regulated in response to functional foods and GWAS hits for common diseases/biological functions.
(A) Gene lists representing all genes significantly regulated in response to dietary interventions with omega-3, flavonoids and resveratrol (see Table 6.1 for study details) were compared against gene lists identified from genome-wide association studies for ageing, breast cancer, cognition, cardiovascular disease (CVD), diabesity, neurodegeneration and psychiatric disorders. Numbers in brackets represent the number of genes within each gene list. GeneOverlap and GeneOverlapMatrix functions available in R were used to calculate and visualise significant overlap between the gene lists tested. Fischer's exact test was used to calculate p-values which are stated within each panel of the grid. Colour key represents odds ratio values. *Significant overlap ($p<0.05$). B-C, Statistical overrepresentation testing was performed using the binomial statistics tool available through PANTHER to identify biological processes (B) and pathway classifications (C) for common genes associated with breast cancer and dietary intervention with omega-3. Uploaded genes were compared to a reference list containing all human genes within the PANTHER database to statistically determine over- or under-representation of PANTHER classification categories using the binomial distribution test (Cho & Campbell, 2000). Associated genes are listed on the right of the bar charts. Abbreviations: *EGF, Epidermal growth factor; FGF, fibroblast growth factor; Flav., flavonoids; HIF, hypoxia-inducible factor; IGF, insulin-like growth factor; mGluR, metabotropic glutamate receptor, Nb, nucleobase; PDGF, platelet-derived growth factor; PKB, protein kinase B; Resv., resveratrol*.

6.7 Summary

Dietary intervention with the functional foods omega-3, flavonoids and resveratrol target important biological and disease pathways, suggesting a potentially important role for these bioactive compounds in the prevention and treatment of dietary-related disease such as cancer.

Chapter 7

Consumer Acceptance of Functional Foods & Personal Genomics

7.1 Consumer acceptance of Functional Foods.

7.1.1 Demographics

The functional food market can be classified into four key target audiences, figure 7.1. *"Undecideds"* who make up the majority of the market (43%) are unsure whether functional food and drink improve health; this group are most likely to disagree that natural foods are just as beneficial as functional foods at promoting health effects (Mintel, 2011). "*Health Buffs"* (20%) are likely to have noticed health improvements in their health due to functional food consumption however commonly purchases food with added health benefits; this group would purchase further products if convinced of the health claim. "*Sprightly Cynics"* (24%) are people who already consume a healthy diet so don't believe incorporating functional foods into their diet would benefit their health; this group are also very sceptical of the health claims made, and believe that these products are overpriced; this target group is mainly made up of 65+ males. Lastly, the *"Impassives"* (13%) are least likely to have noticed an improvement in health after functional food consumption and are the least likely of all groups to purchase foods which promise a functional health benefit.

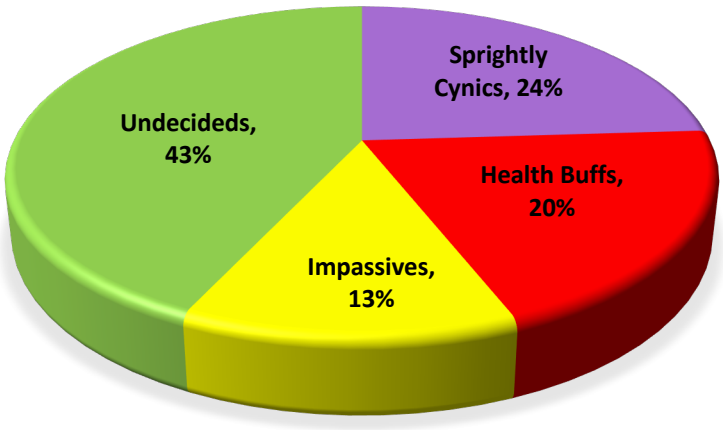

Figure 7.1 Functional Food Target Audiences

7.1.1.1 Ageing population

In 2011, 9.2million (16%) residents in England and Wales were aged 65+, an increase of almost 1 million from 2001. The UK population is estimated to rise to more than 73 million by 2037 (The Office for National Statistics, 2013). The elderly have specific nutritional requirements due to their altered physiology. Furthermore, the composition and activity of their intestinal microbiota differ from younger adults, and their immune function is less efficient. Their nutritional needs offer the functional food industry a niche market to target due to their specific dietary requirements (Ratts *et al,* 2008).

Globally, there is a seven-year gap between life expectancy and healthy life expectancy (Euromonitor International, 2013). To reduce this substantial difference, the ageing population could be targeted with foods that benefit the following areas identified with significant potential: cardiovascular health, bone and joint health, brain health, memory and vision (Cowland, 2014).

Despite functional foods aimed at the ageing population being a niche market, this demographic can help invigorate growth in the functional food market through greater awareness of health benefits, higher disposable income and a desire to live longer in better health. This will help open up a market estimated to be worth $20.6bn. Despite over 577 million consumers globally, sitting in the elderly demographic (aged 65+) they remain extremely under-catered for (Cowland, 2014).

It is possible to assess the interest and ability of potential consumers in specific geographical locations. This is achieved by correlating *per capita* spend on food and drink with *per capita* spend on functional food and drink in the same area (Cowland, 2014). In addition to this, the demographics of these regions are mainly ageing populations. Functional foods aimed at the ageing or the elderly are under catered for, providing an opportunity for market growth. The current products available are minimal with limited use of functional ingredients, The elderly and the ageing are particularly susceptible to malnutrition; therefore nutritional management is a niche market for functional food manufacturers.

Nutricia provide a product portfolio developed to address enhancement of nutrition to improve lifestyle changes and diseases. For example, the product 'FortiFit' is designed to stimulate the rebuilding of muscle to improve muscle strength and function for those suffering from sarcopenia, due to its leucine enriched amino acid mixture. The product 'Souvenaid' is clinically proven to help improve memory for those in the early stage of Alzheimer's disease. 'Nutilis' is an effective product for sufferers of dysphagia allowing adequate nutrition and hydration. Additionally, it includes amylase-resistant features for safer swallowing.

7.1.1.2 Younger generation

Young adults aged 25-34 are considered the key demographic group that will drive functional food and drink sales (Canadean Ltd, 2013). In recent studies, the consensus is that the younger generation is a key demographic for the market as they are more likely than the elderly to have increased their annual purchases of functional foods (Mintel, 2011). However, three-quarters of consumers believe that functional foods are overpriced indicating a key barrier to market growth, a factor which is likely to deter young consumers.

40% of young consumers are familiar with the functional food concept, 27% of them are regular buyers purchasing functional dairy products (Markovina *et al,* 2011). More than half the candidates (51.8%) in the study would purchase functional foods in the future. Female consumers aged 19-30 with higher disposable incomes will be a considerable target audience in the future (Markovina *et al,* 2011). Young adults have a general acceptance of functional foods and are more price sensitive than the elderly consumers (Mitsunori, 2012).

The functional food market at present is directly aimed at female consumers over 45 (Mintel, 2011). Younger consumers and males in particular, are under catered for and could be the focus of new product innovation and marketing activity. Younger consumers could lead the market to substantial growth. This potential market could be targeted by manufacturers conveying greater lifetime value to the benefits that consuming functional foods can provide.

7.1.1.3 Income levels

Higher disposable income increases purchasing power and allows consumers to be more adventurous by increasing their propensity to purchase foods with functional health improvement benefit. Global annual disposable income per household by the end of 2014 reached 1.2%, a considerable growth from the 0.4% recorded in 2013 and the 0.3% in 2012 (Euromonitor International, 2014). This growth is a reflection of improved economic performance and a lower unemployment rate, which has dropped to 6.9% in the UK (BBC C, 2014).

Functional foods often carry a price premium compared to standard alternatives, with products costing up to eight times more. Higher disposable incomes allow consumers to afford the higher price premiums associated with functional foods. However, as incomes increase, more economically developed countries spend less on food and more on materialistic items. For example, South Koreans spent 33% of their income on food in 1975, but with increased wages and economic development, this has reduced to just 12% in 2014 (Plumer, 2014). However, an increase in economic prosperity is not necessarily associated with an increase in the quality of health in the population as seen in the global increase of NCDs.

The functional food market has also continued to expand despite economic downturns demonstrating that demand is growing; however, the commercial failure rate of products is significantly higher than other food sectors. This indicates that despite price premiums, consumers still retain a propensity to purchase if the health benefit has been scientifically validated and focuses on a health impediment

that affects the consumer. UK income levels are sufficient for consumers to buy functional foods. However, decisions to purchase will not occur unless the product in question meets intrinsic consumer needs and will likely result in commercial product failure.

7.1.1.4 Reduced scepticism

Customer scepticism is a key challenge for the functional food market. Comprehensive regulations governing the functional food market have challenged the alleged 'exaggerated' claims made on some functional food products and have established greater consumer confidence. The rigorous process in which functional foods claims are accredited could be leveraged more effectively into marketing communications (Mintel, 2011). This would illustrate to customers how functional foods are laboriously tested and have their scientific claims verified by the EFSA under the 'regulation 1924/2006 on nutrition and health claims made on 'food to warrant a health claim'.

Consumers remain sceptical about health claims, this is reflected in the results of a Mintel survey where 65% of functional food users place more trust in naturally sourced foods such as fruit and vegetables than in functional foods. This is partly due to accessibility, affordability and familiarity of fruit and vegetables which are natural and unprocessed, unlike many functional foods. However, this distrust is mitigated when the health benefits claimed are validated by scientific evidence (Mintel, 2011).

7.1.2 Commercial challenges

An online survey recorded the views and opinions towards functional food from 1,005 Americans (The International Food Information Council, 2013). The information recorded, identified the following reasons that frequent functional food consumption is not achieved; price, purity, taste and lack of knowledge. In addition to this, regulation governing the functional food industry limits innovation, development and launches.

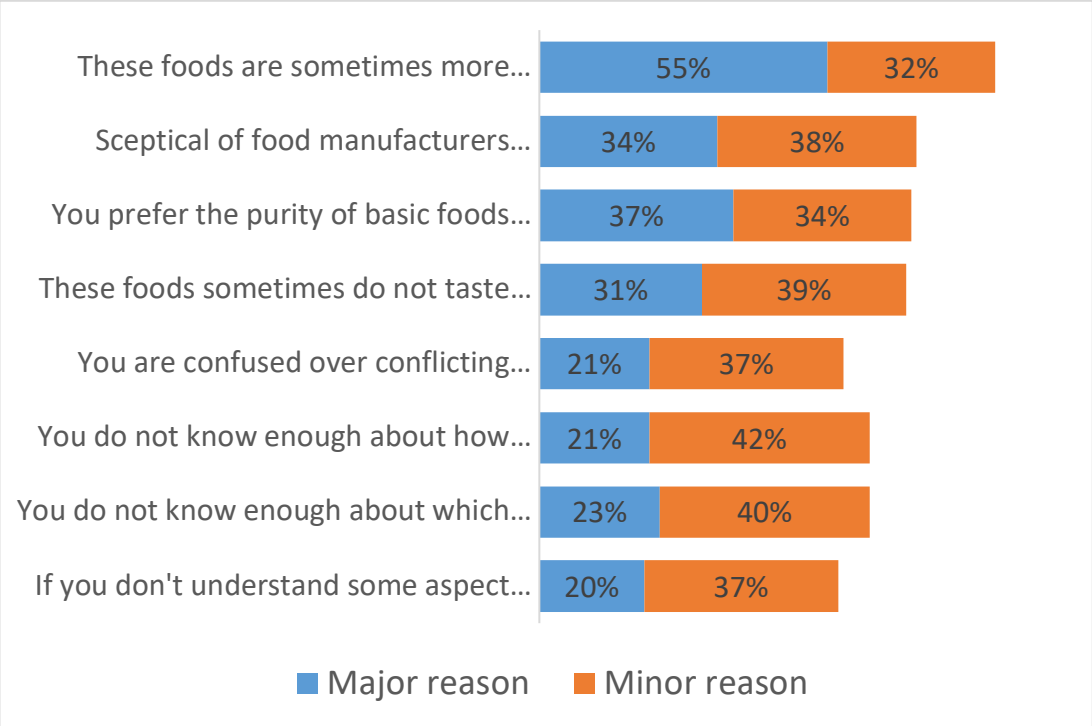

Figure 7.2 Adverse Effects Preventing Sales of Functional foods.

7.1.2.1 Price

The greatest barrier to consumption of functional foods more frequently is price (International Food Information Council, 2013). Figure 7.2 shows that 55% of subjects claim that the higher price charged compared to standard versions is a major reason for preventing purchase. Similarly, 32% state that it is a minor reason. Overall this is the greatest factor affecting sales of functional foods.

The functional food market is afflicted by a damaging level of consumer scepticism. 52% of consumers believe that health claims are merely an excuse for manufacturers to charge a price premium; if doubts are not addressed by the industry, then consumers could become non-users (Mintel B, 2011). The price premium is a considerable barrier for the functional food market with 75% believing that functional foods are overpriced. The sensitivity to price can be related to economic downturn and low disposable income but is also strongly influenced by consumer scepticism (Mintel B, 2011). The premium price premium is a common deterrent across all age and social groups but most common in the 45-54 age groups. Interestingly it is not just low-income consumers, *i.e*. those on salaries of between £15,500 and £24,999 that believe functional foods are overpriced, those earning £50,000+ are equally likely to agree. The purchase of functional foods is not just affected by the financial affordability of the product, but also whether the consumer believes they are receiving value for money. The ability of product to deliver the benefits of stated claims promptly, can be an essential element in providing consumer satisfaction with the product. Despite this, a later study by Mintel (2014), stated that 41% of consumers believe “it’s worth paying more for added benefits, *e.g*. vitamins and minerals”.

The price of functional foods exacerbates consumer suspicion; a study found that 75% believe they are over-priced suggesting reluctance to pay regardless of the improved health benefits (Mintel,2011). Consumers are focused on receiving value for money on all purchases. Greater trust in product efficacy would make it easier for producers to justify the premium.

Despite the premium in this sector, sales continue to grow. The consensus from the general public is that despite higher product cost, when health claims are supported by scientific evidence, then the premium price is considered acceptable (Mintel, 2014).

7.1.2.2 Taste

The competing demand for taste and health is a significant barrier to the food industry. Consumer food choices are driven primarily by the demands of taste, cost, and convenience; however, the major determinant of food selection is taste. Foods that are bitter, acrid or astringent tend to be rejected by the consumer. The instinctive rejection of bitter taste may not be modifiable because it is a key mechanism for survival (Drewnowski & Gomez-Carneros, 2000).

Bitter taste is the main reason for the rejection of food products. The bitterness of cruciferous vegetables is repeatedly linked to their low acceptance. However, not all foods which have bitter characteristics are a deterrent. In selected foods and beverages, a certain degree of bitterness is expected, *e.g*. caffeine or alcohol in coffee, beer, and wine (Drewnowski & Gomez-Carneros, 2000). Many functional ingredients are compounds derived from whole ingredients therefore when consumed within the inherent source are tasteless, but when extracted have a pungent flavour. For example, Stevia leaves in high

concentrations can impart a bitter aftertaste that is similar to liquorice or aniseed and many find this taste unpleasant.

The inclusion and fortification of foods to enhance health represents a major market within the food industry; foods which are tailored to contain increased concentrations of phytonutrients often have a less than a favourable aftertaste. Despite the primary function of food to provide nutrients, its secondary function concerns sensory attributes such as taste and flavour. For the most part, plant-based phytochemicals are bitter and therefore aversive to the consumer (Drewnowski & Gomez-Carneros, 2000). The discovery of a family of ≥50 different bitter-taste receptors confirms the importance of sensitivity to bitter taste was to evolution and survival. Plant-based ingredients present several challenges with sensory qualities having the greatest impact upon food choice; functional food manufacturers however also have to balance the taste aspect with health, texture, appearance and cost.

To bridge the gap between functional products and original versions, manufacturers need to improve on the taste and flavour of products; consumers are continually demanding and taste should not be a sacrifice consumers have to make when purchasing functional food. Also, consumers are not willing to compromise taste for functionality. Only 9% of consumers will accept a functional food concept if the product tastes worse than the conventional counterpart (Saarela, 2011).

7. 2 Consumer acceptance of personal genetic services

The application of genotype-based personalised nutrition could potentially revolutionise health promotion, healthcare and the food industry. If accepted, genomic-based personalised nutrition could directly affect consumer food choice as health will become the

driving factor in food selection (German & Watkins, 2004). Conceptually, nutritional genomics is still in its infancy, and it is unclear how this rapidly developing science will be accepted by society (Castle & Ries, 2007; Ronteltap, van Trijp and Renes, 2007; Moskowitz, German and Saguy, 2005). Studies have investigated the attitudes, knowledge and the interest of people in genetic testing within the US and European countries. However few have focused on the concept of genomic-based personalised nutrition within the UK population.

7.2.1 Previous research

Previous research (Table 7.1) indicates that genetic testing, the basis of genomic-based nutrition, faces many ethical and moral hurdles including an increase in knowledge towards the topic to gain mainstream consumer acceptance. Genetic testing within the American population is viewed positively when the information allows individuals to gain control over their health alongside disease prevention (Rose *et al*., 2005; Catz *et al*., 2005). Concerns around confidentiality, potential misuse of samples and information abuse by employers and insurers resulting in discrimination based on genotype were all expressed by UK consumers (Castle, 2007; Petersen, 2005; Skirton *et al*., 2005). Similar concerns were also reported within the US population regarding the privacy of genetic testing (Rose at al., 2005; Catz *et al*., 2005). A large population study indicated that 21% of consumers within the US were unwilling to undergo any form of genetic testing (Wang *et al*., 2001). In the Netherlands, more positive attitudes towards genetic testing were reported, particularly among individuals who were convinced of the benefits of adapting lifestyle based on their genotype (Henneman and Timmermans, 2006). Similarly, a study in the Netherlands found positive attitudes toward genetic testing among individuals who were younger and had a higher level of education and those with chronic disease (Morren *et al*., 2007).

Studies which have focused on genetic testing regarding well-being, health and personalised nutrition found similar results. The majority of respondents (66%) in the European Union would be willing to undergo genetic testing, and 27% would be willing to follow a personalised diet (Stewart-Knox *et al*., 2008). Another European study focused on the perceived risk-benefit trade-off factors which influence consumer adoption of personalised nutrition services (Berezowska *et al*., 2015). It was reported that consumers intention to adopt personalised nutrition services depends more on perceived personalisation benefit than on perceived privacy risk. A study on the public interest towards internet-based personal genome testing reported that only 13% of respondents were aware of nutritional genomics (Cherkas *et al*., 2010). When considering the cost of the service, 5% were willing to pay the current price of £250. This figure rose to 50% if the service was free of charge. Demographics of age, sex, education, marital status, region of residence, parenthood, college-education are predictors of increased awareness of genetic testing (Roy *et al*., 2016). A study using an Italian sample reported that consumers who were more health consciousness were more aware and accepting towards genetic tests (Oliveri *et al*., 2016).

The benefits and risks of nutritional genomics were identified by members of the public and healthcare professionals in Canada (Morin 2009). For the public, the perceived benefits of nutrigenomics were as follows; removal of guesswork from nutrition, provision of personalised advice, early diagnoses or disease prevention and encouragement of healthier habits. For healthcare professionals, nutrigenomics was perceived to generate wider interest in nutrition, encourage preventive care, and potentially trigger behavioural change, target interventions and may ultimately result in healthcare savings (Morin 2009).

Table 7.1 Review of results of previous investigations into the consumer view of personalised nutrition, results and policy implications					
Author/ Year	Country	Sample size and characteristics	Study design methodology	Outcome measures	Key findings and policy implications
Fischer *et al*. (2016)	Europe 9 countries	N = 9381 Quota sampled Aged 18– 65 yrs Mixed gender	On-line survey	Willingness to pay for PN	One third of sample willing to pay as much as 50% more for PN Greece were willing to pay most and Netherlands willing to pay least, * Regulation to encourage and control commercial PN in Greece * Provide incentive for those in Netherlands to take up PN
Rankin *et al*. (2016)	United Kingdom	N = 32 Aged 18–65 Mixed gender	Focus groups	Theory to inform design of PN	Social Cognitive Theory (SCT) best fit model to describe the public perception of PN Recommend PN services be designed using SCT
Berezowska *et al*. (2015)	Europe 8 countries	N = 9000+ Quota sampled Aged 18–65 yrs	On-line survey	Willingness to pay for PN	Perceived risk associated with data security higher if genetic data considered * Regulate handling of genetic data
Fallaize *et al*. (2015)	United Kingdom and Ireland	N = 73 Aged 18–65 and 30–65 yrs	Focus groups	Attitudes to PN* service delivery	Preference for services to be provided by government and delivered face to face. Payment was associated with increased commitment and motivation to comply with dietary recommendations. UK participants expected PN to be delivered free of charge on the NHS * Provide publicly funded PN in addition to commercial services
Poínhos *et al*. (2014)	Europe 9 countries	N = 9381 Quota sampled Aged 18–65 yrs Mixed gender	On-line survey	Intention to adopt PN	Benefit perception most important determinant of attitude toward adoption of PN. Nutrition self-efficacy a predictor of attitude and intention to take up PN. Perceived risk related to data security had a negative relationship with attitude and an inverse relationship with perceived benefit * Promote benefits of PN. * Regulate data handling.
Berezowska *et al*. (2014)	Europe 8 countries	N = 124 Aged 18–65 yrs Mixed gender	Focus groups	Attitudes to PN services	Face to face interaction was deemed to reduce perceived risk and increase benefit. Qualified experts supported by scientific evidence increased value perception * Recommend off-line communication with qualified health professionals

StewartKnox *et al*. (2013)	Europe 9 countries	N = 126 Aged 18–65 and 30–65 yrs Mixed gender	Focus groups	Attitudes to PN	Positive attitudes toward PN. Benefit: control; anonymity Concerns over data protection, service provider Barriers: social; motivational * Promote benefits of PN. * Regulate data handling. * Policies to enable PN in society
Sanderson *et al*. (2013)	USA (NY)	N = 205 patients Aged 18+ yrs Mixed gender	Structured interviews	Determinants of uptake of genomics to treat dietrelated disease	Reasons for uptake: altruism; benefit to family members; personal health benefit; curiosity; and, understanding. Reasons for rejection: negative perception of research; not personally relevant; negative about procedures; practical barriers; and, fear of results * Promote benefits of PN. * Regulate use of research. * Recommend qualified health professionals communicate results
Wendel *et al*. (2013)	Netherlands	N = 204 Mixed gender M age 38–3 yrs	Survey	Intention to receive /use PN	Usefulness of a system valued PN more and enjoyment valued less when a GP provided advice than if used out of their own curiosity. Trade-off between perceived risk and usefulness * Recommend employment of qualified health professionals
Pavlidis *et al*. (2012)	Greece	N = 1504 51% female Aged < 35 yrs	Survey	Views on nutrigenomics	Majority thought that nutrigenomics should only be offered through health professionals not directly online Concern about results being interpreted incorrectly * Recommend qualified health professionals communicate results
Su and Lu (2012)	Taiwan	N = 258 63% Male	Online survey	Acceptance/preferences for nutrigenomics	Perceived benefit contributed to the acceptance of PN Hospital service provider preferred over direct sale and DIY * Promote benefits of PN. * Provide publicly funded PN in addition to commercial services
Morin (2009)	Canada	N = 90 Mixed gender Age: n/a	Focus groups – discourse analysis	Knowledge Attitudes to PN	Early diagnosis could lead to better diet and disease prevention Concern that validity of tests was not established a Potential breach of privacy of concern * Fund and regulate the use of research in PN. * Regulate data handling
Ronteltap *et al*. (2009)	Netherlands	N = 438 Mixed gender Age 40–60 yrs	Evaluation of videos of PN scenarios	Perceptions and acceptance of PN	Public acceptance of PN is enhanced if perceived a personal benefit, a supportive environment, and PN advice that can be easily incorporated into the daily routine. PN communication is preferred to be delivered by expert stakeholders * Promote benefits of PN. * Recommend qualified health professionals communicate results
Roosen *et al*. (2008)	Germany	N = 452 Mixed gender	Online survey	Attitudes to genetic	45% would agree to a genetic test to receive PN advice * Fund research to encourage the inclusion of nutrigenomic analysis in PN

				profiling and PN	
Brug *et al*. (1999)	Various	N = 8 (studies)	Literature review	Behaviour change theory (motivation, self-evaluation, agency)	Computer-tailored communications were more effective than nontailored interventions particularly for a reduction in dietary fat intake Difficult to draw firm conclusions given the limited number of studies and reliance on self-report data * Policies and research funding to enable digital solutions for PN

7.2.2 Aims and objectives of the VYPR consumer research study

The adoption of nutritional genomics within society faces many hurdles until it can be fully accepted and understood by the public. The current study intends to provide insight into the attitudes and acceptance of the UK population towards genomic-based nutrition, its services and activities, with consideration of how demographic factors may influence acceptance. This was approached by using a series of questions to ascertain consumer acceptance of genetic testing regarding health and wellbeing to elicit a response which can be compared to previous literature.

7.2.2.1 Results

In total, 8816 survey responses were collected from November 2017 and December 2017. Each question released on the VYPR application received a median response of 591±9. The study population between each question consisted of males (15-17%) and females (83-85%). The distribution of age group varied between questions, consisting of 18-24 (9-10%), 25-24 (40-42%), 35-44 (30-32%), 45-54 (11-13%) and 55+ (6-7%) respectively, (see table 7.2).

Table 7.2

Respondent characteristics for each VYPR survey

		Sex		Age				
Survey	n	Male	Female	18-24	25-34	35-44	45-54	55+
Genetic testing is important for the future of health science	585	90 (15%)	495 (85%)	53 (9%)	238 (40%)	183 (31%)	79 (13%)	40 (7%)
I would take a genetic test	590	87 (15%)	503 (85%)	52 (9%)	244 (41%)	185 (31%)	80 (13%)	37 (6%)
I would take a genetic test to improve my health	581	91 (16%)	490 (84%)	57 (10%)	235 (40%)	187 (32%)	74 (13%)	37 (6%)
Clean label food is important	580	86 (15%)	494 (85%)	57 (10%)	235 (40%)	181 (31%)	77 (13%)	38 (7%)
It is important to know what ingredients go into my food	586	87 (15%)	499 (85%)	57 (10%)	237 (40%)	183 (31%)	79 (13%)	38 (7%)
Diet can interact with my genetics to affect my health	586	91 (16%)	495 (84%)	61 (10%)	238 (40%)	182 (31%)	77 (13%)	36 (6%)
I would like to know if I am genetically at risk of a disease	579	89 (15%)	490 (85%)	58 (10%)	234 (40%)	184 (31%)	76 (13%)	36 (6%)
Do you find nutritional genomics (relationship between genome, nutrition and health) appealing	583	94 (16%)	489 (84%)	57 (10%)	237 (40%)	189 (32%)	74 (13%)	35 (6%)
Genetic companies keep all personal information confidential	566	91 (16%)	475 (84%)	55 (10%)	232 (41%)	180 (31%)	71 (12%)	34 (6%)
I would take a genetic test for the purpose of personalised nutrition	577	91 (16%)	486 (84%)	58 (10%)	236 (40%)	185 (32%)	72 (12%)	33 (6%)
Genetic tests for health should be free	571	91 (16%)	480 (84%)	54 (9%)	234 (40%)	184 (32%)	72 (12%)	34 (6%)
I feel I may have a food related intolerance	566	92 (16%)	474 (84%)	56 (10%)	232 (40%)	180 (31%)	71 (12%)	34 (6%)
I am health conscious	569	95 (17%)	474 (83%)	55 (10%)	240 (42%)	179 (31%)	67 (12%)	34 (6%)
I would like to know more about my genetics	570	95 (17%)	475 (83%)	54 (9%)	240 (42%)	182 (32%)	67 (12%)	34 (6%)
I would like to know more about nutritional genomics	565	96 (17%)	469 (83%)	54 (9%)	240 (42%)	179 (31%)	64 (11%)	34 (6%)

Respondents results based on demographics are shown in table 7.2 . Percentages in table 7.3 show agreement level within the different groups. Nearly two thirds (62.21%) of respondents agreed that genetic testing is important for the future of health science. This is supported by previous literature which reported that public interest in genetic testing and research was high (Sanderson *et al*., 2004; Wilde *et al*., 2009). More males (67.79%) than females (60.79%) agreed that genetic testing is important for the future of health science. Participants aged 55+ (67.50%) and 18-24 (66.04%) were more likely to agree that genetic testing is important for the future of health science whereas those aged 45-54 (56.25%) had the lowest agreement percentage of all groups. Just over half (55.11%) of the sample population agreed that they would take a genetic test. More males (57.48%) than females (53.67%) agreed that they would take a genetic test. The age group most likely to agree to a genetic test were those aged 25-34 (58.69%), and the least likely were those aged 45-54 (43.20%). The number of participants who agreed to take part in genetic testing increased when participants were asked if they would take a test to improve their health, with 60.47% in agreement. More males (65.94%) than females (58.17%) agreed that they would take a genetic test to improve their health. Respondents aged 55+ (67.57%) and 25-34 (62.14%) were most likely to agree to take a genetic test to improve their health, whereas respondents aged 45-54 (48.01%) were least likely. However, 55.06% of the population agreed that they would take a genetic test for personalised nutrition. More females (54.83%) agreed that they would take a genetic test for personalised nutrition than males (49.45%). Respondents aged 25-34 (62.29%) were more willing to take a genetic test for personalised nutrition whereas the age group 34-45 (46.19%) were least willing.

Similar studies reported that the younger demographic (18–34 years) were more likely to be interested in genetic testing, which also supports the present study's results which show a high acceptance level with those 18-34 years (Haga *et al*., 2012). Research found that those aged 65+ years had the highest indication of willingness to undergo genetic testing for personalised nutrition, which also supports the present study's results which show a high acceptance level in individuals aged 55+ years (Stewart-Knox *et al*., 2008). However, support and willingness varied little among demographic groups which suggest that the relationship between age and acceptance of genetic testing is complex and may depend on a variety of factors such as the area of genetic testing, location and prior knowledge of genetic research (Kaufman *et al*., 2008).

The motivation for the adoption of genetic testing has been reported to be due to curiosity and intent to find out personal health risks (Gollust *et al*., 2011). Furthermore, willingness to take part in genetic testing had previously been reported to be dependent whether the individual results would be returned to the participants (Kaufman *et al*., 2008). Previous studies also show that the subject area of genetic testing strongly influences public attitudes (Condit, 2010). The present study reports that when personalised nutrition is added as a service to a genetic test, no change in acceptance level occurred, whereas when the improvement of health is suggested, a higher number of participants were willing to take a genetic test. This may suggest that knowledge may be a deciding factor as the population may not be aware of the health benefits of personalised nutrition.

The present study found that around half of the population felt that genetic companies kept all personal information confidential. Almost half of both the male (49.45%) and female (48.85%) population agreed that genetic companies kept their personal information

confidential. Those aged 18-24 (39.99%) were more concerned with the privacy policies of genetic testing companies; whereas those aged 55+ (64.71%) were least concerned with privacy. This is supported by Mählmann *et al*., (2006) who found privacy-related concerns towards personal genomics in adults 60+ years was low. Results from the present study show a significant number of individuals are concerned about the security of their data. This is supported by previous studies who reported concerns about the sharing of their health data without their permission (Haga *et al*., 2012). When public expectations towards the benefits of genetic testing increased, more positive opinions were formed (Henneman *et al*., 2013). Attitudes were influenced by the assessment of the likely validity of genetic test results and its personal healthcare utility (Nicholls *et al*., 2013). Similar concerns were also reported, whereby educational needs in regard to the nature of risk/reward, cost, concerns about confidentiality of medical-related results, and access to personal genomic information affected both acceptance and attitude (Nicholls *et al*., 2013). The results from the present study present possible areas to build public confidence towards genetic testing, opening further market opportunities for the application of nutritional genomics. Similar concerns about the sharing of genetic data suggest the need for more transparency from researchers and organisations, along with more education and public information about genetic research (Lemke *et al*., 2010).

Around three-quarters of respondents (73.48%) agreed that a genetic test should be free. More females (73.38%) than males (69.23%) agree that genetic tests should be free. More participants aged 55+ (88.24%) and 45-54 (76.72%) agreed that genetic tests should be free; whereas participants aged 34-45 (67.22%) had the lowest agreement score. Previous studies support these findings reporting that 5% were willing to pay the current price of £250 for a genetic test whereas when the service was free of charge, this figure rose to 50%

(Cherkas *et al*., 2010). Cost is a key factor which influenced whether people decided to partake in genetic testing in 40% of cases (McGuire *et al*., 2009). The present study reports similar findings, suggesting that to maximise the acceptance of nutritional genomics, price is a key factor to consider.

Around two thirds (68.33%) of the population agreed that clean label food is important. More males (72.10%) than females (67.80%) agreed that clean label food is important. Participants aged 55+ (81.58%) had the highest percentage of agreement that clean label food is important. This was followed by the age group 25-24 (69.36%), whereas, those aged 18-24 (64.91%) had the lowest agreement score. Around three-quarters (76.57%) of participants agreed that it is important to know what ingredients go into their food. More females (78.75%) are in agreement with this statement than males (72.43%). Females may be more aware of the content of food, as statistics indicate that women are more responsible than men for all or most of the household shopping (68% compared with 32%) respectively (Prior, Phillips & O'Driscoll, 2014). Respondents aged 45-54 (82.50%) had the highest percentage of agreement score that it is important to know what ingredients go into your food, whereas participants aged 18-24 (70.18%) were least in agreement out of all the age groups. The results relate to previous studies which show a continuing trend towards food products which fall under the so-called “clean label” umbrella (Varela & Fiszman, 2013; Cheung *et al*., 2016; Joppen, 2006; Zink, 1997).

When participants were asked if they felt like they had some form of underlying food intolerance, 38.69% agreed with this. More females (38.18%) than males (36.95%) felt like they may have an underlying food-related intolerance. A higher percentage of respondents aged 24-35 (41.82%) felt that they may have underlying food-related intolerance whereas

those aged 18-24 (30.36%) had the lowest agreement percentage. Around two-fifths of the sample population reported an underlying food intolerance. Together with the overall prevalence of food allergies increasing globally, the use of genome anayisis may be of great assistance to evaluate the underlying causality relevant to food intolerances. The application of nutritional genomics may become of more importance in this area, especially when incorporating the previously mentioned “clean label” trend into the well-being aspect of health and functional foods (Sicherer & Sampson, 2014; Li, Maggadottir & Hakonarson, 2016).

With the present study reporting that 49.32% of the UK population sample agreed that they were health conscious and with 60.47% willing to undergo genetic testing to improve their health, nutritional genomics may provide a platform to implement more functional foods within an individual’s diet. Males (48.42%) and females (47.99%) similarly felt like they were health conscious. A greater percentage of 55+ year olds (76.47%) agreed that they were health conscious, whereas 18-24 year olds (41.82%) were shown to be the least health conscious.

Around half (54.32%) of the respondents agree that diet can interact with their genetics to affect their health and 51.45% agreed that the science of nutritional genomics was appealing. More males (54.95%) than females (51.71%) agreed that diet can interact with their genetics to affect their health. Participants aged 55+ (55.56%) had the highest agreement percentage towards the statement diet can interact with their genetics to affect their health, whereas participants aged 45-54 (48.70%) had the lowest score of the agreement. Nutritional genomics appealed to more males (54.26%) than females (49.49%). Participants aged 25-34 (56.11%) were more interested in nutritional genomics, whereas

participants aged 35-44 (44.15%) had the lowest interest. Knowledge and awareness are also important with only 13% of respondents aware of nutritional genomics (Cherkas *et al.*, 2010).

Around two thirds (64.99%) of the population would prefer to know if they were genetically at risk of disease. Both males (65.17%) and females (64.09%) would like to know if they were genetically at risk of disease. Of the age groups, more participants aged 55+ (72.22%) preferred to know if they were genetically at risk of a disease, whereas the age group 45-54 (50.65%) were less likely to want to know. Public views on genetics and genetic testing in Belgium reported that respondents expressed moderate interest in predictive genetic testing, with 39.1% willing to learn about their predisposition to diseases through genetic testing and 49.5% showing interest in getting tested specifically for preventable/treatable diseases (Chokoshvili *et al*., 2017).

The present study indicates a greater uptake when asked about the genetic risk of disease within a UK population. A total of 63.95% of the population agreed they would like to know more about their genetics and 53.80% of the population agreed they would like to know more about nutritional genomics. Both females (63.22%) and males (62.11%) agreed that they would like to know more about their genetics. More 25-34 year olds (69.16%) agreed they would like to know more about their genetics than all other groups. Participants aged 18-24 (55.56%) were least likely to want to know more about their genetics. Around half of females (53.41%) and males (51.05%) agreed that they would like to know more about nutritional genomics. More 55+ year olds (61.76%) agreed they would like to know more about nutritional genomics, whereas the age group least in agreement were those aged 35-44 (45.51%). These results show that more than half of the population sample may be willing to consider or participate in nutritional genomics. Knowledge and education may be a crucial

factor in gaining higher approval rates in the UK population. However, based on these results, attitudes towards testing and the ethics surrounding genetic testing resonate positively with a large portion of the sample population. Overall, public attitudes towards genetics are largely positive, albeit complex. Figure 7.3, Questionnaire results.

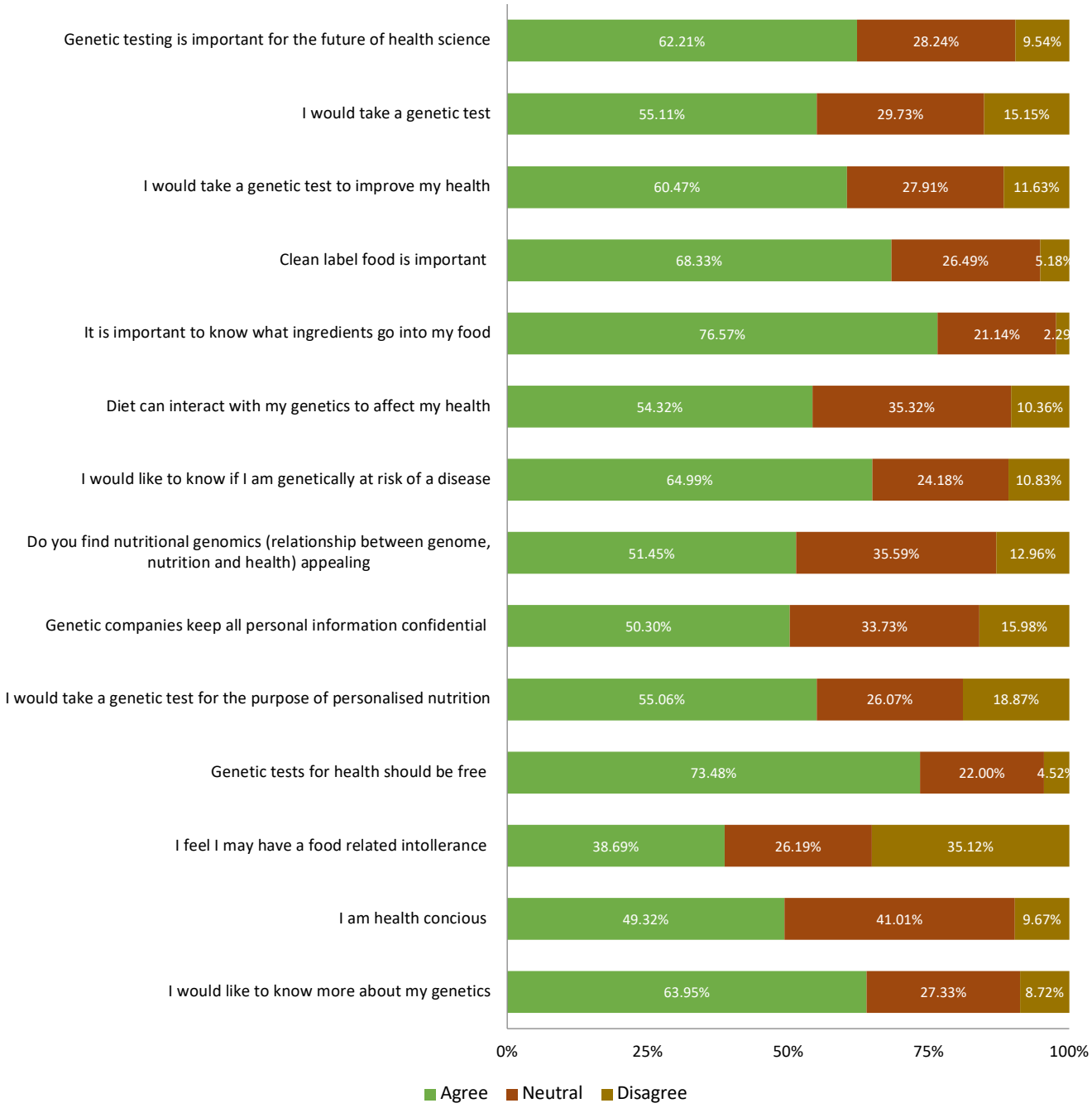

Figure 7.3 Responses to VYPR questions on nutritional genomics, its services and products of the UK population showing percentages for 'agree', 'neutral' and 'disagree'.

Collectively, males (58.45%) and females (57.62%) averaged a similar score throughout the VYPR survey thus indicating no noticeable differences between the two groups (see table 7.3). This result contradicts a previous study reporting that the attitude and awareness

towards personalised nutrition were higher among female participants, however, this study only studied the Indian population (Priyadarshini and Mahjabeen, 2017). Among all the age groups, those aged 55+ (65.50%) showed the highest interest in nutritional genomics and genetic testing, averaging much higher than all other groups; 25-34 (61.07%), 18-24 (55.52%), 35-44 (54.63%) and 45-54 (53.15%), respectively. Knowledge has previously been identified as a contributing factor which affects attitude towards genetic testing, whereby an increased level of knowledge produces more positivity towards genetic testing (Gollust *et al*., 2011; Henneman *et al*., 2004). Previous studies report that age was found to be associated with factual knowledge, with a higher age-related to a lower level of understanding of genetic knowledge (Calsbeek *et al*., 2007).

Table 7.3 Percentage response of 'agree' for each VYPR survey based on age and gender.

	Genetic testing is important for the future of health science	I would take a genetic test	I would take a genetic test to improve my health	Clean label food is important	It is important to know what ingredients go into my food	Diet can interact with my genetics to affect my health	I would like to know if I am genetically at risk of a disease	Do you find nutritional genomics (relationship between genome, nutrition and health) appealing	Genetic companies keep all personal information confidential	I would take a genetic test for personalised nutrition	Genetic tests for health should be free	I feel I may have a food-related intolerance	I am health conscious	I would like to know more about my genetics	I would like to know more about nutritional genomics	Average
Sex																
Male	67.79%	57.48%	65.94%	72.10%	72.43%	54.95%	65.17%	54.26%	49.45%	49.45%	69.23%	36.95%	48.42%	62.11%	51.05%	58.45%
Female	60.79%	53.67%	58.17%	67.80%	78.75%	51.71%	64.09%	49.49%	48.85%	54.83%	73.38%	38.18%	47.99%	63.22%	53.41%	57.62%
Age group (years)																
18-24	66.04%	57.70%	56.14%	64.91%	70.18%	50.83%	65.51%	50.88%	39.99%	56.90%	72.22%	30.36%	41.82%	55.56%	53.71%	55.52%
25-34	63.98%	58.69%	62.14%	69.36%	77.30%	55.03%	66.24%	56.11%	52.16%	62.29%	73.50%	41.82%	51.25%	69.16%	57.08%	61.07%
35-44	59.77%	53.58%	58.06%	66.11%	76.93%	50.27%	65.57%	44.15%	46.36%	46.19%	67.22%	37.99%	42.70%	59.11%	45.51%	54.63%
45-54	56.25%	43.20%	48.01%	69.24%	82.50%	48.70%	50.65%	45.33%	44.45%	47.95%	76.72%	31.95%	42.65%	57.34%	52.31%	53.15%
55+	67.50%	51.35%	67.57%	81.58%	86.84%	55.56%	72.22%	54.29%	64.71%	51.52%	88.24%	38.24%	76.47%	64.71%	61.76%	65.50%

7.2.3 Consumer preferences to functional foods (VYPR)

The majority of consumers were willing to purchase healthy meals which would be delivered to their door. Consumers favouring clean label products preferred homemade food significantly more than processed food made in a factory. Consumers had a neutral view of the use of science within their food, but dislike chemical or scientific nomenclature in the food ingredients lists. Convenience was also an important factor.

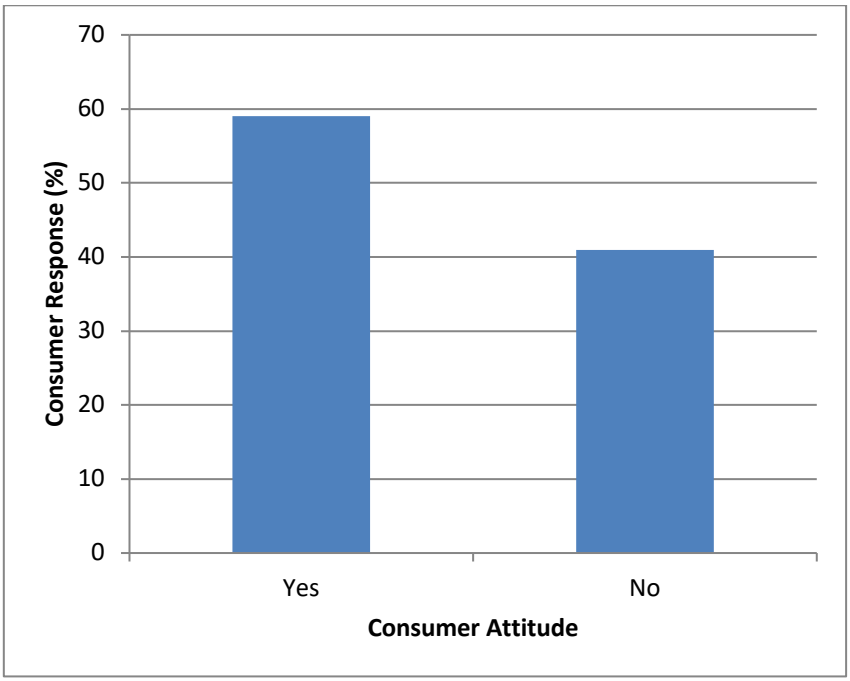

Figure 7.4 Would consumers buy healthy meals delivered to their door?

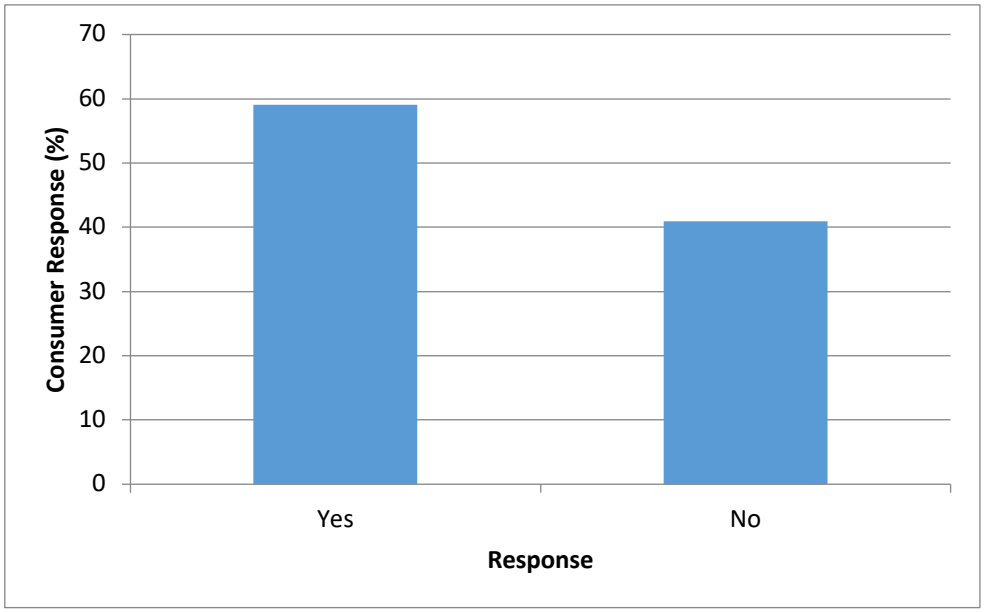

Figure 7.5 Do consumers regard clean label food products to be important?

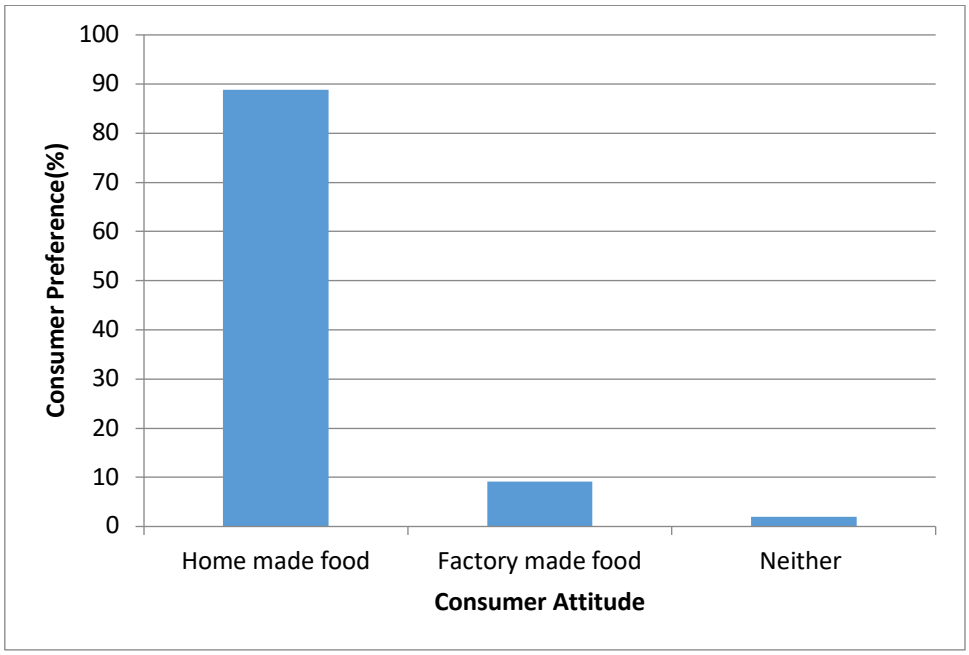

Figure 7.6 Do consumers prefer homemade food, factory made food or neither?

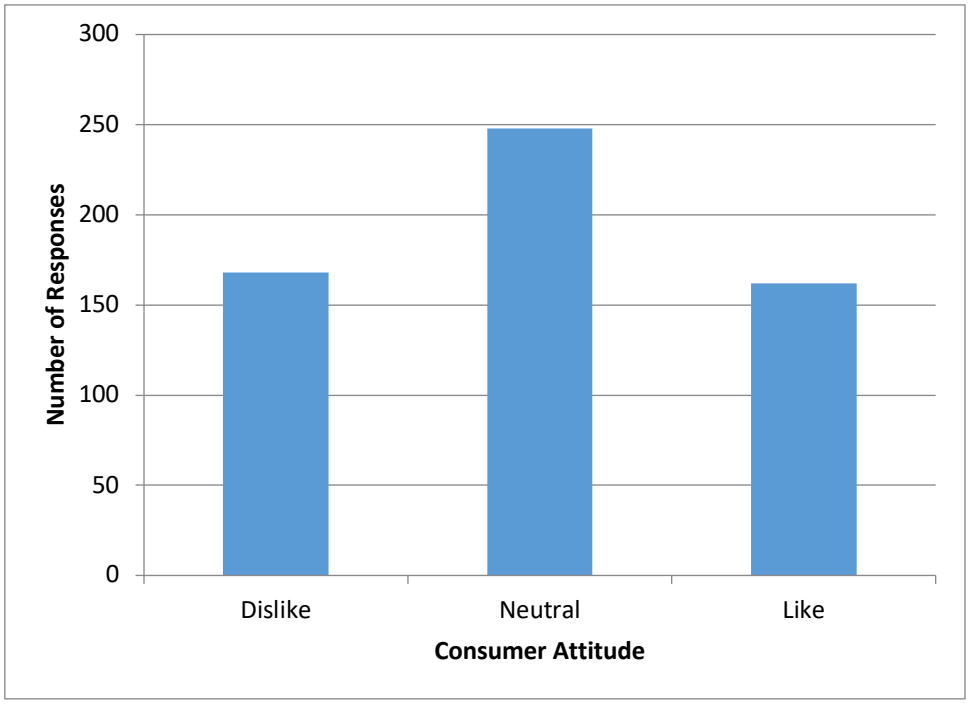

Figure 7.7 How do you consumers feel about the use of science in their food?

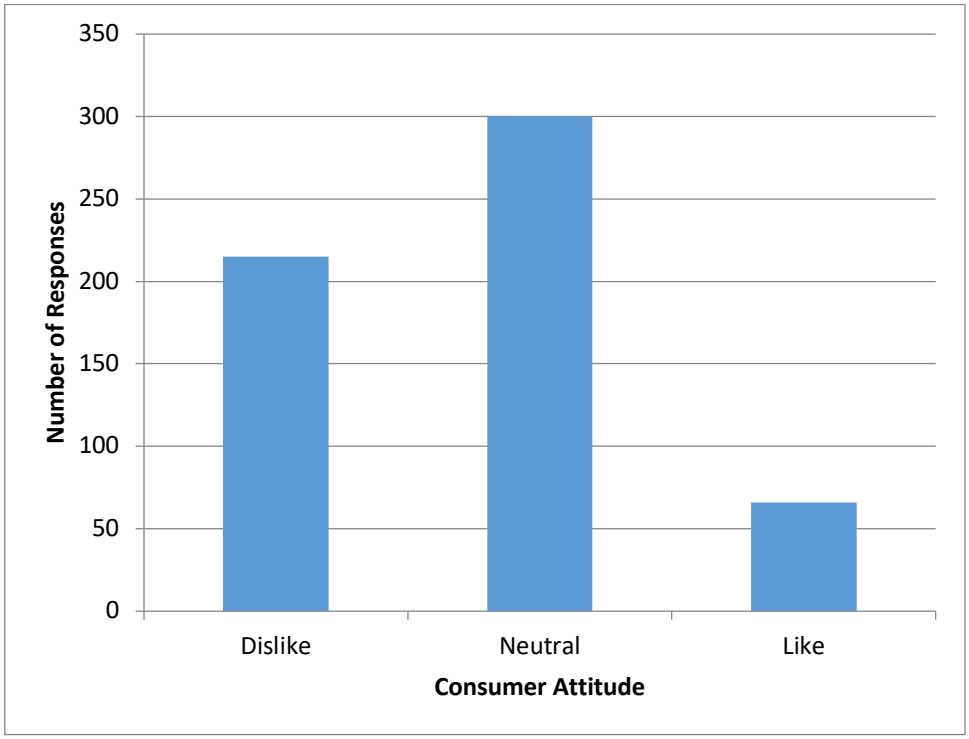

Figure 7.8 How do consumers feel when they see a chemical or scientific name in the food ingredients list?

7.2.4 Consumer preferences to personalised genomic nutrition

Statistical analysis and mean values were used to analyse the collected data from the VYPR questionnaire to identify which demographic is most likely to purchase a nutritional genomic product/service and their preferences for product/service.

Males aged 18-24 were most likely to purchase a nutritional genomic product/service. This was closely followed by males aged 25-34. As age increased the likelihood of buying a nutritional genomics product/service decreased. For the general population, consumers preferred a personalised nutrition plan with healthy meals delivered to their door.

Males aged 18-24 preferred a personalised nutrition plan with healthy meals delivered to their door. Males aged 25-34 also preferred a personalised nutritional plan with healthy meals delivered to their door.

7.2.4.1 Influence of demographic

Respondents answered a series of target questions to assess the effect of age and gender.

7.2.4.1.1 Effect of gender

Males (73.24±12.30%) were more likely to buy a personalised nutrition plan that required genetic testing than females (65.48±12.70%). However, statistical analysis revealed no significant difference between gender groups as determined by an unpaired t-test ($p>0.05$).

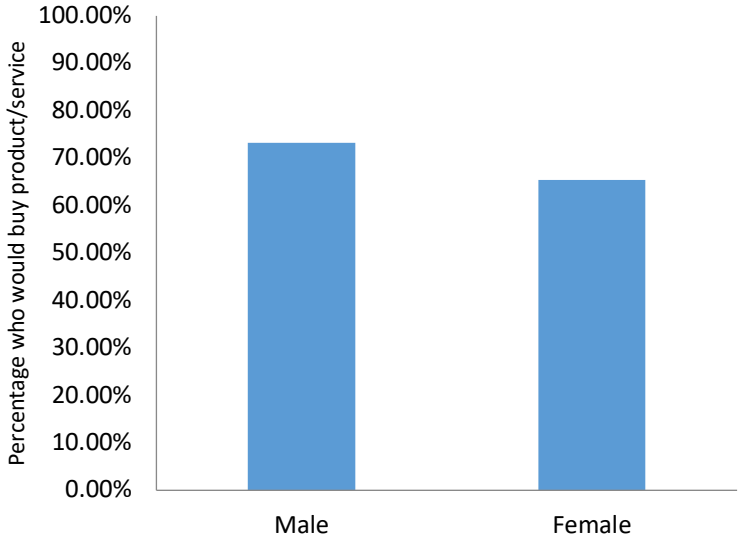

Figure 7.9 Effect of gender on the likelihood of purchasing a nutritional genomics product or service

7.2.4.1.2 Effect of Age

Individuals within the age group of 18-24 (72.81±12.92%) were more likely to buy a personalised nutrition plan that required genetic testing. As age groups increased, the likelihood of buying the product/service decreased; 25-34 (68.08±8.84%), 35-44 (65.33±14.72%) and 45-54 (63.42±16.53%), 55-64 (63.83±20.23%) and 65+ (63.61±26.25%), respectively. However, using a one-way ANOVA test, it was determined that there was no statistically significant difference between groups ($F(5,24) = 0.223$, $p>0.05$).

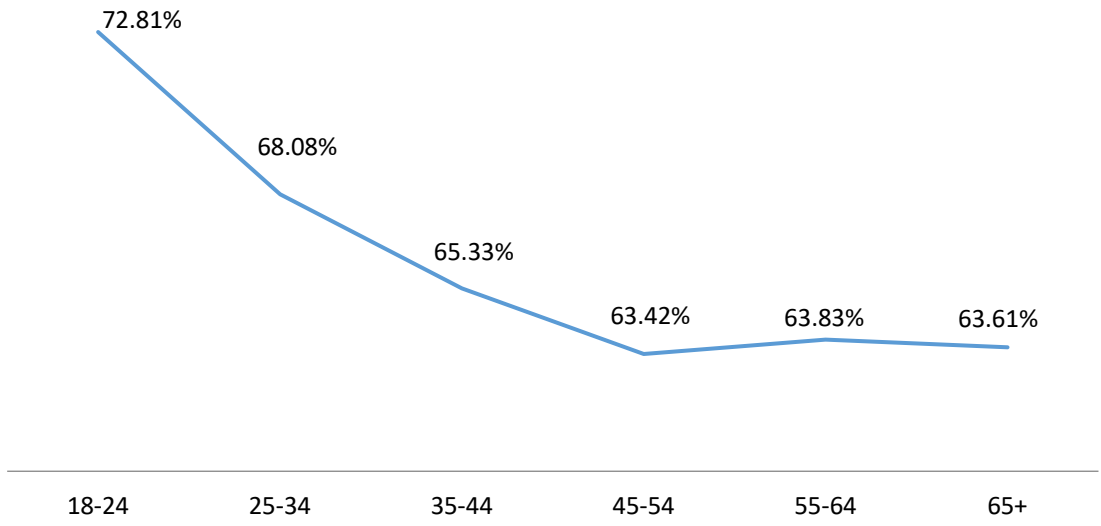

Figure 7.10 Effect of age on the likelihood of purchasing a nutritional genomics product or service

7.2.4.1.3 Gender and Age

Within the male population, those aged 18-24 (78.41±13.78%) were the most likely to buy the product/service. This was closely followed by males aged 25-34 (78.24±7.12%) and subsequently, males aged 35-44 (69.85±18.28%), 55-64 (69.28±24.03%) and 45-54 (62.64±13.92%). Using a one-way ANOVA test, it was determined that there was no statistically significant difference between groups ($F(4,20) = 0.832$, $p>0.05$). Due to the low number of responses for males aged 65+ ($n<5$), this group was not included in the analysis.

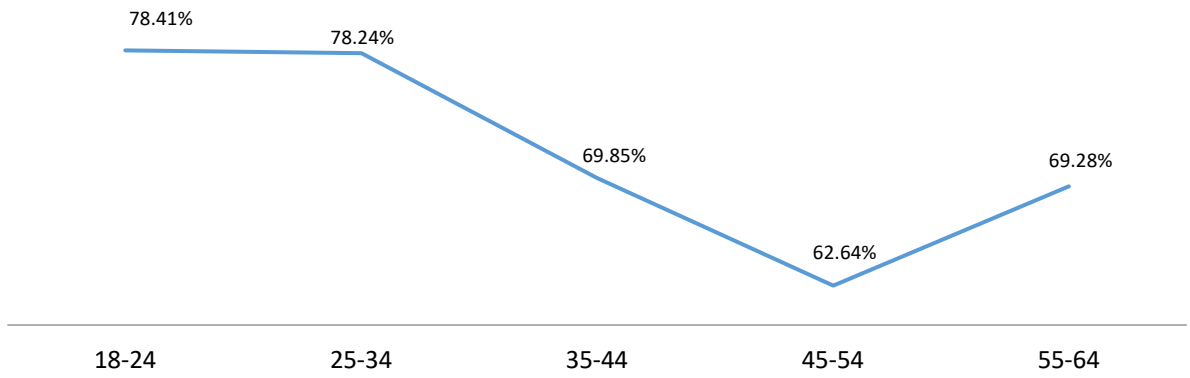

Figure 7.11 Effect of age on the likelihood of purchasing a nutritional genomics product or service within males

Within the female population, those aged 18-24 (70.05±13.55%) were more likely to buy the product/service. Subsequently, as age increased the likelihood of buying the product/service decreased; 25-34 (66.43±9.16%), 35-44 (64.37±14.76%), 45-54 (62.97±17.76%), 55-64 (60.64±20.47%) and 65+ (55.17±27.10%). Using a one-way ANOVA test, it was determined that there was no statistically significant difference between groups ($F(5,24) = 0.399$, $p>0.05$).

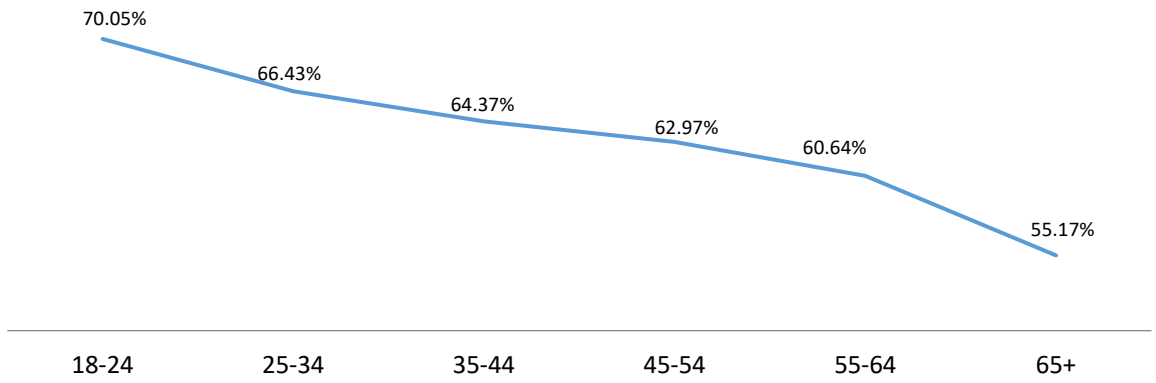

Figure 7.12 Effect of age on the likelihood of purchasing a nutritional genomics product or service within females

Males aged between 18 -24 are most likely to buy a nutritional genomic product/service. Preference tests were used to assess what product/service consumers would choose out of the given options.

Question: What product/service would the general population prefer?

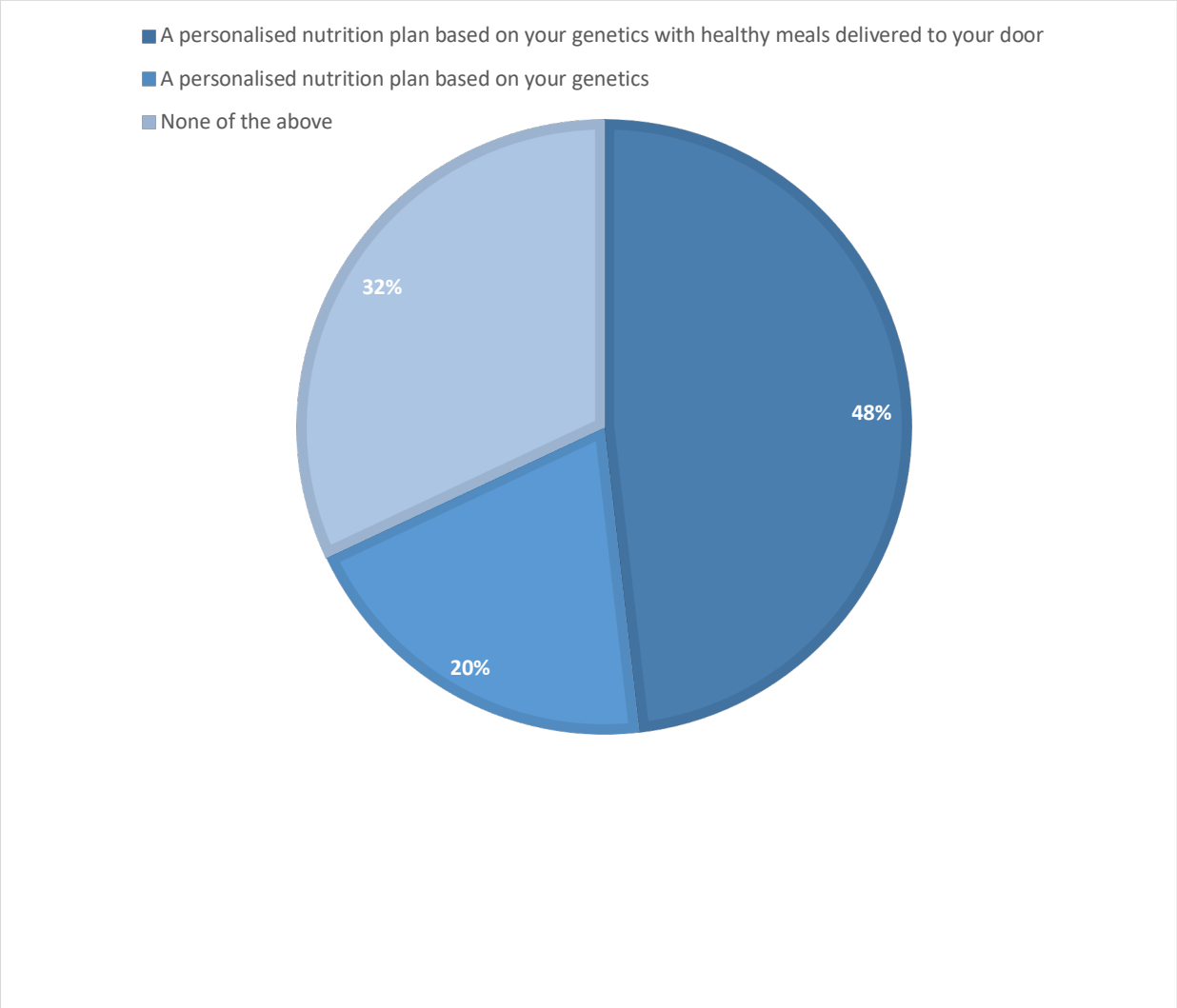

Figure 7.13 Preference of the general population towards a personalised nutrition plan with meals delivered or not delivered.

Question: What product/service would males aged 18-24 prefer?

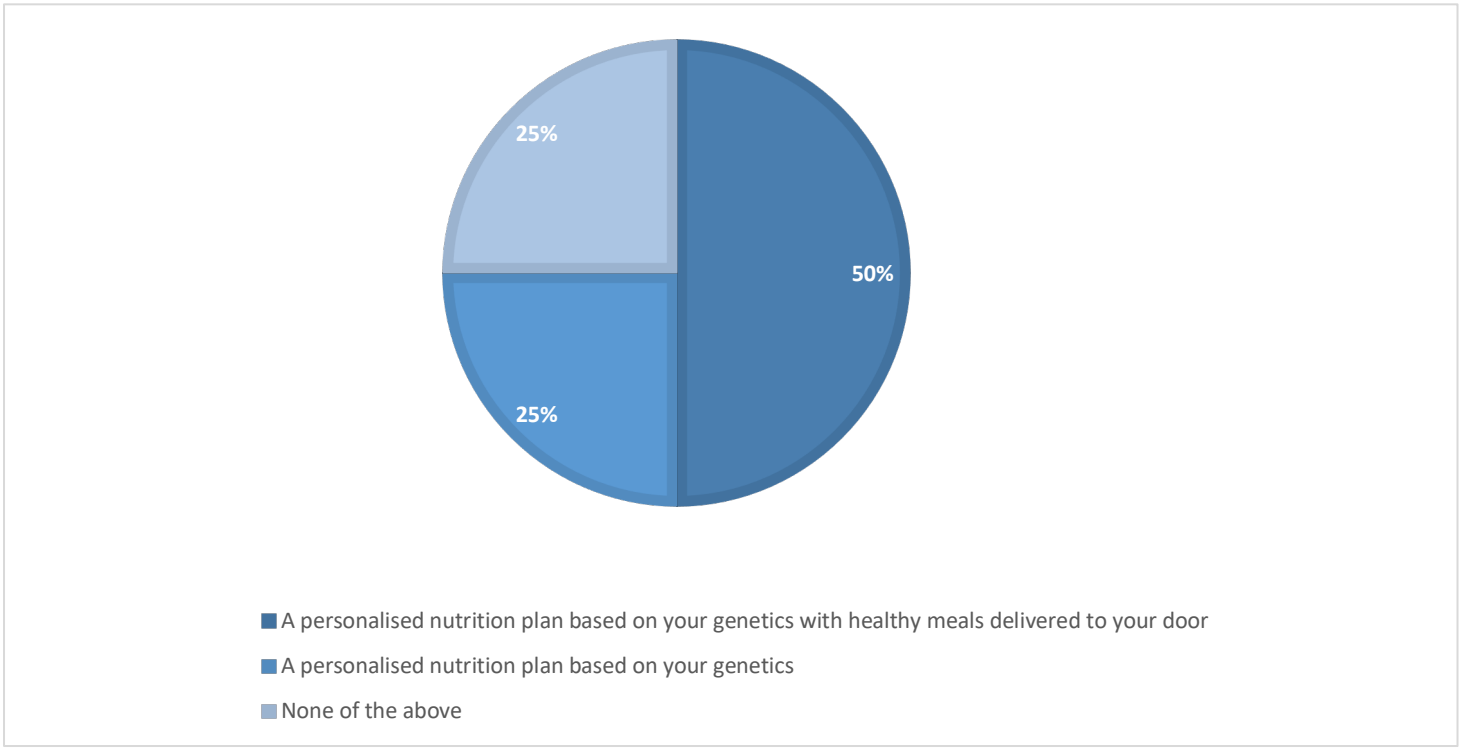

Figure 7.14 Preference of males aged 18-24 towards the option of healthy meal delivery as part of a personalised nutrition plan

Question: What would males aged 25-34 prefer?

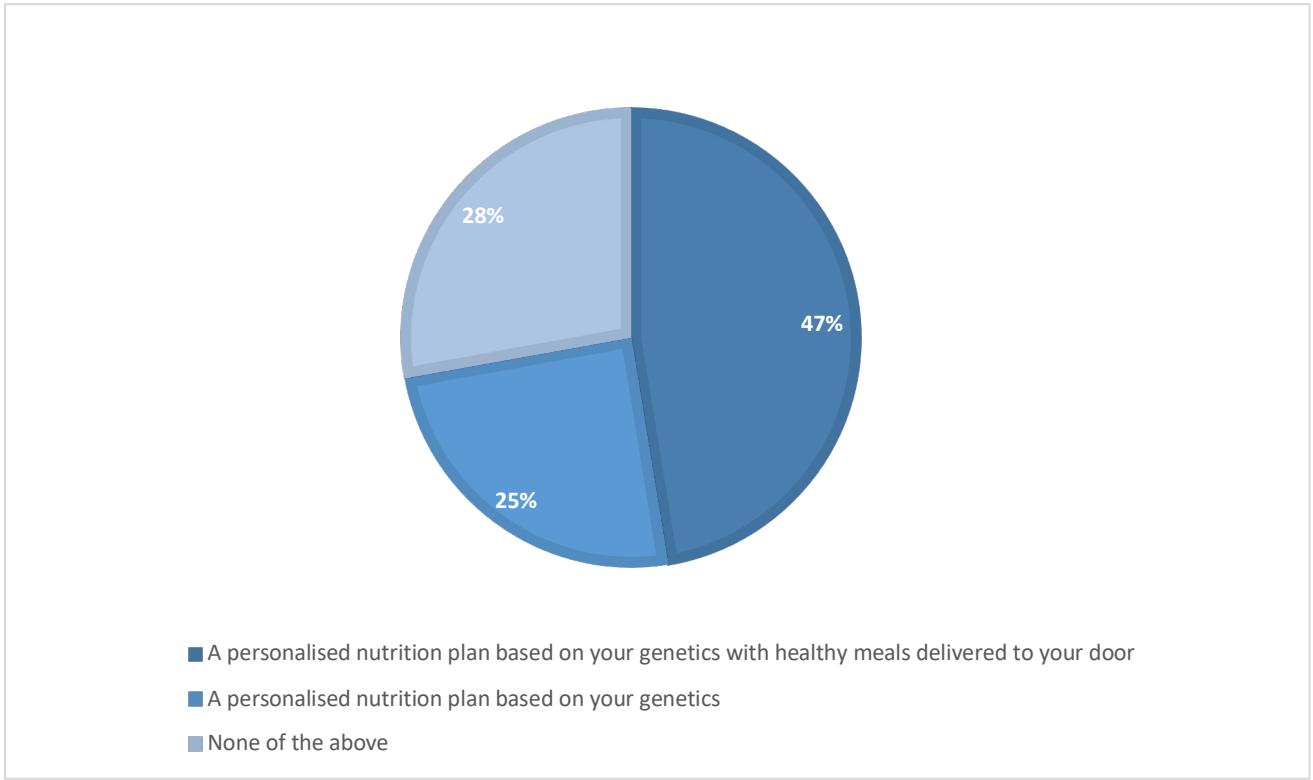

Figure 7.15 Preference of males aged 25-34 towards the option of healthy meal delivery as part of a personalised nutrition plan

The present study aimed to assess UK consumer's attitudes and acceptance towards nutritional genomics, its services and activities with consideration on how demographics affect these factors. Results showed that the UK population had a high level of interest in nutritional genomics. Demographic factors did affect attitudes and acceptance, with those aged 55+ showing the most agreement towards nutritional genomics; this was followed by the age group 25-34 years. Gender did not affect overall response agreement towards the survey. The results of the present study suggest a positive overall outlook towards genomic-based nutrition, its services and activities within the UK population. Previous research suggests that knowledge and education may aid in mainstreaming genomic-based nutrition to the public as knowledge is reported to be a deciding factor in consumer acceptance of genetic testing. This promotes the importance of public health education programs for genomics, which together with the NHS funding more genomic centres may provide potential savings for the NHS through early disease prevention. Education may be imperative to ensure that the public is interested in this continually evolving science which has the potential to bring significant savings to the health service within the UK.

Several limitations are worth noting. This survey was only distributed through a mobile application to individuals who voluntarily participated in the survey research through VYPR. Therefore, it is possible that a selection bias occurred in the sample surveyed. The demographics that responded to the VYPR survey were not evenly distributed, with those between 18-24 representing 9-10% of the sample and ages 55+ representing 6-7% of the population sample. Therefore, the results are not representative and cannot be applied to the general population. Lack of understanding of technology may have prevented elderly

consumers participating in the survey. Similarly, the distribution of gender was unequal with more female's responding to the survey than males.

Further research should consider additional demographic factors such as income, education level and ethnicity to gain a greater understanding of the complex reasons which affect attitudes towards genetic testing and nutritional genomics. A more varied method of distributing the survey would be recommended to avoid sample bias.

7.2.4.2 Market Research

The VYPR platform was used to assess customer attitudes, choice and preference towards the business activities of nutrigenomic companies.

7.2.4.2.1 Consumer needs assessment

Using the VYPR consumer assessment tool and a number of business model parameters were tested. Respondents answered the following question.

Question: Would you purchase a simple DNA test to improve your health?

- Result: Yes: Female 66.8%/ Male 68.5%

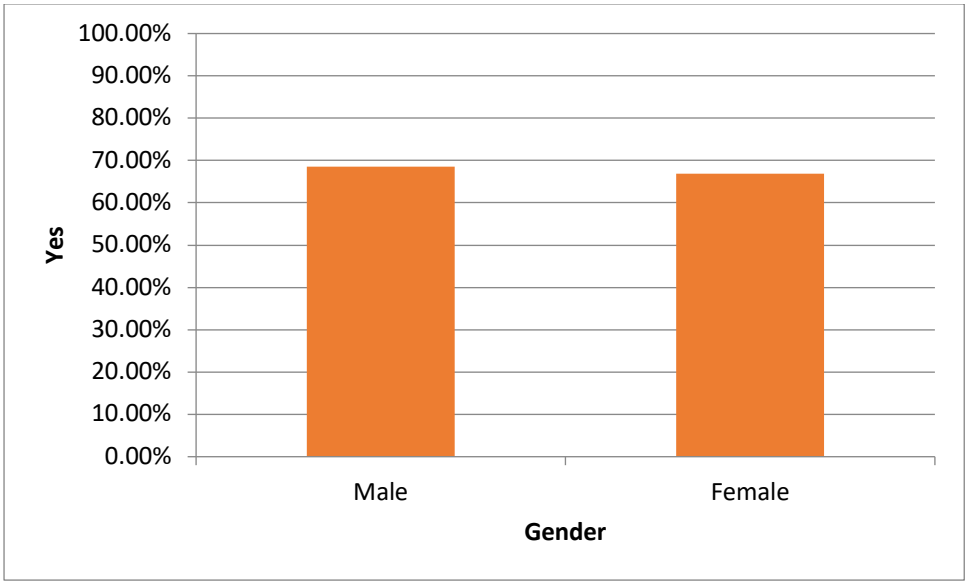

Figure 7.16 Likelihood of trying a free DNA test to improve health based on gender

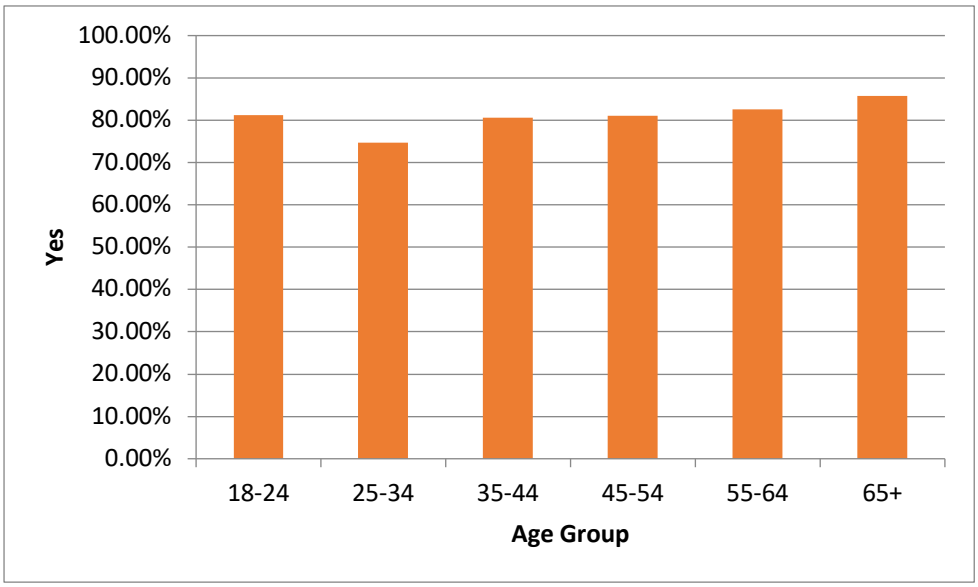

Figure 7.17 Likelihood of trying a free DNA test to improve health based on age group

- Comments: when the DNA test is free more respondents would be willing to try it than when it is not free (67.07%). Age group 25-34 were least likely to try a free DNA test. Males are more likely to try than females.

Question: Take a free simple DNA test that helps you lose weight?

- Result: Yes: Female 77.7%/ Males 85.9%

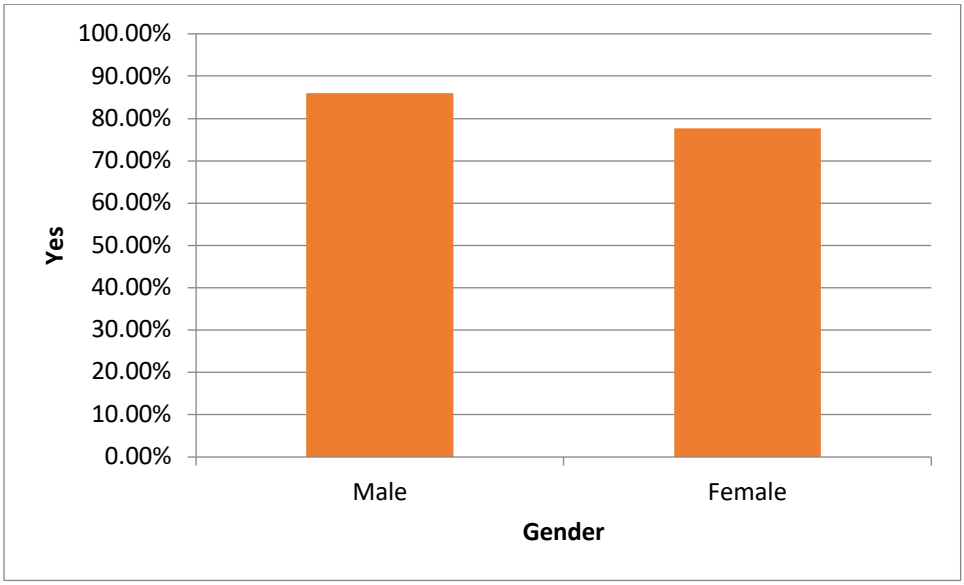

Figure 7.18 Likelihood of trying a free DNA test which helps you lose weight based on gender

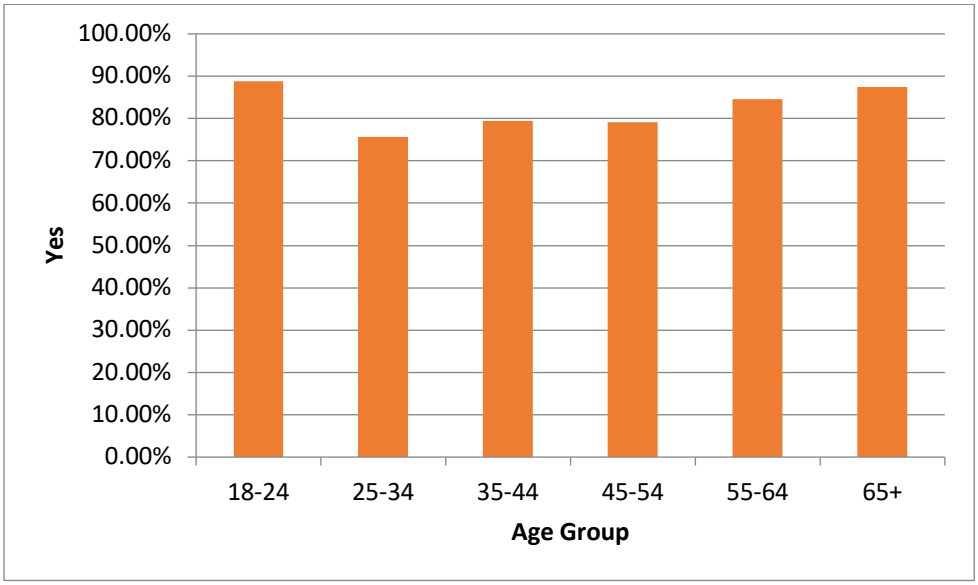

Figure 7.19 Likelihood of trying a free DNA test which helps you lose weight based on age group

- Comments: When weight loss was mentioned, more people would be interested in taking a DNA test. Similarly, age group 25-34 were least likely to try a DNA test. Again, males were more likely to try a DNA test.

Question: Would you purchase a personalised nutrition plan based on your genetics?

- Result: Yes: Female 57.9%/ Male 48.4%

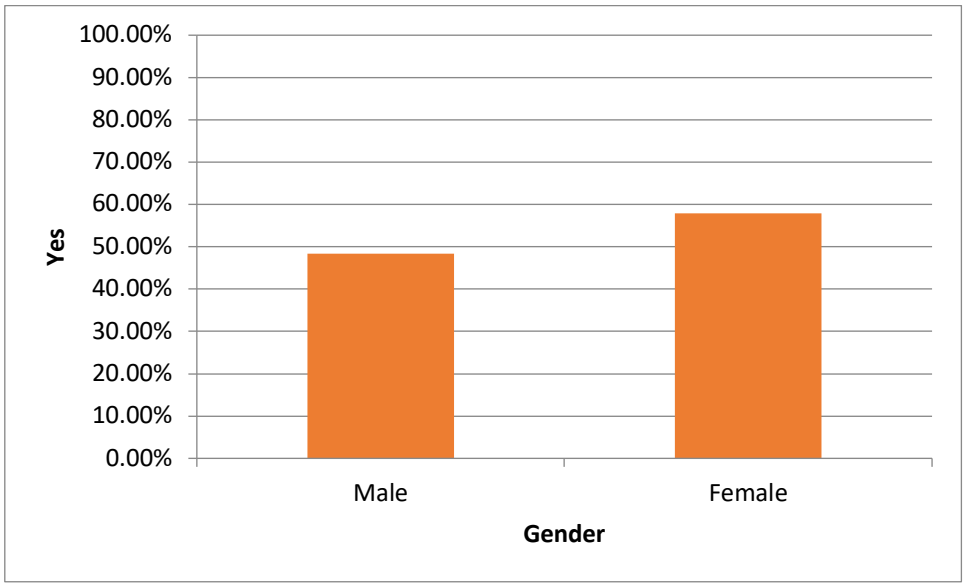

Figure 7.20 Impact of gender on the likelihood of buying a personalised nutrition plan based on genetic test.

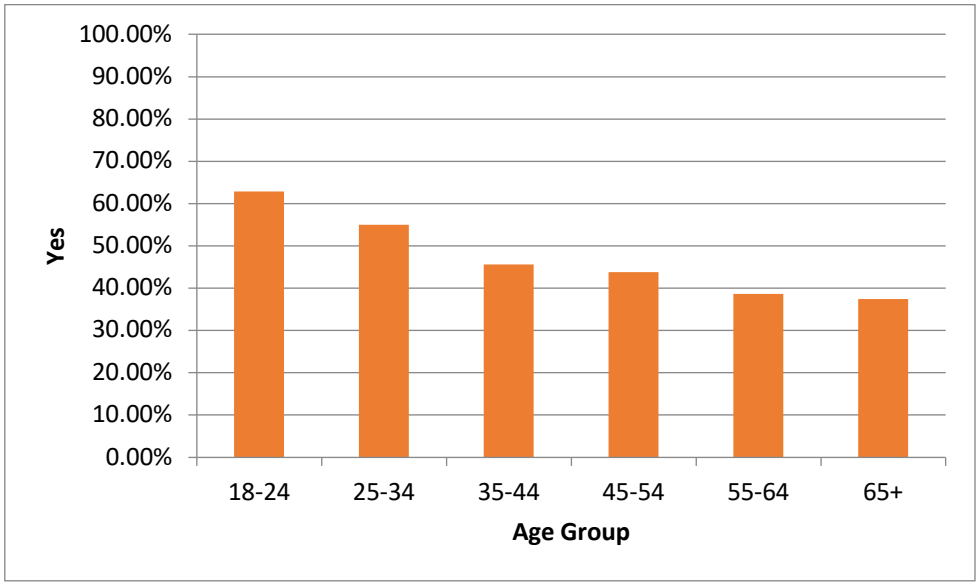

Figure 7.21 Impact of age group on the likelihood of buying a personalised nutrition plan based on genetic test.

Comments: As age increases the likelihood of buying a personalised nutrition plan decreases. Males would be more likely to buy a personalised nutrition plan.

Question: Would you be willing to undergo genetic testing for personalised nutrition?

Result: Yes: Female 67.0%/Male 57.6%

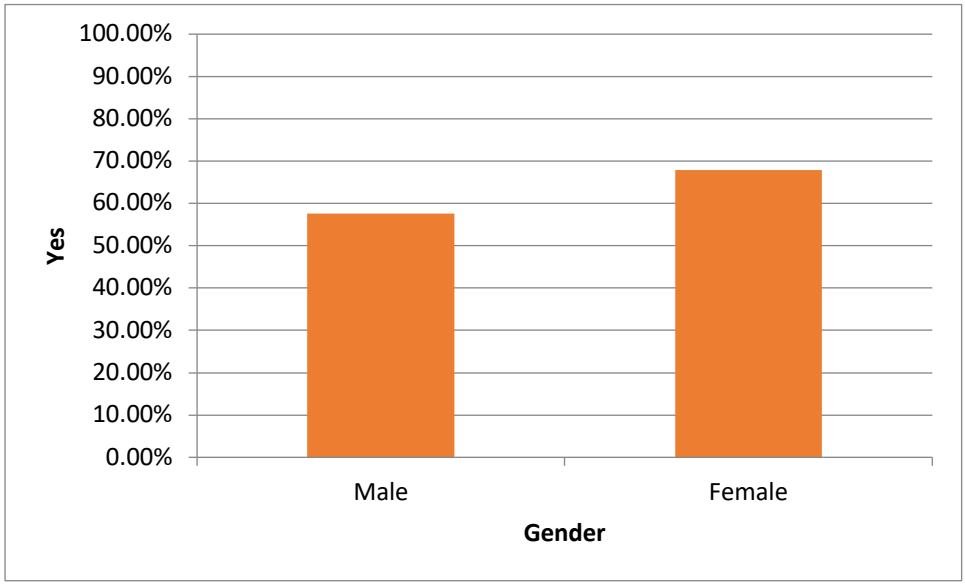

Figure 7.22 Impact of gender on a willingness to undergo genetic testing for a personalised nutrition plan

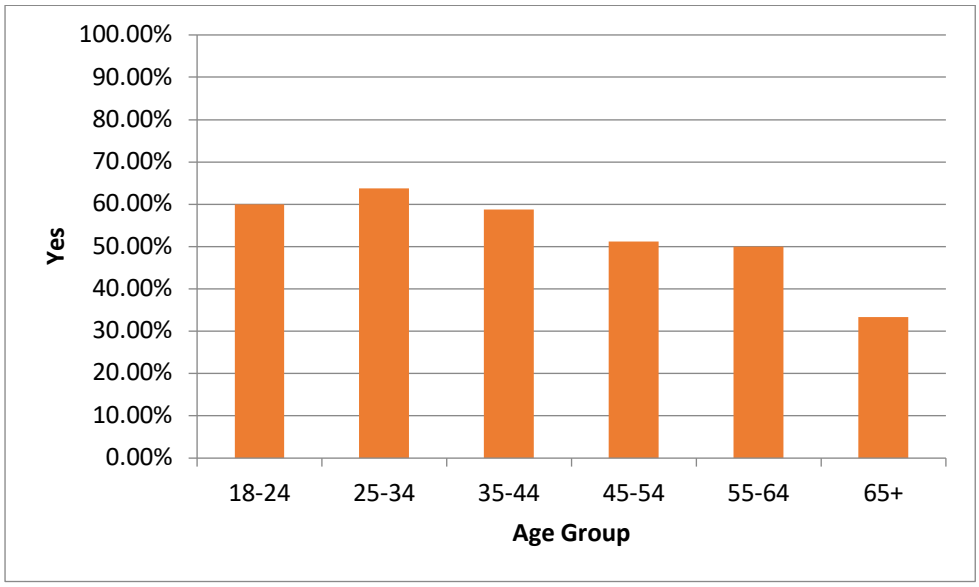

Figure 7.23 Impact of age group on a willingness to undergo genetic testing for a personalised nutrition plan.

- Comments: Females were more likely to undergo a genetic test than males. The age group 25-34 were most likely to undergo a genetic test for a personalised nutrition plan.

Question: Preference tests - out of the following options, which would you prefer?

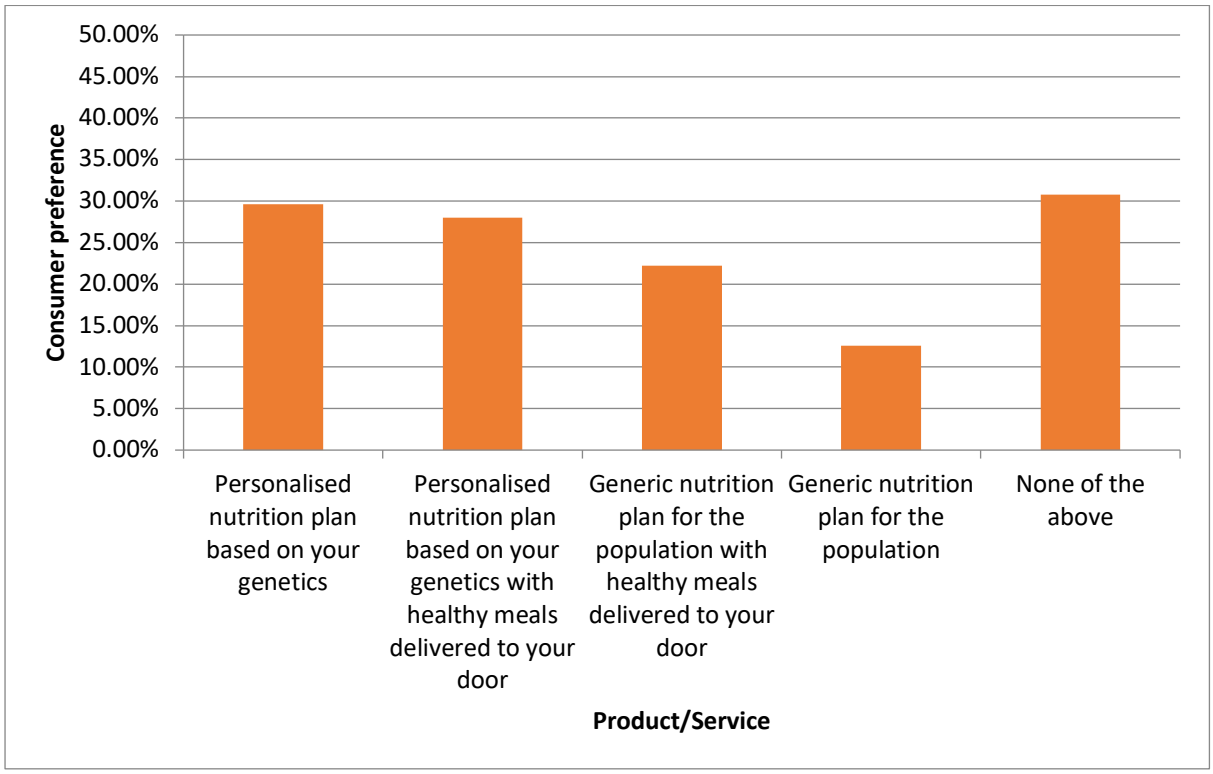

Figure 7.24 Assessment of consumer preference between personal nutrition and a general nutrition plan

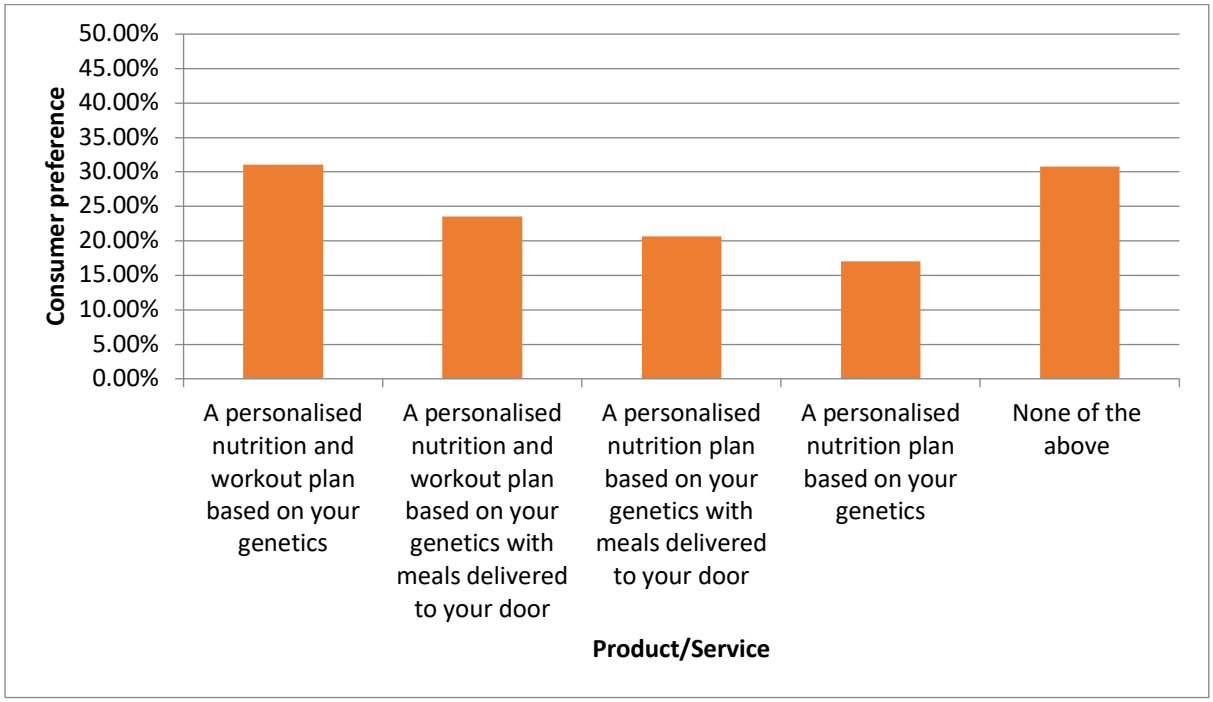

Figure 7.25 Assessment of consumer preference based on different services/products which tail personal nutrition based on genetic testing.

- Comments: In both instances, none of the above was the preferred option. However, out of the services/products available, consumers preferred a personalised nutrition plan over a general nutrition plan. Of the latter survey, consumers preferred a personalised nutrition and workout plan based on their genetics the most. Following this, a personalised nutrition and workout plan based on genetics with meals delivered was the second most preferred option indicating that consumers may prefer a full more encompassing package as opposed to a single service/product. Throughout the surveys, the younger generations seem more accepting and interested in genetic testing for a personalised nutrition plan. When key words were used such as weight loss, the older generations were more accepting of providing genetic information.

Chapter 8

Nutrigenomics Businesses

8.1 Aims of the study

This report assesses the background and business models of personalised nutrition regarding nutrigenomics. Additionally, a brief initial market analysis of customer perception towards nutrigenomics was performed. Finally, elements of a nutrigenomic business model were identified along with a SWOT (strengths, weaknesses, opportunities and threats) analysis. An assessment was undertaken of the potential commerciality for start-up businesses allowing a critique to be formed based on the previously mentioned research highlighting the importance of a strong business model. The evaluation considers the approach of a nutrigenomic start-up company which will provide genetic testing, personalised nutrition, exercise advice and supply of healthy meals.

The focus of the Evaluation

- Information on personalised nutrition in the marketplace.
- Competitor assessment.
- Consumer acceptability of nutrigenomics.
- Review of business activities and business models.
- Hypothetical SWOT analysis of nutrigenomic start-up.

8.2 Personalised nutrition in the market

Personalised nutritional advice based on individual genomics would fit into an emerging trend in the marketplace, where customer-supplier relationships are increasingly moving from a commodity model towards a personalised model (Sutton, 2007). Additionally, in the context of personalised nutritional advice, several studies have suggested that tailoring nutrition

advice may be more efficient for influencing individual dietary behaviour more than mainstream advice (Brug *et al*. 1999, 2003; Elder *et al*. 2009; Oenema *et al*. 2001; Lustria *et al*. 2009). Personalised nutritional advice can be described as a process with four consecutive stages (Vesanen and Raulas 2006).

1. The consumer is willing to release personal information that is sufficiently diagnostic to another party.
2. The other party can use this diagnostic information as a basis for developing personalised nutritional advice.
3. The customer is willing to incorporate that personalised nutritional advice as a basis for food choices.
4. If the consumer believes that the personalised advice is sufficiently rewarding over and above the generic nutritional advice, a learning process can be initiated in which a certain level of habit forming behaviours is likely to occur.

In these stages of interaction between customers and suppliers, personalised nutrition advice can add benefits to the value exchange (Van Trijp and Ronteltap 2007). Furthermore, the latter stages can open opportunities for subscription-based models whereby the consumer may be inclined to continue on a rewarding path avoiding self-interpretation of large amounts of nutrition information. Consumers may also feel a sense of pride and ownership based on the co-design of their personalised diet based on providing their personal and genetic information (Piller & Muller, 2004). Personalised nutrition in the competitive health marketplace may provide a differentiation from commodity type competition, thus generating added value (Ghosh, 2009). The move away from the dominant business model that applies to a population-based nutrition approach to the personalised nutrition model is

at the early stages of commercial success (Saukko *et al*., 2010). According to Ronteltap & Van Triip (2007), for personalised nutritional advice to develop, successful commercialisation of the proposition is essential and the use of genetic profiling to provide personal nutrition advice can encompass two business models:

1. ‘‘Test and run to the finish’’: This business model provides to the consumer relevant feedback on progress towards health improvement on relevant biomarkers, both non-invasive and invasive phenotypic measures. A key feature is an iterative feedback loop that assures follow-up of individual progress and the possibility to adjust the dietary advice accordingly.
2. ‘‘All-in lifestyle guidance’’: This business model extends the ‘‘test and runs to the finish’’ in two directions. It includes genotypic information next to dietary intake data and phenotypic information as a source of personalised advice and monitoring for goal attainment. The personalised advice is also broader in scope; it includes other lifestyle factors in addition to dietary improvements such as activity level or stress/ time management. A key feature is the inclusion of genetic information for holistic change management.

Using a business model approach which delivers; what customers want, how they want it, and how the company should be organised to best deliver those consumers needs while maintaining profitability may be the best route to commercial success.

8.3 Competitor profiling

A selective review of the business models was undertaken for two UK companies, one EU company and two USA company; DNA fit, Fitnessgenes, Karmagene, 23 & Me, and Habit. All with overlapping and differentiated applications of personalised genetic information.

8.3.1 DNA fit

- Segments Served: Individuals who are concerned about fitness and diet and also others who are concerned about their health and well-being.
- Services Offered, Pricings and Discounts: Diet info (£99-199), fitness Info (£149), diet and fitness info (£249). For 23andMe users – Diet info (£49), fitness info (£49-79), diet and fitness info (£119). Fastest turnaround once samples have been provided (10 days).
- Advertising and Campaign Themes: Very sport-based, post their research on social media and promote their sponsorships (Egyptian football team), post a health-related blog.
- Channels of Distribution: Website, social media, offer a free 14-day guide on how genetics impact every aspect of fitness and nutrition. Linked with 23&me and Ancestry.com.

Website - https://www.dnafit.com/.

8.3.2 FitnessGenes

- Segments Served: mainly individuals who are concerned about fitness and diet – more sport focused.
- Services Offered, Pricings and Discounts: Genetic Reload System - *The world's #1 personalised diet and fitness plan based on your DNA - created by FitnessGenes CEO Dr Dan Reardon*. (£199), get fit (starter £149, keep fit £169, complete £199), lose weight (starter £149, momentum £169, complete £199), get lean (starter £169, plus £199, complete £239),

build muscle (starter £199, Scott Herman £279, complete £339). Sign up for the newsletter and receive 10% off next purchase.

- Advertising and Campaign Themes: Q&A by CEO on social media on various health topics, recipe videos.
- Channels of Distribution: Website, social media, newsletter.

Website - https://fitnessgenes.com/

8.3.3 23andMe

- Segments Served: Health and ancestry.
- Services Offered, Pricings and Discounts: Personal genome service - £149.
- Advertising and Campaign Themes: TV adverts.
- Channels of Distribution: Website, social media, newsletter.

Website - https://www.23andme.com/en-gb/

8.3.4 Karmagenes

- Segments Served: Integrates genetics and psychology – career orientation, partnerships and relationship, personalised wellbeing
- Services Offered, Pricings and Discounts: Full personality report ($199), customisable option ($12.95-$19.95).
- Advertising and Campaign Themes: Newsletter.
- Channels of Distribution: Website, social media, newsletter.

Website - http://karmagenes.co/

8.3.5 Habit (US-based company)

Insight into a US-based company which provides a similar service to that proposed in the present report – providing genetic testing, personalised advice and healthy meals.

The protocol Habit provide begins a Nutrition Test kit to assess an individual's DNA, Bloodwork and Body Metrics to see how their body processes food in real time. From this, biology results and a nutrition plan is created which can be tailored to meet consumer activity and goals. After the test results are produced, customers can take advantage of Habit's holistic offering including Fresh Meal delivery tailored to their biology, personal nutrition coaching and digital products.

- Segments Served: Genotype analysis, personalised nutrition programme, fresh personalised meals.
- Services Offered, Pricings and Discounts: Habitcore ($199), meals – $7.99 per breakfast, $13.99 per lunch or dinner (only delivered in the San Francisco Bay Area). Meal plan subscription renews automatically every week. Integrated with Fitbit. Ongoing coaching from dietitians available.
- Advertising and Campaign Themes: Social media, sustainable packaging.
- Channels of Distribution: Website, social media, email letter, blog.

Website - https://habit.com/home2

8.3.6 Other personal genomic companies.

Table 8.1 Further companies within the international marketplace:

Company	Location	URL	Competitors	Price
23andMe	US	https://www.23andme.com/en-gb/	Ancestry/health	Ancestry £79 Ancestry and health £149
23mofang	China	https://www.23mofang.com/	admixture, deep ancestry, health and traits	¥ 399-1499
24genetics	Spain	https://24genetics.com/en/	admixture, exome sequencing, health, paternity, pharmacogenetics, whole genome sequencing	$199-399
African Ancestry	US	http://www.africanancestry.com/home/	deep ancestry	$274-680
Atlas	Russia	https://atlas.ru/	deep ancestry, diet, microbiota,	rub 9900-29900

			health and traits, sport	
Atlasbiome	UK	https://atlasbiomed.com/	microbiome, health, nutrition, ancestry, personal sport traits	£125-149
Dante labs	US/EU	https://www.dantelabs.com/	exome sequencing, health, whole genome sequencing	$250-649
DNA consultants	US	https://dnaconsultants.com/	admixture, deep ancestry	$50-539
DNA worldwide	UK	https://www.dna-worldwide.com/	paternity, immigration, ancestry, extended family, drug & alcohol	£99-429.60
DNAFit	UK	https://www.dnafit.com/	Fitness/diet	£99-249
DNA health	UK	http://www.dna-health.co.uk/home/	Health	£144-540
EasyDNA	UK	https://www.easydna.co.uk/	Paternity, relationship, ancestry, clinical, health, animal	£99-299

Everlywell	US	https://www.everlywell.com/	Health, fitness., well-being	$199-399
Family tree DNA	US	https://www.familytreedna.com/	admixture, deep ancestry, genealogy	$69-199
FitnessGene	UK	https://fitnessgenes.com/	Fitness	£149-339
Genebase	US	https://www.genebase.com/	deep ancestry, genealogy	$119-867
Geneticoncpt	US	https://geneticoncept.com/index.html	Health, fitness, well-being	$134-279
Genos	US	https://genos.co/	whole exome sequencing	$499
Genotek	Russia	https://www.genotek.ru/	admixture, genealogy, diet and fitness, family planning, health, talents and sports	rub 19990-99000
Guardiome	US	https://www.guardiome.com/	admixture, whole genome sequencing and interpretation	$3100
Habit	US	https://habit.com/home2	Diet/meal	$299 Breakfast $8.99

				Lunch/dinner $13.50
Helix	US	https://www.helix.com/	Ancestry, entertainment (DNA scarf), family, fitness, sleep	
Igenea	Germany	https://www.igenea.com/en/home	admixture, deep ancestry, genealogy	£159-1099
Karmagene	US	http://karmagenes.co/	Personality	Basic personality report $199 Full personality report $298
LivingDNA	UK	https://www.livingdna.com/en-gb	admixture, deep ancestry	£99
My heritage	UK	https://www.myheritage.com/dna	admixture, genealogy	£45
Prenetics	China	https://prenetics.com/	Fitness, health, family	
Right-angled	UK	https://www.heartdnatest.com/index.asp	Nutrition, drug response, health	£79-169

Roots for real	UK	http://www.rootsforreal.com/	admixture, deep ancestry	£195-390
Suregenomics	US	http://www.suregenomics.com/	whole genome sequencing and interpretation	$2500
Ubiome	US	https://ubiome.com/about-us	Microbiome	Gut kit $89 Gut time lapse $199 Five site kit $399
Veritas Genetics	US	https://www.veritasgenetics.com/	whole genome sequencing and interpretation	$999
Viome	US	https://www.viome.com/	Microbiome	Annual plan £399
WeGene	China	https://www.wegene.com/	admixture, deep ancestry, health, sports, traits	¥9999
xcode	India	https://www.xcode.in/	Health, fitness, well-being, medicine	$100-150
Yoogene	China	http://www.yoogene.com/	deep ancestry - YSTRs and mtDNA	¥299-2699

8.4 Nutrigenomic business activities – Process flow

Selected Genomic Companies Activities

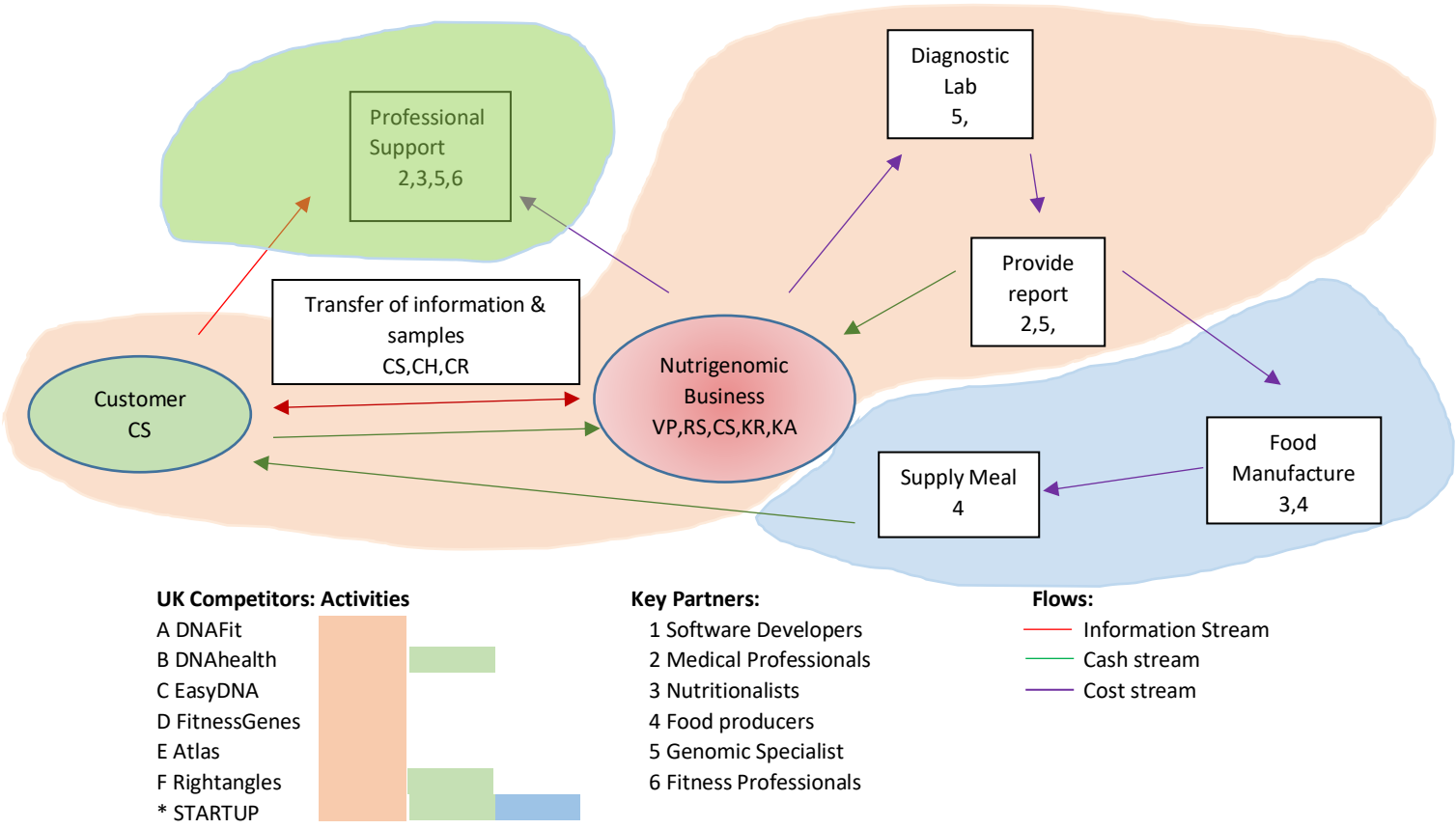

Figure 8.1 Business Model Process Flow.

CS Health conscious, aware of potential health problem, people who want to improve food choices, athletes
CR Individual online relationship
CH Internet
VP Improved health through informed Nutrition, Exercise and Food
RS Subscription to online service, personalised nutritional plan, healthy meals delivered to door
KR Phenotypic and genotypic data interpretation, algorithm for advice/meals
KA Gathering info on dietary intake, self-reported parameters, organising physiological and genotypic sampling and testing, providing advice, monitoring progress, supplying meals
KP Physiological and genomic data analysis specialists, diagnostic labs, dieticians, food producers, couriers, software developers
CS Website/app maintenance, data analysis, personnel, marketing, sales, food production

CS Customer segments, VP Value propositions, CH sales channels, CR Customer relationships, RS Revenue streams, KR Key resources, KA Key activities, KP Key partnerships and CS Cost structure

Figure 8.1. Genomic Business Process Flow

Based on the present report, a SWOT analysis was constructed

8.5 SWOT Analysis of Start-Up

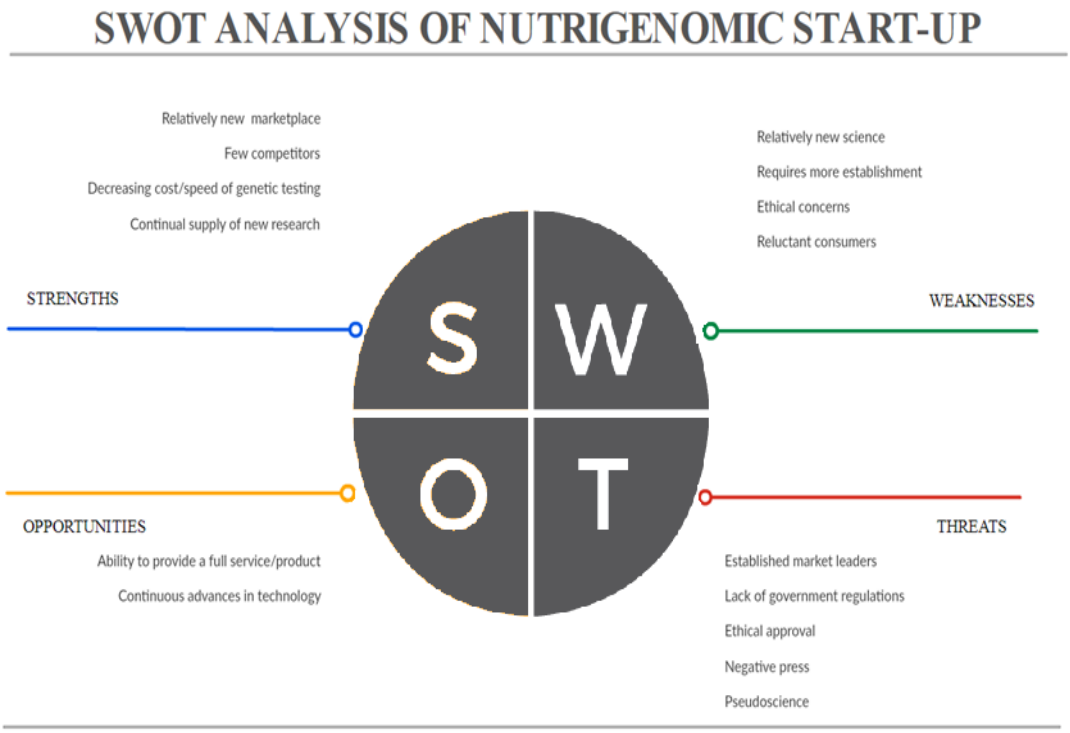

Figure 8.2 SWOT analysis of Genomic Businesses

Chapter 9

Public Health Imperatives

9.1 Regulation of health claims for functional foods

The food industry is heavily governed to ensure the safety of the consumer. However, functional food is a particularly restricted area to protect consumers against false or misleading health claims. As regulatory control continues to tighten over labelling and statements made about functional benefits, this could cause barriers to the development, launch and marketing of functional food products.

The main law governing functional food is REGULATION (EC) No 1924/2006 on Nutrition and Health Claims Made on Foods. This regulation states that the general principles for all claims for functional food products are that the use of nutrition and health claims shall not:

(i) be false, ambiguous or misleading.

(ii) give rise to doubt about the safety and the nutritional adequacy of other foods.

(iii) encourage or condone excess consumption of a food.

(iv) state, suggest or imply that a balanced and varied diet cannot provide appropriate quantities of nutrients in general.

(v) refer to changes in bodily functions which could give rise to or exploit fear in the consumer, either textually or through pictorial, graphic or symbolic representations.

It is becoming increasingly more difficult and costly for manufacturers to launch new products due to requirement for health claims to be scientifically validated and restrictions on the marketing message. Europe is considered the hardest place to have a health claim approved, with only 254 health claims out of the 2,242 submitted being validated. This

makes Europe a hard market to enter for manufacturers however it is also benchmarks the gold standard test for health claims.

Japan on the other hand, are far more lenient with demands for clinical evidence supporting the health claim, making it a relatively east test market for companies. This approach has led Japan to considered the country central to functional innovation both regarding the positive regulatory attitude toward novel ingredients and also the population's disposition to embrace new and unusual functional products (Langley, 2014).

The regulatory landscape is hard to predict however it has been suggested that following the recent scandals within the food industry, tighter regulations will be imposed to illustrate to the public that laws are in place to protect their wellbeing. More specifically large emerging locations which have previously been quite lax are expected to adopt stricter regulations including India, China and Brazil.

Langley (2014) expresses how due to the length of time it takes to collate and express evidence from clinical trials; the approval process can be stalled from 6 to up to 10 years; as this gap closes, more definitive outcomes should be released on a range of new functional ingredients.

In conclusion, the only sufficient way for new products to have their health claims approved successfully, and within a sufficient timespan, is by providing conclusive scientific evidence which supports the health claims the manufacturer is claiming to convey.

9.1.1 Food safety

The general definition of additives is a substance added to something in small quantities, typically to improve or preserve it. Functional foods may contain additives to produce a

health claim; this, however, is a key barrier along with scepticism preventing sales. Mintel (2011) says 50% of consumers avoid foods, particularly functional foods, due to additive inclusion. Even though additives have to be GRAS (Generally Recognised as Safe) before being commercially used, consumers believe they are too new to know the true long-term effects of consumption.

The food additive approval process can take up to 10 years. This consists of five years of trial to ensure safety, two years of EFSA (European Food Safety Authority) analysing data from the testing process, and finally another three years for the additive to be approved for European wide use. Despite this rigour, consumers may still remain sceptical. Functional foods by their very nature contain various functional ingredients and additives to enhance numerous functions including flavour, colour, taste, consistency or to add a health claim.

A study which found that 'health-enhancing food' could be harmful if regularly consumed due to the presence of additives, it is also believed that health claims were a technique to attract customers to the product (Bholah & Neergheen-Bhujun, 2013). Unwillingness to try functional foods related to cost (76.3%), chemical additives (75.8%), preference for natural products (68.8%) and scepticism regarding health improvement efficiency (68%). It is apparent that functional foods which are 'naturally' functional, *e.g*. coconut water, are far more popular with customers due to their pure and unadulterated portrayal. Developments in this market to make products as pure as possible will be far more popular with health conscious consumers.

9.1.2 Lack of knowledge

The rationale and reasoning behind the claims made on functional foods is the greatest barrier to growth. Despite the general trend of consumers being more positive and accepting of functional foods, consumer engagement is impeded by a pervasive attitude of cynicism (Mintel, 2011). In addition to this Mintel (2011) state that 20% of functional food users struggle to understand what health claims mean.

Many consumers do not understand the science behind the health benefits that certain functional foods promote. However, if the information was simplified, displayed in a basic format, consumers may be more inclined to purchase. Much of the public believe that unsubstantiated claims are used as a tactic to charge a price premium which suggests that consumers have low trust in manufacturer's health claims. This is reflected in the results of the survey with 51% of consumers suggesting they would purchase functional food if they were more convinced of the health benefits (Mintel, 2011).

The scepticism stems from functional foods only being governed by one regulation - REGULATION (EC) No 1924/2006 on Nutrition and Health Claims Made on Foods. Claims which are rejected by EFSA, are still allowed to be used for six months prior and withdrawn over the same period, which consumers are understandably aggravated about.

The low trust of manufacturer's claims prevents consumers from believing in functional food, if the rigorous and laborious testing procedure for claim verification was conveyed to the public it might encourage purchases based on greater confidence.

9.1.3 Public perspectives

9.1.3.1 Consumers' perspectives on functional foods

Americans are believed to be overwhelmingly aware and accepting of functional foods and are doing more to include them into their diet. However, consumer studies reported lower healthy food consumption frequency and lower taste preference of healthy foods (Siro *et al.*, 2008). In Europe, food choice of healthiness is continuously growing. however, Europeans are far more critical of new products and technologies. They are suspicious of the safety of novel foods and their production processes. Therefore, it is fair to hypothesise that Europeans' acceptance of functional foods is less unconditional with more concerns and reserves compared to Americans.

Consumer belief in the healthiness of a product greatly depended on the carrier product itself. The healthiness of the carrier products might override the effect of enrichments (Ares & Gambaro 2007). Surveys from different countries demonstrate different consumer attitudes and acceptance towards functional foods. Different socio-demographic parameters, gender, education also have an effect on customer acceptance level on functional foods (Siro *et al*., 2008)

Consumer acceptance of the concept of functional foods has been widely recognised as a key factor for market orientation, consumer-led product development and successful exploitation of market opportunities. However, acceptance is determined by a few factors such as primary health concerns, familiarity with 'functional food' concepts, knowledge of the functional ingredients, the nature of the carrier product, and the manner of health benefit communication, *etc*. Consumer awareness of the health benefits of newly developed functional ingredients may be limited and there is a strong need for specific communication

activities that better inform consumers in this respect. The health benefit information of a specific product should be communicated in a relatively simple way, so that it is more easily understood and trusted by consumers (Siro *et al*., 2008).

9.1.3.2 Consumers' perspectives on health claims and food choice

Some studies have shown that health claims do not influence purchasing decision as much as taste, brand, price, attractiveness and packaging of the product. Among the audited products, 25% of them that have nutritional claims printed on the front-of-pack, and 20% printed on the back-of-pack. In contrast, health claims that include disease risk reduction claims were used much less often on front-of-pack (2%) than back-of-pack (4%) (Wills *et al*., 2012). Wills and her colleagues have created a framework (figure 9.1) on how health claims affect consumers.

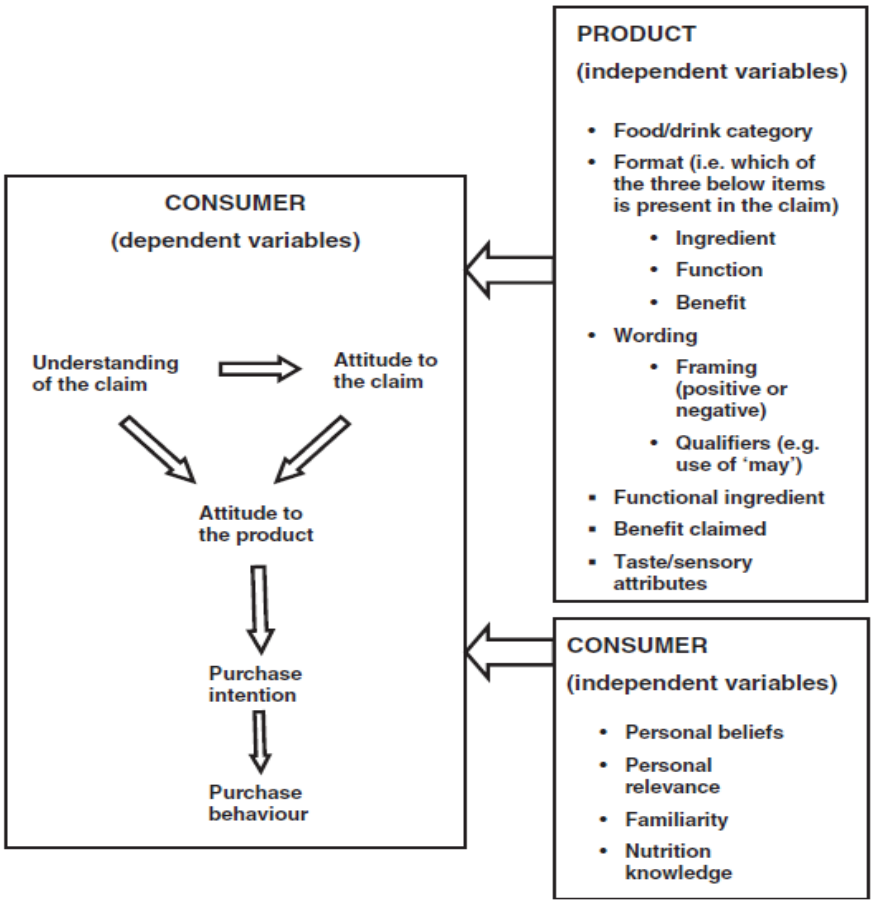

Figure 9.1. Conceptual framework on how health claims affect consumers (Wills *et al*., 2011)

Purchasing intentions and ultimately purchasing behaviour may be affected by understanding and attitudes to the claim. Both product and consumer variables can affect understanding, attitudes and purchasing of the product. Product variables include food and drink category, format and wording of the health claim, the functional ingredient, benefit claimed, taste and sensory attributes of the product. Consumer variables include personal beliefs that are not related to specific claims and products (*e.g*. usefulness of functional food in general), personal relevance (*e.g*. health concerns or problems), familiarity with the functional ingredients that the product contains and nutrition knowledge. Products such as yoghurt and bread with an overall positive health images have a tendency for their health claims to be perceived more positively. However, no consistency exists for the most appropriate type of foods as health claims carrier. Consumers seem to prefer the combination of carrier products with a functional ingredient that is naturally present in the products. Several studies have shown that fortification of functional ingredients in products which are already recognised as healthful by itself, such as yoghurt, honey, juices and low-fat products, do not benefit from carrying health claims. Conversely, products which are perceived as less healthful, such as sweets, spreads and high-fat products, were found to benefit from carrying health claims. Moreover, consumers with a family history of serious diseases such as cancer also positively influence the attitudes toward health claims relating to such disease.

Claims referring to specific food ingredients were found to be more convincing than merely stating the general health benefits of the product. Another factor which influences

the acceptance of the product was the terminology used in a health claim; short and concise wording was found to be more desirable to consumers. To enhance acceptance of the product, short and concise claims can be printed on the front of the package, and detailed explanation can be provided elsewhere on the package. Format and wording of claims impacted less on consumer attitudes than the familiarity and type of claimed benefit of the ingredient. Acceptance of the health claim is stronger when the health benefit of a functional ingredient is well known, *e.g*. calcium is beneficial for osteoporosis, and vitamin K is beneficial for hypertension. Contentment from food products was also observed as a more important factor than the perceived healthiness of the product. Also, no willingness was reported to compromise taste for health in functional products. Some studies showed that previous unpleasant tasting experiences might even have a negative impact on product acceptance in the future. This signifies the importance of taste influencing consumers food choices. Willingness to try functional food is driven by its attractiveness, credibility and uniqueness, which suggests that not only the healthy image of a functional food determines the consumers' acceptance (van Kleef *et al*., 2005).

9.1.4 Regulation of health claims by the European Food Safety Authority (EFSA)

EFSA is responsible for validating submitted claims based on scientific evidence for applicants, who want to use claims that are currently in use or propose claims for authorisation in the EU. Currently, there are 3 types of claims, their structure and function, health claim regulations are explained below:

1) *General function claims*: this is under Article 13.1 of the EC Regulation on nutrition and health claims. Claims that fall into this category are the role of a nutrient or substance in growth, development and body functions, psychological and

behavioural functions, slimming and weight control, satiety or reduction of available energy from the diet. However, claims which are related to child development, health or disease risk reduction are not included in this category (EFSA, 2012).

2) *New function claims*: this is under Article 13/5 of EC Regulation on nutrition and health claims. This category is for those which are based on newly developed scientific and/or for which protection of proprietary data is requested. Authorisation is required for these health claims on each case basis, subsequently the submission of a scientific dossier to EFSA for assessment.
3) *Disease risk reduction and child health and development claim*: this is under Article 14 of EC Regulation on nutrition and health claims. This category includes claims refer to disease risk reduction or children's health and development.

Table 9.1 shows some of the existing functional food products in the current market with approved health claims.

Table 9.1 Examples of products with health claims.

Company	**Product name**	**Functional food / ingredient / nutrients**	**Health claims**
Innocent	Energise	Vitamin B1, B2, B3, B6 and C	"Reduce tiredness and fatigue."
Innocent	Defence	Vitamin C, D and Zinc	"Contribute to the normal function of the immune system."
Innocent	Antioxidant	Vitamin C, E and selenium	"Protection of cells from oxidative stress."
Benecol	Yoghurt drinks, yoghurts and spreads	Plant Stanol Ester	"Proven to lower cholesterol."
Danon	Activia yoghurt	Bifidobacterium lactis	"helps regulate your digestive system."
Quaker	High fibre instant oatmeal	Soluble fibre	"helps reduce cholesterol."
Red Bull	Red Bull Energy drink	Caffeine, Taurine, B-group vitamins and sucrose and glucose	"Vitalizes body and mind."
Fuel	Fuel energy beverage	CoQ10 and Gingko Biloba	"Fire up energy levels."

9.1.4.1 EFSA claim interventions and contraventions (see Appendix 1)

Many of the products included in functional foods are plant/herbal extracts and therefore are regulated as botanicals and only need to be classed as food safe for inclusion in products. However, some of the ingredients used are subject to regulation under the EFSA Article 13 relating to health claims associated with ingredients.

Listed below are examples of the approvals/rejections relating to some of the common functional product ingredients that have been assessed by the NDA (The Panel on Dietetic Products, Nutrition and Allergies): See Table 9.2 Approvals from EFSA / NDA.

Product	Approved by NDA / EFSA	Rejected by NDA / EFSA
Glucomannan (Dietary Fibre)	Reduction of blood cholesterol concentrations	Reduction of post-prandial glycaemic responses
(EFSA-a, 2009; EFSA-b, 2010)	Reduction of body weight	Maintenance of normal blood glucose concentrations
		Maintenance of normal (fasting) blood concentrations of triglycerides
		Maintenance of normal bowel function
		Decreasing potentially pathogenic gastro-intestinal microorganisms
Chromium	Contribution to normal macronutrient metabolism	Contribution to the maintenance or achievement of a normal body weight
(EFSA-c, 2010)	Maintenance of normal blood glucose concentrations	Reduction of tiredness and fatigue
Lecithin	No claims relating to the beneficial effects of Lecithin have been approved	Maintenance of normal blood cholesterol concentrations
(EFSA-d)		Contribution to normal fat metabolism
		Increase in the intestinal absorption of glutamine
		Faster recovery from muscle fatigue after exercise
		Improvement of neuromuscular function
		Contribution to normal cognitive function
		Maintenance of normal neurological function
Conjugated Linoleic Acid	No claims relating to the benefits of CLA have been approved	Contribution to the maintenance or achievement of a normal body weight
(EFSA-e, 2010)		Increase in lean body mass
		Increase in insulin sensitivity
		Protection of DNA, proteins and lipids from oxidative damage
		Contribution to immune defences by stimulation of production of protective antibodies in response to vaccination
Selenium	Maintenance of normal hair	Protection against heavy metals
(EFSA-f, 2010; EFSA-g, 2009)	Maintenance of normal nails	Maintenance of normal joints
	Maintenance of normal thyroid function	The normal function of the heart and blood vessels.
	Protection of DNA, proteins and lipids from oxidative damage	Normal cognitive function
	Maintenance of the normal function of the immune system	Normal prostate function

Product	Approved by NDA / EFSA	Rejected by NDA / EFSA
Iodine	Normal thyroid function	Maintenance of normal vision
(EFSA-h, 2009; EFSA-I, 2010)	Normal production of thyroid hormones	Maintenance of normal hair
	Normal energy-yielding metabolism	Maintenance of normal nails
	Maintenance of normal skin.	
	Contribution to normal cognitive and neurological function	
	Contribution to normal energy-yielding metabolism	
	Contribution to normal thyroid function and production of thyroid hormones	
Niacin	Normal energy-yielding metabolism	Maintenance of normal bone
(EFSA-J, 2009; EFSA-K, 2010)	The normal function of the nervous system	Maintenance of normal teeth
	Maintenance of normal skin and mucous membranes	Maintenance of normal hair
	Reduction of tiredness and fatigue	Maintenance of normal nails
	Contribution to normal psychological functions	Maintenance of normal blood flow (encourages excess consumption)
Caffeine	Increase in endurance performance	Increase in physical performance during short-term high-intensity exercise
(EFSA-k, 2011; EFSA-m, 2011)	Increase in endurance capacity	Increased fat oxidation leading to a reduction in body fat mass
	Reduction in the rated perceived exertion/effort during exercise	Increased energy expenditure leading to a reduction in body weight
	Increased alertness	
	Increased attention	
5 Hydroxytryptophan	No claims relating to the beneficial effects of 5-HTP have been approved	Enhancement of mood
(EFSA-n, 2009; EFSA-o, 2011)		Concentration and reduction of restlessness
		Increase in satiety leading to a reduction in energy intake
Phenylalanine	No claims relating to the beneficial effects of phenylalanine have been approved	Increased alertness
(EFSA-p, 2010)		Enhancement of mood
		Pain relief
		Improvement of memory
Boron	No claims relating to the beneficial effects of Boron have been approved	Maintenance of bone
(EFSA-q, 2009; EFSA-r, 2011)		Maintenance of joints
		Prevention and treatment of prostate cancer
		Maintenance of normal thyroid function
		Contribution to normal cognitive function
L-ornithine	No claims relating to the beneficial effects of L-ornitine have been approved	Contribution to the regulation of the urea cycle.
(EFSA-s, 2011)		Normalization of liver function

Product	Approved by NDA / EFSA	Rejected by NDA / EFSA
L-Arginine	Maintenance of normal ammonia clearance	"Immune system functions."
(EFSA-t, 2011)		Growth or maintenance of muscle mass
		Normal red blood cell formation
		Maintenance of normal blood pressure
		Improvement of endothelium-dependent vasodilation
		"Physical performance and condition."
		"Système nervous"
		Maintenance of normal erectile function
		Contribution to normal spermatogenesis
L-Tyrosine	Contribution to the normal synthesis of catecholamines (stimulatory hormones)	Increased attention
(EFSA-u, 2011)		Contribution to normal muscle function
Vitamin B5/Pantothenic acid	Normal energy-yielding metabolism	Maintenance of normal bone
(EFSA-v, 2009; EFSA-w, 2011)	Normal mental performance	Maintenance of normal teeth
	Normal synthesis and metabolism of steroid hormones, vitamin D and some neurotransmitters.	Maintenance of normal hair
	Reduction of tiredness and fatigue (not deficient in EU)	Maintenance of normal skin
		Maintenance of normal nails.
		Adrenal function
Coenzyme Q10	No claims regarding the beneficial effects of coenzyme Q10 were approved	Contribution to normal energy-yielding metabolism
(EFSA-x, 2010)		Maintenance of normal blood pressure
		Protection of DNA, proteins and lipids from oxidative damage
		Contribution to normal cognitive function
		Maintenance of normal blood cholesterol concentrations
		Increase in endurance capacity and/or endurance performance (ID 1913)
Green Coffee (Chlorogenic Acid)	No claims regarding the beneficial effects of green coffee were approved	Protection of DNA, proteins and lipids from oxidative damage
(EFSA-y, 2011)		Maintenance of normal blood glucose concentrations
		Contribution to the maintenance or achievement of a normal body weight
Cocoa	No claims regarding the beneficial effects of cocoa were approved	Protection of lipids from oxidative damage
(EFSA-z, 2010; EFSA-aa, 2011)		Maintenance of normal blood pressure
		Enhancement of mood
White Kidney bean Extract (Phaseolamine)	No claims regarding the beneficial effects of white kidney bean extract were approved	Inhibit α-amylase activity, hindering the conversion of complex carbohydrate to simple sugars, which are stored as reservoir fats if not immediately utilised by the organism; it results in a lower calories intake, contributing to weight loss

Table 9.2 Approvals from EFSA / NDA

9.1.5 Future functional food information regulation – the new FIR

The European Parliament has approved the new Food Information for Consumers Regulation (FIR) on 6 July 2011, after reviewing both general food and nutrition labelling legislation. The main purpose of the new regulation is to simplify and consolidate the existing labelling legislation, bringing general and nutrition labelling of EU rules together into a single regulation (FSA, 2014)

The main points and key areas of the new Food Information for Consumers Regulation include:

1) Country of origin
2) Nutrition labelling
3) Manufacturers if possible to provide voluntary energy information.
4) Data marking: 'best before' and 'use by'dates.
5) A date of first freezing shown for frozen items, *i.e*. meat and fish.
6) Additional information for children or pregnant and breastfeeding women.
7) The types of vegetable oil used in food products.
8) Allergen information.

9.2 Current issues facing the NHS

Statistics and Segmentation of the rising cost of financing the NHS present a huge healthcare challenge looming within the UK. Resources struggle to cope with demand and costs are escalating as health issues increase within a growing UK population.

9.2.1 Key NHS financial statistics

NHS net expenditure has increased from £78.8bn in 2006/07 to £120.5bn in 2016/17. Planned expenditure for 2017/18 is £123.8bn and for 2018/19 is £126.2bn. In real terms, the budget is expected to increase from £120.5bn in 2016/17 to £123.202bn by 2019/20.

Health expenditure *per capita* in England has risen from £1,879 in 2011/12 to £2,106 in 2015/16. The NHS net deficit for the 2015/16 financial year was £1.8bn. The provider deficit for the 2016/17 financial year has been confirmed at £791m. (NHS statistics, 2017)

9.2.2 Key NHS activity figures

The NHS deals with over 1 million patients every 36 hours. In 2015/16 there were 40% more 'procedures and interventions' (excluding diagnostic testing) completed by the NHS compared to 2005/06, with an increase from 7.2m to 10.1m. There were 16.2m total hospital admissions in 2015/16, 28% more than a decade earlier (12.6m).

In 2016, 507,784 NHS patients were admitted to independent providers for their elective inpatient care. There were 891,717 referrals made by GPs to independent providers for outpatient care during the same period.

At the end of April 2017, there were 3.8 million patients on the waiting list for treatment. 382,618 (10.1%) had been waiting for longer than 18 weeks, compared to 302,901 (8.4%) at the same point in 2016. 90.5% of people with non-admitted pathways were treated or discharged within 18 weeks of referral in April 2017, compared to 92.1% a year earlier. At the end of April 2017, 885,876 patients were on the waiting list for a diagnostic test. Of these, 1.8% had been waiting more than six weeks (NHS statistics, 2017)

9.2.3 Key UK obesity figures

In 2015, 58% of women and 68% of men were overweight or obese. Obesity prevalence increased from 15% in 1993 to 27% in 2015. In 2015/16, over 1 in 5 school children in Reception (4 years old), and over 1 in 3 children in Year 6 (11 years old) were measured as obese or overweight.

In 2015/16 there were 525,000 admissions in NHS hospitals where obesity was recorded as a factor. In 2015/16, there were 6,438 Finished Consultant Episodes (FCE's) in NHS hospitals with a primary diagnosis of obesity and a main or secondary procedure of bariatric surgery. Over three-quarters of bariatric surgery patients were aged between 35 and 54, and over three-quarters of patients were female.

26% of adults ate the recommended 5 or more portions of fruit and vegetables a day in 2015 with females (27%) were more likely to do so than males (24%).

In 2015/16, 26% of adults were classified as inactive (fewer than 30 minutes physical activity a week) £6.1bn p/year spent on obesity-related diseases. (Gov.uk, 2017)

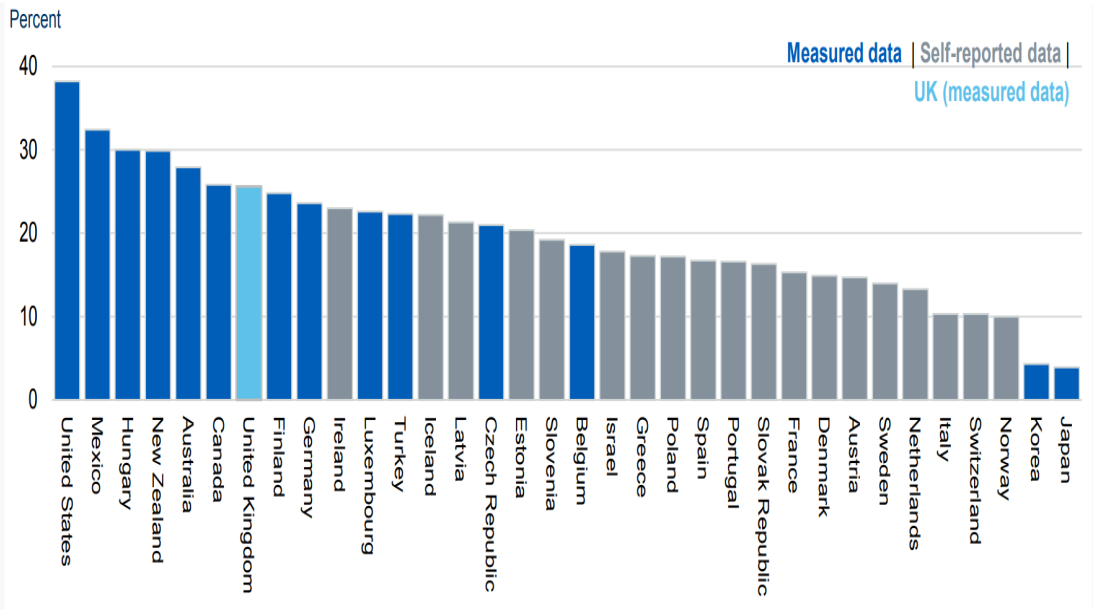

Figure 9.2 Adult obesity: UK Comparison with other OECD (Organisation for Economic Co-operation and Development) countries (Gov.uk, 2017).

9.2.4 Implications for the future of the NHS

Nearly two-thirds of adults (63%) in England were classed as being overweight. Failure to address the epidemic of obesity places an even greater burden on the resources of the NHS. Statistics show that the NHS spent £6.1bn on obesity-related diseases during 2014-2015, with poor nutrition being identified as a large causal factor (Public Health England, 2017). Functional foods have been identified as a promising treatment for obesity and associated comorbidities (Baboota *et al*., 2013). Furthermore, genetic research into the underlying biological mechanisms of functional foods may be informative for the public health sector when advising on nutritional health and disease (Warburton *et al*., 2017).

The NHS suffered a 2016/17 year-end deficit of £791m due to operational challenges and additional financial burden (NHS statistics, 2017). Annual NHS costs for cancer services are reported to be at £5bn (Department of Health and Social Care, 2015). More than £6.8bn was spent on treatment of CVD within the NHS in England in 2012/13 (Bhatnagar *et al*., 2015). The cost of type 2 diabetes was reported to be £8.8bn a year (NHS, 2017). The use of nutritional genomics in the healthcare system has the potential to maximise intervention for individuals with high risk of diseases such as cancer, cardiovascular disease (CVD) and type 2 diabetes (Ordovas & Corella, 2004; Kaput & Rodriguez, 2004; Davis & Milner, 2004).

Research and innovation within the NHS have focused on genomics with the annual report showing an investment in genomics with NHS genomic medical centres increasing from 11 to 13 centres in 2015/16 (NHS England, 2016). This exemplifies the rapid development and importance of understanding public perception of genomic science into society.

Chapter 10

DISCUSSION

10.1 The Effect of diet on health

The role of diet in promoting health and wellbeing is a concept that dates back to over 400 BC: “Let food be thy medicine and medicine be thy food” (Hippocrates). Poor diet is a major cause of global morbidity and mortality from chronic non-communicable diseases as opposed to undernutrition (de Onis *et al*., 2004, Lim *et al*., 2012, Lozano *et al*., 2012, Imamura *et al*., 2015). This worldwide transition to more unhealthy eating patterns is of great concern and highlights the need for improvements to food policies and overall diet quality on a global scale (Hawkes, 2006, Vandevijvere *et al*., 2013). A systematic review of the effects of diet on general health and wellbeing was undertaken and correlated with global dietary patterns and disease trends. Also, pathway analysis was performed to identify major biological processes associated with non-communicable diseases and pathways targeted by the functional foods omega-3, flavonoids and resveratrol to inform on the therapeutic potential of diet in the prevention and treatment of chronic disease.

10.2 The health benefits of the Mediterranean Diet

Since olive oil is an important component of this dietary pattern, much effort has been devoted to understanding the effect of MUFA, the major components of this oil. The replacement of SFA with MUFA reduced metabolic stress and prevented the expression of inflammatory genes in different tissues (PBMC, adipocytes, coronary artery smooth muscle, and human prostate cancer cells), and consequently to a less atherogenic profile and control of tumour progression when compared to other sources of fat.

Minor components of virgin olive oil have been particularly studied and have found that the consumption of virgin olive oil with phenolic compounds, either in a postprandial regimen or over a relatively short period, influences the expression of genes related to atherosclerosis

progression, and that this effect is observed at the moderate doses consumed in this dietary pattern. Whether this action is due to a single, or a combination of phenolic compounds awaits an answer. Not much effort has been devoted to the translation of these transcriptional changes into proteins. However, *in vitro* studies indicate that extracts enriched in hydroxytyrosol, secoiridoids, and lignans can inhibit cell proliferation by modulating cell cycle expression.

Despite being more abundant than phenolic compounds in virgin olive oil, terpenes have received less attention. In animal models, oleanolic and maslinic acids were particularly active in controlling the circadian clock and inflammation genes, respectively.

The results reviewed provide at least a partial molecular basis for the reduced risk of cardiovascular disease, and cancer observed in Mediterranean countries. The nutrient complexity of the Mediterranean diets, differences among the studies and the diversity in the responses depending on the specific genetic makeup makes this field an open arena with enormous possibilities. Increasing and consolidating the nutrigenomic knowledge of this diet warrants further research to provide sound, personalised, and optimised nutritional recommendations.

The Mediterranean Diet is the inspiration for the Gene Overlapping Study in this review assessing the key functional components of the diet.

10.3 Altering dietary patterns to prevent disease

To provide an up-to-date overview on the beneficial and adverse effects of diet on some phenotypic outcomes relevant to general health and wellbeing, and risk of chronic non-communicable disease. The systematic review of the literature confirms that diets high in fruit, vegetables, whole-grains, low-fat dairy, oily fish, protein (including nuts, legumes and

pulses) and certain vitamins and minerals, and low in salt, processed meats, refined grains, sugary beverages and TFAs may have a protective effect against several chronic diseases including CVD, hypertension, high cholesterol, type-2 diabetes, obesity, stroke, cancer and risk of depression (Figures 3.2,3.3,3.4).

10.4 UK dietary trends

UK dietary trends show an over consuming of saturated fat, salt and added sugar, but not enough fruit, vegetables, oily fish and fibre, figure 3.6. This may be reflective of the rising costs of living as it was shown that household income influences what we eat, with lower-income quintiles associating with poorer quality diet (NDNS, 2015). In 2012, the mean cost per unit of healthy and unhealthy products was £7.49 and £2.50, respectively, with the prices of healthier foods and beverages rising at a significantly faster rate than unhealthy products (Jones *et al*., 2014). Low-income households may, therefore, be more at risk of following unhealthy dietary choices. This is a major concern considering the rising trends in adult and child obesity rates in the UK (Stevens *et al*., 2012, LGA, 2014). However, it has been reported that the type of retailer (*i.e*. budget supermarket verses independent shops) plays more of a role in determining the cost of food rather than differences in healthy or unhealthy food choices (Banks *et al*., 2012). This raises opportunities for marketing healthier food choices in the movement towards prevention of dietary-related diseases.

10.5 Global dietary trends

Globally, healthier diet choices are generally increasing. However the consumption of unhealthy food products is outpacing this trend across all income quintiles (Imamura *et al*., 2015). As with high-income countries, the cost of healthier food choices, particularly fruit and vegetables, in emerging economies such as Brazil, China, Korea and Mexico mean that energy-dense processed foods are being consumed at greater rates (ODI, 2015). Adequate

consumption of fruit and vegetables reduces the risk of CVD, diabetes and certain cancer types (Wang *et al*., 2014, Muraki *et al*., 2013, Yokoyama *et al*., 2014, Aune *et al*., 2012, Amir Shaghaghi *et al*., 2013, Liu *et al*., 2014). Global analysis of morbidity and mortality rates for non-communicable diseases showed a positive correlation between lower quality diets based on consumption of more unhealthy products and increased risk of disease burden (Figures 3.8,3.9,3.10). Worldwide estimates of nutritional transitions across the world will help to inform on global food policies and priorities for reducing the health and economic impacts of poor quality diets.

10.6 The role of functional foods

The term 'functional food' originated in Japan in 1984 to describe food products fortified with special constituents that possess advantageous physiological effects (Siro *et al*., 2008). The most recent redefinition of this term states that functional foods are natural or processed foods that contain known or unknown biologically-active compounds; which, in defined, effective non-toxic amounts, provide a clinically proven and documented health benefit for the prevention, management or treatment of chronic disease (Martirosyan, 2015). However the exact biological mechanisms underpinning the functional aspects of these bioactive compounds are little understood.

10.7 The role of nutraceuticals

Nutraceuticals are food products and supplements which have nutritional value and are proven to be beneficial to disease prevention. There is increasing demand for natural products with health benefits. The increase in consumer understanding, attitudes and acceptance of functional food is generating market growth in nutraceuticals. Innovative technology such as microencapsulation and capsule-in-capsule technology is enabling the

creations of new foods and supplements with enhanced organoleptic properties and bioavailability.

10.8 The influence of diet in epigenetics

Epigenetic phenomena are inheritable and can affect gene expression without base pair changes. Abnormalities are believed to occur before the onset of diseases and might contribute to disease pathogenesis. Therefore, it is of great importance to discover markers that might be associated with an increased risk of disease development. Modification of chromatin is critical in controlling DNA transcription, giving rise to a variety of epigenetic effects. Due to its reversible character, epigenetics becomes attractive to nutritional intervention. Some nutrients have been studied and shown that they can modify gene expression via physiological and pathological processes in the epigenetic mechanism. Adjusting these processes by changing diet or consumption of specific nutrients may either prevent diseases, maintain health or promote the development of serious diseases. It is difficult to delineate the specific function for each epigenetic modulation on each type of nutrient. Nevertheless, nutrients with a positive effect health via epigenetic mechanisms have the least harmful effect to the human body; therefore, it is possible to develop nutraceutical products based on an individual's gene expression to construct a personalised treatment and target specific health condition.

10.9 Insights into the overlapping genetics of biology, diet and disease:

Nutrigenomics with dietary composition is not well understood. This study took key nutritional elements from the Mediterranean diet and researched the overlapping genetics of each individually and in combination to assess their synergistic effect.

Pathway analysis using candidate genes for CVD, diabetes and obesity (collectively termed diabesity) was performed to identify disease-specific and common biological processes associated with these conditions. The gene sets were enriched for developmental and immune response pathways relating to angiogenesis and inflammation, respectively, Figure 6.1. Angiogenesis plays a crucial role in the pathogenesis and treatment of cardiovascular disease (Liao *et al*., 2014). The immune system plays an important role in regulating angiogenesis both during development and in adult processes such as wound healing, tissue repair and multiple disease pathways including cancer and disorders of the cardiovascular system (Lavine *et al*., 2014, Epelman et al., 2015, De Martin *et al*., 2000, Tartour *et al*., 2011, Frantz & Nahrendorf, 2014). The bidirectional link between angiogenesis and the immune response is therefore of major clinical importance for the prevention and treatment of chronic diseases which associate with these key biological systems.

Comparative analysis of the CVD and diabesity gene sets identified insulin/IGF-1 signalling as the top significant pathway, Figure 6.3. This pathway has been demonstrated to underlie interactions between nutrition, metabolism and longevity (Cameron *et al*., 2008, van Heemst, 2010). Western dietary patterns typically high in grains, sugar and dairy, have been shown to over-stimulate the insulin/IGF-1 signalling pathway, which has in turn been linked to CVD and cancer (Melnik *et al*., 2011, Xie & Wang, 2013, Kleinridders *et al*., 2014, Massoner *et al*., 2010, Cohen & LeRoith, 2012, Verburgh, 2015, Higashi *et al*., 2012). In contrast, reduced activation of the insulin/IGF-1 pathway is associated with protection against age-related neurotoxicity seen in Alzheimer's disease (Cohen *et al*., 2009, de la Monte, 2012).

There is a growing interest in the role of polyphenol-rich foods in the prevention of chronic diseases (Stoclet *et al*., 2004, Pandey & Rizvi, 2009, Manach *et al*., 2005, Edwards & Kroon, 2014, Sagar *et al*., 2006). The protective role of these bioactive compounds has been

attributed to their antioxidant and anti-apoptotic effects which are in part regulated along the insulin/IGF-1 pathway (Tulio *et al*., 2012, Cameron *et al*., 2008, Wang *et al*., 2003), Figure 6.4. The importance of this pathway in regulating biological processes necessary for normal cellular growth, survival and maintenance, in addition to its overlap into many disease pathways, offers rationale for following diets which avoid persistent over-stimulation of insulin/IGF-1 signalling and exaggerated insulin production, especially in individuals at risk of developing CVD, diabesity and cancer.

Diet not only influences cardiovascular and metabolic processes but can also affect our mental health and wellbeing (Van de Weyer, 2005). Comparative enrichment analysis of gene sets for CVD, diabesity and neurogenesis using the PANTHER pathway analysis suite identified angiogenesis and hormonal signalling pathways to be important (Figure 6.5). Alzheimer's disease and Huntington's disease were identified as common disease pathways associated with the neurogenesis and CVD gene sets, Figure 6.5. Like CVD, neurodegenerative disorders are associated with increasing age and can occur as comorbidities with several pathological conditions, including those affecting the cardiovascular system (Snowdon *et al*., 1997, Blennow *et al*., 1991, Chiu & Alexander, 1982, Lanska *et al*., 1988, Sorensen & Fenger, 1992, Amar *et al*., 1996, Poblador-Plou *et al*., 2014). Several studies have demonstrated overlap in the genetic and biological mechanisms operating between neurodegeneration and disorders of the heart (Li *et al*., 2006, Abildtrup & Shattock, 2013, Melkani *et al*., 2013, van der Burg *et al*., 2009, Bradford *et al*., 2010, Willis & Patterson, 2013). In addition to lowering the risk of CVD, healthy dietary patterns have been associated with a reduced risk of dementia and Alzheimer's disease, and dietary intake has been correlated with the onset of Huntington's disease (Eskelinen *et al*., 2011, Marder *et al*., 2013). A better understanding of

the role that nutrition plays in complex pathological processes is therefore essential for the development of dietary advice for the prevention and management of the disease.

10.10 The therapeutic role of functional foods in the targeting of biological and disease pathways.

The therapeutic potential of the functional foods omega-3, flavonoids and resveratrol in the prevention and treatment of chronic disease has been suggested from the literature (Simopoulos, 2002, Dragan *et al*., 2007, Shukla & Singh, 2011, Petro, 2011, Ramos-Romero *et al*., 2012, Batra & Sharma, 2013, Laviano *et al*., 2013, Tanaka, 2014, Fabian *et al*., 2015). A better understanding of the biological mechanisms underpinning these therapeutic effects would be of great public interest and could be used by medical professions to inform on dietary recommendations as an alternative to pharmaceutical intervention in the treatment of common pathological conditions. Pathway analysis of genes significantly regulated in response to treatment interventions with these functional foods identified several overlapping pathways important for immune responses, between omega-3 and flavonoids, and cell cycle and cancer mechanisms, between omega-3 and resveratrol (Figure 6.6). Filters for immunological disease and cancer were overlaid onto these common pathways (Figures 6.8 and 6.9) which identified several potentially important therapeutic targets, for example, PTEN; a key target gene in cancer therapy (Li *et al*., 2013, Dillon & Miller, 2014). Analysis of disease pathways associated with these functional foods indicated anti-viral, anti-tumorigenic and pro-survival properties for omega-3, Table 6.3, which is supported by the literature (Petrik *et al*., 1999, Notarnicola *et al*., 2013, Cockbain *et al*., 2012). Flavonoids were linked to developmental, immune and inflammatory responses (Table 6.4), whereas resveratrol was enriched for processes involved in cell structure and cell cycle (Table 6.5). Net effects of gene expression changes about toxicity functions suggested a beneficial role for omega-3 in

reducing hypertrophy of the heart and liver cancer, and flavonoids in reducing inflammation of the liver (Table 6.6).

Our diet is influenced by many factors including age, sex, education, ethnicity and socioeconomic pressures. Psychological determinants of diet such as mood and stress levels are also very influential (Adam and Epel, 2007, Torres & Nowson, 2007, Mikolajczyk *et al*., 2009, Ganasegeran *et al*., 2012). To address overlap between nutrition and key biological processes and common diseases potentially modified by dietary interventions, GWAS datasets for ageing, cognition, breast cancer, CVD, diabesity, neurodegeneration and psychiatric disorders were compared against gene expression changes associated with omega-3, flavonoids and resveratrol. Significant overlap was observed between the omega-3 and breast cancer gene sets (Figure 6.10A), emphasising the anti-tumorigenic properties of this functional food.

Dietary intervention with the functional foods omega-3, flavonoids and resveratrol target important biological and disease pathways, suggesting a potentially important role for these bioactive compounds in the prevention and treatment of dietary-related disease such as cancer.

10.11 Consumer attitudes to health interventions based on personal genetic testing.

The present study aimed to assess UK consumer attitudes and acceptance towards nutritional genomics, its services and activities with consideration on how demographics affect these factors. Results showed that the UK population had a high level of interest in nutritional genomics. Demographic factors did affect attitudes and acceptance, with those aged 55+ showing the most agreement towards nutritional genomics; this was followed by the age group 25-34 years. Gender did not affect overall response agreement towards the survey. The results of the present study suggest a positive overall outlook towards genomic-

based nutrition, its services and activities within the UK population. Previous research suggests that knowledge and education may aid in mainstreaming genomic-based nutrition to the public as knowledge is reported to be a driving factor in consumer acceptance of genetic testing. This informs the need for health education programmes to improve consumer understanding of nutrigenomics. The NHS is also funding the expansion of more genomic centres and education may be imperative to ensure the public are better informed of the benefits of this continually evolving science which has the potential to bring significant savings to the health service within the UK.

Further research would be useful to inform on other demographic factors such as income, education level and ethnicity to gain a greater understanding of the complex reasons which affect attitudes towards genetic testing and nutritional genomics. However, there have been a significant number of recent genomic business start-ups that prove that there is a growing consumer demand in this area and this is creating an important market opportunity for the food and healthcare sectors.

10. 12 Genomic business applications

A successful business model is a key to the successful commercialisation of a nutrigenomic start-up business. Combining the right business models with the appropriate consumer research indicates the likely consumer acceptance towards the business activities. A key factor is engagement and clarity with the consumer to reduce avoidance towards genetic testing. A complete business model in the UK which provides a full complete service from genotype testing, personal nutrition advice, to providing a delivered healthy meal based on personal genetic data was not identified. ‘Habit’, a US-based company is the only company identified which currently provides full holistic service.

The public is increasingly reflective in matters of health and willing to adopt health-oriented changes in their eating habits. Developing and marketing new functional food products require significant research efforts. It is a multistage process which involves identifying functional components and their physiological effects; developing a food matrix which is suitable for this compound taking into account its bio-availability, potential changes during food preparation and processing, and finally clinical trials on product efficacy in order to gain approval for health marketing claims (Siro *et al*., 2008).

Education and knowledge level of the product end users is very important in determining the possibility of market success. The health effect message of a specific product should be communicated in a manner which consumers can easily comprehend. Therefore, specialist terminology or medical details should be avoided. Consumers are interested in understanding the benefits, rather than the 'science' behind the product. The role of educating consumers is therefore crucial because, unlike taste and other sensory attributes, consumers cannot perceive the benefit of the product directly and immediately.

10.13 Implications for public health the NHS

The cost of public and private healthcare systems in the UK like many other countries continues to escalate. The cost of the NHS is likely to rise from 7.7% of GDP in 2016 to 12.2% of GDP in 2066 (OECD, 2018). However, the combined public and private cost of healthcare in the UK was 9.7% of GDP in 2016.

Public Health England provides guidance on nutritional levels of salt, sugar and saturated fat and connects with the food industry to reduce the intrinsic amounts of each in processed food. However, these efforts have failed to produce a 'population effect'

improvement in health. In fact, the main NCDs drivers of poor health (CVD, Obesity, Mental Health) are expected to increase.

The UK government has recognised the importance of genetics in healthcare by funding Genomics England to deliver the '100,000 genome project'. The project will collect genetics from patients and families focusing on rare disease and cancer. In parallel, the private sector is using more targeted genomic profiling of selected polymorphisms in commercial ventures in the areas of diet, psychology, exercise and health & wellbeing.

This study provides a rationale for the greater understanding of functional foods, nutrigenomic pathway analysis and consumer acceptance of genomic profiling for health benefits. If a sustainable population effect in health improvement is achievable, then functional nutrition designed from a combination of personalised genetic analysis and functional nutrigenomic interventions represents an important new nexus for improving public health and the future of disease prevention.

Appendices

Appendix 1. Nutrient Interventions for Disease (table 4.7 – Appendix 1)

Disease	*Dietary/Lifestyle Prevention*	*Ingredients Prevention*	*Journal Title*	*Conclusion*	*Year*	*URL*	*Citation*
Metabolic Syndrome, Alzheimer's Disease	-	-	Metabolic Syndrome and Risk for Incident Alzheimer's Disease or Vascular Dementia	The observed relation between high triglycerides, diabetes, and vascular dementia emphasises the need for detection and treatment of vascular risk factors in older individuals to prevent the likelihood of clinical dementia.	2009	https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2606808/	Raffaitin, C., Gin, H., Empana, J.-P., Helmer, C., Berr, C., Tzourio, C., … Barberger-Gateau, P. (2009). Metabolic Syndrome and Risk for Incident Alzheimer's Disease or Vascular Dementia: The Three-City Study. Diabetes Care, 32(1), 169–174. http://doi.org/10.2337/dc08-0272

Alzheimer's	Ketogenic diet,	-	Ketogenic diets and Alzheimer's disease	At present, there is only limited evidence of the usefulness of ketogenic diets in AD. However, this dietary approach seems to be promising and deserves further clinical investigations	2017	https://ac.els-cdn.com/S2213453016301355-s2.0-S2213453016301355-main.pdf?_tid=202fa7c4-d99f-11e7-923a-00000aacb362&acdnat=1512466501_56748ec591062	Lange, K., Lange, K., Makulska-Gertruda, E., Nakamura, Y., Reissmann, A., Kanaya, S. and Hauser, J. (2017). Ketogenic diets and Alzheimer's disease. Food Science and Human Wellness, 6(1), pp.1-9.

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CVD, Alzheimers	-	-	Cardiovascular disease and Alzheimer's disease: common links.	The substantial overlap in risk factors points to new avenues for research and prevention.	2006	https://www.ncbi.nlm.nih.gov/pubmed/16918818	Stampfer MJ. Cardiovascular disease and Alzheimer's disease: Common links. J Intern Med. 2006;260:211–223.
Carotid atherosclerosis, Alzheimer's	-	-	Carotid atherosclerosis promotes the progression of Alzheimer's disease: A three-year prospective study.	The present prospective study showed that carotid atherosclerosis is a predictive factor regarding the progression of cognitive impairment in AD patients, suggesting that early detection and treatment of vascular risk factors may prevent or at least postpone the evolution of the disease.	2017	https://www.ncbi.nlm.nih.gov/pubmed/28810593	Xiang, J. (2017). Carotid atherosclerosis promotes the progression of ofi¿½Alzheimer's disease: A three-year prospective study. Experimental and Therapeutic Medicine.

glucose regulation	aerobic and resistance training,		12 weeks' aerobic and resistance training without dietary intervention did not influence oxidative stress, but aerobic training decreased atherogenic index in middle-aged men with impaired glucose regulation.	Improved lipid profile remained a predictor of decreased MetS score only in NW group, and it seems that Nordic walking has more beneficial effects on cardiovascular disease risks than RT training.	2013	https://www.ncbi.nlm.nih.gov/pubmed/23623841	Venojärvi, M., Korkmaz, A., Wasenius, N., Manderoos, S., Heinonen, O., Lindholm, H., Aunola, S., Eriksson, J. and Atalay, M. (2013). 12 Weeks' aerobic and resistance training without dietary intervention did not influence oxidative stress, but aerobic training decreased atherogenic index in middle-aged men with impaired glucose regulation. Food and Chemical Toxicology, 61, pp.127-135.
alzheimers	Lutein	-	Advances in the researches of lutein and Alzheimer's disease	In this article, we systematically reviewed the literature on the role of lutein in age-related cognitive decline and Alzheimer's disease and	2015	https://www.ncbi.nlm.nih.gov/p	Xu X, Lin X. (2015). Advances in the researches of lutein and Alzheimer's disease. Zhonghua

				its possible mechanisms. It may prove some benefit information for the advanced research and prevention of AD.		ubmed/26081713	Yu Fang Yi Xue Za Zhi.;49(5):456-60.
-	High carb diet, Alzheimer's	-	High carbohydrate diets and Alzheimer's disease.	A change in diet emphasising decreasing dietary carbohydrates and increasing essential fatty acids (EFA) may effectively prevent AD. Interventions that restore lipid homeostasis may treat the disease, including drugs that increase fatty acid metabolism, EFA repletion therapy, and ketone body treatment.	2004	https://www.ncbi.nlm.nih.gov/pubmed/15082091	Henderson, S. (2004). High carbohydrate diets and Alzheimer's disease. Medical Hypotheses, 62(5), pp.689-700.
Diabetes and Alzheimers	-	-	Impairment of synaptic plasticity and memory formation in GLP-1	Results demonstrate that the murine GLP-1R plays an important role in the control of synaptic plasticity and some forms of memory formation.	2009	http://www.sciencedirect.com/science/art	Abbas, T., Faivre, E. and Hölscher, C. (2009). Impairment of synaptic plasticity and memory

			receptor KO mice: Interaction between type 2 diabetes and Alzheimer's disease	The results shed light on the molecular processes that underlie the neuroprotective properties of GLP-1 analogues in animal models of Alzheimer's disease.		icle/pii/S0166432809003970	formation in GLP-1 receptor KO mice: Interaction between type 2 diabetes and Alzheimer's disease. Behavioural Brain Research, 205(1), pp.265-271.
alzheimers	High carbohydrate,	-	A high-glycemic diet is associated with cerebral amyloid burden in cognitively normal older adults.	A high-glycemic diet was associated with greater cerebral amyloid burden, which suggests diet as a potentially modifiable behaviour for cerebral amyloid accumulation and subsequent Alzheimer disease risk.	2017	https://www.ncbi.nlm.nih.gov/pubmed/29070566	Taylor, M., Sullivan, D., Swerdlow, R., Vidoni, E., Morris, J., Mahnken, J. and Burns, J. (2017). A high-glycemic diet is associated with cerebral amyloid burden in cognitively normal older adults. The American Journal of Clinical Nutrition, 106(6), pp.1463-1470.

alzheimers	Ketogenic diet,	-	Neuroprotective and Anti-inflammatory Activities of Ketogenic Diet on MPTP-induced Neurotoxicity	KD was neuroprotective and anti-inflammatory against MPTP-neurotoxicity.	2010	https://link.springer.com/article/10.1007/s12031-010-9336-y#citeas	Yang, X. and Cheng, B. (2010). Neuroprotective and Anti-inflammatory Activities of Ketogenic Diet on MPTP-induced Neurotoxicity. Journal of Molecular Neuroscience, 42(2), pp.145-153.
inflammation	Intermittent fasting,	-	Intermittent fasting during Ramadan attenuates proinflammatory cytokines and immune cells in healthy subjects.	results indicate that RIF attenuates inflammatory status of the body by suppressing proinflammatory cytokine expression and decreasing body fat and circulating levels of leukocytes.	2012	https://www.ncbi.nlm.nih.gov/pubmed/23244540/	Faris, MA., Kacimi, S., Al-Kurd, R., Fararjeh, M., Bustanji, Y., Mohammad, M. and Salem, M. (2012). Intermittent fasting during Ramadan attenuates proinflammatory cytokines and immune cells in healthy subjects. Nutrition Research, 32(12), pp.947-955.

hypocholesterolemic	Negative effect - intermittent fasting,	-	Food restriction by intermittent fasting induces diabetes and obesity and aggravates spontaneous atherosclerosis development in hypercholesterolaemic mice.	the IF regimen induced obesity and diabetes and worsened the development of spontaneous atherosclerosis in LDL-receptor knockout mice. Although being efficient in a wild-type background, this type of food restriction is not beneficial in the context of genetic hypercholesterolaemia.	2014	https://www.ncbi.nlm.nih.gov/pubmed/24176004	Dorighello, G., Rovani, J., Luhman, C., Paim, B., Raposo, H., Vercesi, A. and Oliveira, H. (2013). Food restriction by intermittent fasting induces diabetes and obesity and aggravates spontaneous atherosclerosis development in hypercholesterolaemic mice. British Journal of Nutrition, 111(06), pp.979-986.
- CVD	Mediterranean diet,	-	Mediterranean diet and cardiovascular disease: a systematic review and meta-	Our findings indicate and further quantify that MD exerts a protective effect on the risk of CVD. This inverse association includes CHD and ischemic stroke, but apparently not hemorrhagic stroke.	2017	https://www.ncbi.nlm.nih.gov/pubmed/29177567	Rosato, V., Temple, N., La Vecchia, C., Castellan, G., Tavani, A. and Guercio, V. (2017). Mediterranean diet and cardiovascular disease: a systematic review and meta-

			analysis of observational studies.				analysis of observational studies. European Journal of Nutrition.
- diabetes	-	Yerba mate,	Influence of the traditional Brazilian drink Ilex paraguariensis tea on glucose homeostasis.	this study shows that I. paraguariensis has an anti-hyperglycemic potential role able to improve the diabetic status and is probably a source of multiple hypoglycemic compounds	2012	https://www.ncbi.nlm.nih.gov/pubmed/22795927	Pereira, D., Kappel, V., Cazarolli, L., Boligon, A., Athayde, M., Guesser, S., Da Silva, E. and Silva, F. (2012). Influence of the traditional Brazilian drink Ilex paraguariensis tea on glucose homeostasis. Phytomedicine, 19(10), pp.868-877.

Appendix 2. European Food Safety Association – Assessment of health claims

EFSA- I Panel on Dietetic Products, Nutrition and Allergies (NDA). Scientific Opinion on the substantiation of health claims related to iodine and contribution to normal cognitive and neurological function (ID 273), contribution to normal energy-yielding metabolism (ID 402), and contribution to normal thyroid function and production of thyroid hormones (ID 1237) pursuant to Article 13(1) of Regulation (EC) No 1924/2006. EFSA Journal 2010;8(10):1800. [15 pp.]. doi:10.2903/j.efsa.2010.1800. Available online: www.efsa.europa.eu/efsajournal

EFSA- l Panel on Dietetic Products, Nutrition and Allergies (NDA). Scientific Opinion on the substantiation of health claims related to caffeine and increase in physical performance during short-term high-intensity exercise (ID 737, 1486, 1489), increase in endurance performance (ID 737, 1486), increase in endurance capacity (ID 1488) and reduction in the rated perceived exertion/effort during exercise (ID 1488, 1490) pursuant to Article 13(1) of Regulation (EC) No 1924/2006. EFSA Journal 2011;9(4):2053. [24 pp.]. doi:10.2903/j.efsa.2011.2053. Available online: www.efsa.europa.eu/efsajournal

EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA). Scientific Opinion on the substantiation of health claims related to cocoa flavanols and protection of lipids from oxidative damage (ID 652, 1372, 1506, 3143), and maintenance of normal blood pressure (ID 1507) pursuant to Article 13(1) of Regulation (EC) No 1924/2006. EFSA Journal 2010;8(10):1792. [21 pp.]. doi:10.2903/j.efsa.2010.1792. Available online: www.efsa.europa.eu/efsajournal

EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA). Scientific Opinion on the substantiation of health claims related to caffeine and theobromine in cocoa (*Theobroma cacao* L.) and enhancement of mood (ID 4276) pursuant to Article 13(1) of Regulation (EC) No 1924/2006. EFSA Journal 2011;9(6):2269. [14 pp.]. doi:10.2903/j.efsa.2011.2269. Available online: www.efsa.europa.eu/efsajournal

EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA). Scientific Opinion on the substantiation of health claims related to phaseolamine and reduction in body weight (ID 1701) pursuant to Article 13(1) of Regulation (EC) No 1924/2006. EFSA Journal 2011;9(6):2253. [13 pp.]. doi:10.2903/j.efsa.2011.2253. Available online: www.efsa.europa.eu/efsajournal

EFSA-a Panel on Dietetic Products, Nutrition and Allergies (NDA). Scientific Opinion on the substantiation of health claims related to glucomannan and maintenance of normal blood cholesterol concentrations (ID 836, 1560) pursuant to Article 13(1) of Regulation (EC) No 1924/2006. EFSA Journal 2009; 7(9):1258. [14 pp.]. doi:10.2903/j.efsa.2009.1258. Available online: www.efsa.europa.eu/efsajournal

EFSA-b Panel on Dietetic Products, Nutrition and Allergies (NDA). Scientific Opinion on the substantiation of health claims related to konjac mannan (glucomannan) and reduction of body weight (ID 854, 1556, 3725), reduction of post-prandial glycaemic

responses (ID 1559), maintenance of normal blood glucose concentrations (ID 835, 3724), maintenance of normal (fasting) blood concentrations of triglycerides (ID 3217), maintenance of normal blood cholesterol concentrations (ID 3100, 3217), maintenance of normal bowel function (ID 834, 1557, 3901) and decreasing potentially pathogenic gastro-intestinal microorganisms (ID 1558) pursuant to Article 13(1) of Regulation (EC) No 1924/2006. EFSA Journal 2010;8(10):1798. [27 pp.]. doi:10.2903/j.efsa.2010.1798. Available online: www.efsa.europa.eu/efsajournal

EFSA-c Panel on Dietetic Products, Nutrition and Allergies (NDA). Scientific Opinion on the substantiation of health claims related to chromium and contribution to normal macronutrient metabolism (ID 260, 401, 4665, 4666, 4667), maintenance of normal blood glucose concentrations (ID 262, 4667), contribution to the maintenance or achievement of a normal body weight (ID 339, 4665, 4666), and reduction of tiredness and fatigue (ID 261) pursuant to Article 13(1) of Regulation (EC) No 1924/2006. EFSA Journal 2010;8(10):1732. [23 pp.]. doi:10.2903/j.efsa.2010.1732. Available online: www.efsa.europa.eu/efsajournal

EFSA-d Panel on Dietetic Products, Nutrition and Allergies (NDA). Scientific Opinion on the substantiation of health claims related to soy phosphatidyl choline and maintenance of normal blood cholesterol concentrations (ID 709, 1308, 1630, 1961, 3138, 3187, 4687), contribution to normal fat metabolism (ID 1597), increase in the intestinal absorption of glutamine (ID 4251), faster recovery from muscle fatigue after exercise (ID 4249), improvement of neuromuscular function (ID 4250), contribution to normal cognitive function (ID 710, 1596, 1631, 1983) and maintenance of normal neurological function (ID 1596) pursuant to Article 13(1) of Regulation (EC) No 1924/2006. EFSA Journal 2010;8(10):1741. [26 pp.]. doi:10.2903/j.efsa.2010.1741. Available online: www.efsa.europa.eu/efsajournal

EFSA-e Panel on Dietetic Products, Nutrition and Allergies (NDA). Scientific Opinion on the substantiation of health claims related to conjugated linoleic acid (CLA) isomers and contribution to the maintenance or achievement of a normal body weight (ID 686, 726, 1516, 1518, 2892, 3165), increase in lean body mass (ID 498, 731), increase in insulin sensitivity (ID 1517), protection of DNA, proteins and lipids from oxidative damage (ID 564, 1937), and contribution to immune defences by stimulation of production of protective antibodies in response to vaccination (ID 687, 1519) pursuant to Article 13(1) of Regulation (EC) No 1924/2006. EFSA Journal 2010;8(10):1794. [26 pp.]. doi:10.2903/j.efsa.2010.1794. Available online: www.efsa.europa.eu/efsajournal

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EFSA-J Panel on Dietetic Products, Nutrition and Allergies (NDA). Scientific Opinion on the substantiation of health claims related to niacin and energy-yielding metabolism (ID 43, 49, 54), function of the nervous system (ID 44, 53), maintenance of the skin and mucous membranes (ID 45, 48, 50, 52), maintenance of normal LDL-cholesterol, HDL cholesterol and triglyceride concentrations (ID 46), maintenance of bone (ID 50), maintenance of teeth (ID 50), maintenance of hair (ID 50, 2875) and maintenance of nails (ID 50, 2875) pursuant to Article 13(1) of Regulation (EC) No 1924/2006. EFSA Journal 2009; 7(9):1224. [22 pp.]. doi:10.2903/j.efsa.2009.1224. Available online: www.efsa.europa.eu/efsajournal

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EFSA-p Panel on Dietetic Products, Nutrition and Allergies (NDA). Scientific Opinion on the substantiation of health claims related to L phenylalanine and increased alertness (ID 708, 1629), enhancement of mood (ID 657), pain relief (ID 657) and improvement of memory (ID 658) pursuant to Article 13(1) of Regulation (EC) No 1924/2006. EFSA Journal 2010;8(10):1748. [14 pp.]. doi:10.2903/j.efsa.2010.1748. Available online: www.efsa.europa.eu/efsajournal

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