Is hazelnut-enriched bread an effective vehicle for improving blood glucose response and satiety?

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ABSTRACT

**Background:** Both epidemiological studies and randomised controlled trials report a reduction in cardiovascular disease (CVD) risk with the regular consumption of nuts. Furthermore, nut consumption may improve glycaemic control and increase satiety. These beneficial effects are likely to be due to their low available carbohydrate content, favourable fat and protein profiles as well as the presence of bioactive compounds. Moreover, postprandial events have attracted much attention for the potentially important role they may play in CVD risk and diabetic complications. Importantly, to obtain health benefits, nuts must be consumed regularly and in sufficient amounts. To this end, the National Heart Foundation of New Zealand recommends the daily consumption of 30 g of nuts as a means to reduce CVD. However, the 2008/09 New Zealand Adult Nutrition Survey (2008/09 NZANS) showed that only 6.9% of New Zealanders consumed nuts on the day of the 24-hour diet recall, with a mean population intake of 2.8 g/d. Therefore, innovative strategies are required to increase nut consumption. One such strategy is to incorporate nuts into a staple food such as bread. An important consideration for the formulation of the bread is the form of nut. It is possible that bread enriched with sliced nuts, which require more chewing than ground nuts, may promote satiety, leading to a lower food intake and potentially improved body weight regulation. Conversely, the addition of semi-defatted nut flour, a by-product of nut-oil production, may provide a more cost effective option. To date, only few studies have assessed the effects of different forms of nuts on glycaemic control and no study has incorporated different forms of nuts into the bread. Therefore, it is important to compare the health and satiating effects of these bread forms, bearing in mind the cost effectiveness of such strategies.

**Objective:** To compare the effects of consuming three different forms of hazelnut-enriched bread (finely sliced hazelnut, semi-defatted hazelnut flour and a combination bread containing finely sliced hazelnuts and semi-defatted hazelnut flour) with a control bread without hazelnuts, on postprandial glucose concentrations, satiety, and gastrointestinal tolerance.

**Design:** Thirty-two healthy men and women with a mean (SD) age of 30.2 (11.4) years, and mean body mass index (BMI) (SD) of 24.08 (4.10) kg/m² were recruited to
take part in a 10-week, randomised controlled, 4-arm, single-blinded, cross-over study. The participants were allocated in random order to receive the four different breads: white control bread (no hazelnuts), finely sliced nut bread (30 g finely sliced hazelnuts per 120 g of bread), semi-defatted nut flour bread (30 g hazelnut flour per 120 g of bread) and the combination bread containing finely sliced nuts and semi-defatted nut flour (15 g finely sliced hazelnut and 15 g semi-defatted hazelnut flour per 120 g of bread). All three nut breads were designed to contain 30 g of different forms of hazelnut per 120 g of bread, which is the average daily amount of bread reportedly eaten by bread consumers in the 2008/09 NZANS. Each dietary phase lasted 8 days followed by a one week wash-out period. During each dietary phase, the acute glycaemic response (GR) to the breads was measured (days 1 and 8), along with a satiety test (day 2) where both appetite ratings and subsequent energy intake was assessed. In addition, a further one-day food diary was completed by participants to assess energy and nutrient intake. Participants consumed 120 g of bread for 5 days after days 1 and 2 (i.e. days 3-7), up to the next glycaemic response testing session. For the GR testing, the test breads were provided as portions equivalent to 50 g of available carbohydrate and for the satiety testing day, the amount was equal to the amount of bread the participant’s reported consuming at their usual breakfast. The acute GR of each of the breads was assessed on two separate occasions (to take into account the intra-individual variation in blood glucose response) over a 2-hour postprandial period. Participants were fed the test breads after a 10-12 hour overnight fast. Capillary finger prick blood samples for glucose analysis were obtained at 0, 15, 30, 45, 60, 90, and 120 minutes. Glycaemic responses of all breads were assessed by calculating the incremental area under the 2-hour glucose curve (iAUC) and glycaemic index (GI) of the nut breads was calculated using the white bread as a reference. Each GR testing session was separated by one week. During the first GR session participants also reported any gastrointestinal symptoms using a 100-mm visual analogue scale (VAS) at the same time points as the finger pricks. Appetite ratings were measured on day 2 of each dietary treatment using a 100-mm VAS. The participants consumed the amount of bread they reportedly consumed at a usual breakfast and recorded their appetite ratings at five different points in time; at baseline (pre-bread ingestion), immediately post-bread ingestion, and at 1, 2 and 3 hours post-bread ingestion. In addition, subsequent food intake was measured by weighed diet record (WDR) for the remainder of the day. A further diet record was completed on the Sunday of each treatment period.
**Results:** The incremental glucose area under the curves [mean (95% CI)] for the finely sliced nut bread, semi-defatted nut flour bread, combination bread containing finely sliced nuts and semi-defatted nut flour, and white control bread, were 152 (95% CI: 128, 176), 137 (95% CI: 115, 159), 154 (95% CI: 130, 177) and 179 (95% CI: 146, 212) mmol/L.min, respectively. There was a significant difference in area under the curve (AUC) between the nut breads and the white control bread (p<0.001) with no significant differences between the nut breads (p≥0.130 in all cases). The median GI (Interquartile range) for the finely sliced nut bread, semi-defatted nut flour bread and the combination bread containing finely sliced nuts and semi-defatted nut flour were 83.0 (68.5-120), 78.5 (63.5-110.5), and 85.5 (59.5-129.5) respectively, which is consistent with the AUC for the different breads. There were no overall significant differences in the mean GI between the nut breads (p=0.122). There were no significant differences in either satiety (p≥0.135 in all cases) or gastrointestinal symptoms (p≥0.102 in all cases) between the treatment breads. The consumption of hazelnut-enriched breads improved diet quality compared to the white control bread, namely resulting in an increase in monounsaturated fat, vitamin E and dietary fibre.

**Conclusions:** Our results suggest that consuming hazelnut-enriched bread has beneficial effects on glycaemic control. The finely sliced nut bread, semi-defatted nut flour bread and the combination bread containing finely sliced nuts and semi-defatted nut flour equally improved postprandial glycaemic response (PGR) in the participants, supporting their inclusion in a healthy diet. Hazelnuts in different forms can therefore be incorporated into the usual diet as a means of diminishing PGR. In addition there was no difference in satiety between the breads suggesting no short-term differences in energy regulation. The nut breads did not cause any gastrointestinal discomfort and hence were tolerable. Consuming the nut breads improved diet quality in a manner that would be positively associated with a reduction in CVD. While the findings of this study support a short-term benefit for nuts in terms of postprandial glucose response, more studies are required to determine whether these acute benefits result in a long-term improvements in glycaemic control, as well as a reduction in other markers of CVD. (Clinical Trials number: ACTRN12614000213640)

**Key words:** nuts, hazelnuts; postprandial glycaemic response; satiety; appetite, postprandial glycaemia; coronary heart disease, cardiovascular disease; gastrointestinal symptoms, gastrointestinal tolerance; hazelnut-enriched bread
This project was supervised by Dr. Rachel Brown and Dr. Alex Chisholm, from the Department of Human Nutrition, University of Otago, Dunedin, New Zealand. Dr. Siew Ling (Agnes) Tey provided consultancy on study design. Mr. Andrew Gray from the Department of Preventative and Social Medicine, University of Otago, Dunedin, New Zealand, provided consultancy on study design and expert statistical advice.

This study also involved an aspect where sensory testing of the breads was measured in the sensory laboratory at baseline and the end of the intervention, and at home during the intervention. This aspect of the study was completed by a Master of Dietetics student, and so is not reported in this thesis.

The candidate was the project coordinator and was responsible for the following:

- Development of protocols
- Participant recruitment and screening
- Development of the study booklets
- Communicating with the study participants
- Instructing participants on study protocols and data collection
- Administering questionnaires to study participants
- Packaging of the study breads
- Setting up the GI trolleys and equipment
- Data collection, data entry, data cleaning, data analysis and interpretation of results
- Ensuring the quality of the data collection and data entry
- All study data were entered by the candidate, and double-checked at a later date by the candidate.
- Writing the thesis.
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<td>American Heart Association</td>
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<td>AHS</td>
<td>Adventist Health Study</td>
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<td>AUC</td>
<td>Area under the curve</td>
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<td>BMI</td>
<td>Body mass index</td>
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<td>CAD</td>
<td>Coronary artery disease</td>
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<tr>
<td>CCK</td>
<td>Cholecystokinin</td>
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<tr>
<td>CI</td>
<td>Confidence interval</td>
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<tr>
<td>CHD</td>
<td>Coronary heart disease</td>
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<td>CVD</td>
<td>Cardiovascular disease</td>
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<tr>
<td>EPIC</td>
<td>European Prospective Investigation into Cancer and Nutrition</td>
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<tr>
<td>F</td>
<td>Female</td>
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<tr>
<td>FBG</td>
<td>Fasting blood glucose</td>
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<td>FFQ</td>
<td>Food frequency questionnaire</td>
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<td>GI</td>
<td>Glycaemic index</td>
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<tr>
<td>GLP-1</td>
<td>Glucagon-like peptide 1</td>
</tr>
<tr>
<td>GR</td>
<td>Glycaemic response</td>
</tr>
<tr>
<td>HbA₁c</td>
<td>Haemoglobin A₁C</td>
</tr>
<tr>
<td>HDL-C</td>
<td>High-density lipoprotein cholesterol</td>
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<tr>
<td>HOMA-IR</td>
<td>Homeostatic model assessment of insulin resistance index</td>
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<tr>
<td>HPFS</td>
<td>Health Professionals Follow-up study</td>
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<tr>
<td>HR</td>
<td>Hazards ratio</td>
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<tr>
<td>iAUC</td>
<td>Incremental area under the curve</td>
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<td>ICC</td>
<td>Intra-class coefficient</td>
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<tr>
<td>IHD</td>
<td>Ischemic heart disease</td>
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<tr>
<td>IQR</td>
<td>Interquartile range</td>
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<td>IWHS</td>
<td>Iowa Women’s Health Study</td>
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<td>LDL-C</td>
<td>Low-density lipoprotein cholesterol</td>
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<tr>
<td>Abbreviation</td>
<td>Description</td>
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<tr>
<td>M</td>
<td>Male</td>
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<tr>
<td>MI</td>
<td>Myocardial infarction</td>
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<tr>
<td>mmol/L</td>
<td>Millimoles per litre</td>
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<td>MUFA</td>
<td>Monounsaturated fatty acid</td>
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<td>NCEP II</td>
<td>National Cholesterol Education Program Step II diet</td>
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<td>NCS</td>
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<td>NS</td>
<td>Non-significant</td>
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<td>NZ</td>
<td>New Zealand</td>
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<td>NHS</td>
<td>Nurses’ Health Study</td>
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<tr>
<td>OR</td>
<td>Odds ratio</td>
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<td>PGR</td>
<td>Postprandial glycaemic response</td>
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<td>PHS</td>
<td>Physicians’ Health Study</td>
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<tr>
<td>PREDIMED</td>
<td>Prevención con Dieta Mediterránea</td>
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<td>PUFA</td>
<td>Polyunsaturated fatty acid</td>
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<tr>
<td>RCT</td>
<td>Randomised controlled trial</td>
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<tr>
<td>REE</td>
<td>Resting energy expenditure</td>
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<td>RGR</td>
<td>Relative glycaemic response</td>
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<td>RMR</td>
<td>Resting metabolic rate</td>
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<tr>
<td>RR</td>
<td>Relative risk</td>
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<tr>
<td>SD</td>
<td>Standard deviation</td>
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<tr>
<td>SEM</td>
<td>Standard error of the mean</td>
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<tr>
<td>SFA</td>
<td>Saturated fatty acid</td>
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<tr>
<td>SUN</td>
<td>Seguimiento Universidad de Navarra</td>
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<tr>
<td>TAG</td>
<td>Triacylglyceride</td>
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<tr>
<td>TC</td>
<td>Total cholesterol</td>
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<td>TC: HDL-C ratio</td>
<td>Total cholesterol to high-density lipoprotein cholesterol ratio</td>
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<tr>
<td>T2DM</td>
<td>Type 2 diabetes mellitus</td>
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<tr>
<td>VAS</td>
<td>Visual analogue scale</td>
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WDR  Weighed diet record
1994-96 CSFI/DHKS  1994-96 Continuing Survey of Food Intake by Individuals and Diet and Health Knowledge Survey
2008/09 NZANS  2008/09 New Zealand Adult Nutrition Survey

cm  Centimetres
 g  Grams
 g/d  Grams per day
 kcal  Kilocalories
 kcal/d  Kilocalories per day
 kJ  Kilojoules
 kJ/d  Kilojoules per day
 kg  Kilograms
 m  metres

<  Less than
>  More than
≤  Less than or equal to
≥  More than or equal to
1.0 INTRODUCTION

A tree nut is defined as an edible kernel or a one-seeded dried fruit, which is surrounded by a hard shell (1-3). These include several species such as almonds, Brazil nuts, cashew nuts, hazelnuts, macadamia nuts, pecans, pine nuts, pistachios, and walnuts (2). The nutrient and phytochemical content of nuts can vary considerably by nut type, and genotype (1). Peanuts, which actually belong to the legume family, share a similar nutritional profile with nuts (1, 4).

Despite small variations in their micro- and macro-nutrient profiles, nuts as a whole are regarded as ‘healthy foods’ because of their good fatty acid profile (low in saturated fats and rich sources of cis-unsaturated fatty acids) and low available carbohydrate content, as well as being good sources of vegetable protein, fibre, phytosterols, polyphenols, and several vitamins and minerals (1, 2, 4-6).

Extensive research has been carried out on regular nut consumption and various health outcomes. Evidence from epidemiologic and numerous clinical studies have suggested that frequent nut consumption is associated with favourable plasma lipid profiles, reduced risk of coronary heart disease (CHD), aids weight maintenance and reduces the risk of several other chronic diseases (7-10). As a result, nuts are recommended as an important component of a healthy diet throughout the world (2, 10). The National Heart Foundation of New Zealand recommends that people should consume approximately 30 g of nuts daily as a means to reduce CVD risk (11). Despite these recommendations the prevalence of nut consumption is relatively low. For example, the latest adult nutrition survey (2008/09 NZANS) carried out among New Zealand adults aged 15+ years, reported that only 6.9% of New Zealanders consumed nuts with a mean population intake of 2.8 g/day (12). Therefore, innovative strategies to increase nut consumption are required.

Since nuts are extremely versatile and can be consumed in many different ways, consumers can easily incorporate them into their diets (3). One such strategy to increase nut consumption is to incorporate nuts into a common staple in New Zealand such as bread. The 2008/09 NZANS reported that 71.3% of New Zealanders consumed bread, with an average intake of 120 g/day (13). This strategy may also be beneficial
from a compliance perspective, because it adds variation in the way nuts are consumed and may allow participants to better adhere to the dietary recommendations.

There are several important considerations for designing the formulation of such bread, including the effects on health, fullness and acceptability. Different forms of hazelnuts may be more acceptable and effective than others. It is possible that bread enriched with sliced nuts, which require more mastication than ground nuts, may promote satiety. Conversely, the addition of semi-defatted nut flour, a by-product of nut-oil production, may provide a more cost effective option. It would therefore be of interest to compare breads containing finely sliced nuts (hazelnut), semi-defatted nut flour (hazelnut flour), and a combination of both. The bread containing the semi-defatted nut flour may also provide information on the contributions of the non-lipid components of nuts to health. Including a bread combining both sliced nuts and semi-defatted nut flour may potentially result in an effective, but more affordable loaf.

There is much current interest in the relation of CHD to postprandial events. Postprandial hyperglycaemia has been linked with increased risk of CHD (14). The 2-hour postprandial glycaemia is now recognised as an independent risk factor for CHD (14). Therefore, as a first step in investigating the efficacy of a nut-enriched bread on health, we deemed it important to examine the acute effects of such bread on GR. In addition, we were also interested in measuring satiety. Some previous research has indicated that nuts may increase satiety (15, 16). It is of interest to investigate whether this satiating effect of nuts is also apparent when nuts are added to bread. This information will provide important information for designing large randomised controlled trials (RCTs) to examine the long-term health effects of regular consumption of nut-enriched bread.

Therefore the overall aim of the present study was to investigate the effects of consuming different forms of hazelnut-enriched bread (finely sliced hazelnut, semi-defatted hazelnut flour and a combination bread containing finely sliced nuts and semi-defatted nut flour) on postprandial blood glucose response, satiety and gastrointestinal tolerance in comparison to a white control bread.
1.1 Research Hypothesis

The present study is the first to assess the effect of different forms of hazelnut-enriched bread on postprandial glycaemia, satiety and gastrointestinal tolerance. The hypothesis for this study is that the hazelnut-enriched breads will lower postprandial glycaemic response (PGR) compared to the white bread with no nuts (control bread). In addition we hypothesise that nut-enriched breads will increase satiety (fullness) compared to the control bread due to the higher protein and fibre content.
2.0 LITERATURE REVIEW

2.1 Identification and selection of studies/survey

Literature was searched using MEDLINE, Science Direct, OVID and Google Scholar. Key words used to search for relevant literature included “nuts”, “tree nuts” “body weight”, “satiety”, “glycaemic index”, “cardiovascular diseases”, “glycaemic response”, and “diabetes”. The search was further narrowed down using key words such as “hazelnut”, “almond”, “Brazil nuts”, “cashew nut”, “macadamia nuts”, “peanuts”, “pecan”, “pine nuts”, “pistachio nuts”, and “walnut”. In some cases, articles found through this search strategy referred to other papers that were not identified by the search. All of the articles that were identified via this electronic search were screened independently for suitability and eligibility and were only included if relevant. Furthermore, the reference sections of all papers were examined for additional articles.

Cross-sectional studies, prospective cohort studies, randomised controlled trials and meta-analyses and systematic reviews that assessed a nut-enriched diet (almonds, Brazil nuts, cashew nuts, hazelnuts, macadamia nuts, pecans, pine nuts, pistachio nuts, walnuts and peanuts) and reported on health effects of nuts (on all-cause mortality, cardiovascular diseases, and diabetes), or/and nut intake, and/or anthropometrical outcomes (body weight and body mass index), and/or markers of glycaemic control were included in the literature review. Studies were excluded if data on anthropometrical outcomes, markers of glycaemic control and health effects of nuts were not clearly stated in the selected articles.

2.2 Nutritional Composition of Nuts

Nuts are complex food matrices and sources of energy, a variety of nutrients and bioactive compounds (phytochemicals) such as tocopherols, phytosterols, and phenolic compounds (1, 5, 6, 17). In general, the various nuts have a healthy nutritional profile and are nutrient dense, sharing many similarities in nutrient composition (1, 2, 4, 18). For the purpose of this literature review, the term “nut” encompasses peanuts and all tree nuts (almonds, Brazil nuts, cashew nuts, hazelnuts, macadamia nuts, pecans, pine nuts, pistachio nuts and walnuts) except coconut and chestnut.
Table 2.1. Macronutrient composition for 100 g and one serving (~30 g) of raw nuts

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Hazelnut 100g</th>
<th>30g</th>
<th>Almond 100g 30g</th>
<th>Cashew nut 100g 30g</th>
<th>Brazil nut 100g 30g</th>
<th>Macadamia nut 100g 30g</th>
<th>Pecan 100g 30g</th>
<th>Pistachio nut 100g 30g</th>
<th>Pine nut 100g 30g</th>
<th>Walnut 100g 30g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (kJ)</td>
<td>2550</td>
<td>765</td>
<td>2430</td>
<td>729</td>
<td>2400.0</td>
<td>720</td>
<td>2790</td>
<td>837</td>
<td>2970</td>
<td>891</td>
</tr>
<tr>
<td>Water (g)</td>
<td>4.7</td>
<td>1.4</td>
<td>2.6</td>
<td>0.8</td>
<td>3.5</td>
<td>1.1</td>
<td>8.5</td>
<td>2.6</td>
<td>2.9</td>
<td>0.9</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>14.8</td>
<td>4.4</td>
<td>22.1</td>
<td>6.6</td>
<td>17.0</td>
<td>5.1</td>
<td>12.0</td>
<td>3.6</td>
<td>9.8</td>
<td>2.9</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>5.2</td>
<td>1.6</td>
<td>5.6</td>
<td>1.7</td>
<td>16.8</td>
<td>5.0</td>
<td>3.8</td>
<td>1.1</td>
<td>4.5</td>
<td>1.4</td>
</tr>
<tr>
<td>Dietary fibre (g)</td>
<td>10.4</td>
<td>3.1</td>
<td>11.8</td>
<td>3.5</td>
<td>5.9</td>
<td>1.8</td>
<td>8.0</td>
<td>2.4</td>
<td>9.3</td>
<td>2.8</td>
</tr>
<tr>
<td>Sugar (g)</td>
<td>4.2</td>
<td>1.3</td>
<td>4.9</td>
<td>1.5</td>
<td>5.5</td>
<td>1.7</td>
<td>1.6</td>
<td>0.5</td>
<td>3.8</td>
<td>1.1</td>
</tr>
<tr>
<td>Total fat (g)</td>
<td>59.8</td>
<td>17.9</td>
<td>52.8</td>
<td>15.8</td>
<td>49.2</td>
<td>14.8</td>
<td>68.2</td>
<td>20.5</td>
<td>73.7</td>
<td>22.1</td>
</tr>
<tr>
<td>SFA (g)</td>
<td>5.7</td>
<td>1.7</td>
<td>4.0</td>
<td>1.2</td>
<td>8.4</td>
<td>2.5</td>
<td>17.4</td>
<td>5.2</td>
<td>11.0</td>
<td>3.3</td>
</tr>
<tr>
<td>MUFA (g)</td>
<td>42.4</td>
<td>12.7</td>
<td>33.7</td>
<td>10.1</td>
<td>31.1</td>
<td>9.3</td>
<td>22.4</td>
<td>6.7</td>
<td>58.2</td>
<td>17.5</td>
</tr>
<tr>
<td>PUFA (g)</td>
<td>8.7</td>
<td>2.6</td>
<td>12.7</td>
<td>3.8</td>
<td>7.5</td>
<td>2.3</td>
<td>25.4</td>
<td>7.6</td>
<td>1.3</td>
<td>0.4</td>
</tr>
</tbody>
</table>

1 Nutrient data are from The Concise New Zealand Food Composition Tables, 10th Edition 2013 (Slightly Revised, August 2014) (20), for 100 g portion only.
Abbreviation used: MUFA= Monounsaturated fatty acids; PUFA= Polyunsaturated fatty acids and SFA= Saturated fatty acids.
This is because peanuts have a very similar nutrient profile to tree nuts while coconut and chestnut, on the other hand, differ in nutrient composition to tree nuts (2, 19). Coconuts are relatively high in saturated fatty acid (SFA) (29.7 g/100 g), and chestnuts are high in carbohydrate (53.2 g/100 g). The two nuts (coconut and chestnut) have a very high water content of up to 45 g/100 g and also contain very little protein (<4 g/100 g) (18, 20, 21). Hence, these characteristics make their nutrient composition different from other nuts, which have been included in this literature review.

Tables 2.1 and 2.2 summarise the macro- and micro-nutrient distribution of the different nuts. Although nuts are relatively high in total fat content, their fatty acid profile is mostly monounsaturated (MUFA) or polyunsaturated (PUFA) (2, 4, 19, 22). Nuts are also low in saturated fat (1, 2, 22). The highest total fat content is exhibited by macadamia nut, followed by pecans, pine nuts, Brazil nuts, walnuts, hazelnuts, almonds, peanuts, pistachios, and cashew nuts (1, 21). The MUFA content predominates over the PUFA content in most nuts such as, almonds, cashew nuts, macadamia nuts, pecans, pistachios and hazelnuts, except for walnuts, pine nuts and Brazil nuts which are high in PUFA (1, 21). The MUFA content in edible portions of nuts ranges from 12.4 g/100 g in walnuts to 58.2 g/100 g in macadamia nuts, while PUFA vary from 1.3 g/100 g in macadamia nuts to 42.5 g/100 g in walnuts (1). As a rich source of fatty acids, nuts are considered energy dense foods (1, 2, 15). Most nuts provide between 2093-2931 kJ/100 g (500-700 kcal/100 g) of energy per edible portion (1).

Nuts are also a good source of dietary fibre and plant protein and yield about 5-11% by weight of dietary fibre (ranging from 2.4 to 33.5 g/100 g of dietary fibre in edible portion when consumed with seed skins) and provide 10-25% by weight in protein (1, 22).

In spite of their similarities in fat content, nuts have a wide range of chemical composition (21). Different nuts supply several important vitamins, such as vitamin E, folate, vitamin B6, vitamin K, thiamin, riboflavin, and niacin and minerals such as zinc, phosphorus, sodium, and potassium (22).

In addition, some nuts are also high in trace elements which are crucial in the diet (21). These elements including chromium (Cr), copper (Cu), and iron (Fe) act as cofactors for many physiological and metabolic functions (1, 21).
<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Hazelnut 100g</th>
<th>Almond 100g</th>
<th>Cashew nut 100g</th>
<th>Brazil nut 100g</th>
<th>Macadamia nut 100g</th>
<th>Pecan 100g</th>
<th>Pistachio nut 100g</th>
<th>Pine nut 100g</th>
<th>Walnut 100g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium (mg)</td>
<td>180.0</td>
<td>54.0</td>
<td>260.0</td>
<td>78.0</td>
<td>34.0</td>
<td>10.2</td>
<td>180.0</td>
<td>54.0</td>
<td>70.0</td>
</tr>
<tr>
<td>Iron (mg)</td>
<td>2.0</td>
<td>0.6</td>
<td>3.7</td>
<td>1.1</td>
<td>5.0</td>
<td>1.50</td>
<td>2.8</td>
<td>0.8</td>
<td>2.4</td>
</tr>
<tr>
<td>Phosphorous (mg)</td>
<td>280.0</td>
<td>84.0</td>
<td>480.0</td>
<td>144.0</td>
<td>530.0</td>
<td>159.0</td>
<td>590.0</td>
<td>177.0</td>
<td>140.0</td>
</tr>
<tr>
<td>Potassium (mg)</td>
<td>900.0</td>
<td>270.0</td>
<td>710.0</td>
<td>213.0</td>
<td>550.0</td>
<td>165.0</td>
<td>760.0</td>
<td>228.0</td>
<td>370.0</td>
</tr>
<tr>
<td>Sodium (mg)</td>
<td>0.0</td>
<td>0.0</td>
<td>1.0</td>
<td>0.3</td>
<td>11.0</td>
<td>3.3</td>
<td>2.0</td>
<td>0.6</td>
<td>5.0</td>
</tr>
<tr>
<td>Zinc (mg)</td>
<td>2.1</td>
<td>0.6</td>
<td>3.1</td>
<td>0.9</td>
<td>5.5</td>
<td>1.65</td>
<td>4.2</td>
<td>1.3</td>
<td>1.7</td>
</tr>
<tr>
<td>Vitamin C (mg)</td>
<td>1.0</td>
<td>0.3</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.7</td>
<td>0.2</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Thiamin (mg)</td>
<td>0.5</td>
<td>0.1</td>
<td>0.2</td>
<td>0.1</td>
<td>0.6</td>
<td>0.19</td>
<td>1.0</td>
<td>0.3</td>
<td>0.4</td>
</tr>
<tr>
<td>Riboflavin (mg)</td>
<td>0.1</td>
<td>0.0</td>
<td>1.0</td>
<td>0.3</td>
<td>0.2</td>
<td>0.06</td>
<td>0.1</td>
<td>0.0</td>
<td>0.1</td>
</tr>
<tr>
<td>Niacin (mg)</td>
<td>6.7</td>
<td>2.0</td>
<td>7.0</td>
<td>2.1</td>
<td>7.3</td>
<td>2.19</td>
<td>4.3</td>
<td>1.3</td>
<td>3.8</td>
</tr>
<tr>
<td>Vitamin B6 (mg)</td>
<td>0.2</td>
<td>0.1</td>
<td>0.0</td>
<td>0.4</td>
<td>0.11</td>
<td>0.2</td>
<td>0.1</td>
<td>0.1</td>
<td>0.2</td>
</tr>
<tr>
<td>Folate (µg)</td>
<td>110.0</td>
<td>33.0</td>
<td>50.0</td>
<td>15.0</td>
<td>25.0</td>
<td>7.5</td>
<td>22.0</td>
<td>6.6</td>
<td>11.0</td>
</tr>
<tr>
<td>Vitamin A (µg)</td>
<td>2.7</td>
<td>0.8</td>
<td>0.2</td>
<td>0.1</td>
<td>1.0</td>
<td>0.3</td>
<td>1.5</td>
<td>0.5</td>
<td>0.0</td>
</tr>
<tr>
<td>Vitamin E (µg)</td>
<td>17.0</td>
<td>5.1</td>
<td>26.0</td>
<td>7.8</td>
<td>0.7</td>
<td>0.22</td>
<td>7.2</td>
<td>2.2</td>
<td>0.4</td>
</tr>
</tbody>
</table>

1Nutrient data are from The Concise New Zealand Food Composition Tables, 10th Edition 2013 (Slightly Revised, August 2014) (20), for 100 g portion only.
The order of element levels in nuts typically follows the pattern; Magnesium (Mg) > Calcium (Ca) > Iron (Fe) > Copper (Cu) > Chromium (Cr) > Arsenic (As) > Selenium (Se) (1, 21).

Hazelnuts have an important place among the different types of nuts in terms of nutrition and health (1, 23). They are a rich source of the most common MUFA, oleic acid, as well as protein and vitamin E (1). Hazelnuts are low in sodium (undetectable levels) and contain significant amounts of antioxidants, such as flavan-3-ols and anthocyanins (6, 22, 1). Moreover, they also contain substantial amounts of some of the B-vitamins, such as folate, and niacin, as well as smaller amounts of other vitamins such as thiamine, vitamin B6, riboflavin, and vitamin C (1, 6).

2.3 Health effects of nuts: An overview

In the preceding years, nuts have attracted the attention of many investigators for their potential health benefits. Based on the current scientific evidence, nuts appear to play an important role in improving the risk factors for many chronic diseases. Although not the basis of this thesis, it is important to set the scene on the potential health benefits of regular nut consumption. This section of the chapter provides an overview of the major epidemiological and intervention trials which describe the relationship between nut consumption and some current major health concerns such as all-cause mortality, cardiovascular diseases (CVDs), and type 2 diabetes mellitus (T2DM).

2.3.1 Nuts and all-cause mortality

Despite the inverse association observed between nut intake and several major chronic diseases, only eight studies have investigated the effects of nut consumption in relation to total mortality (7, 24-30) (Table 2.3). An inverse association has been observed between nut intake and all-cause mortality in most studies conducted to date (7, 24-29).

Compared to non-nut consumers, a 14% (95% CI: 5%, 23%), 18% (95% CI:4%, 30%), 20% (95% CI: 14%, 27%), 39% (95% CI:17%, 55%) and 40% (95% CI: 0%-70%) reduction in risk for all-cause mortality were observed (in the highest nut consumption category) in the studies by Baer et al., 2011; Fraser et al., 1997, Bao et al., 2013, Guasch-Ferre et al., 2013 et al, and Fraser et al., 1997, respectively (7, 24, 25, 28, 29).
Table 2.3. Observational studies evaluating the effects of nut consumption on risk of all-cause mortality

<table>
<thead>
<tr>
<th>Author (year)</th>
<th>Study name</th>
<th>Study design</th>
<th>Number of participants</th>
<th>Duration of follow-up</th>
<th>Exposure assessment</th>
<th>Endpoint</th>
<th>Nut intake category</th>
<th>HR (95% CI)</th>
<th>P for trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baer et al. (2011)</td>
<td>The NHS³</td>
<td>Prospective cohort</td>
<td>50, 112 (F)</td>
<td>19 years</td>
<td>FFQ</td>
<td>All-cause mortality</td>
<td>None</td>
<td>0.90 (0.87-0.93)</td>
<td>&lt;0.050</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>≤1 time/week</td>
<td>0.86 (0.77-0.95)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>≥2 times/week</td>
<td>0.75 (0.65-0.86)</td>
<td></td>
<td>&lt;0.010</td>
</tr>
<tr>
<td>Fraser et al. (1997)</td>
<td>The AHS¹</td>
<td>Prospective cohort</td>
<td>34, 198 (M, F)</td>
<td>12 years</td>
<td>FFQ</td>
<td>All-cause mortality</td>
<td>&lt;1 time/week</td>
<td>0.71 (0.54-0.93)</td>
<td>0.012</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1-4 times/week</td>
<td>0.6 (0.4-0.9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>≥5 times/week</td>
<td>0.6 (0.3-1.0)</td>
<td></td>
<td>&lt;0.050</td>
</tr>
<tr>
<td>Bao et al. (2013)</td>
<td>The NHS³ and The HPFS⁶</td>
<td>Prospective cohort</td>
<td>76, 464 (F)</td>
<td>30 years</td>
<td>FFQ</td>
<td>Total and All-cause mortality</td>
<td>Never</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt;1 time/week</td>
<td>0.93 (0.90-0.96)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1/week</td>
<td>0.89 (0.86-0.93)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2-4 times/week</td>
<td>0.87 (0.83-0.90)</td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>5-6 times/week</td>
<td>0.85 (0.79-0.91)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>≥7 times/week</td>
<td>0.80 (0.73-0.86)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Guasch-Fere et al.</td>
<td>PREDIMED trial⁵</td>
<td>Prospective cohort</td>
<td>7216(M,F)</td>
<td>4.8 years</td>
<td>Mediterranean diet with nuts</td>
<td>Total mortality</td>
<td>Never</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>(2013)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1-3 times/week</td>
<td>0.71 (0.54-0.93)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&gt;3 times/week</td>
<td>0.61 (0.45-0.83)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fraser et al. (1997)</td>
<td>The AHS¹</td>
<td>Prospective cohort</td>
<td>1668 (M,F)</td>
<td>9 years</td>
<td>FFQ</td>
<td>All-cause mortality</td>
<td>&lt;1 time/week</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>(24)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1-4 times/week</td>
<td>0.6 (0.4-0.9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>≥5 times/week</td>
<td>0.6 (0.3-1.0)</td>
<td></td>
<td>&lt;0.050</td>
</tr>
<tr>
<td>Mann et al. (1997)</td>
<td>-</td>
<td>Prospective cohort</td>
<td>10, 802 (M, F)</td>
<td>13.3 years</td>
<td>FFQ</td>
<td>Total mortality</td>
<td>≤ once a week</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>(26)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1-4 times/week</td>
<td>0.99 (0.79-1.25)</td>
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<td></td>
<td></td>
<td></td>
<td>≥5 times/week</td>
<td>0.77 (0.58-1.01)</td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>Van den Brandt (2011)</td>
<td>The NCS⁷</td>
<td>Case-cohort analysis</td>
<td>120, 852 (M, F)</td>
<td>9 years</td>
<td>FFQ</td>
<td>All-cause mortality</td>
<td>IQR: 25th percentile</td>
<td>0.92 (0.87-0.98) M</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>75th percentile</td>
<td>0.95 (0.90-1.00) F</td>
<td></td>
<td>**</td>
</tr>
</tbody>
</table>

Abbreviations used: CI=confidence interval; F=female; FFQ=food frequency questionnaire; HR=hazard ratio; IQR=interquartile range; M=male; NS=non-significant; RCT=randomised controlled trial
¹The Adventist Health Study; ³The Nurses’ Health Study; ⁵The Prevención con Dieta Mediterránea trial; ⁶The Health Professionals Follow-up Study; ⁷The Netherlands cohort study;
** specifics not available
Additional information, in a meta-analysis performed by Luo et al., 2014, it was observed that for a 1-serving/day increment in nut intake, there was a 17% (95% CI: 9%, 24%) reduction in risk for all-cause mortality (P=0.032). Furthermore, the pooled relative risk for all-cause mortality for the comparison of extreme quantiles of nut intake was 0.85 (95% CI: 0.79, 0.91; P=0.005) (30).

Alternatively, there have also been studies that have produced mixed results. In a study by Mann et al., 1997, no association was observed between nut consumption and total mortality while van den Brandt, (2011), found an association for men but not women (26, 27).

The different results could reflect confounding by unmeasured or poorly measured factors. Moreover, this could also be due to inadequacies of the instrument for measuring dietary intake which in these cases were food frequency questionnaires. There could have been changes in dietary habits which the investigators may not have been aware of. This could have led to measurement errors which may have attenuated the associations. Moreover, the observed differences may be the result of a different hormonal milieu between the sexes or just due to chance.

Overall the majority of the studies show an inverse association between nut intake and all-cause mortality. This finding has been observed among a number of populations, including both males and females (7, 28, 29), Black Americans Seventh Day Adventists (24) and elderly non-Hispanic White Seventh Day Adventists (25). These consistent findings may be regarded as a strength as similar results were observed in different population groups. However, given the observational nature of the studies, it is not possible to firmly conclude that the inverse relationship between nut consumption and all-cause mortality reflects cause and effect. Nevertheless, there are some major strengths to the studies such as, large sample size, relatively long duration, and thorough ascertainment of mortality as an outcome in these prospective observational assessments.

The weight of the evidence available to date supports the notion that nut consumption reduces all-cause mortality risk in a number of different population groups.
<table>
<thead>
<tr>
<th>Author (year)</th>
<th>Study name</th>
<th>Number of participants</th>
<th>Duration of follow-up</th>
<th>Exposure assessment</th>
<th>Endpoint</th>
<th>Nut intake category</th>
<th>RR (95% CI)</th>
<th>P for trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fraser et al. (1992)</td>
<td>The AHS¹</td>
<td>31,208 (M,F)</td>
<td>6 years</td>
<td>FFQ</td>
<td>Fatal CHD</td>
<td>&lt;1 time/week</td>
<td>1.00</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td>1-4 times/week</td>
<td>0.73 (0.54-0.99)</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>≥5 times/week</td>
<td>0.62 (0.44-0.90)</td>
<td>&lt;0.010</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Non-fatal MI</td>
<td>&lt;1 time/week</td>
<td>1.00</td>
<td></td>
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<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>1-4 times/week</td>
<td>0.74 (0.49-1.11)</td>
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<td></td>
<td></td>
<td>≥5 times/week</td>
<td>0.52 (0.30-0.87)</td>
<td>&lt;0.010</td>
</tr>
<tr>
<td>Prineas et al. (1993)</td>
<td>The IWHS²</td>
<td>41,837 (F)</td>
<td>5 years</td>
<td>FFQ</td>
<td>Fatal CHD</td>
<td>Never</td>
<td>1.00</td>
<td></td>
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<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>1-3 times/month</td>
<td>0.60 (0.44-0.89)</td>
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<td></td>
<td>1 time/week</td>
<td>0.75 (0.44-1.27)</td>
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<td></td>
<td></td>
<td>2-4 times/week</td>
<td>0.43 (0.19-0.93)</td>
<td>0.060</td>
</tr>
<tr>
<td>Kushi et al. (1996)</td>
<td>The IWHS</td>
<td>34,486 (F)</td>
<td>7 years</td>
<td>FFQ</td>
<td>Fatal CHD</td>
<td>Never</td>
<td>1.00</td>
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<td></td>
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<td></td>
<td></td>
<td>1 or 2 times/week</td>
<td>0.98 (0.57-1.68)</td>
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<td></td>
<td></td>
<td>3 or 4 times/week</td>
<td>0.96 (0.56-1.64)</td>
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<td></td>
<td></td>
<td></td>
<td>&gt;4 times/week</td>
<td>0.60 (0.36-1.01)</td>
<td></td>
</tr>
<tr>
<td>Hu et al. (1998)</td>
<td>The NHS³</td>
<td>86,016 (F)</td>
<td>14 years</td>
<td>FFQ</td>
<td>Total CHD</td>
<td>Almost never</td>
<td>1.00</td>
<td></td>
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<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>≤ once a week</td>
<td>0.91 (0.81-1.02)</td>
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<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>2-4 times/week</td>
<td>0.77 (0.61-0.97)</td>
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<td></td>
<td></td>
<td>≥5 times/week</td>
<td>0.65 (0.47-0.89)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Fatal CHD</td>
<td>Almost never</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>≤ once a week</td>
<td>0.75 (0.61-0.93)</td>
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<td></td>
<td>2-4 times/week</td>
<td>0.56 (0.36-0.89)</td>
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<td></td>
<td></td>
<td></td>
<td>≥5 times/week</td>
<td>0.61 (0.35-1.05)</td>
<td>0.007</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Non-fatal MI</td>
<td>Almost never</td>
<td>1.00</td>
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<td></td>
<td></td>
<td></td>
<td>≤ once a week</td>
<td>1.00 (0.87-1.16)</td>
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<td></td>
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<td></td>
<td></td>
<td>2-4 times/week</td>
<td>0.87 (0.66-1.14)</td>
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<td></td>
<td></td>
<td></td>
<td>≥5 times/week</td>
<td>0.68 (0.47-1.00)</td>
<td>0.040</td>
</tr>
<tr>
<td>Albert et al. (2002)</td>
<td>The PHS¹</td>
<td>21,454 (M)</td>
<td>17 years</td>
<td>FFQ</td>
<td>Total CHD death</td>
<td>≤ once a month</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
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<td></td>
<td></td>
<td></td>
<td>1-3 times/month</td>
<td>0.89 (0.67-1.16)</td>
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<td></td>
<td>1 time/week</td>
<td>0.90 (0.67-1.22)</td>
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<td></td>
<td></td>
<td>≥2 times/week</td>
<td>0.70 (0.50-0.98)</td>
<td>0.060</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td>Sudden death</td>
<td>≤ once a month</td>
<td>1.00</td>
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<td></td>
<td></td>
<td></td>
<td>1-3 times/month</td>
<td>0.80 (0.52-1.23)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>1 time/week</td>
<td>0.60 (0.36-1.00)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>≥2 times/week</td>
<td>0.53 (0.30-0.92)</td>
<td>0.010</td>
</tr>
</tbody>
</table>

Abbreviations used: CHD = coronary heart disease; CI = confidence interval; F = female; FFQ = food frequency questionnaire; M = male; MI = myocardial infarction; RR = relative risk
¹The Adventist Health Study; ²The Iowa Women's Health Study; ³The Nurses' Health Study; ⁴The Physicians' Health Study
2.3.2 Nut consumption and cardiovascular disease (CVD) risk

2.3.2.1 Epidemiological evidence for nut consumption and CVD risk

Perhaps the area that has received the most attention and is most consistent with regard to demonstrating the health effects of nuts is cardiovascular disease (CVD). CVD is a significant cause of morbidity and mortality world-wide and considerable research has suggested that nuts have beneficial effect on CVD risks (31, 32). A summary of the epidemiological studies on the effects of nut consumption on CHD is provided in Tables 2.4 and 2.5.

To date, four major cohort studies; namely, The Adventist Health Study (AHS), The Iowa Women’s Health Study (IWHS), The Nurses’ Health Study (NHS), and The Physicians’ Health Study (PHS), have shown a clear dose-response relation between nut consumption and reduced CHD risk (8, 32-36).

The results from these four cohort studies are remarkably consistent, and in general, report that frequent nut consumption lowers the risk of CVD, fatal and non-fatal CHD, non-fatal myocardial infarction (MI) and sudden cardiac death (8, 32-36).

In the AHS, an inverse association was observed between frequent nut consumption and definite fatal CHD and non-fatal MI events. In this cohort, subjects who consumed nuts 1-4 times per week and 5 or more times per week, experienced a 27% (95% CI: 1%, 46%) and 38% (95% CI: 10%, 56%) reduction in fatal CHD events, respectively, P for trend < 0.01 (32, 33). Similar inverse associations were also present between frequent nut consumption and non-fatal MI where subjects who consumed nuts frequently (more than five times per week) exhibited a 48% reduction in risk (95% CI: 13%, 70%; P for trend <0.010) (33).

In the IWHS, Prineas et al., 1993, found that the multivariate relative risks for fatal CHD across categories of nut consumption (no nuts, nuts 1-3 times/month, once a week, and 2-4 times/week) were 1.0, 0.60 (95% CI:0.4, 0.89), 0.75 (95% CI:0.44, 1.27), and 0.43 (95% CI:0.19-0.93), respectively, (P for trend = 0.060), after five years of follow up (32, 34). This inverse association was also observed after seven years of follow-up of the IWHS by Kushi et al, 1996. The risk of fatal CHD was reduced by 2% in those who consumed nuts 1 or 2 times/week, 4% in those who consumed nuts 3 or 4
times/week while 40% in those who consumed nuts more than four times per week compared with subjects who never consumed nuts; P for trend=0.016 (32, 35).

Furthermore, frequent nut consumption was also associated with a reduced risk of both fatal CHD and non-fatal MI in the NHS (36). After adjusting for a wide range of CHD risk factors, Hu et al., 1998, concluded that compared with women who rarely ate nuts (almost never or less than once per month), those with frequent consumption of 2-4 times per week and five or more times per week, had a 23% (95% CI: 3%, 39) and a 35% (95% CI: 11%, 53%) lower risk of total CHD, respectively; P for trend<0.001. The magnitude of risk reduction was similar for fatal CHD with a 25% (95% CI: 7%, 39%), 44% (95% CI: 11%, 64%), and 39% reduction in risk for those who consumed nuts ≤1 time/week, 2-4 times/week and five or more times/week, respectively (P for trend=0.007). Similar reductions in the risk for non-fatal MI were also observed. Those who consumed nuts 2-4 times/week and five or more times/week experienced a 13% and 32% reduction in non-fatal MI risks, respectively; P for trend<0.04 (32, 36).

Likewise, after adjusting for known cardiac risk factors and other dietary habits, nut intake was associated with a significantly reduced risk of sudden cardiac death in the PHS (8). Nut intake was inversely related to death from CHD and sudden cardiac death for men who consumed nuts two or more times per week. Compared with men who rarely consumed nuts, those who consumed nuts 2 or more times per week had a 47% (95% CI: 8%, 70%; P for trend=0.010) reduced risks of sudden cardiac death and 30% (95% CI: 2%, 50%; P for trend=0.060) reduced risks of total CHD death, respectively (8).
Table 2.5. Meta-analyses and systematic reviews: The effects of nut consumption on risk of coronary heart disease (CHD)

<table>
<thead>
<tr>
<th>Author (year)</th>
<th>Number of participants</th>
<th>Number of studies included</th>
<th>Endpoint</th>
<th>Pooled RR (95% CI)</th>
<th>P for trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kelly and Sabate, (2006) (37)</td>
<td>153, 604 (M, F)</td>
<td>4 cohort studies</td>
<td>CHD</td>
<td>0.63 (0.51-0.83)</td>
<td>&lt;0.050</td>
</tr>
<tr>
<td>Kris-Etherton et al. (2008) (38)</td>
<td>173, 164 (M, F)</td>
<td>4 cohort studies</td>
<td>CHD</td>
<td>0.65 (0.47-0.89)</td>
<td>&lt;0.050</td>
</tr>
<tr>
<td>Zhou et al. (2014) (39)</td>
<td>179, 885 (M,F)</td>
<td>9 cohort studies</td>
<td>CAD</td>
<td>0.83 (0.74-0.93)</td>
<td>0.010</td>
</tr>
<tr>
<td>Luo et al. (2014) (30)</td>
<td>3, 278, 552 (M, F)</td>
<td>18 cohort studies</td>
<td>Non-fatal MI</td>
<td>0.70 (0.43-1.17)</td>
<td>0.020</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Fatal IHD</td>
<td>0.66 (0.55, 0.78)</td>
<td>0.020</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>CVD death</td>
<td>0.70 (0.60, 0.81)</td>
<td>0.270</td>
</tr>
<tr>
<td>Afshin et al. (2014) (40)</td>
<td>501, 791 (M, F)</td>
<td>5 cohort studies</td>
<td>1 RCT</td>
<td>Fatal IHD</td>
<td>0.78 (0.67, 0.92)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Non-fatal IHD</td>
<td>0.76 (0.69, 0.84)</td>
<td>0.227</td>
</tr>
</tbody>
</table>

Abbreviations used: CAD= coronary artery disease; CHD= coronary heart disease; CI=confidence interval; CVD=cardiovascular disease; F=female; IHD= ischemic heart disease; M=male; MI=myocardial infarction; RCT=randomised controlled trial; RR=relative risk
Meanwhile, reviews by Kelly and Sabate, 2006 and Kris-Etherton et al., 2008, have produced similar conclusions (37, 38). The analyses of the four cohorts by Kelly and Sabate, 2006 showed that for each weekly 30 g serving of nut consumption, there was an 8.3% reduction in the risk of CHD death. In comparison to those who never or seldom consumed nuts, those who consumed nuts more than four times per week had a 37% (95% CI: 17%, 49%) reduction in risk of death from CHD (37).

Similarly, the pooled analysis of the same four cohorts, by Kris-Etherton et al., 2008, demonstrated that in comparison to little or no nut consumption, the highest intake group for nut consumption had approximately a 35% (95% CI: 11%, 53%) reduction in the risk of CHD incidence. Moreover, the beneficial effects of nut consumption were observed to be similar for different clinical outcomes such as non-fatal MI, fatal CHD, and sudden cardiac death (38).

Similar results have also been observed in three different systematic reviews and meta-analyses performed recently by Zhou et al., 2014, Luo et al., 2014, and Afshin et al., 2014 (30, 39, 40). Zhou et al., 2014, investigated the association of nut consumption with coronary artery disease (CAD), stroke, hypertension, and T2DM. After evaluation of the nine cohort studies from 7 publications, they found that the highest intake of nuts was inversely associated with risk of CAD. Compared with that of the lowest intake group, those with the highest intake had a 17% (95% CI: 7%, 26%) reduced risk of CAD; P=0.010. After the sensitivity analysis, the relative risks (95% CIs) ranged from 0.86 (0.77, 0.96) to 0.81 (0.71, 0.91). Furthermore, the dose-response relationship suggested a 19% (95% CI: 9%, 28%) average risk reduction of CAD for each 1-serving/day increment in nut intake; P=0.018 (39).

Moreover, Luo et al., 2014 quantified the relation between nut consumption and risk of type 2 diabetes, CVD, and all-cause mortality. They found that nut intake was inversely associated with ischemic heart disease (IHD) and overall CVD. For a 1-serving/day increment in nut consumption, there was a 28% (95% CI: 19%, 36%) reduction in risk of IHD (P for trend=0.640) and 29% (95% CI: 15%, 41%) reduction in overall CHD risk; P for trend=0.120. The pooled relative risk for the comparison of extreme quantiles of nut intake were 0.66 (95% CI: 0.55, 0.78; P=0.020) for IHD and 0.70 (95% CI: 0.60, 0.81; P=0.270) for CVD, respectively (30).
Afshin et al., 2014, examined the associations between nut and legume intakes and incidence of IHD, stroke and diabetes. On the analyses of seven prospective cohorts and one RCT, the investigators concluded that consumption of nuts was inversely associated with fatal IHD and non-fatal IHD. They showed that a 4 weekly, 28.4g serving of nuts was associated with a 24% (95% CI: 16%, 31%; P=0.227) lower risk of fatal IHD and 22% (95% CI: 8%, 33%; P=0.463) lower risk of non-fatal IHD (40).

While, the results from the different studies suggest that a dose-dependent, inverse association exists for nut consumption and CHD, epidemiologic studies are not the best way to analyse the effect of nut on different clinical outcomes. As nut consumers tend to follow a healthy dietary pattern, observational studies cannot exclude the effects of unknown confounding factors (unmeasured or residual) (30, 39). It is difficult to separate the independent effects of nut consumption from other dietary factors in observational analyses.

Furthermore, the majority of the studies used nut consumption at baseline as the dietary exposure and the participants self-reported their nut intake frequencies using a semi-quantitative food frequency questionnaire (FFQ). FFQs have limitations regarding assessing dietary patterns (41). During the follow up period, some individuals might have changed their dietary habits (30). Thus, the misclassification of exposure might have biased the results. Also, the studies adjusted for covariates that the investigators regarded important but not all studies adjusted for all covariates (30, 39, 40). Additionally, the definitions of nuts were not identical across studies, and most studies did not account for nuts consumed as an ingredient within other foods (39). This in itself could have attenuated the true magnitude of the effect.

Even though there are some limitations, it must be acknowledged that most of the epidemiological studies conducted were prospective cohort studies with large sample sizes and long-term follow-up periods. In addition, the results are consistent across different population groups, suggesting the observations to be generalisable. Collectively, the findings so far provide strong and compelling evidence of the cardioprotective benefit of nut consumption.
2.3.2.2 Intervention studies on risk factors for CVD

Evidence indicates that the cardio-protective effect of nuts is largely due to their favorable effects on plasma lipids and lipoproteins (5, 42, 43). Besides population-based observations, numerous clinical studies have been conducted to evaluate the effects of nut consumption in reducing CVD risk factors (9). These studies differ in the type and amount of nuts consumed, study design, subject selection criteria, and duration (9, 43).

Studies have been conducted in well-controlled dietary conditions or with free-living subjects on self-selected diets who were given either specific instructions to follow regarding nut consumption or daily allotments of nuts to consume (5, 43-45). Despite these differences in study design, the results remain largely consistent.

So far, several studies have demonstrated that regular nut intake improves blood lipid parameters such as, total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) concentrations (a predictor of CHD) and the LDL: HDL ratio in both men and women with normal- and hyper-cholesterolemia (5, 9, 23, 43-46).

A recent pooled analysis of 25 clinical studies on various kinds of nuts showed a dose-response cholesterol lowering effect (9). Specifically, a mean daily consumption of 67g of nuts resulted in estimated mean reductions of 0.28 mmol/L (10.9mg/dL) (5.1% change) in TC, 0.26 mmol/L (10.2mg/dL) (7.4% change) in LDL-C, 0.2 (8.3% change) in ratio of LDL-C to HDL-C, and 0.2 (5.6% change) in ratio of TC to HDL-C (9).
### Table 2.6. Meta-analyses: The effects of nut consumption on CVD risk (blood lipid)

<table>
<thead>
<tr>
<th>Author (year)</th>
<th>Number of participants</th>
<th>Number of studies included</th>
<th>Endpoint</th>
<th>Pooled RR (95% CI)</th>
<th>P for trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sabate, Oda &amp; Ros, 2010</td>
<td>583 (M, F)</td>
<td>25 RCT</td>
<td>TC</td>
<td>-10.9 (-14.1 - -7.8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>LDL-C</td>
<td>-10.2 (-13.1 - -7.4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>HDL-C</td>
<td>0.09 (1.0- 1.19)</td>
<td>0.880</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>LDL-C- HDL-C ratio</td>
<td>-0.2 (-0.3 - -0.1)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>TC/HDL-C</td>
<td>-0.2 (-0.3 - -0.1)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>TAG</td>
<td>-3.1 (-7.2 - 1.2)</td>
<td>0.150</td>
</tr>
<tr>
<td>Banel and Hu, 2009</td>
<td>365 (M,F)</td>
<td>13 RCT</td>
<td>TC</td>
<td>-10.29 (-14.76 - -5.83)</td>
<td>0.630</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>LDL-C</td>
<td>-9.23 (-13.10 - -5.36)</td>
<td>0.650</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>HDL-C</td>
<td>-0.20 (-1.79-1.38)</td>
<td>0.800</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>TAG</td>
<td>-3.86 (-11.92-4.29)</td>
<td>0.990</td>
</tr>
</tbody>
</table>

**Abbreviations used:** CI=confidence interval; F=female; HDL-C=high density lipoprotein cholesterol; LDL-C=low density lipoprotein cholesterol; M=male; RCT=randomised controlled trial; RR=relative risk; TAG=triacylglyceride; TC=total cholesterol
The reductions in CVD risk in the aforementioned pooled analysis by Sabate, Oda & Ros, (2010) is very similar to those obtained in a meta-analysis of walnut consumption studies by Banel and Hu, (2009). The meta-analysis of 13 clinical trials indicated that diets supplemented with walnuts were associated with a 0.27 mmol/L (10.3 mg/dL) and 0.24 mmol/L (9.2 mg/dL) decrease in TC and LDL–C, respectively (46). The results summary of the two studies is provided in Table 2.6.

Additionally, the conclusive findings of the epidemiological studies for CVD risks were remarkably similar to the conclusion drawn in a recent RCT (The PREDIMED trial) (Prevención con Dieta Mediterránea) by Estruch et al., 2013. The primary endpoint of the study was a composite of MI, stroke, and death from cardiovascular events. The investigators found that among persons at high CVD risk, a Mediterranean diet supplemented with nuts reduced the incidence of major cardiovascular events by 28% (95% CI: 3%, 46%; p=0.030) for the group assigned to a Mediterranean diet with nuts (83 events), versus the control group (low fat diet) (109 events) (47). So far, these studies provide the best estimate of the effects of nut consumption on risk factors for CVD.

Collectively, the findings from the intervention studies confirm the results of the epidemiological studies showing that nut consumption lowers CVD risk and supports the inclusion of nuts in dietary interventions for improving blood lipid levels and lipoproteins and for lowering CVD risks. It is evident from the existing data that nuts can be included in, and even used to design, cholesterol-lowering diets that have desirable fat contents and fatty acid profiles. Because of their unique fatty acid composition, nuts can be used to reduce the SFA content of the diet by replacing the energy from SFA with energy from unsaturated fatty acids, while maintaining the amount of dietary fat. This may be an effective strategy for improving the overall lipid profile and hence lower CVD risks.
Table 2.7. Prospective cohort studies evaluating the effects of nut consumption on risk of type 2 diabetes mellitus

<table>
<thead>
<tr>
<th>Author (year)</th>
<th>Study name</th>
<th>Number of participants</th>
<th>Duration of follow-up</th>
<th>Exposure assessment</th>
<th>Endpoint</th>
<th>Nut intake category</th>
<th>HR (95% CI)</th>
<th>P for trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jiang et al. (2002)</td>
<td>The NHS III</td>
<td>83,818 (F)</td>
<td>16 years</td>
<td>FFQ</td>
<td>Type 2 Diabetes</td>
<td>Never/almost never</td>
<td>1.00</td>
<td>0.92 (0.85-1.00)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt; Once a week</td>
<td>0.92 (0.85-1.00)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1-4 times/week</td>
<td>0.84 (0.76-0.93)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>≥5 times/week</td>
<td>0.73 (0.60-0.89)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pan et al. (2013)</td>
<td>The NHS III and The NHS II</td>
<td>58,063 (F)</td>
<td>10 years</td>
<td>FFQ</td>
<td>Type 2 Diabetes</td>
<td>Never/rarely</td>
<td>1.00</td>
<td>0.96 (0.92-1.01)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt; Once a week</td>
<td>0.96 (0.92-1.01)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Once a week</td>
<td>0.95 (0.89-1.02)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2-4 times/week</td>
<td>0.89 (0.80-0.98)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>≥5 times/week</td>
<td>0.84 (0.75-0.93)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kochar et al. (2010)</td>
<td>The PHS III</td>
<td>20,224 (M)</td>
<td>19.2 years</td>
<td>FFQ</td>
<td>Type 2 Diabetes</td>
<td>Never/rarely</td>
<td>1.00</td>
<td>1.01 (0.89-1.15)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt; Once a week</td>
<td>1.01 (0.89-1.15)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Once a week</td>
<td>1.03 (0.90-1.18)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2-4 times/week</td>
<td>0.87 (0.73-1.02)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>5-6 times/week</td>
<td>0.88 (0.67-1.16)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>≥7 times/week</td>
<td>0.68 (0.48-0.97)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parker, Hamack &amp; Folsom, (2003)</td>
<td>The IWHS II</td>
<td>134,486 (F)</td>
<td>11 years</td>
<td>FFQ</td>
<td>Type 2 Diabetes</td>
<td>Once a month</td>
<td>1.00</td>
<td>0.98 (0.87-1.10)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt; Once a week</td>
<td>0.98 (0.87-1.10)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1-4 times/week</td>
<td>1.06 (0.93-1.22)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>≥5 times/week</td>
<td>1.51 (1.13-2.04)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations used: CI=confidence interval; F=female; FFQ=food frequency questionnaire; HR=hazard ratio; M=male

2 The Iowa Women’s Health Study; 3 The Nurses’ Health Study; The Physicians Health Study; 11 The Nurses’ Health Study II
2.3.3 Nut consumption and type 2 diabetes mellitus (T2DM)

2.3.3.1 Epidemiological evidence available on T2DM

A number of large cohort studies have investigated the association of nut consumption with type 2 diabetes mellitus (T2DM). However, the effect of nut consumption on the risk of developing T2DM is not conclusive as studies have reported conflicting results.

To date, four epidemiological studies and three meta-analyses have evaluated the effect of nut consumption on the risk of developing T2DM (30, 39, 40, 48-52) (Tables 2.7-2.8). Five out of the seven studies (three of the four epidemiological studies and two of the three meta-analyses) reported a positive association between nut consumption and diabetes (30, 40, 48, 50, 51).

A dose-dependent inverse association was observed in the study by Jiang et al., 2002. The results from the NHS cohort reflected a 16% (95% CI: 7%, 24%) and 27% (95% CI: 11%, 40%) reduction in risk of developing diabetes in individuals who consumed nuts 1-4 times per week and 5 or more times per week compared with those who rarely or never ate nuts, respectively, (P for trend<0.001) (48). Furthermore, a positive association was also observed in the studies by Pan et al., 2013 and Kochar et al., 2010. The investigators found that individuals in the highest nut intake category had a 16% (95% CI: 7%, 25%; p<0.001) and 32% (95% CI: 3%, 52%; P for trend=0.017) reduction in risk of diabetes, respectively (50, 51).

Additionally, in a pooled analyses of four studies, an inverse association between nut intake and diabetes was observed. For a 1-serving/day increment in nut consumption, there was a 12% (95% CI: 8%, 16%) reduction in diabetes risk (P for trend=0.600) (30). Similarly, an inverse association was also observed between nut intake (per 4 weekly servings) and incident diabetes in a pooled analysis of five prospective cohorts and one RCT. Overall, 4-weekly servings of nuts was associated with a 13% (95% CI: 6%, 19%) lower risk of incident diabetes (P=0.269) (40).
<table>
<thead>
<tr>
<th>Author (year)</th>
<th>Number of participants</th>
<th>Number of studies included</th>
<th>Endpoint</th>
<th>Pooled RR (95% CI)</th>
<th>P for trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>Luo et al. (2014)</td>
<td>2,982,852 (M, F)</td>
<td>5 prospective cohort studies</td>
<td>Type 2 diabetes</td>
<td>0.88 (0.84-0.92)</td>
<td>0.600</td>
</tr>
<tr>
<td>Afshin et al. (2014)</td>
<td>230,216 (M, F)</td>
<td>5 prospective cohort studies &amp; 1 RCT</td>
<td>Incident diabetes</td>
<td>0.87 (0.81-0.94)</td>
<td>0.269</td>
</tr>
<tr>
<td>Zhou et al. (2014)</td>
<td>342,213 (M, F)</td>
<td>6 prospective cohort studies</td>
<td>Type 2 diabetes</td>
<td>0.92 (0.78, 1.09)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Abbreviations used: CI=confidence interval; F=female; M=male; RCT=randomised controlled trial; RR=relative risk.
On the contrary, data from the IWHS cohort, and the meta-analysis by Zhou et al., 2014, failed to show a link between nut intake and the risk of developing T2DM (39, 49). This could be due to certain limitations, such as, the over-adjustments of variables in the studies which may have contributed to major changes in the multivariate analysis. Also the non-specificity of self-reported diabetes might have contributed to attenuation of the results in the studies.

From the recent evidence available, it is difficult to make firm conclusions on the association between frequent nut intake and the risk of type 2 diabetes mellitus. Moreover, in order to understand the role of nuts in prevention and management of diabetes, more long-term interventions that examine the role of nuts in pre-diabetic and diabetic patients are justified.


2.3.3.2 Nut intervention trials on T2DM

This section of the chapter will focus on diabetes incidence only and the effects of nuts on glycaemia will follow later in the chapter. So far two dietary intervention studies have analysed the effects of nut consumption on the incidence of diabetes (53, 54).

Salas-Salvado et al., 2011, examined the effects of two Mediterranean diet intervention versus a low fat diet on incidence of diabetes in the PREDIMED (Prevencion con Dieta Mediterranea) study. After a median follow-up of four years, diabetes incidence was 10.1% (95% CI: 5.1-15.1%), 11.0% (95% CI: 5.9-16.1%), and 17.9% (95% CI: 11.4-24.4%) in the Mediterranean diet with olive oil group, the Mediterranean diet with nuts group, and the control group, respectively. The investigators found that compared with the control group, a Mediterranean diet supplemented with nuts reduced the incidence of diabetes by 52% (95% CI: 4-76%) (53).

However, in a subsequent sub-group analysis of the same cohort, a non-significant inverse association was observed. Compared with the control diet, the multivariate-adjusted hazards ratio for the Mediterranean diet supplemented with nuts was 0.82 (95% CI: 0.61-1.10) (54).

There are some limitations to these studies. Firstly, the trial was conducted in adults aged over 65 years who consumed a Mediterranean diet which customarily had a high
nut content, and who were at high risk of cardiovascular diseases. The study findings may not be generalisable to younger, and/or healthier individuals from other geographical locations. Secondly, the latest study by Salas-Salvado et al., 2014, was conducted as a secondary analysis, where diabetes incidence was a secondary end point, making these analyses exploratory in nature (54). Furthermore, there was also a high rate of attrition in the control group. Participants who dropped out had a worse CVD risk profile at baseline than those who remained in the study, suggesting a bias towards benefit in the control group. Finally, measurement errors affecting physical activity and other dietary intake habits cannot be discounted. For example, non-report of changes in physical activity and diet patterns.

While the epidemiological evidence to some degree suggest a favorable impact of nuts on incidence of diabetes, results are not conclusive for the intervention trials. Since not many intervention studies are available on effects of nuts on incidence of T2DM, more randomised intervention trials are needed to reach firm conclusions.

2.3.4 Summary of the evidence for health effects of nuts

Overall, while the data on diabetes is more conflicting, the protective effects of nuts is evident for all-cause mortality and diseases such as CVD. An inverse, dose-dependent relationship has been observed between nut intake and the different clinical outcomes such as CVD, CHD and all-cause mortality. Intervention studies have also shown improvement in risk factors of CVD such as, the lipid profile. The similarity of the results obtained by the different methodologic approaches for the different health outcomes confirms the validity of the findings.

Although, it is difficult to come to firm conclusions on the basis of epidemiological studies alone, long term, double blind, RCTs would provide the best evidence on the effects of nut on the different chronic diseases. However, this is not practical especially where the endpoint is death or any chronic disease leading to morbidity because of the length and subsequent cost of such studies.

As a significant body of consistent evidence has supported the beneficial effects of frequent nut intake on various chronic diseases, it is plausible that nuts are protective against many diseases and hence should be included as part of a healthy diet.
2.4 Glycaemia and health

Postprandial events, including postprandial glycaemia have been implicated in the development of chronic diseases, such as, obesity, T2DM, and CVD (14, 55).

There are indications that fluctuations in postprandial glycaemic concentrations may be particularly important in the aetiology of chronic diseases (55). Postprandial hyperglycaemia has been associated with increased risk of CHD and diabetes mellitus (14). Additionally, the 2-hour postprandial glycaemia is recognised as an independent risk factor for CHD (14).

The carbohydrate content of a food is the main dietary component affecting glycaemia (55). Postprandial glycaemia is affected by the quantity and type of carbohydrate present in a food or meal (55, 56).

Dietary glycaemic index (GI) has been shown to reliably predict the relative postprandial glycaemic responses to meals (57, 58). The concept of GI was introduced as a method for classifying the available carbohydrate in different foods according to their post-ingestion glycaemic response effect (55, 59).

While the GI is a potentially useful concept, it is also deceptively complex (60). Glycaemic responses are known to vary from day-to-day within individuals (56, 61). Furthermore, there is a large possibility of not detecting a true difference due to type II errors if the number of subjects being studied is not large and the expected difference is small (56, 61). However, GI is clinically useful, at least qualitatively for determining the ranking of the glycaemic responses of meals (60, 61).

The GI is defined as the incremental area under the curve (iAUC) for the blood glucose response after consumption of a 50 g available carbohydrate portion of a test food, expressed as a percentage of the response to an equivalent amount of carbohydrate from a reference food (57, 59, 62). The test and reference foods are both ingested by the same subject where glucose or white bread is used as the reference food (59, 62). The foods are categorised as low GI (<55), medium GI (55-70) or high GI (>70) (15). High GI foods have been proposed as a dietary factor that favours the development of chronic diseases (63). Some evidence suggests that consuming low GI foods and reducing the GI of a diet can positively affect postprandial plasma glucose excursions.
and as such, potentially diminish the risk of CVDs and improve blood glucose control in diabetics (64-66).

Furthermore, the glycaemic response to a food may influence regulation of food intake (56). The rate of digestion of the food is an important determinant of glycaemic response. It has been suggested that low GI foods are characterised by a slow rate of digestion and absorption, thereby eliciting a low glycaemic response (15, 63).

2.5 Effects of nut consumption on markers of glycaemic control

The ability of nuts to improve blood lipid profile and attenuate the risk of cardiovascular diseases has been investigated and the beneficial effects are now well recognised (67). Some research also supports the notion that the regular consumption of nuts may also help improve both short and long-term markers of glycaemic control.

Some nut feeding studies (acute and long-term intervention trials) indicate that when eaten on their own, nuts have minimal impact on the rising postprandial blood glucose levels. In addition, when nuts are consumed in combination with carbohydrate rich foods, the postprandial glycaemic response (PGR) of the resulting composite meal is attenuated (58, 64). This reduction in postprandial glycaemia has been observed in both normoglycaemic as well as in individuals with type 2 diabetes (58, 64).
Table 2.9. Effect of nut consumption on markers of glycaemic control in acute clinical interventions

<table>
<thead>
<tr>
<th>Author</th>
<th>Study design/ Objective</th>
<th>Number of subjects</th>
<th>Study population</th>
<th>Intervention</th>
<th>Outcome measures</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jenkins et al. (2006) (68)</td>
<td>Randomised cross-over clinical trial</td>
<td>15 (M/F)</td>
<td>Healthy</td>
<td>Control (white bread) Vs. 60g raw un-blanched almonds with white bread Vs. Parboiled rice with butter and cheese Vs. Instant mashed potatoes with butter and cheese</td>
<td>4-hour post-prandial glucose</td>
<td>The GI for the rice and almond meals were less than that for the mashed potato meal (p&lt;0.003).</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7 men 8 women</td>
<td>Age: 19-52 years</td>
<td>BMI: 17.4-29.5 kg/m²</td>
<td>4-hour post-prandial serum insulin</td>
<td>No significant difference in GI between almond and rice meals (p=0.25)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Healthy</td>
<td>Control (white bread) Vs. 60g raw un-blanched almonds with white bread Vs. Parboiled rice with butter and cheese Vs. Instant mashed potatoes with butter and cheese</td>
<td>GI</td>
<td>Lower postprandial glucose and serum insulin responses for almond and rice meal compared to potato meal and control (p&lt;0.001).</td>
</tr>
<tr>
<td>(2007) (14)</td>
<td></td>
<td></td>
<td>Healthy</td>
<td>Control (white bread) Vs. White bread with 30g almonds Vs. White bread with 60g almonds Vs. White bread with 90g almonds</td>
<td>Fasting glucose</td>
<td>The addition of almonds to white bread resulted in a significant reduction in GI of the composite meal in dose-dependent manner for all 3 nut doses (p=0.001)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>9 (M/F)</td>
<td>Age: 21-39 years</td>
<td>BMI: 20.3-31.4 kg/m²</td>
<td>GI</td>
<td>The control meal had the highest glucose response, followed by the 30g, the 60g, and 90g almond meals</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7 men 2 women</td>
<td>Healthy</td>
<td>Control (white bread) Vs. White bread with 30g almonds Vs. White bread with 60g almonds Vs. White bread with 90g almonds</td>
<td></td>
<td>The GI of the 90g almond meal was significantly lower than the GI of the control (p=0.009) and the 30g almond meals (p&lt;0.001)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Healthy</td>
<td>Control (white bread) Vs. White bread with 30g almonds Vs. White bread with 60g almonds Vs. White bread with 90g almonds</td>
<td></td>
<td>The GI of 60g almond meal was significantly lower than 30g almond meal (p&lt;0.017)</td>
</tr>
</tbody>
</table>

Abbreviations used: BMI=body mass index; F=female; GI=glycaemic index; HbA1c=haemoglobin A1c; M=male; RCT= randomised controlled trial
2.5.1 Nuts and glycaemic control in acute studies

To date, eight acute feeding trials have examined the impact of consuming different types of nuts on PGR (Table 2.9). Six of these studies (14, 58, 68-71) have included only healthy individuals while the other two have included a combination of individuals with type 2 diabetes and healthy participants (64, 72).

Jenkins et al., 2006 and Josse et al., 2007, have reported that almonds, eaten alone or in combination with carbohydrate-rich foods, reduced postprandial glucose and insulin excursions compared to the control meal and as such, play a beneficial role in glycemic control (14, 68).

Furthermore, similar results have also been observed in the two studies by Kendall et al. (58, 64). Kendall et al., 2011, found that consuming mixed nuts (almonds, macadamias, walnuts, pistachios, hazelnuts and pecans in equal proportions by weight) alone or in combination with white bread led to a reduction in glycaemic response (GR). Mixed nuts of varying doses (30, 60 and 90 g) when consumed alone significantly reduced the GR in both healthy and diabetic individuals in comparison to the white bread, (p<0.001), although, no clear dose-response effect was observed in the study (64).

Nevertheless, in the same study, addition of mixed nuts to white bread progressively reduced the GR of the nut-bread meal by 11.2%, 29.7 %, and 53.5% for the corresponding 30, 60, and 90 g nut doses (p = 0.354, p= 0.031, and p <0.001, respectively) in the study conducted in normoglycaemic individuals. In addition, the reduction in GR was only significant for 60 and 90 g nut-bread meal in the normoglycaemic and only for the 90g nut-bread meal in the diabetic individuals (64).

Subsequently, in another study, Kendall and colleagues, further tested the potential impact of pistachio nuts on postprandial glycaemia in two studies. The first part of the study assessed the dose-response effect of pistachios on postprandial blood glucose when consumed alone and consumed with a white bread meal. The second part of the study assessed the effect of 56 g of pistachio on postprandial blood glucose excursion when consumed with other commonly consumed carbohydrate-rich foods (58).
<table>
<thead>
<tr>
<th>Author (year)</th>
<th>Study design/objective</th>
<th>Number of subjects</th>
<th>Study population</th>
<th>Intervention</th>
<th>Outcome measures</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kendall et al. (2011) (64)</td>
<td>Randomised cross-over clinical trial</td>
<td>14 (M/F)</td>
<td>Healthy, normoglycaemic</td>
<td>Control (white bread) Vs.</td>
<td>2-hour post-prandial glucose</td>
<td>Compared to control, all 3 nut doses when fed alone significantly reduced RGR in both study populations (p&lt;0.001)</td>
</tr>
<tr>
<td></td>
<td>Objective: To examine the effects of nuts alone and in combination with white bread on postprandial glycaemia</td>
<td>5 men 9 women</td>
<td>Age: 36±4 years BMI: 21.8±0.6 kg/m²</td>
<td>30g mixed nuts Vs. 60g mixed nuts Vs. 90g mixed nuts</td>
<td>RGR</td>
<td>The 60g and 90g nut-bread meal significantly reduced the RGR in normoglycaemic, (p=0.031, p&lt;0.001, respectively)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10 (M/F)</td>
<td>Type 2 diabetics</td>
<td>White bread with 30g mixed nuts Vs. White bread with 60g mixed nuts Vs. White bread with 90g mixed nuts</td>
<td></td>
<td>A significant reduction in the RGR in the diabetic patients was only seen for the 90g nut bread meal (p=0.015)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6 men 4 women</td>
<td>Age: 68±2 years BMI: 26.5±0.5 kg/m²</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kendall et al. (2011) (58)</td>
<td>Study 1: Randomised cross-over clinical trial</td>
<td>10 (M/F)</td>
<td>Healthy Overweight</td>
<td>White bread Vs.</td>
<td>2-hour post-prandial glucose</td>
<td>A significant reduction in RGR for all doses of nuts consumed alone compared to white bread (p&lt;0.001)</td>
</tr>
<tr>
<td></td>
<td>Objective: To assess the dose-response effect of pistachios on post-prandial blood glucose when consumed alone and consumed with a white bread meal</td>
<td>3 men 7 women</td>
<td>Age: 48.3±6.4 years BMI: 28.0±4.8 kg/m²</td>
<td>28g pistachio nuts Vs. 56g pistachio nuts Vs. 84g pistachio nuts</td>
<td>RGR</td>
<td>A significant reduction in RGR for the 56g (p=0.009) and 84g (p&lt;0.001) nut-bread meal but not 28g nut bread meal</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>White bread with 28g pistachio Vs. White bread with 56g pistachio Vs. White bread with 84g pistachio</td>
<td></td>
<td>Significant reduction in RGR for rice and pistachio meal compared to rice alone (p=0.031), and pasta and pistachio meal in comparison to pasta alone (p=0.025) but not mashed potato meal (0.063)</td>
</tr>
<tr>
<td></td>
<td>Study 2: Randomised cross-over clinical trial</td>
<td></td>
<td></td>
<td>White bread Vs. Parboiled rice Vs. Rice with 56g pistachio Pasta Vs. Pasta with 56g pistachio Instant mashed potato Vs. Mashed potato with pistachio</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations used: BMI=body mass index; F=female; HbA1c=haemoglobin A1C; M=male; RCT=randomised controlled trial; RGR=relative glycaemic response
In study 1, the relative glycaemic response (RGR) of pistachios consumed alone (for all doses of nuts- 28 g, 56g, 84 g) was reduced compared to the white bread (p<0.001). The addition of pistachio to white bread significantly reduced the RGR of the 56 g (p=0.009) and 84 g (p<0.001) nut-bread meal but not for the 28 g nut-bread meal (p=0.100). Furthermore, a significant reduction in RGR was also observed in Study 2, for composite meals of rice with pistachios and pasta with pistachios in comparison to their respective controls (p<0.05 in both cases), but not for the mashed potato with pistachios meal compared to mashed potato control (p=0.063) (58).

Contrary to some of the observations made by Kendall et al., 2011 (64), in a study by Cohen & Johnston, (2011), it was found that consumption of almonds in combination with other foods improved glycemic control in patients with type 2 diabetes (p=0.043) but not in the healthy participants (p=0.638) (72). It may be that individuals with diabetes, have a much higher blood glucose excursions and hence a dramatic improvement in glucose concentrations was observed in comparison to healthy individuals with normal blood glucose levels.

Reis et al., 2011, evaluated the effect of nut processing on glycaemic response (GR) using 63 g of peanuts and found that ground roasted peanuts without skin had a lower GR compared to raw peanuts with skin (p=0.020). However, no significant differences in GR were observed between the control meal of cheese sandwich, and raw peanuts with skin, and roasted peanuts without skin (69). This is not surprising, given the fat and protein content of cheese, which is likely to reduce the GR. Perhaps, a control bread only would have been a more useful comparison.

Following on from this study, Reis and colleague (2013) conducted another study that assessed the effects of peanut consumption (whole peanuts or peanut butter) on first and second meal glucose metabolism in obese women with high risk of type 2 diabetes (70). The GR of a second meal eaten during the postprandial period is influenced by the GI of the preceding meal (56). For example, ingesting a high GI food between meals increases the GR of a subsequent meal. This is called the ‘second meal effect’ (56). Reis and colleague (2013) added 42.5 g of whole peanuts without skin, peanut butter or no peanuts (control) to a 75 g available carbohydrate-matched breakfast meal. They found that the GI of the whole peanut (without skin) and peanut butter containing meals were reduced compared to the control meal (carbohydrate meal with no peanuts).
<table>
<thead>
<tr>
<th>Author (year)</th>
<th>Study design/objective</th>
<th>Number of subjects</th>
<th>Study population</th>
<th>Intervention</th>
<th>Outcome measures</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reis et al (2011) (69)</td>
<td>Randomised cross-over clinical trial</td>
<td>13 (M/F)</td>
<td>Healthy</td>
<td>63g raw peanuts with skin Vs. 63g roasted peanuts without skin Vs. 63g ground, roasted peanuts without skin Vs. Control (cheese sandwich)</td>
<td>2-hour postprandial glucose</td>
<td>The GR for the ground roasted peanuts without skin was significantly lower than raw peanuts with skin (p=0.02)</td>
</tr>
<tr>
<td></td>
<td>Objective: To evaluate the effect of peanut processing on glycaemic response, energy and nutrient intakes</td>
<td>4 men 9 women</td>
<td>Age: 28.5±10 years BMI: 22.7±2.5 kg/m²</td>
<td></td>
<td>GR</td>
<td>No significant differences in GR between control meal, ground roasted peanuts without skin and roasted peanuts without skin</td>
</tr>
<tr>
<td>Cohen &amp; Johnston (2011) (72)</td>
<td>Randomised cross-over clinical trial</td>
<td>12 (M/F)</td>
<td>Healthy</td>
<td>Treatment meal (white bagel, berry juice, butter) Vs. Treatment meal with 28g almonds</td>
<td>2-hour postprandial glucose</td>
<td>A significant reduction in postprandial glycaemia in the diabetic patients (p=0.043) but not in healthy subjects (p=0.638) in comparison to respective controls</td>
</tr>
<tr>
<td></td>
<td>Objective: To determine if acute almond ingestion altered postprandial glycaemia and insulinaemia, and plasma GLP-1 concentrations after meal ingestion in healthy and type 2 diabetes individuals</td>
<td>2 men 12 women</td>
<td></td>
<td></td>
<td>1-hour postprandial insulin</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>7 (M/F)</td>
<td>Type 2 diabetics</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>4 men 3 women</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations used: BMI=body mass index; F=female; GLP-1 – glucagon like peptide 1 hormone; GR=glycaemic response; HbA₁c=haemoglobin A₁C; M=male; RCT=randomised controlled trial
The peanut butter meal resulted in a significantly lower second meal GR when compared to the control meal with no peanuts (p=0.030). Also, the first meal glycaemic responses for all test meals were significantly lower than the second meal glycaemic responses (p<0.030) (70).

Finally, Johnston, Trier, and Fleming, (2013), examined the acute effect of peanut (23g) and grain bar (40g) preloads on glycaemia in 15 healthy adults. In the study, blood glucose did not differ significantly between the treatment groups (p=0.901). However, blood glucose was significantly elevated an hour after ingestion of grain bar compared to peanut and control meal (p<0.001). Furthermore, no significant differences in serum insulin between treatment groups were observed (71).

Overall, the available data from short-term studies demonstrate that adding nuts to a carbohydrate-rich meal lowers postprandial glycaemia. However, there are some limitations that need to be addressed in regards to the interpretation of the studies conducted so far. Firstly, all of the acute studies had a small sample size (as few as 9 participants, range 9-15). The relatively small sample sizes may have led the studies to be inadequately powered and thus not being able to show significant clinical differences in the outcome measures. Also, the intra-individual response in blood glucose is relatively high, suggesting that repeating the test may be beneficial. Secondly, the results from the acute studies may not be generalisable to people with varied health status. All of the short-term studies, except for two recruited otherwise healthy subjects (64, 72). Furthermore, it must be pointed out that the results of the studies may only apply to the foods tested in the studies. Results may be different for foods varying in composition and physical form (58), for example, the GR of a meal may differ where nuts are added to liquid meals or where beverages such as tea, coffee, milk or juice are given with test meals. Also the macronutrient composition of the different test meals may influence the glycaemic and insulinaemic responses in the different studies. For example, the high protein content of meals which contain cheese may be responsible for high postprandial insulin and lower blood glucose.

Finally, the effects of nuts on glycaemia were tested using a wide range of nut quantities (30-90 g). While, the acute trials, to some extent, showed a dose-response effect, with a reduction in short-term glycaemic markers with increasing nut amounts, it would be interesting to see the effects of only consuming one serving of nuts (~30g) on glycaemic responses.
### Table 2.9. Effect of nut consumption on markers of glycaemic control in acute clinical interventions continued

<table>
<thead>
<tr>
<th>Author, (year)</th>
<th>Study design/objective</th>
<th>Number of subjects</th>
<th>Study population</th>
<th>Intervention</th>
<th>Outcome measures</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reis et al., (2013)</td>
<td>Randomised cross-over clinical trial</td>
<td>13 (F)</td>
<td>Obese women with high type 2 diabetes risk</td>
<td>Control (cream of wheat &amp; orange juice) Vs. 42.5g whole peanuts without skin with cream of wheat &amp; orange juice Vs. 42.5g peanut butter with cream of wheat &amp; orange juice</td>
<td>GI 0-240 minute (1st meal) &amp; 240-490 minute (2nd meal): Postprandial glycaemic response</td>
<td>The GI of the peanut and peanut butter containing meals were reduced The first meal glycaemic response did not differ between the test meals (p=0.480) The peanut butter meal resulted in a significantly lower 2nd meal glycaemic response compared to control meal (p=0.030) The 1st meal glycaemic responses for all test meals were significantly lower than the 2nd meal glycaemic responses (p&lt;0.030)</td>
</tr>
</tbody>
</table>

| Johnston, Trier, & Fleming (2013) | Randomised cross-over clinical trial | 15 (M/F) | Healthy Non-smoker | 23g peanut with 1 cup water before food (bagel, juice & margarine) Vs. 1 grain bar with 1 cup water before food (bagel, juice & margarine) Vs. 1 cup water before food (bagel, juice & margarine) | Serum fasting glucose Serum fasting insulin 2-hour postprandial glucose | The 2-hour postprandial glucose did not vary between the treatment groups Blood glucose was significantly elevated an hour after ingestion of grain bar compared to peanut and control meal (p<0.001) No significant differences in serum insulin between treatment groups (p=0.268) |

Abbreviations used: BMI=body mass index; F=female; GI=glycaemic index; HbA1c=haemoglobin A1C; M=male; RCT=randomised controlled trial
2.5.2 Nuts and glycaemic control in long-term intervention studies

The effects of nut consumption on markers of glycaemic control have also been investigated in numerous long-term feeding trials that have included healthy, obese, diabetic subjects and patients with metabolic syndrome (73, 74). While acute studies are supportive of nuts lowering postprandial glycaemia, evidence of improved glycemic control from long-term RCTs are less conclusive.

To date, twelve studies have specifically examined effect of nuts on markers of glycaemic control in long-term clinical trials (45, 71-73, 75-82) (Table 2.10). Six studies showed a reduction in GR (45, 71, 72, 76, 78, 81), three studies showed an increase (79, 80, 82) and three studies showed no change (73, 75, 77).

A number of studies have failed to show an effect of nut consumption on blood glucose regulation. Lovejoy et al., 2002, assessed the effects of almond-enriched diets on insulin sensitivity and lipids in patients with normoglycaemia (Study 1) or type 2 diabetes (Study 2). The investigators reported no significant changes in fasting and 2-hour glucose and insulin responses after subjects who were healthy, consumed 100 g/day of almonds for 4 weeks (Study 1). In Study 2, four different diets (high-fat/high-almond (57-113g/d almonds), low-fat/high-almond (57-113g/d almonds), high-fat/control, and low-fat/control) were fed to 30 volunteers with type 2 diabetes. In this study, no significant reductions in levels of haemoglobin A1C (HbA1c) and effect of fat level (high fat compared with low fat) or fat source (almond compared with oil) were seen on plasma glucose and insulin levels during a 2-hour oral glucose tolerance test on the diabetic subjects. The addition of almonds to either high fat or low fat diets in individuals with type 2 diabetes had no effect on fasting or postprandial glucose or insulin concentration (73). It is likely that a nut intervention longer than 4 weeks is required to modify insulin sensitivity and glycaemic control as assessed by HbA1c (72, 83).

Scott et al., 2003, randomised 35 patients with the metabolic syndrome or type 2 diabetes to the contemporary American Heart Association (AHA) diet (15% protein, 30% fat, and 15% MUFA) or HiPro-HiMono diet (a diet higher in protein (25%), total fat (40%) and MUFA (22%) for 42 weeks.
Table 2.10. Effect of nut consumption on markers of glycaemic control in long-term clinical interventions

<table>
<thead>
<tr>
<th>Author (year)</th>
<th>Length of study/ Objective</th>
<th>Study design</th>
<th>Number of subjects</th>
<th>Study population</th>
<th>Intervention</th>
<th>Outcome measures</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lovejoy et al. (2002)</td>
<td>4 weeks</td>
<td>Study 1: Not controlled</td>
<td>20 (M/F)</td>
<td>Healthy</td>
<td>100g/d almonds with habitual diet</td>
<td>Fasting glucose and insulin</td>
<td>No significant changes in any of the parameters compared to baseline</td>
</tr>
<tr>
<td></td>
<td>Objective: To assess effects of almond-enriched diets on insulin sensitivity and lipids in patients with normoglycaemia (Study 1) and type 2 diabetes (Study 2)</td>
<td>Study 2: Randomised, double blind, 4-arm, crossover study</td>
<td>30 (M/F)</td>
<td>Type 2 diabetics</td>
<td>High-fat, high almond (With 57-113g/d almonds) Vs. Low-fat, high almond (With 57-113g/d almonds) Vs. High-fat control Vs. Low-fat control</td>
<td>Fasting glucose and insulin</td>
<td>No significant difference between groups for any of the parameters</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>17 men 17 women (14 post-menopausal)</td>
<td>Age: 30-65 years</td>
<td>BMI: 20-40 kg/m²</td>
<td>2-hour post-prandial glucose</td>
<td>Study duration not long enough to show changes in HbA₁c</td>
</tr>
</tbody>
</table>

Abbreviations used: AHA diet=American Heart Association Diet; BMI=body mass index; F=female; HbA₁c=haemoglobin A₁c; M=male; MUFA=monounsaturated fatty acid; RCT=randomised controlled trial
**Studies are with free-living populations unless stated otherwise**
Although trends in risk factors slightly favored the HiPro-HiMono diet, changes were not significantly different between the AHA and the HiPro-HiMono groups for fasting glucose (-2.2 vs. -3.2 mmol/L; p=0.153) (75).

Furthermore, Tapsell et al., 2004, compared three dietary advice groups (low fat diet, modified low-fat diet and modified low-fat diet with 30 g walnut/day) and found no significant differences in HbA1c between the treatment groups (77).

In terms of the potential effects of nuts on long-term markers of glycaemic control, six long-term studies have shown significant improvements in at least one marker of glycaemic control (45, 71, 72, 76, 78, 81).

A study by Estruch et al., 2006, showed a significant reduction in fasting glucose, fasting insulin and homeostatic model assessment of insulin resistance index (HOMA-IR) after consuming two Mediterranean diets containing either olive oil (p=0.017, p=0.001 and p<0.001, respectively) or mixed nuts (p=0.039, p<0.001, and p<0.001, respectively) in comparison to a control group (low fat diet) (78). Studies by Tapsell et al., 2009 and Casas-Agustench et al., 2011 found significant between-treatment effects with the regular consumption of nuts on fasting insulin but not for fasting glucose in comparison to their respective control diets (76, 81). The latter study by Casas-Agustench et al., 2011, also found improvements in HOMA-IR in the nut group compared to the control group (p<0.013) (81). Similar results were also observed by Li et al., 2011 where the investigators compared National Cholesterol Education Program Step II diet (NCEP II) and a diet containing almonds (56g). They found that there was a reduction in fasting insulin (p=0.018) and glucose (p=0.024) in the almond diet group in comparison to the control diet. Moreover, HOMA-IR was lower in the almond group than in the control group (p=0.004) (45).

Six out of the twelve trials have also analysed HbA1c, which is an established marker of long-term glycaemic control (71, 72, 73, 76, 77, 80). However, only two studies out of the six have shown improvement in HbA1c in their respective studies (71, 72). Johnston, Trier, & Fleming, (2013) examined the long-term satiating effects of daily peanut ingestion (28g) on body mass over an 8-week period in overweight individuals. The study showed significant improvement in HbA1c (71).
Table 2.10. Effect of nut consumption on markers of glycaemic control in long term clinical interventions continued

<table>
<thead>
<tr>
<th>Author (year)</th>
<th>Length of study/objective</th>
<th>Study design</th>
<th>Number of subjects</th>
<th>Study population</th>
<th>Intervention</th>
<th>Outcome measures</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scott et al. (2003) (75)</td>
<td>42 weeks</td>
<td>Parallel RCT</td>
<td>35 (M/F)</td>
<td>Metabolic syndrome or type 2 diabetes patients</td>
<td>AHA diet Vs. High protein, high fat, MUFA diet with almond</td>
<td>Fasting glucose</td>
<td>No significant differences between the treatment groups (p=0.153)</td>
</tr>
<tr>
<td>Tapsell et al. (2004) (77)</td>
<td>6 months</td>
<td>Parallel RCT</td>
<td>58 (M/F)</td>
<td>Type 2 diabetics</td>
<td>Low fat diet Vs. Modified low fat diet Vs. Modified low fat diet with 30 g walnuts/day</td>
<td>HbA1c</td>
<td>No significant differences between groups for changes in the outcome measure</td>
</tr>
</tbody>
</table>

**Objective:**
To examine the effect of a moderate-fat diet inclusive of walnuts on blood lipid profiles in patients with type 2 diabetes

**Limitations:**
High attrition rate and small sample size

Limitations:
Not generalisable to other population groups

Biased randomisation (low cholesterol in walnut group)

Abbreviations used: AHA diet=American Heart Association Diet; BMI=body mass index; F=female; HbA1c=haemoglobin A1c; M=male; RCT=randomised controlled trial
In the study by Cohen and Johnston, (2011), while fasting glucose and fasting insulin did not differ between the treatment groups (28g almond vs. 2 cheese sticks), there were significant reduction in HbA1c at 12 weeks in the almond group in comparison to the control group (cheese group); p=0.005 (72)

The results from these long-term nut feeding trials have not been consistent. No studies to date have shown improvements in all aspects of glycaemic control. In addition three studies have reported increases in fasting glucose concentrations with long-term consumption of nuts (100 g of walnut and cashew nuts, 56 g walnuts and 30 g of mixed nuts, respectively) (79, 80, 82).

Mukkudem-Petersen et al., 2007, reported that fasting glucose increased significantly in the cashew nut diet group compared to the control diet group while no between treatment differences in postprandial glucose and serum fructose-amine were observed in any of the treatment groups (79). Similar results were also observed by Ma et al., 2010. While no changes were observed in the control group, fasting glucose significantly increased in the walnut group compared to baseline. There were also no significant differences in fasting glucose and HbA1c in comparison to the control diet (80). Furthermore, Lasa et al., 2014, found that the fasting glucose was significantly reduced in the low fat diet group compared to the two Mediterranean diets with olive or nuts. Also the adiponectin/HOMA-IR ratio was significantly increased in the olive diet group. However, there were also no significant differences in HOMA-IR between or within the treatment groups (82).

Overall, long-term intervention trials investigating the regular consumption of nuts have produced inconsistent results. These discrepancies may be related to differences in study design. For example, differences in the results may be attributed to the small sample size, variations in the health status of the study participants, length of the trials, the outcome measures, meal composition and finally, the dose of nuts used in the interventions. The sample sizes in the different studies varied, ranging from 13 to 772 people and the majority of the long-term studies involved a variety of participants e.g. people who were at high risk of developing CVD, people with type 2 diabetes, hyperlipidaemic subjects, and patients with metabolic syndrome. Therefore, the results from specific population groups may not be extrapolated to other sub-populations.
Table 2.10. Effect of nut consumption on markers of glycaemic control in long-term clinical interventions continued

<table>
<thead>
<tr>
<th>Author et al. (year)</th>
<th>Length of study/ objective</th>
<th>Study design</th>
<th>Number of subjects</th>
<th>Study population</th>
<th>Intervention</th>
<th>Outcome measures</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estruch et al. (2006) (78)</td>
<td>3 months</td>
<td>Parallel RCT</td>
<td>772 (M/F)</td>
<td>Asymptomatic persons with high CVD risks</td>
<td>Low fat diet Vs. Mediterranean diet with olive oil (1L/week) Vs. Mediterranean diet with mixed nuts (30g/d)</td>
<td>Fasting glucose Fasting insulin</td>
<td>The measured parameters were significantly lower in the two Mediterranean diets compared to the low fat diet.</td>
</tr>
</tbody>
</table>

Objective:
To compare the short-term effects of 2 Mediterranean diets versus those of a low-fat diet on intermediate markers of cardiovascular risk.

| Mukuddem-Petersen et al. (2007) (79) | 8 weeks | Parallel RCT | 64 (M/F) | Metabolic syndrome | Control diet Vs. Walnut diet (100g) Vs. Unsalted cashew nut diet (100g) | Plasma glucose 2-hour post-prandial glucose Serum fructose-amine | Fasting glucose increased significantly in the cashew nut diet group compared to the control diet group and the walnut diet group. No significant effect on serum fructose-amine in the walnut and cashew group compared to the control group. No between treatment differences in 2-hour glucose and serum fructose-amine. |

Objective:
To examine effects of a high walnut diet and high unsalted cashew nut diet on selected markers of metabolic syndrome.

Limitations:
Study did not focus on clinical outcomes.

Abbreviations used: F=female; HOMA-IR=homeostasis model assessment of insulin resistance index; M=male; RCT=randomised controlled trial.
In some of the studies, the duration of the trial may not have been adequate to assess certain outcome measures, such as HbA1c which is a primary marker of glycaemic control (73). Measurement of HbA1c assesses the degree of glycaemia over a period of 2-3 months (83). Thus, study length of less than 12 weeks may not be of adequate duration to show changes in the HbA1c. Furthermore, the long-term interventions have not examined the dose-response relationship. Establishing a dose-response relationship is important before making nutritional recommendations for the general public.

2.5.3 Meta-analysis: Nuts and glycaemic control

A systematic review and meta-analysis of 12 randomised controlled trials (RCTs) assessing the effects of tree nuts on markers of glycaemic control has been conducted recently (83). RCTs with a duration of 3 or more weeks that were conducted in individuals with diabetes and compared the effect of diets emphasising tree nuts to isocaloric diets without tree nuts on haemoglobin A1c (HbA1c), fasting glucose, fasting insulin, and HOMA-IR were selected and included in the analyses. The investigators found that a median dose of 56 g/d significantly lowered HbA1c by -0.07% (95% CI: -0.10, -0.03%; P<0.001) and fasting glucose by -0.15mmol/L (95% CI: -0.27, -0.02mmol/L; P<0.03) compared with the control diet. Furthermore, no significant treatment effects were observed for fasting insulin and HOMA-IR. However, the direction of the effect favoured tree nuts (83).

While the pooled analyses by Viguiliouk et al., 2014, shows that tree nuts improve glycaemic control in individuals with T2DM, the majority of the studies included in the meta-analyses were of short duration. Therefore, owing to the uncertainties in the analyses, there is need for longer, higher quality trials.
<table>
<thead>
<tr>
<th>Author (year)</th>
<th>Length of study/ Objective</th>
<th>Study design</th>
<th>Number of subjects</th>
<th>Study population</th>
<th>Intervention</th>
<th>Outcome measures</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tapsell et al. (2009) (76)</td>
<td>1 year</td>
<td>Parallel RCT</td>
<td>50 (M/F)</td>
<td>Type 2 diabetics</td>
<td>Control diet (low fat dietary advice) Vs. Low fat dietary advice with 30g/d walnuts</td>
<td>Fasting glucose and HbA1c</td>
<td>No between treatment differences in fasting glucose and Haemoglobin A1c. Greater reduction in fasting insulin in the walnut group compared to the control (p=0.046). A significant (time-effect) reduction in all parameters at 12 months in both groups compared to baseline.</td>
</tr>
<tr>
<td>Ma et al. (2010) (80)</td>
<td>8 weeks</td>
<td>Randomised controlled, single blind, cross-over trial</td>
<td>24 (M/F)</td>
<td>Type 2 diabetics</td>
<td>Ad libitum diet (control) Vs. Ad libitum diet with 56g/d walnut</td>
<td>Fasting glucose and HbA1c</td>
<td>Fasting glucose increased significantly in the walnut group compared to baseline (p=0.04). No significant difference in fasting glucose in comparison to control diet (p&gt;0.05). No significant between or within group changes in HbA1c and insulin sensitivity (p&gt;0.05).</td>
</tr>
</tbody>
</table>

**Limitations:**
The control diet was not standardised. Small sample size.

**Abbreviations used:** BMI=body mass index; F=female; HbA1c=haemoglobin A1c; HOMA-IR=homeostasis model assessment of insulin resistance index; M=male; RCT=randomised controlled trial.
2.5.4 Summary of the findings from the intervention trials on nuts and glycaemia

There were twenty intervention trials (short (n=8) and long-term (n=12)) that examined the effects of nut consumption on markers of glycaemic control in individuals with varied health status. The doses of nuts consumed in the studies ranged from 28 to 90 g in the short-term intervention trials and 28 to 113 g in the long-term intervention clinical trials.

The data from the acute studies are more consistent, and indicate that nuts diminish the rise in blood glucose levels when consumed with carbohydrate rich foods. Also, a dose-dependent reduction in the glycemic response to the meal has been shown to some extent. These studies suggest that, in addition to cholesterol lowering, nut consumption lowers postprandial glycaemia and insulinaemia and hence, may contribute to decreased risk of chronic diseases associated with consistent hyper-glycaemia. While the findings support a short-term benefit of nuts in postprandial glucose response, more studies are required to determine whether these acute benefits translate to long-term improvements in glycaemic control, as six out of the twelve long-term nut feeding trials have failed to demonstrate improvements in markers of glycaemic control. The inconsistencies in the findings make it difficult at this stage to reach definitive conclusions on the role of nuts on glycemic control over the long term. Some of these inconsistencies may be attributed to the variations in the health status of the study population, sample size, the duration of trial, outcome measures and dose of nuts used.

In general, the trials that used 28-56 g of nuts in their respective studies showed improvements in the markers of glycaemic response (45, 71, 72, 76, 78, 81).

Therefore, the present findings warrant further research, in the form of larger and longer RCTs that measure appropriate long-term glycaemic markers such as HbA1c, into the role of regular nuts consumption in the prevention and management of chronic diseases. Also, future trials that are adequately powered should examine the impact of chronic ingestion of different types of nuts of varying doses and including the recommended amount of 30 g/d on outcome measures in people with varied health status. For people with specific health issues, establishing the optimal dose could be important before making nutritional recommendations.
<table>
<thead>
<tr>
<th>Author (year)</th>
<th>Length of study/objective</th>
<th>Study design</th>
<th>Number of subjects</th>
<th>Study population</th>
<th>Intervention</th>
<th>Outcome measures</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casas-Agustench et al. (2011) (81)</td>
<td>12 weeks</td>
<td>Parallel RCT</td>
<td>50 (M/F)</td>
<td>Metabolic syndrome patients</td>
<td>Healthy diet advice Vs. Healthy diet advice with 30g/d raw mixed nuts</td>
<td>Fasting glucose</td>
<td>Fasting insulin and HOMA-IR were significantly reduced in the nut group compared to the control group (p&lt;0.013)</td>
</tr>
<tr>
<td></td>
<td><strong>Objective:</strong></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td>To assess the effects of a qualitative diet enriched with nuts versus a control diet on serum lipid profile, insulin resistance, energy metabolism and circulating inflammatory biomarkers in patients with metabolic syndrome</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>28 men 22 women</td>
<td>Age: 18-65 years</td>
<td>BMI: &lt;35kg/m²</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cohen &amp; Johnston, (2011) (72)</td>
<td>12 weeks</td>
<td>Parallel RCT (pilot study)</td>
<td>13 (M/F)</td>
<td>Type 2 diabetics</td>
<td>28g of almonds (5d/week) Vs. 2 cheese sticks (5d/week)</td>
<td>Fasting glucose</td>
<td>Baseline parameters did not differ between the two treatment groups</td>
</tr>
<tr>
<td></td>
<td><strong>Objective:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>To assess the impact of chronic almond ingestion (5x/week) on markers of glycaemic control in type 2 diabetes individuals</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>7 men 6 women</td>
<td>Age: 66 years</td>
<td>BMI: 35 kg/m²</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations used: BMI=body mass index; F=female; HbA₁c=haemoglobin A₁c; HOMA-IR=homeostasis model assessment of insulin resistance index; M=male; RCT=randomised controlled trial
2.5.5 Mechanisms affecting glycaemic response

There is reason to believe that the nutrient composition of nuts may help improve markers of glycaemic control. Nuts are low in available carbohydrate, have a healthy fatty acid profile and are high in protein and fibre (2, 85).

When nuts are eaten alone or in combination with mixed meals, the low available carbohydrate content of nuts may help lower the postprandial glucose and insulin responses and as such, play a favorable role in glycaemic control (58, 85).

Nuts may displace the carbohydrate in the diet/meal which is likely to effectively decrease the glycaemic load (85). The fatty acid composition of nuts may also play a role in modifying markers of glycaemic control. Nuts are high in MUFAs and PUFAs and it is understood that diets high in these fats can improve glycaemic control (85, 86). MUFAs have been shown to improve beta cell efficiency by enhancing the secretion of glucagon-like peptide-1 (GLP-1) hormone. GLP-1 is known to help in the regulation of postprandial glucose clearance and insulin sensitivity (86).

Furthermore, nuts also provide a significant amount of fibre (both soluble and insoluble) and antioxidants such as flavonoids, polyphenols and tocopherols (1, 6). A high intake of dietary fibre (soluble), has been shown to improve glycaemic control (14, 15). Dietary antioxidants in nuts may also play an important role in modulating markers of glycaemic control, such as insulin resistance by restoring the ratio of plasma oxidized glutathione to reduced glutathione (GSSG/GSH), to a more appropriate concentration and improving β-cell response to glucose and insulin action (87).
### Table 2.10. Effect of nut consumption on markers of glycaemic control in long-term clinical interventions

<table>
<thead>
<tr>
<th>Author (year)</th>
<th>Length of study/objective</th>
<th>Study design</th>
<th>Number of subjects</th>
<th>Study population</th>
<th>Intervention</th>
<th>Outcome measures</th>
<th>Results</th>
<th>Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Li et al. (2011)</td>
<td>12 weeks</td>
<td>Randomised cross-over clinical trial</td>
<td>20 (M/F)</td>
<td>Type 2 diabetics with mild hyperlipidemia</td>
<td>NCEP step II diet (control) Vs. Almond diet (56g/day)</td>
<td>Fasting insulin</td>
<td>The almond diet significantly reduced the fasting insulin (p=0.018) and glucose (p=0.024) compared to the control diet.</td>
<td>Small sample size</td>
</tr>
<tr>
<td></td>
<td><strong>Objective:</strong></td>
<td></td>
<td>9 men</td>
<td>Age: 58 years</td>
<td></td>
<td></td>
<td>HOMA-IR was lower in the almond group than the control group (p=0.0039)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>To investigate the extent by which incorporation of almonds into the NCEP step II diet improves insulin sensitivity and lipid profile in Chinese T2DM patients</td>
<td></td>
<td>11 women (post-menopausal)</td>
<td>BMI: 26 kg/m²</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Johnston, Trier, &amp; Fleming, (2013)</td>
<td>8 weeks</td>
<td>Parallel RCT</td>
<td>44 (M/F)</td>
<td>Healthy Overweight</td>
<td>28g peanut 1 hour prior to food (including low-fat diet advice) Vs. Grain bar (1.4oz) 1 hour prior to food (including low-fat diet advice)</td>
<td>Fasting serum glucose</td>
<td>HbA₁c was significantly reduced in both test meals with a higher reduction in the grain bar group (p=0.001)</td>
<td>High rate of attrition; Self-reported health status; Results not generalisable to other population groups</td>
</tr>
<tr>
<td></td>
<td><strong>Objective:</strong></td>
<td></td>
<td>16 men</td>
<td>Age: 20-65 years</td>
<td></td>
<td></td>
<td>No significant differences in serum insulin and glucose between group nor within when compared to baseline, (p=0.226, p=0.254, respectively)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>To examine the long-term satiating effect of daily peanut ingestion on (28g/d) body mass over an 8 week period in overweight adults</td>
<td></td>
<td>28 women</td>
<td>BMI: &gt;25kg/m²</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Limitations:**

- Small sample size

**Abbreviations used:**

- BMI=body mass index; F=female; HbA₁c=haemoglobin A₁C; HOMA-IR=homeostasis model assessment of insulin resistance index; M=male;
- NCEP step II diet=National cholesterol education program step II diet; RCT=randomised controlled trial; T2DM=type 2 diabetes mellitus
Table 2.10. Effect of nut consumption on markers of glycaemic control in long-term clinical interventions continued

<table>
<thead>
<tr>
<th>Author (year)</th>
<th>Length of study/objective</th>
<th>Study design</th>
<th>Number of subjects</th>
<th>Study population</th>
<th>Intervention</th>
<th>Outcome measures</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lasa et al. (2014) (82)</td>
<td>1 year</td>
<td>Secondary analysis/ longitudinal assessment of a RCT (PREDIMED trial)</td>
<td>191 (M/F)</td>
<td>Type 2 diabetics</td>
<td>Low fat diet Vs. Mediterranean diet with olive oil (1L oil/week) Vs. Mediterranean diet with mixed nuts (30g/d nuts)</td>
<td>Fasting glucose</td>
<td>Fasting glucose reduced significantly in the low fat diet group compared to the olive oil and nut groups. No significant differences in HOMA-IR between or within the groups. Significant increase in adiponectin/HOMA-IR ratio in the olive diet group compared to control and nut diet group (p=0.027).</td>
</tr>
</tbody>
</table>

Limitation: Results cannot be extrapolated to other population groups. Study not a RCT.

Abbreviations used: BMI=body mass index; F=female; HbA1c=haemoglobin A1c; HOMA-IR=homeostasis model assessment of insulin resistance index; M=male; RCT=randomised controlled trial.
2.6  Nut consumption and body weight

The many health benefits of nuts have prompted recommendations to increase their consumption. However, nuts are viewed as energy dense foods with a high fat content. This has led to misconceptions among consumers that increased nut consumption may lead to unwanted gain in body weight (16). Nonetheless, the available scientific evidence supports the theory that there appears to be no adverse effects of frequent nut consumption on body weight, despite an increase in total energy intake.

To date, sixteen studies (three cross-sectional studies, two prospective cohort studies, ten nut feeding intervention trials and one meta-analysis) have specifically evaluated the role of nuts on body weight (16, 81, 88-100) (Tables 2.11 and 2.12).

2.6.1 Epidemiological evidence available on body weight

Three cross-sectional studies conducted recently have indicated an inverse association between the frequency of nut consumption and the risk of body weight gain (84, 98, 100).

In a cross-sectional assessment of 847 participants from the PREDIMED study, Casas-Agustench et al. (2011) found that nut consumption was inversely associated with adiposity measures such as, BMI and waist circumference, independent of other lifestyle factors in the elderly Mediterranean population who were at high cardiovascular disease risk. Adjusted models from the cross-sectional analysis predicted that for each 30 g serving of nuts, BMI and waist circumference decreased by 0.78 kg/m² (p=0.002) and 2.10 cm (p=0.002), respectively (84).

In another subsequent cross-sectional analysis of the participants from the same cohort of the PREDIMED study, Ibarrola-Jurado et al., 2013, evaluated the association between frequency of nut consumption and prevalence of cardio-metabolic risk factors (obesity, metabolic syndrome, type 2 diabetes, hypertension and dyslipidaemia) in 7210 elderly men and women at high risk of CVDs.
### Table 2.11. Epidemiological evidence on effect of nut consumption on body composition parameters

<table>
<thead>
<tr>
<th>Author (year)</th>
<th>Length of study/objective</th>
<th>Study design (study name)</th>
<th>Number of subjects</th>
<th>Exposure assessment</th>
<th>Nut intake category</th>
<th>Point estimate (95% CI)</th>
<th>P for trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bes-Rastrollo et al. (2007) (102)</td>
<td>28 months</td>
<td>Prospective cohort (The SUN Project)†</td>
<td>8865 (M/F) FFQ</td>
<td>Never/almost never</td>
<td>1-3 times/month</td>
<td>1.00</td>
<td>0.006</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3700 Men</td>
<td>1-3 times/month</td>
<td>Once a week</td>
<td>0.95 (0.77-1.11)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>5165 Women</td>
<td>≥2 times/week</td>
<td></td>
<td>0.69 (0.53-0.90)</td>
<td></td>
</tr>
<tr>
<td>Bes-Rastrollo et al. (2009) (92)</td>
<td>8 years</td>
<td>Prospective cohort (NHSII)‡</td>
<td>51,188 (F) FFQ</td>
<td>Never/almost never</td>
<td>1-3 times/month</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Women only</td>
<td>1-3 times/month</td>
<td>Once a week</td>
<td>1.00 (0.91-1.10)</td>
<td>0.003</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>≥2 times/week</td>
<td>0.87 (0.79-0.96)</td>
<td></td>
</tr>
<tr>
<td>Casas-Agustench et al. (2011) (84)</td>
<td>Objective: To examine dietary determinants of adiposity in the PREDIMED study cohort</td>
<td>Cross-sectional study (The PREDIMED Study)§</td>
<td>847 (M/F) FFQ</td>
<td>30g nuts/day</td>
<td>**</td>
<td>**</td>
<td>**</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>375 men</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>472 women</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Abbreviations used:** CI=confidence interval; F=female; FFQ=food frequency questionnaire; HR=hazards ratio; M=male; OR=odds ratio

†The Seguimiento Universidad de Navarra; ‡The Nurses’ Health Study II; §The Prevención con Dieta Mediterránea Study;

*Multivariate-adjusted odds ratio; †Multivariate adjusted hazard ratios

** detail on specifics not available
Compared to participants who consumed less than one serving of nuts per week, those who consumed more than three servings of nuts per week had a 39% (OR: 0.61; 95% CI: 0.54-0.68; P for trend <0.001) and 32% (OR: 0.68; 95% CI: 0.60-0.79; P for trend <0.001) lower risk of obesity and abdominal obesity, respectively (98).

Furthermore, similar results were also observed in the 803 adults from the Adventist Health Study 2 (AHS 2) (100). In this cross-sectional analysis, Jaceldo-Siegl et al., 2014 examined the association between nut consumption, metabolic syndrome and obesity. They assessed intake of total nuts, tree nuts and peanuts and also classified subjects into low tree nut/low peanut (LT/LP), low tree nut/high peanut (LT/HP), high tree nut/high peanut (HT/HP), high tree nut/low peanut (HT/LP) groups. Jaceldo-Siegl and colleagues reported that obesity was 37% (OR: 0.63; 95% CI: 0.40-0.99) and 46% (OR: 0.54; 95% CI: 0.34-0.88) lower in HT/HP and HT/LP consumers compared with LT/LP consumers, respectively, (P for trend <0.006) (100).

Overall, the three cross-sectional studies have shown that nut consumption is associated with lower BMI (84) and obesity risk (98, 100). While the outcome of the cross-sectional studies suggest that the incorporation of nuts into the diet does not lead to a greater body weight gain and may help in weight control, caution needs to be taken when interpreting the results. Because cross-sectional studies only examine the exposure at one particular time point, the temporality of nuts and BMI cannot be established, and cross-sectional analyses cannot assess the cause and effect relationship (101).

The findings from the cross-sectional studies are further supported by two long-term prospective cohort studies, with sample sizes of 8865 and 51,188, respectively (92, 102). The Seguimiento Universidad de Navarra (SUN) study investigated nut consumption and its association with either risk of weight gain of five or more kilograms or the risk of becoming overweight or obese in a Mediterranean population (102). The cohort consisted of 8865 adult men and women (university graduates in Spain) who completed a follow-up questionnaire with validated semi-quantitative FFQ after a median of 28 months. The prospective study of the SUN cohort reported a significant inverse association between nut consumption and weight gain.
Table 2.11. Epidemiological evidence on effect of nut consumption on body composition parameters *continued*

<table>
<thead>
<tr>
<th>Author</th>
<th>Length of study/objective</th>
<th>Study design</th>
<th>Number of subjects</th>
<th>Exposure assessment</th>
<th>Nut intake category</th>
<th>Point estimate (95% CI)</th>
<th>P for trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ibarrola-Jurado et al. (2013) (98)</td>
<td><strong>Objective:</strong> To evaluate the associations between frequency of nut consumption and prevalence of cardio-metabolic risk factors in a Mediterranean population at high cardiovascular risk</td>
<td>Cross-sectional study (the PREDIMED Study)³</td>
<td>7210 (M/F)</td>
<td>FFQ</td>
<td>&lt;1 serving/week 1-3 servings/week &gt;3 servings/week</td>
<td>OR (95% CI)¹</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.00</td>
<td>0.80 (0.71-0.90)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3067 men</td>
<td></td>
<td></td>
<td>0.61 (0.54-0.68)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4143 women</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jaceldo-Siegl et al. (2014) (100)</td>
<td><strong>Objective:</strong> To examine the relationship of nut consumption, metabolic syndrome and obesity in the Adventist Health Study 2</td>
<td>Cross-sectional study (the AHS 2)⁴</td>
<td>803 (M/F)</td>
<td>FFQ</td>
<td>Low tree nut/low peanut (LT/LP)</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Low tree nut/high peanut (LT/HP)</td>
<td>0.89 (0.53-1.48)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>286 men</td>
<td></td>
<td>High tree nut/high peanut (HT/HP)</td>
<td>0.63 (0.40-0.99)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>High tree nut/low peanuts (HT/LP)</td>
<td>0.54 (0.34-0.88)</td>
<td>0.006</td>
</tr>
</tbody>
</table>

Abbreviations used: CI=confidence interval; F=female; FFQ=food frequency questionnaire; M=male; OR=odds ratio; ³The Prevención con Dieta Mediterránea Study; ⁴The Adventist Health Study 2

¹Multivariate-adjusted odds ratio
Bes-Rastrollo et al., 2007 found that compared with those who never or almost never consumed nuts, participants who consumed nuts two or more times per week had a 31% (OR: 0.69; 95% CI: 0.53-0.90; P for trend=0.006) lower risk of gaining five or more kilograms during the 28 month follow up period. Also, individuals who never ate nuts gained on average 0.424 kg (95% CI: 0.102-0.746 kg; P for trend= 0.018) more weight following adjustments for potential confounders than frequent nut eaters after a median follow up of 28 months. Furthermore, similar trends were also observed when nut consumption categories were split into just two categories of two to four times per week and at least five times per week. The crude odds ratio (OR) for weight gain of five or more kilograms was 0.63 (95% CI: 0.47-0.85) for individuals who consumed nuts two to four times per week and 0.58 (95% CI: 0.38-0.88) among those who consumed nuts at least five times per week (102).

Inverse associations between nut consumption and weight gain were also reported in the Nurses’ Health Study II (NHS II) (92). Bes-Rastrollo et al., 2009 examined the long-term association between nut consumption and weight change over an 8-year period in a free-living population of women from the NHS (n=51188). The results indicated that a higher nut consumption was not associated with greater body weight gain during the 8 year follow-up period in healthy middle-aged women. In this women only study, participants who reported eating nuts two or more times per week experienced a slightly lower mean weight gain (mean ± SE) (5.04 ±0.12 kg) than did women who rarely ate nuts (5.55±0.04 kg), P for trend <0.001. In fact, greater nut consumption of two or more times per week was associated with a slightly lower risk of weight gain and obesity (HR: 0.77; 95% CI: 0.57-1.02; P for trend=0.003). Moreover, among women who were not obese at baseline, the risk of developing obesity during the 8-year follow up period was lower (HR= 0.62; 95% CI: 0.39-0.99; P for trend= 0.001) for women consuming two or more servings of nuts per week than those who rarely ate nuts. Also, when the total nut consumption was sub-divided into peanuts and tree nuts, an inverse association was observed. The results were also similar for normal weight, overweight and obese participants (92).
Table 2.12. Effect of nut consumption on body composition parameters in nut feeding intervention trials

<table>
<thead>
<tr>
<th>Author (year)</th>
<th>Length of study/objective</th>
<th>Study design</th>
<th>Number of subjects</th>
<th>Study population</th>
<th>Intervention</th>
<th>Outcome measure</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alper &amp; Mattes (2002) (16)</td>
<td>30 weeks</td>
<td>Objective: To investigate the effects of chronic peanut consumption on energy balance and hedonics</td>
<td>Randomised, double blind, 3-arm crossover, intervention study</td>
<td>15 (M/F)</td>
<td>Healthy, normal weight</td>
<td>8 weeks of FF &amp; instructions to consume peanuts daily (50% dietary fat provided based on energy &amp; fat intake) (habitual diet)</td>
<td>Dietary intake</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>8 men</td>
<td>Age: 33±9 years</td>
<td>3 weeks ADD (50% of dietary fat from peanuts added to a prescribed diet iso-caloric to participants energy and fat intake), individualised meal plan &amp; an exchange booklet as a reference manual</td>
<td>Body weight</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>7 women</td>
<td>BMI: 23±1.8kg/m²</td>
<td>8 weeks SUB (decreased fat intake by 50%, and replacement of fat from peanuts), individualised meal plan &amp; an exchange booklet as a reference manual</td>
<td>REE</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Appetitive indices</td>
<td>Hedonics</td>
</tr>
<tr>
<td></td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations used: ADD=(addition phase) nuts added to modified calorie controlled diet; BMI=body mass index; F=female; FF=free feeding phase; M=male; REE=resting energy expenditure; SUB=(substitution phase) nuts incorporated into a calorie controlled diet

**Studies are with free-living populations unless stated otherwise**
While the evidence from the observational studies are in agreement, cautious interpretation of the results is required as observational studies cannot determine cause and effect and the results may be affected by some unknown confounders. Residual confounding should be taken into account. The factors unaccounted for in the questionnaires that may imply a healthier lifestyle could mediate the inverse association between nut intake and body weight. The participants with frequent nut consumption could have reported healthier lifestyles and a better overall diet than those who rarely ate nuts. Although the possibility of residual or unmeasured confounding cannot be ruled out, it is unlikely to fully explain the inverse associations observed in the studies. Potential measurement error in the assessment of dietary nut consumption based on FFQs are also inherent in nutritional epidemiology. As nut intake was assessed using FFQs, participants could have over- or under-reported their respective nut intakes in the studies. Because of the nature of the studies, causal relationships cannot be proven. The possibility of reverse causation bias also exists as an increased BMI could be a reason for individuals to decrease intake of fatty foods, among them nuts. Furthermore, the generalisability of the studies is also questionable. Two of the studies were conducted in highly educated group, one of the studies was only conducted in women while two cross-sectional studies were conducted in an elderly population who customarily had a high nut intake (92, 98, 100, 102). Therefore, the results may not be generalisable to other population groups.
<table>
<thead>
<tr>
<th>Author (year)</th>
<th>Length of study/objective</th>
<th>Study design</th>
<th>Number of subjects</th>
<th>Study population</th>
<th>Intervention</th>
<th>Outcome measure</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fraser et al. (2002) (89)</td>
<td>12 months (2 sequential 6 month diet periods)</td>
<td>Randomised, cross-over study</td>
<td>81 (M/F)</td>
<td>Healthy, overweight</td>
<td>1st six month period – control (No formal intervention/no almonds)</td>
<td>Body weight</td>
<td>No significant difference in body weight during almond feeding period in comparison to control period (p=0.09)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>43 men 38 women</td>
<td>Age: 25-70 years</td>
<td>2nd six month period (54.3g almond/day (76.4kJ) (raw or dry roasted) provided to account for 15% of daily energy for each individual, with no dietary advice)</td>
<td></td>
<td>A significant increase in body weight of 0.65kg in men in the almond feeding period (p&lt;0.01) but not in women (p=0.79) when compared to control period</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>A statistically significant inverse association between baseline BMI and change in weight was observed in men alone (p&lt;0.005) and when both genders were combined (p=0.05) but not in women alone (p=0.49)</td>
</tr>
<tr>
<td>Wein et al. (2003) (90)</td>
<td>24 weeks</td>
<td>Prospective RCT</td>
<td>65 (M/F)</td>
<td>Overweight</td>
<td>Self-selected complex carbohydrates LCD (CHO-LCD)-control</td>
<td>Body composition</td>
<td>A significant reduction in weight/BMI, waist circumference, and fat mass in the almond-LCD compared to CHO-LCD (p=0.0001, p&lt;0.05 and p&lt;0.05, respectively)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>28 men 37 women</td>
<td>Age: 27-79 years</td>
<td>Almond LCD (84g/day) (almond-LCD)</td>
<td>Metabolic parameter</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations used: BMI=body mass index; CHO-LCD=complex carbohydrate rich low calorie diet; F=female; LCD=low calorie diet; M=male; RCT=randomised controlled trial

**All studies are with free-living populations unless stated otherwise**
2.6.2 Nut intervention trials on body weight

Ten dietary intervention studies using different types of nuts have explored the effects of nut consumption on body weight (nine RCTs and one uncontrolled trial) (16, 88-91, 93-97).

The participants in these studies included healthy (16, 88, 93-95) and/or overweight and obese individuals (89-91, 96) and patients of metabolic syndrome (97). The duration of the studies was between three and twelve months. In these intervention trials, five studies used almonds (88-90, 93, 96), one study used peanuts (16), two studies used pistachios (94, 97), one used walnuts (91) and another hazelnuts (95). Although differing in methodology and dietary control, collectively these investigations provide substantial evidence that consumption of moderate to large amounts of nuts lead to no weight gain or less weight gain than predicted.

Six out of the ten clinical studies (88, 89, 91, 93, 95, 97) specifically investigated the effects of supplementing the habitual diets of free-living subjects with nuts without constraints on energy balance on body weight.

Fraser et al., 2002 (89) and Sabate et al., 2005 (91) added nuts (almonds and walnuts, respectively) to the participants’ diet, at intakes equivalent to a proportion of their usual daily intake. In both these studies, participants were advised not to make any dietary changes other than to include nuts in their daily diets.

Fraser et al. (2002) reported that almond supplementation had minimal effects on body weight. Eighty-one healthy and overweight adults were provided with 42–70 g (averaging 1340 kJ or 320 kcal/day) of raw or dry-roasted almonds/d for six months with no specific dietary instructions other than eating the nuts. The almond supplement provided 15% of daily energy requirements for each person. While the expected uncompensated weight gain was predicted as 6.4 kg from the extra calories consumed from almonds, there was only a non-significant weight gain of 0.4 kg for the group overall.
Table 2.12. Effect of nut consumption on body composition parameters in nut feeding intervention trials

<table>
<thead>
<tr>
<th>Author</th>
<th>Length of study/objective</th>
<th>Study design</th>
<th>Number of subjects</th>
<th>Study population</th>
<th>Intervention</th>
<th>Outcome measure</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sabate et al.</td>
<td>12 months (2 sequential 6 month diet periods)</td>
<td>Randomised cross-over field trial</td>
<td>90 (M/F)</td>
<td>Healthy and Over-weight</td>
<td>1st six month period – control (Habitual diet- no walnut)</td>
<td>Body weight</td>
<td>No significant changes in body weight and BMI when the control-walnut sequence group incorporated walnuts in their usual diet for 6 months</td>
</tr>
<tr>
<td>(2005) (91)</td>
<td></td>
<td>Sequence 1</td>
<td>40 men 50 women</td>
<td>BMI: &lt;35kg/m²</td>
<td>2nd six month period- walnut supplemented diet (12% of daily energy intake added to diet provided)</td>
<td>Body composition</td>
<td>Significant decrease in fat mass by 0.4kg and percentage body fat by 0.6% and increase in fat-free mass by 0.58kg in the control-walnut sequence group (p=0.04, p&lt;0.0001, and p&lt;0.0001, respectively)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sequence 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>A significant decrease in body weight, BMI, fat mass and percentage by 0.5kg, 0.2kg/m², 0.8kg and 1% when the walnut-control sequence group stopped consuming walnuts after 6 months (p=0.004, p&lt;0.001, p&lt;0.001,p&lt;0.001, respectively).</td>
</tr>
<tr>
<td>Hollis &amp; Mattes</td>
<td>23 weeks</td>
<td>Randomised cross-over study</td>
<td>20 (F)</td>
<td>Healthy</td>
<td>Control group (no almond, usual diet for 10 weeks with no other dietary advice)</td>
<td>Body weight</td>
<td>No significant changes in body weight, percent fat, fat mass or fat free mass within or between the groups; (p&gt;0.05)</td>
</tr>
<tr>
<td>(2007) (88)</td>
<td></td>
<td>Women only</td>
<td></td>
<td>Mean age: 24±9 years</td>
<td>Almond group (1440 kJ portion of raw, unsalted almonds daily with no other dietary advice) for 10 weeks</td>
<td>Body composition</td>
<td>While fat intake increased significantly in the almond group (p&lt;0.05), inclusion of almonds in the diet did not lead to a statistically significant increase in food intake at any time point (p&gt;0.05)</td>
</tr>
</tbody>
</table>

Abbreviations used: BMI=body mass index; F=female; M=male

**All studies are with free-living populations unless stated otherwise**
After 6 months of consuming the almond supplement, men gained only 0.65 kg (p<0.010) while weight in women did not change significantly (0.11kg; p=0.790) compared with a 6-month control period. Furthermore, only the lean subjects in the lowest tertile of baseline BMI gained weight during the almond phase of the study, and women in the highest-baseline-BMI tertile actually lost weight with almond supplementation. Thus, the daily incorporation of a modest quantity of almonds for 6 months did not lead to clinically important or statistically significant (for women) changes in body weight (89).

Sabate et al, (2005) on the other hand, showed that a small but significant increase in body weight occurred in the men and women consuming approximately 35 g of walnuts daily compared to the control diet. Ninety free-living men and women were assigned to either a walnut supplemented diet (28–56 g walnuts/d) corresponding to 12% of their daily energy intake or habitual diet for 6 months. Participants were not given any dietary specific instructions other than to eat the allotted amount of nuts. At the end of 6 months, the theoretical weight gain was predicted to be 3.1 kg. Although daily energy intake increased by 557 kJ (133 kcal), the weight gain was only 0.4 kg. After adjusting for energy differences between the control and the walnut-supplemented diets, no significant differences were observed in body weight or body composition parameters, except for BMI (91).

Hollis and Mattes, (2007) added to the evidence specifically investigating body weight changes after introducing a moderate consumption of nuts into the daily diet of free-living individuals. In their study, twenty free living, overweight, adult women were provided with 60 g/d raw, unsalted almonds (1440 kJ/d) to be incorporated into their diets for 10 weeks or no almonds for another 10 weeks without further dietary instructions in a randomised cross-over study with a 3-week washout period between the experimental periods. After this intervention, participants did not experience a significant change in body weight or body composition measures in comparison to the control group (88).

Zaveri and Drummond (2009) (93) and Tey et al. (2011) (95) adopted a rather similar approach where, a conventional snack (such as cereal bar, chocolate, and potato crisp) or a non-conventional snack (nuts- almonds and hazelnuts) was provided to assess the impact on body weight and appetite indices.
Table 2.12. Effect of nut consumption on body composition parameters in nut feeding intervention trials continued

<table>
<thead>
<tr>
<th>Author (year)</th>
<th>Length of study/objective</th>
<th>Study design</th>
<th>Number of subjects</th>
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<th>Intervention</th>
<th>Outcome measure</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zaveri &amp; Drummond (2009) (93)</td>
<td>12 weeks Objective: To assess the impact of providing either a conventional snack (cereal bar) or a non-conventional snack (almonds) on eating frequency, hunger rating, dietary intake, body weight and blood lipids</td>
<td>Non randomised, dietary intervention study</td>
<td>36 (M) Men only</td>
<td>Healthy Age: 25-50 years BMI: 25-35kg/m²</td>
<td>Control + general healthy eating advice (n=12) Cereal bar (60g) + general healthy eating advice (n=13) Almond (56g) + general healthy eating advice (n=11)</td>
<td>Body weight BMI</td>
<td>There were no significant differences in body weight, BMI, percent body fat or waist circumference within or between the groups</td>
</tr>
<tr>
<td>Li et al. (2010) (94)</td>
<td>12 weeks Objective: To study the effects of pistachio snack consumption on body weight and lipid levels in obese participants under real world conditions</td>
<td>Randomised cross-over study</td>
<td>59 (M/F) 2 men 57 women</td>
<td>Healthy Age: 20-65 years BMI: 27-35kg/m²</td>
<td>Iso-caloric weight reduction diets (meal plans): 56g of salted pretzels daily (n=28) 53g salted pistachio nuts daily (n=31)</td>
<td>Body weight</td>
<td>Significant reduction in body weight in both groups compared to their respective baseline (p&lt;0.01) No significant differences in body weight in the nut group compared to the pretzel group (p=0.09) Significant decrease in BMI in both groups in comparison to baseline at 12 weeks (p&lt;0.05) with a greater reduction in the pistachio group (p&lt;0.05)</td>
</tr>
</tbody>
</table>

Abbreviations used: BMI=body mass index; F= female; M=male
**All studies are with free-living populations unless stated otherwise
Zaveri & Drummond (2009) conducted a three-arm intervention (control group, cereal bar group and almond group) in 36 men by providing the participants with 60 g cereal bar or 56 g almonds or no snacks to the control group. All groups were provided with advice on healthy eating. This men only study found that while the almond snack group had a significantly higher eating frequency than the control group (p≤0.050) and the cereal bar group (p≤0.010), this did not result in higher energy intake, body weight or percentage body fat in the almond snack group. However, the energy intake from the almonds was significantly higher than from the cereal bar (1430 kJ vs. 950 kJ, respectively). Thus, this discrepancy in energy intakes makes comparison difficult between the two groups (93).

Similarly, Tey et al. (2011) assessed the effects of providing daily portions of hazelnuts, chocolate or potato crisps (~1100 kJ/d) or no snacks (control) for 12 weeks on body weight while no dietary advice was given. The investigators concluded that there were no significant differences in the changes of the anthropometric measurements from baseline to 12 weeks between the groups (p≥0.106). Furthermore, a significant reduction in waist circumference for those with higher BMI in the nut group (p=0.005) and in the potato crisp group (p=0.032) in comparison to the control group was also observed. Hence, consuming a number of different snack foods, including nuts did not result in a change in body weight compared to a control group provided with no snack foods (95).

Moreover, Wang et al (2012) investigated the impact of different dosages of pistachio nuts (42 g, or 70 g or no pistachios) on body weight for 12 weeks in ninety adult Chinese men and women with metabolic syndrome. They reported that the daily ingestion of either 42 g or 70 g of pistachios for 12 weeks did not lead to weight gain or an increase in waist-to-hip ratio in comparison to the control group (97).

Alper and Mattes (2002) examined the effects of peanut consumption on energy balance and the hedonic ratings for peanuts and other snack foods. Fifteen adults with normal weight were provided with 89 g of peanuts (2113 kJ/d or 500 kcal/d) under 3 experimental conditions: a free-feeding phase of 8 weeks in which participants were given peanuts without dietary guidance, followed by a 3 week addition phase in which subjects were asked to add peanuts to their baseline habitual diet, and finally an 8 week substitution phase during which peanuts replaced an equal amount of other fats in the diet.
### Table 2.12. Effect of nut consumption on body composition parameters in nut feeding intervention trials continued

<table>
<thead>
<tr>
<th>Author (year)</th>
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<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tey et al. (2011) (95)</td>
<td>12 weeks</td>
<td>Parallel, randomised, controlled, 4-arm, study</td>
<td>118 (M/F) 55 men 63 women</td>
<td>Healthy Age: 18-65 years BMI: &lt;30kg/m²</td>
<td>42g Hazelnut -1100kJ/d with no dietary advice 50g chocolate - 1100kJ/d with no dietary advice 50g potato crisp - 1100kJ/d with no dietary advice Control (no additional food)</td>
<td>Body weight Appetitive indices RMR</td>
<td>No statistically significant differences in the changes of the anthropometric measurements and RMR from baseline to 12 weeks between the groups (p≥0.106) A significant reduction in waist circumference for those with higher BMI in the nut group (p=0.005) and in the potato crisp group (p=0.032) in comparison to the control group</td>
</tr>
</tbody>
</table>

| Wang et al. (2012) (97) | 12 weeks | Parallel RCT | 90 (M/F) 41 men 49 women | Patients of metabolic syndrome Age: 25-65 years BMI: 28 kg/m² | 42g of pistachios daily 70g of pistachios daily No pistachios (control) | Body weight BMI | No significant changes in body weight or BMI in any groups during the study nor any change from baseline at any time point in any group No significant differences in waist-to-hip ratio among the groups or any change from baseline in any group |

**Abbreviations used:** BMI = Body mass index; F = female; M = male; RCT = randomised controlled trial; RMR = resting metabolic rate

**All studies are with free-living populations unless stated otherwise**
The phases of the study were separated by 4-week washout periods. No weight gain was observed in the substitution phase while during the free-feeding phase, the observed weight gain was 1.0 kg, which was significantly lower than the theoretical, expected weight gain of 3.6 kg, based on the additional calories provided. Similarly, weight gain was also significantly lower during the addition phase than predicted. Subjects gained only 0.6 kg, while 1.4 kg had been predicted (16).

While Alper and Mattes, (2002) concluded that peanut consumption does not lead to increased body weight, they did not include a control group where, no peanuts were consumed by the participants. Collectively, these studies have shown that incorporating nuts in the diet either leads to no weight gain or less weight gain than predicted.

Finally, three out of the ten intervention studies which assessed body weight and regular nut consumption included nuts as part of an energy controlled weight loss diet (90, 94, 96).

The weight reduction study by Wien et al. (2003) (90) which included overweight and obese subjects, showed a 62% greater weight loss in the nut consuming group compared to the control group. Sixty-five overweight and obese individuals were randomised into either a complex carbohydrate-enriched or an almond-enriched low calorie diet for 24 weeks under free-living conditions. Approximately 84 g/d of almonds was provided to the almond enriched diet group, which was equivalent to 39% energy from fat as opposed to 18% energy from fat in the carbohydrate enriched low calorie diet.

Participants were advised to begin a walking program (20– 30 minutes, 3–5 times per week) starting in week 5 of the dietary intervention. As expected, both diet groups lost weight. However, the almond group had more favorable reductions in body weight and BMI (18 vs. 11%; p<0.0001), waist circumference (14 vs. 9%), and fat mass (30 vs. 20%) (p<0.050) at the end of 24 weeks. The almond group experienced a sustained and 62% greater weight reduction for the 24-week duration than the carbohydrate group. This study suggests that in the context of low caloric diets, including nuts may enhance weight loss and improve body composition indices (90).
Table 2.12. Effect of nut consumption on body composition parameters in nut feeding intervention trials continued

<table>
<thead>
<tr>
<th>Author (year)</th>
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<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Foster et al. (2012) (96)</td>
<td>18 months</td>
<td>Parallel RCT</td>
<td>123 (M/F)</td>
<td>Overweight and obese</td>
<td>Hypocaloric nut free diet (NFD) + instructions on traditional behavioural methods of weight control</td>
<td>Body weight</td>
<td>A statistically significant weight reduction at 6 months in both groups compared to baseline with a greater reduction in NFD group (p=0.04)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>11 men</td>
<td>Age: 18-75 years</td>
<td>Hypocaloric, almond-enriched diet (AED) + instructions on traditional behavioural methods of weight control (28 g/d almond)</td>
<td>Body composition</td>
<td>No significant differences in weight loss at 12 months in the two groups (p=0.120)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>112 women</td>
<td>BMI: 27-40 kg/m²</td>
<td></td>
<td></td>
<td>No significant differences in body composition between the two groups at 6 or 12 months</td>
</tr>
</tbody>
</table>

Abbreviations used: BMI=Body mass index; F=female; M=male; RCT=randomised controlled trial
**All studies are with free-living populations unless stated otherwise**
Similarly, in a low calorie weight loss trial comparing the effects of pistachios or pretzels served as an afternoon snack for 12 weeks, Li et al., (2010) found a significantly greater reduction in the BMI of participants consuming pistachios compared to the participants in the pretzel group (94).

Furthermore, Foster et al. (2012) compared the effects of a hypo-caloric, almond-enriched diet with a hypo-caloric nut-free diet on body weight and CVD risk in 132 overweight and obese individuals in the context of an 18-month behavioural weight management program. Those on the hypo-caloric, almond-enriched diet lost slightly but significantly less weight than those on hypo-caloric nut free diet at 6 months (5.5 kg vs. 7.4 kg, respectively; p=0.040). The participants in both the hypo-caloric almond-enriched diet and the hypo-caloric nut free diet experienced clinically significant and comparable weight loss at 18 months. There was however, no difference in weight loss between the two diets (hypo-caloric almond-enriched diet and the hypo-caloric nut free diet) at 18 months (3.7 kg vs. 5.9 kg, respectively; p=0.120) (96).

Overall, the clinical trials reveal little or no weight change with inclusion of various types of nuts in the diet. In the studies that added nuts to the regular diet of participants without controlling for energy intake, actual weight variations were less than predicted. Hence, these clinical trials suggest a limited impact of nut consumption on body weight.

It is important to interpret the findings of the studies discussed above in light of several limitations. The majority of the studies used crossover study design, and some did not randomise the treatment order (93). Crossover trial is unlikely to be the best study design when the primary outcome is bodyweight. Crossover study relies heavily on the ability of the wash-out period to return outcomes back to baseline values. It is unclear how long would be required to see wash-out treatment effects on body weight. In addition, some of the intervention studies were of short duration (e.g. 12 weeks) (93-95, 97) or had relatively small numbers of participants (e.g. 15 participants) (16). Also some of the studies may not be generalisable as they used specific populations such as females (88) or males (93) only, or only those who were obese or overweight (89-91, 93, 94, 96, 97). However given the consistent results, collectively the studies suggest that the regular consumption of nuts results in less than predicted weight gain among males and females, and among normal weight and overweight and obese individuals.
These findings are in agreement with a systematic review and meta-analysis of 33 published, randomised nut-feeding trials which estimated the effects of nut consumption on adiposity measures performed by Flores-Mateo et al (2013). Pooled results indicated a non-significant effect on body weight, BMI, or waist circumference of diets including nuts compared with control diets. The meta-analysis of clinical trials showed that nut consumption was associated with a non-significant decrease in body weight of 0.47 kg, BMI of 0.40 kg/m², and waist circumference on 1.25 cm, respectively. Thus, Flores-Mateo and colleagues concluded that nut rich diets compared with different control diets do not increase body weight, BMI or waist circumference (99).

The findings by Flores-Mateo confirm the results of the epidemiologic studies and the intervention trials that suggest regular nut consumption does not lead to increased body weight. While the meta-analysis shows that body weight is not affected by nut consumption, it included not only the studies where weight gain was the primary outcome but also other studies for which body composition data were available. Therefore, it would be interesting to see if the relationship would exist if only the intervention trials that had body composition parameters as the primary outcome were exclusively analysed.

2.6.3 Summary of evidence: Nuts and body weight

Overall, epidemiologic studies indicate that incorporating nuts into the diet on a regular basis does not compromise, and may aid, weight maintenance whereas the findings from the intervention trials show that the effects of nut consumption on body weight is varied, but on the whole, result in less weight gain than predicted. When nuts were added to an existing diet without controlling for energy intake, there were no significant changes in body weight or body weight increased in comparison to the control groups, although to a much lesser extent than theoretically predicted. Moreover, controlled feeding studies using various nuts (almonds, walnuts, and hazelnut) indicate that nut consumption does not cause changes in body weight when energy intake is adjusted (90). However, it must be noted that four of these studies were relatively short-term
trials (93-95, 97)) and three (16, 88, 93) had limited power to detect small changes in body composition parameters.

Although there are reports of small, but significant increases in body weight with nut consumption (91), the preponderance of evidence indicates that under controlled or free living situation, nut consumption does not promote weight gain. To date, the intervention studies conducted have tested the effects of nuts on body weight using larger quantities of nuts rather than the recommended daily serving of 30 g. Therefore, there is a need for long-term randomised intervention studies to establish the effects of nut consumed daily in realistic quantities on maximal and sustainable weight loss.

It is also evident that more clinical data gleaned from greater numbers of participants and in studies of longer durations with body weight as the primary outcome are needed. This would permit more robust conclusions to be drawn before claiming that the ad libitum addition of nuts to the habitual diet of free-living individuals does not affect energy balance and finally body weight.

### 2.6.4 Potential Mechanisms of weight control

While the reasons for the less than predicted weight gain remain unclear, there are a number of mechanistic hypotheses that could explain this finding. The mechanisms by which nuts influence body weight include increased satiety levels (15, 16, 19, 103-108), an incomplete digestion and absorption leading to increased fecal fat (16, 91, 108-112) and increased resting energy expenditure (16, 88, 89, 91, 95, 113-115).

#### 2.6.4.1 Satiety

Satiety is the process that inhibits further eating, causing a reduction in hunger, and an increase in fullness after a meal is eaten (15). Nuts are known to induce satiation (reduction in the total amount of food eaten in a single meal) and satiety (reduction in the frequency of meals) (15, 108). Studies have shown that nut consumption moderates appetite postprandially and that the inclusion of almonds and peanuts in the diet suppress hunger and desire to eat and increase fullness rating after ingestion (103, 105, 107, 108).
Dietary compensation seems to be a major reason for the lack of predicted weight gain in long-term nut-supplemented diets (104). A substantial amount of energy provided by nuts can be compensated by a lower intake of other energy-dense foods due to nutrient displacement, satiation or satiety (19, 104). It has been estimated that inclusion of nuts in the diet may offset approximately 65-75% of the energy provided from nuts by means of dietary compensation due to strong satiety effects (104).

In a 6 month almond supplementation study, Fraser et al., 2002, estimated that 54-78% of the extra energy from almonds was displaced by reduction in other foods (89). Also, in an acute preload study, consumption of 2092 kJ of peanuts exerted a strong suppression of hunger and energy compensation (103). Furthermore, in another feeding study of 20 men and women, increased level of satiety and sense of fullness were observed after consumption of 48 g walnut containing shake for breakfast in comparison to a control walnut-free shake (105).

The dietary components of nuts have also been implicated in their effects on satiety. Nuts are energy dense foods with high dietary fibre, and protein content and low glycaemic index, all of which are dietary factors that have been shown to increase satiety (116-118).

Having to shell nuts prior to consumption has been shown to reduce energy intake as compared with nuts which have been pre-shelled (15). The act of de-shelling the nuts may slow the rate of consumption by permitting greater metabolic feedback during ingestion or may increase satiety by increasing perception of the quantity consumed (15, 104). Whole nuts require an effort of mastication, which may promote satiety, since the metabolic effects of consumption of high fat foods are modulated by oral exposure (15). Thus the form of nut may be important for satiety. Whole and sliced nuts may result in higher satiety levels compared to ground nuts due to the degree of mastication required (119). Studies have shown that ingestion of nut oil elicits a weaker compensatory dietary response in comparison to whole nuts. Dietary compensation scores of 50-78% has been reported in previous studies using whole nuts (16, 88, 115). In comparison, weaker dietary compensation scores have been reported in two studies where nut oil was used.
Following a diet rich in peanut oil, Iyer et al., 2006, reported a dietary compensation score of 46% in healthy adults, and Coelho et al., 2006 reported dietary compensation scores of 66%, but in lean adults only, and 4% in overweight adults (120, 121).

Furthermore, the high content of fibre in nuts may lead to delayed gastric emptying and reduced absorption (116). Moreover, the macronutrient profile of nuts is also associated with increased release of glucagon-like peptide 1 (GLP-1), cholecystokinin (CCK), and gastrointestinal hormones that affect satiety (15, 100).

2.6.4.2 Poor digestion and absorption

Decreased fat absorption may be another explanation for the minimal, and less than expected weight gain with regular nut consumption. The physical form in which nuts are consumed may be important as it has been demonstrated that whole nuts are inefficiently absorbed due to incomplete mastication (111, 119, 122). It is likely that fatty acid availability from nuts is decreased because of incomplete digestion and absorption. As such, some of the fat contained in the nuts becomes unavailable (111). Additionally, the parenchymal cell wall of nuts is resistant to microbial and enzymatic degradation. Thus, cells that are not ruptured as a result of insufficient mastication may pass through the gastrointestinal tract without releasing the fatty acids and nutrients contained within (15, 111, 112, 123).

With the help of electron microscopy, it has been demonstrated that the cell walls of almond remain intact in fecal samples, decreasing the bio-accessibility of intracellular fatty acids contained in the almonds and leading to a three-fold increase in percent fecal fat excretion (111). Visual inspection showed that stools contained intact portions of the nuts indicating that absorption was compromised (111, 123, 124).

A number of studies have evaluated the efficiency of energy absorption from nuts. All showed substantive increases in fecal fat loss with nut consumption, with the values ranging from 5% to more than 20% (16, 81, 109-112).

As nuts are rather high in total fat content, increased faecal fat loss could protect nut consumers from gaining weight (125). It has recently been shown in two studies that Atwater factors, when applied to two different nuts resulted in an over-estimation of their measured energy contents (126, 127).
The measurement of the metabolisable energy value for whole pistachio nuts (126), and whole almonds (127) were overestimated by 5% and 32%, respectively. Moreover, it would be interesting to see whether the observed discrepancy measured in whole nuts such as almonds and pistachios would be consistent for other types and forms of nuts and the respective nut butters or sliced nuts.

Thus, the excretion of fat in the stools and the displacement of foods from habitual diets together may account for the lack of weight gain among nut consumers (125). This mechanism of poor absorption has been tested in mostly almonds only and therefore, needs to be verified in other nuts as well.

2.6.4.3 Resting energy expenditure (REE)

Increased energy expenditure may be another mechanism by which nut consumers maintain weight. It has been suggested that the combination of unsaturated fat and a high protein content found in nuts may influence diet-induced thermogenesis by increasing resting energy expenditure (REE) (16, 88, 121). However, only few trials have explored the effects of nut consumption on REE.

It has been shown in two studies that REE increased following peanut consumption. Regular consumption of peanuts for 19 weeks resulted in an 11% increase in REE when compared with the baseline measurement (16). Similarly, Coelho et al., 2006, reported a 5% increase in resting metabolic rate (RMR) in participants who consumed peanut oil containing milk shakes for 8 weeks (121). On the contrary, however, studies of varied lengths ranging from four days to 12 months, using different nuts (almonds, walnuts, mixed nuts, hazelnuts), have reported no changes in REE, or RMR post-nut consumption (76, 81, 88, 95, 105). Some evidence suggest that MUFA and PUFA present in nuts are more readily oxidised than saturated fatty acids, leading to reduced fat accumulation (92, 113).

While there is only limited evidence of nuts influencing energy expenditure, the studies that have shown effect were only conducted on one nut type (peanuts), therefore, it is difficult to extrapolate the results to other nut types.
Increased satiety with nut consumption, the displacement of foods from the habitual diet, and increased faecal fat excretion appear to be plausible mechanisms by which regular nut consumption does not adversely influence body weight despite the fact that nuts are energy dense. Mechanistic studies indicate this is largely attributable to the high satiety and low metabolisable energy (poor bio-accessibility leading to inefficient energy absorption) properties of nuts (113).

However, there is only limited data to suggest that routine nut consumption is associated with elevated REE and the thermogenic effect of feeding, resulting in dissipation of a portion of the energy they provide. Therefore, the effects of nut consumption on diet-induced thermogenesis and REE need further substantiation. Inclusion of nuts in energy-restricted diets may help adherence to the diet, facilitate weight loss, and improve measures of body composition. Future studies need to further explore the potential mechanisms by which nuts prevent weight gain.

2.7 Methods of measuring satiety

Hunger, satiation and satiety are regarded as the three components of appetite (15, 128). Since appetite is a subjective concept, it is not open to direct measurement (129). There are a number of methods used to measure appetite and satiety. Among these are assessment of eating patterns/food intake, and questionnaires (15, 104, 129, 130). Studies that have assessed satiety using subjective ratings of appetite, or by measuring actual energy intake have shown that appetite ratings correlated with food intake in a standardised setting (105, 106).

One of the experimental techniques used to study the short-term regulation of food intake is the preload test meal paradigm (129, 130). Pre-load studies are conducted using a within-subject repeated measures design. It uses double-blind, controlled conditions which includes a non-preload or placebo treatment. In these studies, subjects are presented with precisely prepared food matched for taste, appearance and other sensory properties but varying in energy and macronutrient composition.
After a variable time delay, the effects of the preload on spontaneous food intake are measured through accurately monitored test meals, or alternatively subjects might self-report their own food intake (129, 130). Subjective measures of appetite are usually taken prior to, and at pre-determined time intervals after the preload and the test meal (130). Subjects are required to record responses to appetite rating questions, such as, ‘how hungry do you feel right now’, ‘how strong is your desire to eat right now?’, ‘how much food could you eat right now?’, ‘how full do you feel right now’, and ‘do you have any preoccupation with thoughts of food right now?’. In many of these experiments, food intake for the remainder of the day is also self-recorded by the subjects. It must be acknowledged however that the preload design is particularly prone to type II errors and consequently, the time of day at which preload is offered and the appropriateness of the food for that time of the day need to be considered (129, 130).

In order to capture self-reports of appetite-related feelings, uni- and bi-polar structured and unstructured lines, verbal categories and numerical scoring questionnaires can be used. The most common response format to assess subjective rating of appetite is the unipolar unstructured line method that uses VAS (130). Appetite related self-reports include a range of measures intended to capture perceived general state of hunger over a given period. In general, these scales are completed before and after consumption of the test food, and then at regular time intervals usually for 3-5 hours or to the start of the next meal (129, 130). The appetite rating questionnaires are easy to design, easily applied and unambiguously interpreted by investigators and subjects. It demonstrates repeatability with regard to group mean data and comparisons of specific foods, even over several months or years. Appetite scores measured through VAS can be reproduced and are therefore feasible tools to measure appetite and satiety sensations (129, 130).

2.8 Nut intake in New Zealand and world-wide

Nuts are now recommended as part of a healthy diet in many dietary guidelines globally (2, 131). In New Zealand, the National Heart Foundation recommends that people should consume up to 30g of nuts regularly (11).
However, there is little information on the percentage of people within the population who are consuming this amount of nuts regularly (131). In addition, very little data exists on nut consumption in terms of prevalence, mean population intake and mean portion sizes in different population groups. Data on the patterns of nut consumption within and between population groups are scarce (12, 131, 132).

To date, only three observational studies have evaluated the prevalence and patterns of nut consumption in different geographical regions; namely The European Prospective Investigation into Cancer and Nutrition (EPIC) study, the National Health and Nutrition Examination Survey (NHANES) and the 2009/09 NZANS (12, 132-135). The method of the three studies have been described and published elsewhere and will be summarised below (13, 132, 135).

The EPIC study, a large, multi-center prospective cohort study that was conducted in 10 Western European countries, assessed nut and seed intake from various dietary sources using detailed 24-hour dietary recalls in a subset of the EPIC study population (n=36994) (131, 132). The EPIC study analysed the population mean intake and mean portion sizes in individuals reporting intake of nuts and seeds consumed on the day that the 24-hour diet recall was administered. The intake of nuts and seeds was reported as that consumed as whole, derived from hidden sources, and or from spreads (132).

The intake of total nuts consumed whole, from hidden sources and in spreads were 2.23 g/d, 1.43 g/d and 0.34 g/d for the entire population (consumers and non-consumers combined). Whereas, the average portion sizes among nut consumers for total nuts consumed as whole, from hidden sources and spreads were 30.8 g/d, 7.7 g/d and 15.8 g/d, respectively (132).

Overall, for all countries combined, 6.9%, 20.7% and 2.2%, of subjects consumed total nuts (tree nuts (almonds, Brazil nuts, cashews, hazelnuts, macadamia nuts, pecans, pine nuts, pistachios and walnuts)), peanuts, and unspecified nuts consumed as whole, from hidden sources and spreads, respectively (132).

Furthermore, the EPIC study showed that nut intake varied widely with geographical region. The mean intake of total nuts varied approximately by 8 fold from northern to southern Europe, ranging from 0.61 g/d (lowest intake) in Sweden to 4.83 g/d (highest intake) in Spain. Moreover, among tree nut consumers, the mean portion size of total tree nuts varied from 20.3 g/d in northern Europe to 29.1 g/d in Southern Europe (132).
The consumption of total nuts and total tree nuts (consumed as whole) was highest in Spain where 11.9% and 8.3% of the population consumed about 40 g and 34.7 g of total nuts and total tree nuts, respectively, on the day that the 24-hour diet recall was administered (131, 132). Additionally, in the EPIC cohort differences by sex were also apparent for both total nuts and total tree nuts intakes. Men reported consuming a significantly larger portion size of total nuts and total tree nuts (38.3 g/d and 28.5 g/d, respectively) than women (28.7 g/d and 23.1 g/d, respectively). However, on the day of the 24-hour diet recall, a higher percentage of women (7.2% and 4.8%) indicated consumption of total nuts and total tree nuts than men (6.4% and 3.7%) (131, 132).

In the United States, nut consumption data has been derived from NHANES 1999-2004, which uses cross-sectional data. The nut intakes were determined using the 24-hour diet recall method (133-135). Studies have investigated the prevalence of nut consumption in the US (134); the association of tree nut consumption and nutrient intake and diet quality (135) and the association of out of hand nut consumption with nutrient intake, diet quality, and the prevalence of risk factors for cardiovascular disease and metabolic syndrome (133).

The NHANES survey uses a nationally representative sample of adults. Data from adults 19 years and older participating in the NHANES from 1999-2004 have been combined for analyses (n=13, 292). In the NHANES 1999-2004, the percentage of individuals, 19–50 years and 51 years and over, consuming all nuts and nut butters on the day of the recall was 18.6% and 21.0%, respectively (134). The prevalence of tree nut consumers and tree nut butter consumers was 5.5% for individuals aged 19-50 years (n=7,049) and 8.4% in individuals aged 51+ years (n=6,243) (134).

Among consumers, the mean intake of tree nuts and tree nut butters was 36.6 g/d and 33.8 g/d, respectively (134, 135). Tree nuts were significantly more likely to be consumed by adults 19-50 years of age, ‘whites’, and those individuals with a higher income and education. Unlike the EPIC cohort, no difference in tree nut consumption were observed between the sexes (135).

Data from 24-hour diet recalls from individuals aged 2 years or greater (n=24385) was used in a subsequent analysis of the NHANES 1999-2004, to determine the association of out of hand nut consumption with nutrient intake, diet quality, and the prevalence of risk factors for CVD and metabolic syndrome (133).
Out-of-hand nuts were defined as those nuts consumed solely as nuts and not as part of products, for example, in breads, cereals, or candy bars. In this study it was found that prevalence of nut consumption was low, and was positively associated with age. The prevalence of nut consumption was 6.5% and 9.6% in those aged 19-50 and 51+ years. The consumption of nuts and seeds by consumers aged 2-11, 12-18, and 19+ years was 97 g (3.24 ounce), 110.2 g (3.89 ounce), and 114 g (4.01 ounce), respectively (133).

Most recently, the 2008/09 NZANS, a cross-sectional study investigated the prevalence and predictors of nut consumption in 4721 New Zealanders (12). Similar to the previous two studies, data from 24-hour diet recalls was used to identify nut consumers, sources and predictors of nut intake in the 2008/09 NZANS. The percentage of consumers on the day of the diet recall, for whole nuts, nut butters, and nuts from hidden sources were 6.9%, 7.2%, and 19.2%, respectively. Among consumers, the mean portion size of whole nuts, nut butters, nuts from hidden sources, and all nut sources combined were 40.3 g, 12.9 g, 7.8 g, and 17.9 g, respectively. Furthermore, the population mean intakes were relatively low for whole nuts (2.8 g), nut butters (0.9 g) and nuts from hidden sources (1.5 g). There was an association between nuts and age. Participants aged 15-18 years had the lowest whole nut consumption but the highest peanut butter consumption. The consumption of whole nuts was inversely associated with BMI while consumption of total nuts was positively associated with level of education and socio-economic status (SES) (12).

The studies conducted to date have provided an overview of the population mean intakes and average daily portion sizes in subjects reporting intake of nuts consumed in different forms in the diet (whole, from hidden sources and or spreads). It is clear that the prevalence of nut consumption is relatively low in the different population groups studied so far (12, 131-135). It appears that the recommended amount of 30 g of nuts/day is being met by very few individuals within the population. The data from the three studies show some degree of homogeneity for nut consumption, i.e. the prevalence of nut intake and population mean intakes in the three studies are very similar. However, the data also shows a clear geographical differences in nut intake especially in the EPIC study where the Southern European countries had a higher nut intake compared to the Northern European countries (131, 132).
There are several limitations to take into account when interpreting this data. Firstly, nut intake was assessed by 24-hour diet recall method. The 24-hour dietary recalls may not accurately reflect the usual dietary intake patterns and participants rely on their memory to self-report dietary intakes. Therefore, data are prone to non-sampling errors, where subjects may over- or under-report usual intake and energy. However, in studies using large sample sizes, single 24-hour diet recalls produce reasonably accurate group estimates of nutrient intake (133-135). Therefore this concern is particularly applicable to the NZ population where the sample size was smaller (13).

In summary, the prevalence and serving size of nut intake appears to be consistent among these different population groups. The low prevalence of nut consumption in these populations is of concern and new strategies to increase nut consumption are required.

2.9 Overall Summary

Collectively, evidence to date suggests that nut consumption has many health benefits such as decreased all-cause mortality risk and CVD risks. It is also appears that nut consumption does not lead to body weight gain and if anything helps in the maintenance of weight. While the evidence on the effect of nut consumption on diabetes and glycaemia is not as conclusive, the direction of the effect favours nuts. However, this warrants further research in the form of larger and longer RCTs that measure the appropriate outcomes. Even though nuts are recommended as part of a healthy diet, it is very clear that the prevalence of nut consumption is relatively low in the general population. Moreover, few people are meeting the daily recommended amount of 30 g of nuts per day and innovative strategies to increase nut consumption may be required. One strategy to increase nut consumption is to incorporate nuts into a common staple such as bread. Therefore, the aim of this study is to assess and compare the effect of adding different forms of hazelnuts to a bread meal on postprandial glycaemic response, satiety and gastrointestinal tolerance.
Figure 3.1. Overview of the study design
3.0 METHODS

3.1 Study design

This study was conducted using a randomised, controlled, 4-arm, single blinded, crossover design with four different breads over a period of 10 weeks. The treatments comprised of a control bread with no hazelnuts, bread containing finely sliced hazelnuts, bread containing semi-defatted hazelnut flour, and bread containing a combination of the finely sliced nuts and semi-defatted nut meal. For the purpose of blinding and randomisation, the breads were coded as B1 (control bread with no nuts), B2 (finely sliced nut bread), B3 (semi-defatted nut flour bread) and B4 (combination bread containing finely sliced nut and semi-defatted nut flour). An overview of the study design is presented in Figure 3.1.

In this study, a crossover design was used to allow each participant to act as his/her own control. Participants were randomly allocated a group (G1, G2, G3 and G4). This was to ensure that the participants received the breads in a random order.

The groups were balanced by period so only one bread was used by one group in each period. Each bread had one group where it was preceded by each other bread. The different groups (G1, G2, G3, and G4) received the breads in the following order over the 10-week period:

G1: Bread B1, Bread B2, Bread B3, Bread B4
G2: Bread B2, Bread B4, Bread B1, Bread B3
G3: Bread B3, Bread B1, Bread B4, Bread B2
G4: Bread B4, Bread B3, Bread B2, Bread B1

All participants were randomly allocated to receive one of the four test breads for a week, followed by a one week wash-out period. This was repeated until all participants had consumed all four test breads.
3.2 Ethical approval

The study protocol was approved by the University of Otago Human Ethics Committee (Health) (UOHEC (Health), 14 February, 2014 (Reference: H14/004) (Appendix A). All participants were given an information sheet (Appendix B) outlining the aim and the requirements of the study. The information sheet was also verbally explained to the participants. All participants gave written informed consent, co-signed by a witness (Appendix C) in the clinic. The trial was registered for inclusion in the Australian New Zealand Clinical Trials Registry (ANZCTR) on 27th February, 2014, and the allocated code is ACTRN12614000213640 (Appendix D).

3.3 Participants

3.3.1 Recruitment

Thirty-three healthy participants, aged 18-65 years (10 males and 23 females) were recruited from the general public in Dunedin, New Zealand. However, four participants of the thirty-three recruited, withdrew at the beginning of the study due to personal reasons unrelated to the study. Therefore, three more participants were further recruited to make a total of 32 participants. The recruitment process involved displaying posters on the notice boards around the campus. A copy of the recruitment poster is outlined in Appendix E.

Prospective participants interested in the study contacted the investigator by email, phone or text messages. All prospective participants were sent the participant information sheet (Appendix B), study consent form (Appendix C), and the recruitment questionnaire (Appendix F) via email. The recruitment questionnaire included questions related to contact details, demographics, relevant health details and also on usual bread intake and exercise practices.

All participants were allocated a study ID and a day for the GR clinic (either Tuesday, or Wednesday, or Thursday). The participants were given the opportunity to choose the day of GR clinic most suited and convenient to them. An email containing details
of participant study ID, chosen day of GR clinic, the GR protocol (Appendix G) and a map of the venue (GR clinic) was sent out to the participants. A reminder email and text message was sent out to participants a day before the GR clinic days (Monday, Tuesday and Wednesday).

3.3.2 Inclusion criteria

The inclusion criteria were as follows:

i. Healthy males and females aged 18-65 years who regularly consumed at least 3 slices of bread per day.

The exclusion criteria were:

i. People with diabetes mellitus

ii. Disorders of carbohydrate metabolism

iii. Intolerances or allergies to the test products i.e. hazelnuts, breads, and wheat/gluten

iv. Smokers

v. Pregnant and lactating women

3.4 Study protocol

In brief, the study participants were asked to follow an 8-day protocol for each treatment phase. A GR testing was held on days 1 and 8 of each treatment phase. Participants underwent a satiety test on day 2 where they consumed breads in amounts equivalent to their usual breakfast amounts. For days 3-7, participants consumed a fixed amount (120g) of the study bread. Participants also completed a WDR on day 2 of each treatment phase and the Sunday of each treatment phase. Each treatment phase was separated by a one-week wash-out period. During the one-week wash-out period, participants did not consume any study bread and were asked to consume their usual diet without any changes to physical activity patterns. The schematic of the study protocol is presented in Figure 3.2. All forms completed from days 2-7 were
provided in a booklet given to participants on day 1 of each treatment phase during the GI testing session. As part of the present study, acceptance and the sensory testing of the hazelnut-enriched breads were also examined. For this reason, the participants had to consume the bread for a week. This topic, however, was part of another student’s Master of Dietetics thesis, so therefore is not discussed in this thesis. For the purpose of this thesis, only GR and GI testing, gastrointestinal symptoms, body weight, satiety testing and diet records are discussed. During each treatment phase, participants were encouraged to follow their usual dietary and physical activity patterns. For the duration of the study, participants were permitted to continue to consume nuts and nut products in their diets. They were also instructed in the first instance to consume their allocated study bread and to then consume their usual breads if they required.

<table>
<thead>
<tr>
<th>Time</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 4</th>
<th>Day 5</th>
<th>Day 6</th>
<th>Day 7</th>
<th>Day 8</th>
<th>Day 9</th>
<th>Day 10</th>
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<th>Day 12</th>
<th>Day 13</th>
<th>Day 14</th>
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<tr>
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<td>Satiety testing</td>
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<tr>
<td>Questionnaire on how bread was consumed</td>
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</table>

The 33 participants will be divided into 3 groups of 11.

*To standardise all groups, diet record 2 will be completed on a Sunday which will be on day 4 or 5 depending on group.

Days 1 to 14 will be repeated for each bread.

**Figure 3.2. Schematic for study protocol**
Table 3.1. Proximate nutrient analysis using AOAC methods

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Energy (kJ/100g)</th>
<th>Moisture (%)</th>
<th>Protein (%)</th>
<th>Total fat (%)</th>
<th>Carbohydrate (%)</th>
<th>Dietary fibre (%)</th>
<th>Ash (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>White control bread</td>
<td>912</td>
<td>44.0</td>
<td>8.3</td>
<td>1.2</td>
<td>42.7</td>
<td>2.9</td>
<td>0.9</td>
</tr>
<tr>
<td>Finely sliced hazelnut bread</td>
<td>1196</td>
<td>36.5</td>
<td>9.3</td>
<td>14.8</td>
<td>28.9</td>
<td>9.4</td>
<td>1.2</td>
</tr>
<tr>
<td>Semi-defatted hazelnut flour bread</td>
<td>1019</td>
<td>37.9</td>
<td>13.8</td>
<td>6.0</td>
<td>33.1</td>
<td>7.3</td>
<td>1.8</td>
</tr>
<tr>
<td>Combination bread</td>
<td>1119</td>
<td>36.9</td>
<td>11.3</td>
<td>10.60</td>
<td>31.5</td>
<td>8.2</td>
<td>1.5</td>
</tr>
<tr>
<td>Semi-defatted hazelnut flour</td>
<td>1328</td>
<td>8.9</td>
<td>32.8</td>
<td>20.0</td>
<td>1.8</td>
<td>31.8</td>
<td>4.7</td>
</tr>
</tbody>
</table>

*Percent weight
3.5 The study test breads

Four different test breads (Breads B1, B2, B3 and B4) were consumed during the study. The National Heart Foundation of New Zealand recommends the daily consumption of 30 g of nuts, thus, the hazelnut-enriched breads were designed to contain 30 g of hazelnuts per 120 g of bread (11). One-hundred and twenty grams of bread was chosen because this was the mean amount consumed by bread consumers in 2008/09 NZANS (13).

The test breads consisted of a white control bread with no nuts (B1), finely sliced hazelnut bread containing 30 g of nuts per 120 g (B2), semi-defatted hazelnut flour bread containing 30 g of semi-defatted nut flour per 120 g (B3), and a combination bread containing 15 g of finely sliced nuts and 15 g of semi-defatted hazelnut flour per 120 g (B4) bread. The breads were analysed for nutrient composition by the Massey University Nutrition Laboratory (Appendix H). Fat was measured using the AOAC methods (Table 3.1). The basic recipe was similar for all breads, with nuts added to the hazelnut-enriched breads. All breads contained flour, salt, sugar, yeast and water. The appropriate nut form was added to the different types of bread in amounts equivalent to 30 g per 100 g of bread.

The hazelnuts used in the study breads were purchased from Uncle Joe’s Walnuts and Hazelnuts (Blenheim, New Zealand). The test breads were made by Gilbert’s Fine Foods (Dunedin, New Zealand). The breads were collected from the bakery by a member of the research team every month. The breads were all stored in a -20 °C freezer to maintain freshness.

For GI testing, the white bread with no nuts was the used as the reference. During GI testing, each of the four breads were provided as portions providing 50 g of available carbohydrate, defined as total carbohydrate by difference minus dietary fibre (59). This equated to 117 g of white bread (Bread B1), 173 g of finely sliced hazelnut bread (B2), 151 g of semi-defatted hazelnut flour bread (B3) and 159 g of the combination bread (B4) (finely sliced hazelnut and semi-defatted hazelnut flour).

The order in which the four breads were consumed was randomised. All breads were tested in duplicate to account for intra-individual variability in GI measurements.

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During each treatment phase, participants were provided with their allocated breads for home consumption for days 2 to 7. On day 2, participants were given the study breads, in the amounts equivalent in weight (calculated amount) to their usual breakfast bread consumption. This portion was used to measure satiety. On days 3 to 7 of the protocol, participants were provided with 120 g of the test bread which contained 30 g of nuts.

3.6 Glycaemic Response/Index protocol

The GR of all the breads was measured twice to take into account the large intra-individual variation in GR reported previously (56). Participants arrived at the GR clinic at 6.30 am after a 10-12 hour overnight fast (no food or beverages consumed except water). They were asked to consume the same type of meal at the same time on the previous evening, avoid alcohol consumption and maintain the same exercise pattern for the day before the testing. It was recommended that participants travel to the GR clinic by vehicle, however, if they travelled by bicycle or on foot, it was recommended that they rest for 10 minutes prior to commencing the testing session. On day one of the first testing (phase 1) upon arrival at the clinic, both height and weight measurements were taken for the participants.

The participants then gave two capillary blood samples which were obtained from finger-pricks using Unistik® 3 Normal single use safety lancets (Owen Mumford Ltd) and were analysed immediately with a HemoCue® Glucose 201 Analyser (Helsingborg, Sweden). After the fasting blood samples (two baseline measurements) were taken, participants ate the allocated test bread at a comfortable pace within 15 minutes and had six further blood samples taken at 15, 30, 45, 60, 90, and 120 minutes after starting to eat. If the two baseline measurements taken were more than 0.5 mmol/L apart, then a third measurement was taken. The median of the three measurements was recorded. Test breads were served with 300 ml of water. The finger-prick procedure, the method of blood sampling and the glucose measurement procedures were standardised for the duration of the study. For instance, the same research assistant took blood samples from the same participants throughout the study using the same HemoCue® Glucose 201 Analyser (Helsingborg, Sweden). Prior to each GR session the HemoCue® Glucose 201 Analyser (Helsingborg, Sweden) was
tested for quality control by the investigator. Each glycaemic response testing session was separated by a week (Appendix G). The white control bread was used as the reference for calculating the GI of the nut breads. Thus, the GI for the nut breads was calculated by dividing the mean GR of each nut bread by the mean GR of the white bread.

3.7 The gastrointestinal symptom reporting questionnaire

At the beginning of each new phase during the GI testing session, participants reported their experiences of the gastrointestinal symptoms at eight different points in time; firstly at baseline (pre-bread ingestion), then immediately post bread ingestion, and then at 15, 30, 45, 60, 90 and 120 minutes post bread ingestion. Each questionnaire took the form of an A4 sheet of paper with seven 100-mm VAS that corresponded to the seven questions (Appendix I). In relation to each question, there were extreme statements anchored at either end of the line. The questions asked were: experiences of “Belching?” (No problem/ very severe problem); “Stomach bloating?” (No problem/ very severe problem); “Stomach cramping?” (No problem/ very severe problem); “Flatulence?” (No problem/ very severe problem); “Diarrhoea?” (No problem/ very severe problem); “Nausea?” (No problem/ very severe problem); and, “Stomach ache?” (No problem/ very severe problem).

3.8 Satiety testing

On day 2 of each new phase, participants underwent a satiety test at home. Participants completed a questionnaire at baseline which asked how much bread they usually consumed during breakfast. Each participant received this portion of bread to consume for the satiety test. The participants reported their appetite at five different points in time; firstly at baseline (pre-bread ingestion), then immediately post-bread ingestion, and then at 1, 2 and 3 hours post-bread ingestion. Each questionnaire took the form of an A4 sheet of paper with five 100-mm VAS that corresponded to the five appetite questions listed below. In relation to each question, there were extreme statements anchored at either end of the line (Appendix J).
The questions asked were: “How hungry do you feel right now?” (Not at all hungry/extremely hungry); “How strong is your desire to eat right now?” (Strong desire not to eat/ strong desire to eat); “How much food could you eat right now?” (Nothing at all/the most that I have ever eaten); “How full do you feel right now?” (Not at all full/extremely full); “Do you have any preoccupation with thoughts of food right now?” (No thoughts of food/very preoccupied, difficult to concentrate). These ratings follow the methodology of Blundell et al., 1979 (136). Participants also completed a food diary on the day of satiety testing to examine any differences in nutrient and energy intake between the different bread types.

3.9 Dietary assessment

3.9.1 The two day weighed diet record (WDR)

Weighed diet records (WDR) of all foods and beverages consumed, both in and out of the home were collected from participants on two separate days during each study phase, using electronic kitchen food scales (Salter Electronic, Salter Housewares Ltd., Kent, UK), accurate to ± 1 gram. Detailed instructions on how to complete/keep a food diary were verbally explained to the participants by the researcher and written instructions were also included in the booklet (Appendices K and L). The diet records were kept on one week-day and one weekend day (Sunday) for each of the phases. For each individual, the diet records were kept on the same days’ through-out the study for the four treatment phases. The diet records were checked and reviewed by the researcher at the time of collection, for completeness and accuracy.

3.9.1.1 Satiety testing food diary

As previously mentioned, participants underwent a satiety test on day 2 of each treatment phase. Participants were provided amounts of bread equivalent to their usual intake at breakfast. Food record were kept for all food and beverages consumed during this 24-hour period. This WDR was used to examine whether energy and nutrient intake on the day of the satiety test was different between the bread treatments.
3.9.1.2  Sunday food diary

Participants were provided with 120 g of test bread. A WDR was kept for all food and beverages consumed during the 24 hours. The 120 g of bread consumed reflects the mean bread intake of New Zealand adults as reported in the 2008/09 NZANS (13).

3.9.2  Dietary analysis

All diet records were analysed to provide an estimate of average energy and nutrient intakes. Dietary analysis was performed using the dietary assessment software, Kai-calculator [v1.11i] (Department of Human Nutrition, University of Otago, Dunedin, New Zealand). Kai-calculator software uses the food composition data from New Zealand food composition database “NZ FOODfiles”. All diet records were entered by a single trained researcher to ensure consistency in data-entry decisions when substitutions had to be made. Once all the diet records had been entered, the entries were double checked by the same researcher at a later date to ensure accuracy.

3.10  Statistical analysis

A 4-arm, cross-over study design was used to compare the effects of four different breads (white control bread, finely sliced nut bread, semi-defatted nut flour bread, and combination bread containing finely sliced nuts and semi-defatted nut flour) on blood glucose response, satiety and gastrointestinal tolerance. In order to detect moderate to large differences in GI values between any two arms of the trial (0.6 SD, equivalent to differences in GI values of 12.6 based on a SD of 21 for white bread from Venn et al. 2006)(137) and assuming an intra-class coefficient (ICC) of 0.3 for the two repeated measures at each time point (based on a conservative rounding upwards of an ICC of 0.27 for white bread from Williams et al. 2008) (138), 29 participants were needed to provide 80% power when using a two-sided test at the 0.05 level. In order to allow for 10% loss of data to attrition, 33 participants were needed at the start of the study. Baseline characteristics of participants are presented as arithmetic means and standard deviations.
Mean outcomes are presented alongside their associated confidence intervals. Where outcome were highly skewed (i.e. the GI results) the medians and interquartile ranges (IQR) are presented. The outcomes were modelled using mixed models with random participant and random participant-intervention effects to accommodate the two levels of repeated measures. Where the overall test for differences between treatments was significant, pairwise comparisons between groups was performed. For gastrointestinal tolerance and satiety, mixed models were used to compare outcomes at each time point between treatments, controlling for baseline values. All analyses were performed using Stata 12.1 (StataCorp. 2011. Stata Statistical Software: Release 12. College Station, TX: StataCorp LP.) and all tests were performed at the two-sided 0.05 level. The data are reported as mean, standard deviation, 95% confidence intervals, and percentages or number.
4.0 RESULTS

4.1 Participant retention

Figure 4.1 illustrates the flow of the participants through the study. Initially, thirty-three individuals were assessed for eligibility, recruited and randomised into one of the four groups which decided the order in which the participants were to consume the four different breads.
Out of the 33 eligible participants, four of the randomised subjects withdrew at the beginning of the study either due to illness not related to the study (n=1), over-commitment (n=2) and personal reasons (n=1).

The withdrawals were from groups 1 (n=3) and 4 (n=1). Therefore, three more participants were further recruited to make a total of 32 participants. Thirty-two subjects (11 men and 21 women) completed the entire 4-arm crossover, 10-week intervention study (including the washout periods) and were included in the final analysis.

### 4.2 Participant characteristics

A total of 32 people (11 men and 21 women) participated in the study. The baseline characteristics of the study participants are presented in Table 4.1. Participants were healthy men and women, aged between 19 and 64 years with an overall mean age (SD) of 30.2 (11.4) years. The study population comprised of New Zealand European (37.5%), twenty-two percent Chinese, three percent Maori and other ethnicities (Indian, African, and Arab descent) (37.5%). Furthermore, approximately, two-thirds of the study populations were females. The mean (SD) height at baseline was 1.69 (0.09) m with a range 1.54 to 1.91m. Mean (SD) weight was 68.37 (12.33) kg with a range 48.30 to 106.85 kg. Mean (SD) BMI was 24.08 kg/m² with a range 19.83-39.99 kg/m².

| Table 4.1. Baseline characteristics of the study participants (n=32) |
|-------------------|-------------------|-------------------|
|                  | Mean (SD)*        | Range             |
| Sex (%)           | Sex (%)           |                   |
| M                 | 34                | -                 |
| F                 | 66                | -                 |
| Age (years)       | 30.2 (11.4)       | 19-64             |
| Height (metres)   | 1.69 (0.09)       | 1.54-1.91         |
| Weight (kg)       | 68.37 (12.33)     | 48.30-106.85      |
| BMI (kg/m²)       | 24.08 (4.10)      | 19.83-39.99       |

*Date are expressed as mean (standard deviation) unless otherwise indicated

Abbreviations used: BMI=body mass index (calculated as body weight in kilograms divided by the square of height in meters); n=number of subjects; F=female; M=male
4.3 Mean incremental blood glucose responses, area under the curve and glycaemic index

The mean incremental blood glucose responses to the different breads with and without nuts are shown in Figure 4.2. The incremental glucose area under the curves [mean (95% CI)] for the finely sliced nut bread, semi-defatted nut flour bread, combination bread containing finely sliced nuts and semi-defatted nut flour and white control bread were, 152 (95% CI: 128, 176), 137 (95% CI: 115, 159), 154 (95% CI: 130, 177) and 179 (95% CI: 146, 212) mmol/L.min, respectively.

![Figure 4.2](image.png)

**Figure 4.2.** Mean (95% CI) incremental blood glucose responses to the white bread, finely sliced nut bread, semi-defatted nut flour bread and the combination bread containing finely sliced nuts and semi-defatted nut flour

There was a significant difference in AUC between the breads (p<0.001). Pairwise comparisons showed that compared to the white control bread, the AUC of the finely sliced nut bread, the semi-defatted nut flour bread and the combination bread containing sliced nuts and semi-defatted nut flour were statistically significantly lower.
(p=0.012, p<0.001, p=0.008, respectively). However, there were no statistical differences in AUC between the three nut-enriched breads (all p≥0.130) (Figure 4.3).

The mean GI for the breads was also calculated. For the finely sliced nut bread, semi-defatted nut flour bread and the combination bread containing finely sliced nuts and semi-defatted nut flour, the mean GIs (95% CI) were 98 (95% CI: 82, 114), 89 (95% CI: 73, 105), and 101 (95% CI: 83, 119), respectively. The distribution of the mean GIs was highly skewed. Therefore, the median GI of the breads was calculated. The median (IQR) for the finely sliced nut bread, semi-defatted nut flour bread and the combination bread containing finely sliced nuts and semi-defatted nut flour were 83.0 (68.5-120), 78.5 (63.5-110.5), and 85.5 (59.5-129.5), respectively. These values are more consistent with the AUC results for the different breads, which were significantly lower compared to the white control bread. There were no overall significant differences in the mean GI between the nut breads (p= 0.122).
Table 4.2. Appetite ratings in response to the satiety test

<table>
<thead>
<tr>
<th>Appetite rating questions</th>
<th>White bread*</th>
<th>Sliced nut bread*</th>
<th>Nut flour bread*</th>
<th>Combination bread*</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q1. How <strong>hungry</strong> do you feel right now?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline (pre-consumption)</td>
<td>66.3 (56.5, 76.1)</td>
<td>66.2 (57.7, 74.7)</td>
<td>62.7 (53.5, 71.9)</td>
<td>65.9 (57.3, 74.5)</td>
<td>-</td>
</tr>
<tr>
<td>Immediately post consumption</td>
<td>33.6 (24.3, 42.9)</td>
<td>27.8 (18.7, 37.0)</td>
<td>28.1 (19.1, 37.2)</td>
<td>31.1 (21.0, 41.2)</td>
<td>0.534</td>
</tr>
<tr>
<td>1 hour after consumption</td>
<td>36.1 (27.2, 45.0)</td>
<td>30.0 (20.2, 45.0)</td>
<td>27.3 (18.9, 35.6)</td>
<td>25.6 (17.6, 33.6)</td>
<td>0.180</td>
</tr>
<tr>
<td>2 hours after consumption</td>
<td>39.3 (30.2, 48.5)</td>
<td>34.0 (24.3, 43.7)</td>
<td>36.3 (28.0, 45.1)</td>
<td>32.2 (23.0, 41.4)</td>
<td>0.570</td>
</tr>
<tr>
<td>3 hours after consumption</td>
<td>47.3 (38.3, 56.3)</td>
<td>46.6 (36.1, 57.1)</td>
<td>43.8 (34.3, 53.4)</td>
<td>42.4 (30.7, 54.2)</td>
<td>0.846</td>
</tr>
<tr>
<td>Q2. How strong is your <strong>desire to eat</strong> right now?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline (pre-consumption)</td>
<td>69.6 (60.6, 78.6)</td>
<td>69.5 (61.0, 78.0)</td>
<td>68.2 (59.4, 77.0)</td>
<td>68.2 (60.5, 75.9)</td>
<td>-</td>
</tr>
<tr>
<td>Immediately post consumption</td>
<td>36.3 (27.1, 45.4)</td>
<td>30.3 (21.6, 38.9)</td>
<td>29.7 (20.5, 30.8)</td>
<td>32.8 (23.3, 42.4)</td>
<td>0.391</td>
</tr>
<tr>
<td>1 hour after consumption</td>
<td>38.6 (29.4, 47.7)</td>
<td>29.4 (20.6, 38.3)</td>
<td>30.5 (22.5, 38.5)</td>
<td>29.3 (21.9, 37.6)</td>
<td>0.227</td>
</tr>
<tr>
<td>2 hours after consumption</td>
<td>42.0 (32.5, 51.5)</td>
<td>34.9 (25.0, 44.8)</td>
<td>37.8 (29.1, 46.4)</td>
<td>33.5 (24.0, 42.0)</td>
<td>0.507</td>
</tr>
<tr>
<td>3 hours after consumption</td>
<td>47.6 (39.0, 56.3)</td>
<td>47.5 (36.7, 50.3)</td>
<td>48.8 (39.2, 58.4)</td>
<td>45.8 (34.1, 57.5)</td>
<td>0.960</td>
</tr>
<tr>
<td>Q3. <strong>How much food could you eat</strong> right now?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline (pre-consumption)</td>
<td>63.2 (56.3, 70.0)</td>
<td>61.0 (54.7, 67.4)</td>
<td>60.4 (52.3, 68.5)</td>
<td>60.6 (53.8, 67.4)</td>
<td>-</td>
</tr>
<tr>
<td>Immediately post consumption</td>
<td>38.4 (30.2, 46.6)</td>
<td>31.3 (22.7, 40.0)</td>
<td>30.0 (21.4, 38.7)</td>
<td>32.5 (23.8, 41.3)</td>
<td>0.281</td>
</tr>
<tr>
<td>1 hour after consumption</td>
<td>42.3 (34.7, 50.0)</td>
<td>32.6 (25.1, 40.2)</td>
<td>32.3 (24.0, 40.6)</td>
<td>31.6 (23.2, 40.1)</td>
<td>0.142</td>
</tr>
<tr>
<td>2 hours after consumption</td>
<td>47.9 (39.4, 56.4)</td>
<td>36.3 (27.3, 45.2)</td>
<td>42.2 (33.0, 51.5)</td>
<td>37.2 (28.6, 45.8)</td>
<td>0.150</td>
</tr>
<tr>
<td>3 hours after consumption</td>
<td>47.7 (39.0, 56.4)</td>
<td>45.8 (36.2, 55.3)</td>
<td>46.6 (37.1, 56.1)</td>
<td>42.3 (32.1, 52.6)</td>
<td>0.818</td>
</tr>
<tr>
<td>Q4. How <strong>full</strong> do you feel right now?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline (pre-consumption)</td>
<td>20.3 (12.6, 28.1)</td>
<td>25.4 (18.0, 32.8)</td>
<td>24.4 (16.5, 32.3)</td>
<td>27.1 (17.9, 36.2)</td>
<td>-</td>
</tr>
<tr>
<td>Immediately post consumption</td>
<td>60.8 (53.0, 68.7)</td>
<td>65.2 (57.5, 73.0)</td>
<td>66.3 (59.0, 73.5)</td>
<td>59.0 (48.7, 69.3)</td>
<td>0.431</td>
</tr>
<tr>
<td>1 hour after consumption</td>
<td>53.4 (45.7, 61.2)</td>
<td>63.6 (56.1, 71.0)</td>
<td>61.3 (54.3, 68.3)</td>
<td>63.9 (56.6, 71.2)</td>
<td>0.135</td>
</tr>
<tr>
<td>2 hours after consumption</td>
<td>49.3 (41.7, 56.9)</td>
<td>53.0 (44.2, 61.8)</td>
<td>49.1 (40.8, 57.4)</td>
<td>52.0 (43.2, 60.8)</td>
<td>0.773</td>
</tr>
<tr>
<td>3 hours after consumption</td>
<td>42.6 (33.8, 51.4)</td>
<td>44.1 (34.7, 51.4)</td>
<td>40.4 (32.3, 48.6)</td>
<td>41.1 (31.4, 50.8)</td>
<td>0.848</td>
</tr>
<tr>
<td>Q5. Do you have any <strong>preoccupation with thoughts of food</strong> right now?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline (pre-consumption)</td>
<td>57.4 (48.8, 66.0)</td>
<td>56.8 (47.1, 66.4)</td>
<td>48.2 (39.4, 56.9)</td>
<td>50.0 (41.2, 58.8)</td>
<td>-</td>
</tr>
<tr>
<td>Immediately post consumption</td>
<td>33.5 (25.3, 41.7)</td>
<td>28.1 (19.5, 36.7)</td>
<td>25.6 (16.0, 34.4)</td>
<td>29.6 (20.3, 38.9)</td>
<td>0.480</td>
</tr>
<tr>
<td>1 hour after consumption</td>
<td>33.5 (24.5, 42.5)</td>
<td>27.1 (19.0, 35.3)</td>
<td>24.3 (15.7, 32.9)</td>
<td>25.0 (16.7, 33.8)</td>
<td>0.603</td>
</tr>
<tr>
<td>2 hours after consumption</td>
<td>39.0 (28.9, 48.3)</td>
<td>33.3 (24.5, 42.0)</td>
<td>31.3 (22.7, 39.8)</td>
<td>29.0 (19.0, 38.9)</td>
<td>0.694</td>
</tr>
<tr>
<td>3 hours after consumption</td>
<td>43.3 (34.1, 52.5)</td>
<td>37.9 (27.6, 48.2)</td>
<td>43.1 (33.7, 52.4)</td>
<td>44.3 (32.8, 55.8)</td>
<td>0.357</td>
</tr>
</tbody>
</table>

* Data are expressed as mean (95% confidence interval) unless otherwise indicated

*p-values are adjusted for baseline
4.4 Perceptions of appetite

After controlling for baseline values, there were no significant differences between the breads for ratings of ‘how hungry do you feel right now’, ‘how strong is your desire to eat right now’, ‘how much food could you eat right now’, ‘how full do you feel right now’ and ‘do you have any preoccupation with thoughts of food right now’ at any of the time points (p≥0.135 in all cases) (Table 4.2).

4.5 Gastrointestinal symptoms

After controlling for baseline values, the gastrointestinal symptoms for ‘belching’, ‘stomach bloating’, ‘stomach cramping’, ‘flatulence’, ‘stomach ache’, ‘diarrhoea’ and ‘nausea’ did not differ between breads at any of the time points (immediately post ingestion, at 15 minutes, at 30 minutes, at 45 minutes, at 60 minutes, 90 minutes and 120 minutes); p≥0.102 in all cases. (Table 4.3). The mean peak ratings of the different measures of intolerance for the different breads were very low, with no bread reporting a peak of greater than 5.6 mm out of 100 mm.

Overall, the peak rating for ‘belching’, ‘stomach bloating’, ‘stomach cramping’, ‘flatulence’, ‘stomach ache’, ‘diarrhoea’ and ‘nausea’ was ≤ 3.5 mm, ≤ 5.6 mm, ≤ 3.5mm, ≤ 3.4 mm, ≤ 4mm, ≤ 3.3 mm, and ≤ 3.7 mm, respectively.
Table 4.3. Prevalence of gastrointestinal symptoms ratings in response to bread consumption

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>White bread n (%)</th>
<th>Sliced nut bread n (%)</th>
<th>Nut flour bread n (%)</th>
<th>Combination bread n (%)</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Belching</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline (pre-consumption)</td>
<td>5 (16)</td>
<td>7 (22)</td>
<td>5 (16)</td>
<td>8 (25)</td>
<td></td>
</tr>
<tr>
<td>Immediately post ingestion</td>
<td>9 (28)</td>
<td>7 (22)</td>
<td>10 (31)</td>
<td>10 (31)</td>
<td>0.477</td>
</tr>
<tr>
<td>15 minutes post ingestion</td>
<td>9 (28)</td>
<td>9 (28)</td>
<td>9 (28)</td>
<td>10 (31)</td>
<td>0.947</td>
</tr>
<tr>
<td>30 minutes post ingestion</td>
<td>9 (28)</td>
<td>9 (28)</td>
<td>7 (22)</td>
<td>11 (34)</td>
<td>0.786</td>
</tr>
<tr>
<td>45 minutes post ingestion</td>
<td>10 (31)</td>
<td>7 (22)</td>
<td>8 (25)</td>
<td>8 (25)</td>
<td>0.432</td>
</tr>
<tr>
<td>60 minutes post ingestion</td>
<td>8 (25)</td>
<td>5 (16)</td>
<td>7 (22)</td>
<td>6 (19)</td>
<td>0.370</td>
</tr>
<tr>
<td>90 minutes post ingestion</td>
<td>5 (16)</td>
<td>7 (22)</td>
<td>8 (25)</td>
<td>9 (28)</td>
<td>0.771</td>
</tr>
<tr>
<td>120 minutes post ingestion</td>
<td>6 (19)</td>
<td>5 (16)</td>
<td>8 (25)</td>
<td>9 (28)</td>
<td>0.421</td>
</tr>
<tr>
<td>Stomach bloating</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline (pre-consumption)</td>
<td>9 (28)</td>
<td>12 (38)</td>
<td>8 (25)</td>
<td>8 (25)</td>
<td></td>
</tr>
<tr>
<td>Immediately post ingestion</td>
<td>9 (28)</td>
<td>9 (28)</td>
<td>11 (34)</td>
<td>13 (41)</td>
<td>0.200</td>
</tr>
<tr>
<td>15 minutes post ingestion</td>
<td>9 (28)</td>
<td>9 (28)</td>
<td>10 (31)</td>
<td>14 (44)</td>
<td>0.140</td>
</tr>
<tr>
<td>30 minutes post ingestion</td>
<td>9 (28)</td>
<td>9 (28)</td>
<td>12 (38)</td>
<td>13 (41)</td>
<td>0.207</td>
</tr>
<tr>
<td>45 minutes post ingestion</td>
<td>9 (28)</td>
<td>10 (31)</td>
<td>12 (38)</td>
<td>12 (38)</td>
<td>0.577</td>
</tr>
<tr>
<td>60 minutes post ingestion</td>
<td>10 (31)</td>
<td>8 (25)</td>
<td>7 (22)</td>
<td>11 (34)</td>
<td>0.265</td>
</tr>
<tr>
<td>90 minutes post ingestion</td>
<td>7 (22)</td>
<td>8 (25)</td>
<td>9 (28)</td>
<td>12 (38)</td>
<td>0.314</td>
</tr>
<tr>
<td>120 minutes post ingestion</td>
<td>6 (19)</td>
<td>7 (22)</td>
<td>6 (19)</td>
<td>11 (34)</td>
<td>0.227</td>
</tr>
<tr>
<td>Stomach cramping</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline (pre-consumption)</td>
<td>7 (22)</td>
<td>7 (22)</td>
<td>7 (22)</td>
<td>8 (25)</td>
<td></td>
</tr>
<tr>
<td>Immediately post ingestion</td>
<td>7 (22)</td>
<td>6 (19)</td>
<td>9 (28)</td>
<td>9 (28)</td>
<td>0.750</td>
</tr>
<tr>
<td>15 minutes post ingestion</td>
<td>7 (22)</td>
<td>7 (22)</td>
<td>8 (25)</td>
<td>10 (31)</td>
<td>0.949</td>
</tr>
<tr>
<td>30 minutes post ingestion</td>
<td>8 (25)</td>
<td>7 (22)</td>
<td>8 (25)</td>
<td>10 (31)</td>
<td>0.912</td>
</tr>
<tr>
<td>45 minutes post ingestion</td>
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<td>7 (22)</td>
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<tr>
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<td>9 (28)</td>
<td>5 (16)</td>
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</tr>
</tbody>
</table>

n (%)=number and (percentage) of participants who reported having gastrointestinal symptoms; **p-value could not be estimated
*p-values are adjusted for baseline
Table 4.3. Prevalence of gastrointestinal symptoms ratings in response to the bread consumption continued

<table>
<thead>
<tr>
<th>Flatulence</th>
<th>Baseline (pre-consumption)</th>
<th>Immediately post ingestion</th>
<th>15 minutes post ingestion</th>
<th>30 minutes post ingestion</th>
<th>45 minutes post ingestion</th>
<th>60 minutes post ingestion</th>
<th>90 minutes post ingestion</th>
<th>120 minutes post ingestion</th>
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<tr>
<td></td>
<td>6 (19)</td>
<td>9 (28)</td>
<td>8 (25)</td>
<td>9 (28)</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flatulence (post-consumption)</td>
<td>Immediately post ingestion</td>
<td>6 (19)</td>
<td>6 (19)</td>
<td>8 (25)</td>
<td>11 (34)</td>
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<td>Diarrhea</td>
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<td>5 (16)</td>
<td>7 (22)</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diarrhea</td>
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<tr>
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<td>4 (13)</td>
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<tr>
<td>Diarrhea</td>
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<tr>
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<td>4 (13)</td>
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<td>Baseline (pre-consumption)</td>
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<td>5 (16)</td>
<td>6 (19)</td>
<td>10 (31)</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nausea</td>
<td>Immediately post ingestion</td>
<td>6 (19)</td>
<td>6 (19)</td>
<td>6 (19)</td>
<td>9 (28)</td>
<td>0.999</td>
<td></td>
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<tr>
<td>Nausea</td>
<td>15 minutes post ingestion</td>
<td>5 (16)</td>
<td>5 (16)</td>
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<td>9 (28)</td>
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<tr>
<td>Nausea</td>
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<td>5 (16)</td>
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<tr>
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</tr>
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<td>60 minutes post ingestion</td>
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<td>5 (16)</td>
<td>5 (16)</td>
<td>7 (22)</td>
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<tr>
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<td>5 (16)</td>
<td>4 (13)</td>
<td>7 (22)</td>
<td>**</td>
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<td></td>
</tr>
<tr>
<td>Nausea</td>
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<td>4 (13)</td>
<td>4 (13)</td>
<td>7 (22)</td>
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<td>Baseline (pre-consumption)</td>
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<td>11 (34)</td>
<td>6 (19)</td>
<td>9 (28)</td>
<td>-</td>
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<tr>
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<td>8 (25)</td>
<td>9 (28)</td>
<td>9 (28)</td>
<td>0.462</td>
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<td>4 (13)</td>
<td>7 (22)</td>
<td>8 (25)</td>
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<td>6 (19)</td>
<td>8 (25)</td>
<td>9 (28)</td>
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<td>8 (25)</td>
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<td>7 (22)</td>
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<td>Stomach ache</td>
<td>90 minutes post ingestion</td>
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<td>5 (16)</td>
<td>6 (19)</td>
<td>10 (31)</td>
<td>0.345</td>
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<tr>
<td>Stomach ache</td>
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<td>6 (19)</td>
<td>7 (22)</td>
<td>7 (22)</td>
<td>0.295</td>
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</tr>
</tbody>
</table>

n (%)=number and (percentage) of participants who reported having gastrointestinal symptoms; **p-value could not be estimated; *p-values are adjusted for baseline
Table 4.4. Mean nutrient intakes for the satiety test day from the diet record during each dietary treatment

<table>
<thead>
<tr>
<th>Nutrient intakes</th>
<th>White bread Mean (95% CI)</th>
<th>Sliced nut bread Mean (95% CI)</th>
<th>Nut flour bread Mean (95% CI)</th>
<th>Combination bread Mean (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total energy (TE) (kJ)</td>
<td>8832 (7376, 10288)</td>
<td>8699 (7735, 9664)</td>
<td>8904 (8034, 9773)</td>
<td>7749 (6730, 8767)</td>
<td>0.102</td>
</tr>
<tr>
<td>Energy from bread alone (EB) (kJ)</td>
<td>1054 (945, 1163)</td>
<td>1518 (1343,1693)</td>
<td>1310 (1167, 1453)</td>
<td>1343 (1160,1526)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>253 (213, 292)</td>
<td>238 (206, 269)</td>
<td>243 (210, 277)</td>
<td>209 (174, 243)</td>
<td>0.101</td>
</tr>
<tr>
<td>% of total energy</td>
<td>48.3 (43.9, 52.8)</td>
<td>43.8 (40.6, 47.0)</td>
<td>43.2 (39.7, 46.6)</td>
<td>43.1 (39.4, 46.9)</td>
<td>0.048</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>86 (68, 104)</td>
<td>87 (75, 99)</td>
<td>94 (80, 108)</td>
<td>85 (72, 98)</td>
<td>0.429</td>
</tr>
<tr>
<td>% of total energy</td>
<td>16.4 (14.8, 18.0)</td>
<td>17.1 (15.6,18.6)</td>
<td>18.0 (16.0,20.0)</td>
<td>19.0 (16.9, 20.7)</td>
<td>0.005</td>
</tr>
<tr>
<td>Total Fat(g)</td>
<td>82 (63, 102)</td>
<td>87 (74,100)</td>
<td>86 (76, 97)</td>
<td>74 (62, 97)</td>
<td>0.301</td>
</tr>
<tr>
<td>% of total energy</td>
<td>31.9 (27.7, 36.1)</td>
<td>36.7 (33.6, 39.9)</td>
<td>36.8 (33.2, 39.8)</td>
<td>35.0 (32.0, 38.0)</td>
<td>0.100</td>
</tr>
<tr>
<td>SFA (g)</td>
<td>11.8 (9.9, 13.8)</td>
<td>10.8 (9.2, 12.3)</td>
<td>11.2 (9.5, 13.0)</td>
<td>10.4 (8.7, 12.0)</td>
<td>0.341</td>
</tr>
<tr>
<td>% of total energy</td>
<td>29.9 (22.2, 37.6)</td>
<td>38.9 (33.5, 44.2)</td>
<td>30.7 (25.5, 35.9)</td>
<td>29.4 (24.1, 34.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>MUFA (g)</td>
<td>11.8 (9.6, 13.9)</td>
<td>16.7 (15.0, 18.4)</td>
<td>12.9 (11.1, 14.7)</td>
<td>13.9 (12.3, 15.5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>% of total energy</td>
<td>12.9 (9.5, 16.3)</td>
<td>14.1 (11.8,16.3)</td>
<td>15.5 (11.7, 19.3)</td>
<td>12.4 (10.0, 14.8)</td>
<td>0.394</td>
</tr>
<tr>
<td>PUFA (g)</td>
<td>5.2 (4.2, 6.2)</td>
<td>6.1 (5.3, 6.9)</td>
<td>6.4 (4.9, 8.0)</td>
<td>6.0 (5.0, 7.0)</td>
<td>0.327</td>
</tr>
<tr>
<td>% of total energy</td>
<td>25.3 (21.3, 29.4)</td>
<td>32.3 (28.0, 37.0)</td>
<td>35.1 (30.3, 40.0)</td>
<td>28.8 (24.0, 33.6)</td>
<td>0.002</td>
</tr>
<tr>
<td>Dietary fibre (g)</td>
<td>843.0 (566.3, 1119.7)</td>
<td>701.9 (541.9, 861.9)</td>
<td>665.1 (504.4, 825.9)</td>
<td>736.6 (534.5, 938.6)</td>
<td>0.270</td>
</tr>
<tr>
<td>Calcium (mg)</td>
<td>312.2 (262.7, 361.7)</td>
<td>299.6 (258.8,340.3)</td>
<td>314.4 (265.4,363.4)</td>
<td>300.5 (250.4,350.7)</td>
<td>0.902</td>
</tr>
<tr>
<td>Magnesium (mg)</td>
<td>1.4 (1.0, 1.9)</td>
<td>1.3 (0.9, 1.7)</td>
<td>1.6 (0.8, 2.5)</td>
<td>1.3 (0.8, 1.8)</td>
<td>0.320</td>
</tr>
<tr>
<td>Thiamine (mg)</td>
<td>1.9 (1.3, 2.4)</td>
<td>1.5 (1.2, 1.8)</td>
<td>1.7 (1.1, 2.3)</td>
<td>1.6 (1.2, 2.0)</td>
<td>0.410</td>
</tr>
<tr>
<td>Riboflavin (mg)</td>
<td>1.9 (1.4, 2.4)</td>
<td>1.7 (1.1, 2.1)</td>
<td>1.9 (1.5, 2.2)</td>
<td>1.7 (1.4, 2.0)</td>
<td>0.683</td>
</tr>
<tr>
<td>Vitamin B6 (mg)</td>
<td>274.0 (234.5, 313.5)</td>
<td>296.5 (265.7, 327.0)</td>
<td>359.3 (255.8, 462.7)</td>
<td>313.8 (226.7, 400.9)</td>
<td>0.341</td>
</tr>
<tr>
<td>Folate (µg)</td>
<td>8.6 (6.0, 11.3)</td>
<td>13.4 (11.9, 14.8)</td>
<td>14.1 (9.9, 18.4)</td>
<td>12.1 (9.9, 14.2)</td>
<td>0.019</td>
</tr>
</tbody>
</table>

Data are expressed as mean (95% confidence interval) unless otherwise indicated

Values with different superscript are statistically significantly different, p<0.05

Abbreviation used: TE= total energy; EB=energy from the test breads alone; SFA=saturated fatty acids; MUFA=monounsaturated fatty acids; PUFA=polyunsaturated fatty acids
4.6 Dietary data

4.6.1 Comparison of nutrient intakes for the satiety testing (day 2 of each treatment phase) and food diary day (Sunday of each treatment phase) for the four dietary phases

Participants reported their intakes using a WDR during the day of satiety testing (day 2 of each treatment phase) and during each Sunday of each treatment period. The nutrient intakes for the satiety test day and the Sunday of each treatment phase are presented in Tables 4.4-4.5, respectively.

4.6.1.1 Energy and nutrient intakes on the satiety test day

The amount of bread provided for the satiety test was the amount participants reported they normally consumed during breakfast as reported in the recruitment questionnaire. Therefore, the amount of bread provided was based on weight and not energy, and differed for each participant. The mean weight of bread provided was 123 g which equated to 1054 kJ, 1518 kJ, 1310 kJ, and 1343 kJ for the white control bread, finely sliced nut bread, semi-defatted nut flour bread and combination bread, respectively. The energy provided from the breads during the satiety tests significantly differed between treatments (p<0.001). Pairwise comparisons showed that compared to the white control bread, the energy derived from the finely sliced nut bread, the semi-defatted nut flour bread and the combination bread were statistically significantly higher (p<0.001 in all cases). In addition, the energy from the semi-defatted nut flour bread and the combination bread were significantly lower compared to the finely sliced nut bread (p=0.002, p=0.007, respectively). However, there was no difference in between the energy from the semi-defatted nut flour bread and the combination bread (p=0.700).

When total daily energy intake was calculated there was statistically significant difference between the breads (p=0.102). Also, no significant differences were observed for the carbohydrate, protein, total fat and PUFA (in grams) between the four breads (p ≥0.101 in all cases).
Statistically significant overall differences were observed for the MUFA and SFA (in grams) between the different breads (p=0.013, p=0.026, respectively). Pairwise comparisons showed that the absolute intake of MUFA from the finely sliced nut bread was significantly higher in comparison to the white control bread, the semi-defatted nut flour bread and the combination bread (p=0.006, p=0.016, p=0.004, respectively). No significant differences were seen in the MUFA intakes between the other breads (p≥0.649 in all cases). For absolute SFA, pairwise comparisons showed that in comparison to the white control bread, the SFA intake from the combination bread was statistically significantly lower (p=0.003).

While overall statistically significant differences for the percentage of total energy derived from carbohydrate (p=0.048), protein (p=0.005) and MUFA (p<0.001) were observed, there were no significant differences in the percentage of total energy derived from total fat (p=0.301), PUFA (p=0.327) and SFA (p=0.314) between the treatments. Pairwise comparisons showed that compared to the white control bread, the percentage of total energy from carbohydrate for all the nut breads were statistically significantly lower (p≤0.039 in all cases). No significant differences were observed between the nut breads (p≥0.728 in all cases).

For the percentage of total energy derived from protein, pairwise comparisons showed that in comparison to the white control bread, percentage of total energy from protein for the semi-defatted nut flour bread, and the combination bread were statistically significantly higher (p=0.028, p=0.001, respectively). The percent of energy from protein was also higher in combination bread when compared to finely sliced nut bread (p=0.018). Pairwise comparisons showed that the percentage of total energy derived from MUFA for the finely sliced nut bread was significantly higher compared to all other breads (all p ≤ 0.007). In addition, the percent energy from MUFA for the combination bread was significantly higher compared to the white control bread (p=0.025).

The daily intakes of calcium, magnesium, thiamine, riboflavin, vitamin B6 and folate were not significantly different between the treatment groups (p≥0.270 in all cases). However, there were significant differences in the dietary fibre, and vitamin E intakes between the treatments (p≤0.016 in both cases). Pairwise comparisons showed that the dietary fibre intake from the finely sliced nut bread and the semi-defatted nut flour
bread were significantly higher than the white control bread (p=0.010, and p<0.001, respectively). Higher fibre intakes were also observed for the semi-defatted nut flour bread in comparison to the combination bread (p=0.016).

For vitamin E, pairwise comparisons showed that intakes from the finely sliced nut bread and the semi-defatted nut flour bread were statistically significantly higher than the white control bread (p=0.014, p=0.003, respectively). There was a tendency for a higher vitamin E intake for the combination bread in comparison to the white control bread (p=0.086). There were no statistically significant differences in vitamin E intake between the three nut breads (p≥0.220 in all cases).

4.6.1.2 Energy and nutrient intakes on Sunday during each treatment

All participants received 120 g of the treatment bread on days 3 to 7 (which included the Sunday food diary day) which equated to 1122 kJ, 1436 kJ, 1224 kJ, and 1343 kJ for white bread, finely sliced nut bread, semi-defatted nut flour bread and the combination bread, respectively. The energy provided from the breads during the Sunday food diary day significantly differed between treatments (p<0.001). Pairwise comparisons showed that compared to the white control bread, the energy derived from the finely sliced nut bread, the semi-defatted nut flour bread and the combination bread were statistically significantly higher (p≤0.001 in all cases). Lower energy intakes were apparent for the semi-defatted nut flour bread and the combination bread in comparison to the finely sliced nut bread and also for the semi-defatted nut flour bread in comparison to the combination bread (p<0.001 in all cases).

While, the total energy intake for the semi-defatted nut flour bread (8214 kJ) was the highest compared to the white control bread (7878 kJ), finely sliced nut bread (8100 kJ) and the combination bread (7319 kJ), there were no significant differences in total energy between the treatments (p=0.321).
Table 4.5. Mean nutrient intakes for the food diary day (Sunday) from the diet record during each dietary phase

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>White bread Mean (95% CI)</th>
<th>Sliced nut bread Mean (95% CI)</th>
<th>Nut flour bread Mean (95% CI)</th>
<th>Combination bread Mean (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total energy (TE) (kJ)</td>
<td>7878 (6691, 9065)</td>
<td>8100 (7002, 9199)</td>
<td>8214 (7095, 9333)</td>
<td>7319 (6254, 8385)</td>
<td>0.321</td>
</tr>
<tr>
<td>Energy from bread alone (EB) (kJ)</td>
<td>1122 (1057, 1187)</td>
<td>1436 (1426, 1446)</td>
<td>1224 (1214, 1233)</td>
<td>1343 (1329, 1356)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>231 (198, 264)</td>
<td>216 (178, 253)</td>
<td>237 (201.2, 273)</td>
<td>195 (164, 226)</td>
<td>0.075</td>
</tr>
<tr>
<td>% of total energy</td>
<td>48.9 (44.4, 53.3)</td>
<td>42.2 (38.0, 46.3)</td>
<td>47.0 (42.9, 50.9)</td>
<td>43.3 (39.2, 47.5)</td>
<td>0.009</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>85 (69, 102)</td>
<td>82 (70, 93)</td>
<td>91 (78, 104)</td>
<td>86 (67, 105)</td>
<td>0.633</td>
</tr>
<tr>
<td>% of total energy</td>
<td>18.3 (16.2, 20.4)</td>
<td>17.6 (15.5, 19.7)</td>
<td>19.1 (17.6, 20.7)</td>
<td>19.8 (16.9, 22.6)</td>
<td>0.220</td>
</tr>
<tr>
<td>Total fat (g)</td>
<td>69 (54, 84)</td>
<td>83 (69, 98)</td>
<td>72 (59, 85)</td>
<td>70 (57, 83)</td>
<td>0.210</td>
</tr>
<tr>
<td>% of total energy</td>
<td>30.6 (26.8, 34.4)</td>
<td>37.8 (34.1, 41.5)</td>
<td>31.7 (28.2, 35.1)</td>
<td>34.8 (31.0, 38.6)</td>
<td>0.004</td>
</tr>
<tr>
<td>SFA (g)</td>
<td>23.4 (17.8, 29.0)</td>
<td>26.6 (20.0, 33.3)</td>
<td>22.1 (17.6, 26.7)</td>
<td>23.3 (17.0, 29.7)</td>
<td>0.526</td>
</tr>
<tr>
<td>% of total energy</td>
<td>10.6 (8.8, 12.4)</td>
<td>11.6 (9.3, 13.8)</td>
<td>9.8 (8.4, 11.3)</td>
<td>11.2 (9.1, 13.3)</td>
<td>0.351</td>
</tr>
<tr>
<td>MUFA (g)</td>
<td>26.5 (19.5, 33.6)</td>
<td>36.1 (30.1, 42.0)</td>
<td>24.8 (18.6, 30.9)</td>
<td>25.8 (20.9, 30.7)</td>
<td>0.002</td>
</tr>
<tr>
<td>% of total energy</td>
<td>11.6 (9.6, 13.6)</td>
<td>16.9 (14.7, 19.0)</td>
<td>10.5 (8.6, 12.4)</td>
<td>13.0 (11.3, 14.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PUFA (g)</td>
<td>12.3 (8.6, 16.1)</td>
<td>13.2 (10.6, 15.8)</td>
<td>12.7 (8.8, 16.6)</td>
<td>11.2 (8.7, 13.7)</td>
<td>0.772</td>
</tr>
<tr>
<td>% of total energy</td>
<td>5.3 (4.2, 6.4)</td>
<td>6.1 (5.2, 7.0)</td>
<td>5.2 (4.0, 6.5)</td>
<td>5.7 (4.5, 6.9)</td>
<td>0.522</td>
</tr>
<tr>
<td>Dietary fibre (g)</td>
<td>24.0 (19.0, 29.0)</td>
<td>29.9 (26.4, 33.4)</td>
<td>32.9 (28.8, 37.0)</td>
<td>25.0 (21.1, 29)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Calcium (mg)</td>
<td>739.4 (531.7, 947.1)</td>
<td>736.5 (531.7, 941.4)</td>
<td>658.5 (479.6, 837.4)</td>
<td>772.1 (502.3, 1042.0)</td>
<td>0.752</td>
</tr>
<tr>
<td>Magnesium (mg)</td>
<td>324.6 (254.5, 394.8)</td>
<td>292.4 (249.2, 335.6)</td>
<td>297.5 (247.3, 347.7)</td>
<td>253.0 (214.5, 291.5)</td>
<td>0.027</td>
</tr>
<tr>
<td>Thiamine (mg)</td>
<td>1.3 (0.9, 1.7)</td>
<td>1.4 (1.0, 1.8)</td>
<td>1.4 (0.9, 1.9)</td>
<td>1.3 (0.7, 1.9)</td>
<td>0.938</td>
</tr>
<tr>
<td>Riboflavin (mg)</td>
<td>1.7 (1.3, 2.1)</td>
<td>1.5 (1.1, 1.9)</td>
<td>1.7 (1.3, 2.2)</td>
<td>1.5 (1.0, 1.9)</td>
<td>0.424</td>
</tr>
<tr>
<td>Vitamin B6 (mg)</td>
<td>1.9 (1.4, 2.3)</td>
<td>1.8 (1.4, 2.1)</td>
<td>1.9 (1.5, 2.2)</td>
<td>1.4 (1.1, 1.7)</td>
<td>0.073</td>
</tr>
<tr>
<td>Folate (µg)</td>
<td>288.6 (227.0, 350.1)</td>
<td>294.3 (237.6, 351.0)</td>
<td>313.3 (252.2, 374.4)</td>
<td>261.2 (216.7, 305.7)</td>
<td>0.357</td>
</tr>
<tr>
<td>Vitamin E (mg)</td>
<td>9.0 (6.9, 11.0)</td>
<td>13.2 (11.0, 15.4)</td>
<td>10.5 (8.3, 12.8)</td>
<td>10.2 (9.1, 11.3)</td>
<td>0.004</td>
</tr>
</tbody>
</table>

Data are expressed as mean (95% confidence interval) unless otherwise indicated
Values with different superscript are statistically significantly different, p<0.05
Abbreviation used: TE= total energy; EB=energy from the test breads alone; SFA=saturated fatty acids; MUFA=monounsaturated fatty acids; PUFA=polyunsaturated fatty acids
The carbohydrate, protein, total fat, SFA intakes, and PUFA intakes (in grams) from the four different breads were not statistically significantly different (p ≥0.075 in all cases) with the exception of MUFA (p=0.002).

Pairwise comparisons showed that the MUFA intake from the finely sliced nut bread was higher than all the other breads (p≤0.005 in all cases) with no differences between the remaining three breads (p≥0.597 in all cases). While, statistically significant differences between treatments for the percentage of total energy derived from carbohydrate (p=0.009), total fat (p=0.004) and MUFA (p<0.001) were observed, the percentage of total energy derived from protein, PUFA and SFA did not differ significantly between treatments (p≥0.220 in all cases). Pairwise comparisons showed that compared to the white control bread, percentage of total energy derived from carbohydrates for the finely sliced nut bread and the combination bread were statistically significantly lower (p=0.002, and p=0.018, respectively). The percentage of total energy from carbohydrates was also statistically significantly lower for the semi-defatted nut flour bread in comparison to the finely sliced nut bread (p=0.028).

For the percentage of total energy derived from total fat, pairwise comparisons showed that compared to the white control bread, the percentage of total energy from total fat for the finely sliced nut bread was statistically significantly higher (p=0.001). Higher fat intakes were also observed for the finely sliced nut bread in comparison to the semi-defatted nut flour bread (p=0.004). Moreover, no significant differences were observed for the percentage of total energy from total fat for the semi-defatted nut flour bread and the combination bread in comparison to white control bread (p=0.721, and p=0.080, respectively), the combination bread in comparison to finely sliced nut bread (p=0.129), and the combination bread in comparison to the semi-defatted nut flour bread (p=0.163).

The pairwise comparison of the percentage of total energy derived from MUFA showed that the percentage of MUFA from the finely sliced nut bread was significantly higher in comparison to the other three breads (p≤0.036 in all cases). In addition, the percent energy from MUFA was significantly higher in the combination bread compared to the semi-defatted nut flour bread treatment (p=0.036).

The calcium, thiamine, riboflavin, vitamin B6, and folate intakes were not significantly different between the treatment groups (p≥0.073). However, there were significant
differences in the dietary fibre, magnesium and vitamin E intakes between the treatments (p≤0.027 in all cases).

Pairwise comparisons showed that dietary fibre intake from the finely sliced nut bread and the semi-defatted nut flour bread were significantly higher than the white control bread (p=0.008, and p<0.001, respectively). Also, fibre intakes were significantly lower for the combination bread in comparison to the semi-defatted nut flour bread (p<0.001) and the finely sliced nut bread (p=0.026).

For magnesium, pairwise comparisons showed that intakes for the combination bread were statistically significantly lower than the white control bread (p=0.003), with a tendency of lower intakes compared to the semi-defatted nut flour bread (p=0.052). In terms of vitamin E, pairwise comparisons showed that intakes from the finely sliced nut bread was statistically significantly higher than all other breads (p≤0.029 in all cases). No difference were observed between the remaining three breads (p≥0.183 in all cases).

4.7 Lifestyle questionnaire

The lifestyle questionnaire completed at the end of the study indicated no change in lifestyle for the majority of participants. Furthermore, 16% reported taking medication or supplements during the study. However, there were no changes in dosage or frequency of the medication being taken.
5.0 DISCUSSION

5.1 Study design, subject characteristics, study conditions, and research hypothesis

For the purpose of this study, a randomised controlled, 4-arm crossover study design was used. A crossover study design allows the participants to serve as their own controls. Not only does this study design reduce variance but also allows for the use of a smaller sample size to observe the true differences that are statistically and clinically valid (139). In this study, each dietary phase lasted for 8 days with a one week wash-out period between the dietary phases.

A total of 32 healthy men and women completed the study. The GR component of the study (days 1 and 8 of each phase) was conducted in a standardised laboratory setting, while the remainder of the study (days 2 to 7 of each phase) was conducted in a free-living situation. For each phase, participants were provided with a one-week supply of the different breads and were instructed to consume the breads in any manner they chose. Participants were asked to complete an appetite rating questionnaire on day 2 of each study phase and also keep a diet record for day 2 (week day) and the Sunday of each treatment phase. As the study participants were free-living, it allowed for natural eating behaviours and contributed to the external validity of the study (103).

This study assessed the postprandial effects of three different forms of hazelnut-enriched breads (finely sliced nut bread, semi-defatted nut flour bread and combination bread containing finely sliced nut and semi-defatted nut flour) (all containing 30 g of hazelnuts) and white control bread (with no nuts) on blood glucose response and satiety. The current recommendation by the New Zealand Heart Foundation is to consume 30 g of nuts per day (11).

We hypothesised that the hazelnut-enriched breads would have a lower postprandial glycaemic response (PGR) and exhibit increased satiety (fullness) compared to the white control bread. The results of this study yield a number of important findings discussed below.
5.2 Effects of the nut-enriched bread on postprandial glycaemia

Firstly, the data indicate that ingestion of the hazelnut-enriched breads significantly blunted the PGR in comparison to the white control bread in the study participants. The results of the present study confirm previous findings from acute studies that show when eaten in combination with carbohydrate rich foods, nuts attenuate postprandial blood glucose (58, 64). Contrary to the findings of the present study, however, in these studies, significance was only observed where larger quantities of nuts were ingested. In our study, the testing was performed on only one serving of nuts (a fixed 30 g quantity), where we were able to observe significant reductions in postprandial glycaemia.

Josse et al (2007) observed a significant reduction in the peak 2-hour postprandial blood glucose concentration in healthy adults when 90 g of almonds was eaten with bread containing 50 g available carbohydrate. In this study, smaller quantities of almonds (30 g and 60 g) did not significantly impact postprandial glycaemia (14).

In the study by Jenkins et al. (2006), where participants were provided with two bread control meals (97 g each) and three test meals: almond-bread meal (60 g); parboiled rice (68 g); and instant mashed potatoes (62 g), balanced in carbohydrate, fat and protein, using butter and cheese, the postprandial blood glucose response was also lower for the almond-bread meal (~60 g) compared to the control and the mashed potato meals (68).

Moreover, Kendall et al., 2011a, found that consuming mixed nuts with white bread progressively reduced the GR in both normoglycaemic (when consuming 60 g and 90 g of nut-bread meal) and diabetic individuals (when consuming 90 g of nut-bread meals only). No reduction in GR was observed for the 30 g nut-bread meal in either groups (64). Likewise, in a subsequent analysis by Kendall and colleagues, ingestion of pistachio with white bread significantly reduced the RGR of the 56 g and 84 g nut-bread meal but not for the 28 g nut-bread meal (58). Furthermore, these studies suggested that nuts progressively reduce the GR in a dose-dependent manner.

While these studies show that higher doses of nut consumption blunts postprandial glycaemia, the studies were conducted in a small sample size (ranging from 9-15
people). Furthermore, our study was slightly different in the study design compared to the previous studies mentioned. The nuts in our study were incorporated into the bread by cooking/baking, whereas in the previous studies, the nuts were co-ingested raw with white bread rather than the nuts being combined in a meal by cooking. The differences in the study design could have been responsible for the observed results where cooking methods may affect postprandial glycaemia (56). Also, the study by Jenkins et al (2006) monitored blood glucose for 4-hours postprandially whereas other studies assessed blood glucose for 2 hours only (68).

Additionally, there have been some long-term studies that have shown improvement in markers of glycaemic control when consuming nut-enriched diets (45, 71, 76, 78, 81). In the majority of the long-term studies, improvement in the markers of glycaemic control were observed where smaller quantities of nuts were consumed (28 g and 30 g nuts).

So far only two studies have shown improvement in HbA1c, which is an established marker of long-term glycaemic control (71, 72). Postprandial glycaemia is significantly correlated with HbA1c and is responsible for 50-70% of the overall diurnal hyperglycaemia when HbA1c values are less than 8.5 (72). The two studies that assessed HbA1c were in fact not very long. One of these studies was only 8 weeks long (71) and the other 12 weeks in duration (72). Since HbA1c reflects mean glycaemia for the previous 3 months, it is not certain whether this is long enough to diminish any potential carry-over effects in these cross-over studies. A greater lowering of HbA1c by nuts has been observed in trials of 12 or more weeks in comparison to trials of less than 12 weeks (83). These results suggest that nut consumption over a longer period may lead to greater improvements in glycaemic control.

Meanwhile, while there have been studies that have shown a significant reduction in GR, there have also been some long-term studies which show neither a reduction nor a deleterious effect of nut consumption on markers of glycaemic control (73, 75, 77). Some limitations associated with previous studies include absence of a control group (73), a small sample size (14, 45, 58, 64, 68-72) and a relatively short follow up period (71, 73, 79, 80).

Several mechanisms may explain the reduction in GR found in the present study and others. PGR can be affected by several factors, including the type of carbohydrate,
method used to process starch, the amount of dietary fibre, fat and protein present in a meal, the digestibility of the carbohydrate present in the meal and also the cooking methods (56). Nuts are rich in fibre, fat and protein, which may act synergistically to promote a reduction in the postprandial glycaemic response (1). The ability of nuts to improve glycaemic control may relate to a carbohydrate displacement mechanism by which nuts reduce the glycaemic load of the diet by displacing high glycaemic index carbohydrates (83). In addition, magnesium, dietary fibre, MUFA, and polyphenols in nuts are also some potential underlying contributors to the improvement in glycaemic control (45).

Some investigators have attributed the anti-glycaemic effect of nuts to a theoretical reduction in the gastric emptying rate (56). Nuts are high energy foods, and it has been recognized that gastric emptying is reduced by a high fat and energy load; the pylorus tends to regulate the flow of energy into the duodenum (58, 140). In the present study, the protein and fat content (based on weight) of the hazelnut-enriched breads ranged from 9.3-13.8% and 6.0-14.8%, respectively. In comparison, the white control bread contributed about 8.3% protein and 1.2% fat per weight.

In addition, in some of the glycaemic studies, nuts were not cooked with the carbohydrate foods but consumed along with other foods, whereas in the present study, the nuts were added in the breads and baked and eaten as one. Therefore, the postprandial glycaemic responses could be affected by the food matrices, cooking methods and how the different foods were eaten together (56).

Since nuts are high in fat, greater fat availability may also reduce the gastric emptying rate, decrease carbohydrate absorption rate, and increase insulin secretion mediated by intestinal hormones (glucose-dependent insulinotropic polypeptide and glucagon like peptide 1 (GLP-1)) and hence favour a reduction in glycaemic response (70).

It is also believed that the differences in the physical form of consumed nuts can affect the PGR (14, 70, 112). Changes in the physical form of nuts by processing may rupture the cell wall structure and may result in greater fat and fat-soluble nutrient bioaccessibility (111, 112). The high fat availability in the intestinal lumen may decrease the rate of carbohydrate absorption (by delayed gastric emptying), favouring a reduced glycaemic response (56, 70, 140). However in our study we found no significant differences between the nut forms. This could be due to the fact that we chose to
compare finely sliced hazelnuts with semi-defatted hazelnut flour. The latter has a lower fat content compared to finely sliced hazelnuts (20 g/100g vs 60 g/100g), which may have attenuated any additional effect on glycaemic response given the greater rupture of the cell wall.

Overall, the data suggest that the addition of nuts to a staple such as bread reduces the glycaemic response. As this study was conducted in a healthy, non-obese population, it would be interesting to replicate this study in other population groups such as in individuals with diabetes and in an obese population.

5.3 Effects of the nut-enriched bread on glycaemic index

The mean GI of the finely sliced nut bread, semi-defatted nut flour bread and the combination bread were 98, 89, and 101, respectively. This was somewhat surprising, considering that the AUC for PGR of the hazelnut-enriched breads was statistically significantly lower than the white control bread.

There are limitations associated with glycaemic index measurements. The GI is the ratio of two independent variable numbers. As the variability of the values from which any ratio is calculated increases, the distribution of the ratio becomes skewed and the mean increases (56, 57). In the present study, closer inspection of the data revealed that the GI ratios were considerably skewed. Therefore, the median GI which is suggestive of a typical response was also calculated. The median GI (IQR) was 83 (68.5-120), 78.5 (63.5-110.5), and 85.5 (59.5, 129.5) for the finely sliced nut bread, semi-defatted nut flour bread and the combination bread, respectively. The median GI values of the breads were found to be consistent with the AUC glycaemic response in the present study. Furthermore, the glycaemic index is a complex tool where large variations are known to exist in the glycaemic response within individuals (56, 61). Additionally, there is also a possibility of not being able to detect a true difference when the sample size and the expected difference is small (56, 60, 61).

There is always concern over compliance and accuracy of data collection in studies where participants are asked to adhere to certain behaviour patterns. While all care was taken to standardise the GI testing procedure, and the participants being instructed to follow a certain behavioural pattern for the GI testing, it is possible that there may have
been some changes in dietary or physical activity patterns of the participants. It is a possibility that the results could have been affected due to ‘second meal effects’. Altering the macronutrient content (fat and carbohydrate) of the evening meal could produce varied response and affect the GI determination the following morning. However, it has been shown that varying the fat and carbohydrate contents of the evening meal or consuming alcohol in moderate amounts the night before the GI testing, does not significantly affect the glycaemic response or GI (141, 142).

Most GI studies are usually conducted in a small sample size (as few as ten subjects) (143). While inclusion of ten subjects may provide useful results, large improvements in power and precision would require two to three times more subjects (143). Previous acute studies that have assessed the effects of nut-bread meals on postprandial glycaemia have had a sample size of 9 to 15 people (14, 58, 64, 68). The present study had 32 participants, and measured each bread twice which would have provided a reasonable degree of power and precision for measuring and detecting small differences in GI.

5.4 Effects of the nut-enriched bread on appetite

Nuts are energy dense, high in protein with a low GI; characteristics that may influence satiety and energetics (104). According to Bornet et al (2007), due to slower rate of digestion and absorption, the consumption of foods with a lower glycaemic response favours an increase in satiety (144).

Alternatively, Anderson and colleagues proposed that high glycaemic carbohydrates promote satiety in the short-term (one hour after ingestion) whereas low glycaemic carbohydrates are associated with a delayed satiety (2-3 hours post-ingestion) consistent with their delayed impact on blood glucose concentrations (145). Accordingly, they propose satiety is maximised when blood glucose concentrations are high (71, 145).

In the present study, although the AUC glycaemic response and GI (median GI) for the hazelnut-enriched breads were lower than the white control bread, there were no significant differences in appetite ratings between the treatments over a three hour period.
The appetitive properties of nuts have been assessed through preload studies and compensatory dietary responses (107, 115, 130, 136). Studies have assessed satiety using subjective ratings of appetite, or by measuring actual energy intake (130). In the present study, satiety was measured by the following methods. Firstly, participants were provided with the amount of bread that they usually reported consuming for breakfast (day 2 of each dietary phase), and were required to complete a questionnaire to assess subjective ratings of appetite. Participants also completed a WDR in order to compare energy intake between treatments. In addition, total energy intake was also measured by WDR when a fixed amount of 120 g of bread was consumed.

One consideration when interpreting the results from the satiety tests, is the different energy content provided by the breads. Nuts contain fat and protein and therefore the nut breads in the present study contained more energy than the control bread (white bread=912 kJ/100 g vs. finely sliced nut bread=1196 kJ/100; semi-defatted nut flour bread=1019 kJ/100 g and combination bread = 1119 kJ/ 100 g). We could have manipulated the satiety tests so that they provided either the same amount of energy or the same weight of bread. Previous literature involving the covert manipulation of the energy content of a food or meal revealed that individuals consume a consistent weight of food, independent of the energy content (146-148). For this reason, in the present study, the treatment breads were given to the participants by weight and not by calorie count.

It was somewhat unexpected that the reported satiety levels were not different among the four groups. We hypothesised that the nut-enriched breads would have a higher satiety effect in comparison to the white control bread. Our study showed that the reported satiety levels after the different bread consumption was not statistically significantly different across the treatment groups.

While our study test breads were not iso-energetic, our finding is in line with another study which reported no differences in satiety when an iso-energetic meals rich in PUFA from walnuts, MUFA from olive oil and SFA from dairy products were compared (149).

Contrary to our findings, three studies have observed increased level of satiety and sense of fullness following consumption of nut containing diets (68, 105, 106). A diet containing walnuts was found to increase satiety ratings over a 3-4 day period when
compared with a control diet (105). Moreover, in a 5-arm, crossover study, whole almonds (~ 42.5 g) were found to significantly increase satiety when added to a breakfast meal matched for carbohydrate, protein, fat and fibre in comparison to meals containing almond flour, almond butter, almond oil, or no almonds (106). Furthermore, in the study by Jenkins and colleagues, the satiety of the almond meal was greater than that of the control white bread meal for 2 hours and 4-hours postprandially where participants were provided with two bread control meals (97 g each) and three test meals: almonds-bread meal (60 g); parboiled rice (68 g); and instant mashed potatoes (62 g), balanced in carbohydrate, fat and protein, using butter and cheese. (68).

It is important to point out that while our study took place in a home setting, Brennan and colleagues, provided the test meals in the controlled environment of an in-patient setting where diet and activity was closely monitored (105). It is also possible that the difference in satiety is not evident over several hours. The present study assessed satiety after only one meal. In the study by Brennan et al., 2010, differences in satiety only started achieving significance after 3 days of walnut consumption (105). It is possible that mechanisms by which nuts increase satiety may not manifest in an extremely short time frame and are difficult to assess in an uncontrolled environment. Moreover, further studies examining the role of preloads in promoting post-meal satiety are needed to elucidate mechanisms.

The possible effects of nut consumption on satiety needs to be addressed in future studies not only with the subjective VAS method, but also with blood markers related to satiety, e.g. GLP-1, will provide useful physiological information regarding mechanisms (149).

As well as measuring appetite rating, we also calculated energy intake over the day. As mentioned above, the treatment breads were provided on the basis of weight, meaning the calories provided by the nut-enriched breads were higher than the control bread. However, despite giving the participants more calories, when consuming the hazelnut test bread in comparison to the control bread, the mean total energy was not statistically significantly different between the treatments, suggesting the participants’ may have partially compensated. Several previous studies have reported that participants who incorporated nuts into their diet daily compensated for the majority of these calories by displacement of other foods in the diet leading to an overall reduction in energy intake.
(88, 89, 93, 104, 112). Studies have also shown that compensation is strong, ranging from about 65-75% of the energy contributed by the nuts (104).

5.5 Effects of the nut-enriched bread on gastrointestinal symptoms

When formulating a product, in this case, bread, enriched with nuts, it is important to test if the bread is well tolerated. The mean peak ratings of the different breads for a number of gastrointestinal symptoms including belching, stomach bloating, stomach cramping, flatulence, stomach ache, diarrhoea, and nausea were 5.6 mm or less on a scale of 100 mm. There were no differences in gastrointestinal symptoms between breads. Therefore, all the breads appear to be well tolerated by the participants.

5.6 Dietary intervention

5.6.1 Nutritive value of hazelnut and the hazelnut-enriched breads

Hazelnuts are high in total fat (17.9 g/30 g) and contain predominantly MUFAs (12.7 g/30 g). Hazelnuts are also a rich source of protein (4.4 g/30 g), dietary fibre (3.1 g/30 g), calcium (54 g/30 g), vitamin E (5.1 µg/30 g), folate (33 µg/30 g), phosphorous (84 mg/30 g) and potassium (270 mg/30 g) (Tables 2.1 and 2.2). The protein quality of hazelnuts is high (66.6%) in comparison to many proteins of plant origin. In comparison, semi-defatted hazelnut flour contains approximately 6 g/30 g in total fat, 9.84 g/30 g protein, and 9.54 g/30 g dietary fibre (150).

For the purpose of the current study, 30 g of different forms of hazelnut was incorporated into 120 g bread. The amount of nuts used in the present study was based on current recommendations (11). Laboratory analyses on samples of the hazelnut-enriched breads indicate based on weight, a high protein content (≥ 9.3%), fat content (≥ 6.0%), and dietary fibre content (≥ 7.3%). Carbohydrate content per weight was ≥28.9 %. (Appendix H)
When the participants were provided with breads in amounts equivalent to their usual breakfast intake (satiety test), the white control bread, finely sliced nut bread, semi-defatted nut flour bread and the combination bread contributed about 11.9%, 17.4%, 14.7%, 17.3% of total energy intake, respectively. Similarly, the white control bread, finely sliced nut bread, semi-defatted nut flour bread and the combination bread contributed about 14.2%, 17.7%, 14.8%, and 18.3% of total energy intake when provided with 120g of bread.

In the present study, the favourable characteristics of hazelnut on diet quality was repeatedly observed, where MUFA, dietary fibre, and vitamin E intakes were substantially higher with the consumption of hazelnut-enriched bread in comparison to the white control bread consumption period. This was particularly apparent for the finely sliced hazelnut bread. Diets with such fatty acid quality are associated with improved insulin sensitivity compared to low fat or high SFA diets (81, 151). In addition, long-term consumption is likely to improve markers of CVD.

### 5.6.2 Nutrient profile and diet quality

#### 5.6.2.1 Satiety testing day (measured on usual intake)

Numerous studies have shown an improvement in diet quality with the regular inclusion of nuts in the diet (95, 133, 135, 152). Epidemiological studies have shown that nut consumers have a superior diet quality and lower CVD risk factors than non-nut consumers (133, 134). As with the present study, in the 1999-2004 NHANES, it was found that nut consumption was associated with a higher overall diet improved nutrient intake (135). Similarly, in the Continuing Survey of Food Intake by Individuals and Diet and Health Knowledge Survey (1994-96 CSFI/DHKS), it was found that peanut consumers had improved diet quality over non-peanut consumers (152). Griel et al. (2004), found that peanut consumers had higher intakes of protein, total fat, PUFA, MUFA, dietary fibre, vitamin A, vitamin E, folate, calcium, magnesium, zinc and iron. They also found that dietary cholesterol of peanut consumers was lower for all population groups. It has been reported that nut consumers had higher intakes of MUFA, PUFA, dietary fibre, vitamins A, C, and E, calcium, magnesium and potassium(152). In addition, RCTs which have incorporated
nuts into the diet had improvements in nutrient profiles (77, 95). However, no study to date has assessed diet quality when nuts are added to a staple such as bread.

In this study, during the satiety testing day, a statistically significant difference between the dietary treatments was detected for the percent of energy derived from carbohydrate, protein, MUFA and absolute amounts of SFA (g), MUFA (g), dietary fibre (g), and vitamin E (mg).

These differences were similar, but not entirely consistent across all nut breads. The percentage of energy derived from carbohydrate was significantly lower during all of the nut-enriched bread treatments compared to the control treatment. This finding is consistent with numerous intervention studies that have added nuts to the usual diet (95). The energy derived from protein has increased in some studies where nuts have been incorporated into the diet (133, 152), but not others (77, 95, 135). In the present study the energy derived from protein was significantly higher in the semi-defatted nut flour bread and the combination bread treatments than the control bread, but not the finely sliced nut bread treatment. Conversely, the percentage of energy derived from MUFA was significantly higher only for the finely sliced nut bread compared to the control bread. This is likely due to the higher MUFA content of the sliced hazelnuts (45.6 g/100 g) compared to the semi-defatted hazelnut flour (20.0 g /100 g) (20). The Vitamin E content of the diets containing the finely sliced nut bread and the semi-defatted nut flour bread was significantly higher, than the control bread, with a tendency for a higher intake in the combination bread compared to the control. The same pattern was seen for dietary fibre.

Overall, our study is in agreement with previous epidemiological studies and clinical trials which have reported that the regular consumption of nuts results in an improvement in diet quality that is likely to improve CVD risk. Our study suggests that adding nuts to a staple food does not appear to compromise this enhanced diet quality.

5.6.2.2 Sunday food diary day (measured on 120g)

We also measured nutrient intakes on a day where participants consumed 120 g of the breads, an amount which provided the Heart Foundation recommendations of 30 g of nuts. Changes in nutrient profiles were similar to those seen during the satiety testing day, with perhaps a slightly more favourable effect observed for the finely sliced nut
bread. The total energy derived from carbohydrate was significantly lower for the finely sliced nut bread and the combination bread compared to the control, however the protein contribution did not differ significantly between treatments. Again the contribution of energy from MUFA was significantly higher in the finely sliced nut bread treatment compared to all other treatments, as was the vitamin E intake. Dietary fibre intake was significantly higher in the finely sliced nut bread and semi-defatted nut flour bread compared to the other two bread treatments.

The results from the two days are relatively consistent and suggest that adding nuts to bread results in positive effects on diet quality. Using bread as a vehicle, among the general population provides additional options for increasing nut consumption which may promote adherence to a healthy diet that reduces risk of chronic diseases. Importantly, similar to other studies (95, 133, 135, 152), we have also observed improvements in diet quality with the addition of nuts to the diet without any further healthy eating advice.

5.7 Strengths and limitation

5.7.1 Strengths

The present study is the first to investigate the effects of incorporating different forms of hazelnut into bread on PGR and satiety. This study was a RCT with a crossover design. As the participants served as their own controls, the influence of confounding covariates were reduced. This design allowed for minimal confounding biases (139, 153).

This 4-arm, randomised, controlled crossover study was sufficiently powered at 80% to detect moderate to large differences in GI. Four participants of the thirty-three recruited, withdrew at the beginning of the study, and therefore, three more participants were recruited. The participant retention rate was excellent as thirty-two participants completed the 10-week study.

For testing the GR, the breads were tested in duplicate (tested twice at the beginning and end of each dietary phase) to account for intra-individual variability in GR measurements. In addition our sample size was larger than most previous studies.
investigating the GR to nuts and nut containing meals. The testing took place using standardised procedures and in a controlled laboratory setting. The remainder of the study (i.e. days 2-7 of each phase), was designed to mimic a real life scenario in which participants were simply instructed to consume the nut containing breads without changing or altering their usual eating habits.

All participants received emails and text reminders from the investigator during the study period. Studies have shown that regular contact between the investigators and study participants (such as phone calls or meetings) showed greater dietary adherence (154-156). The participants were allowed to eat the breads in any manner they chose. It has been shown that choice can positively influence food acceptability and intake (157, 158). Furthermore, the present study had a control group which allowed comparison with a no nut option. Finally, the study sample should be considered representative of the general population and may be generalisable to a wider population group. The present study reported data from 32 healthy individuals; there was a good representation of people of different ethnicities (European, Maori, Asian and Middle-Eastern descent) and a wide distribution of age and sex.

5.7.2 Limitations

Our study has some limitations to bear in mind when interpreting the results. We evaluated only the acute effects of the hazelnut-enriched breads on glycaemia and satiety, thus the results provide no insight on what might occur during chronic intake. A future trial with a long-term follow up period is warranted where other markers of GR such as HbA1c can be monitored. For this, a study of 3 months or longer is needed. Other variables such as body weight and markers of CVD should also be measured.

Secondly, accurate measurement of food intake is challenging. In this study, food intake was measured by WDR on two days in each phase. The burden on the participants to keep diet record can lead to altered food patterns (159). There may have been some measurement errors associated with limitations in nutrient databases and reliance on self-reported dietary intakes, which are susceptible to report bias, though, WDR were used as these are regarded as the ‘gold standard’ for measuring food intake.
However, food intake was only measured on two days during each phase due to the already high participant burden.

Moreover, there may be some limitations associated with testing in a home setting. One limitation is the lack of control. While the study took place in a home setting for days 2-7 of each phase, it might have been wiser to provide the test breads in the controlled environment of an in-patient setting where diet and activity could be closely monitored for when satiety was measured. In addition, we only measured satiety ratings on one day. Given the evidence from previous research that differences in satiety may take several days (105), we could have measured appetite over a number of days. However, we were mindful of participant respondent burden, which was already high given our study protocol. It is possible that physical activity levels may have differed between the dietary treatment phases. However, information from a post-study questionnaire (Appendix M) indicated that there were no large changes in physical activity patterns among participants.

5.8 Areas for future research

This was a short-term study investigating the effects of consuming different forms of hazelnut-enriched breads on PGR and satiety in comparison to a white control bread.

Future, long-term intervention trials of three or more months are warranted to examine whether improved markers of glycaemic control can be achieved and sustained over a longer period of time. Also future research could assess the effects of the hazelnut-enriched breads on body weight and acceptance over a long period of time. Other markers of CVD risk factors such lipid profiles should also be measured.

As part of the present study, a Master of Dietetics student examined acceptance and the sensory testing of the hazelnut-enriched breads. It was found that the participants favoured the finely sliced nut bread over the other three breads. Therefore, it would also be interesting to compare the acceptance of the test breads over a longer period. This is important because long-term acceptance will influence compliance to consume these breads in sufficient quantities.

Moreover, a study should be conducted where barriers to nut consumption such as costs of nuts and nut-enriched breads are addressed. It is known that dietary choices are
primarily influenced by considerations, such as, taste, cost, convenience and nutritional value of a food (160). Food pricing is an important component of the eating environment and cost is often a reported barrier to nut consumption. For this reason it is important to determine how much the hazelnut-enriched breads would cost in comparison to other specialty breads such as gluten free and other multi-grain and seed breads. We found that a 750 g loaf of finely sliced-hazelnut bread would cost approximately $5.20, a semi-defatted nut flour bread would cost approximately $4.18 and the combination bread (finely sliced nut and semi-defatted nut flour) would cost $4.69. These prices are in line with the price of other multigrain breads and cheaper than other special breads such as gluten free breads which range in cost from $6.99 to $8.39. Cost is an important consideration, given that those most likely to gain benefits from nut breads are likely to have a more restricted food budget. Also, increasing knowledge on the health benefits of nuts should be encouraged.

Furthermore, previous studies have shown that higher doses of nuts result in a more pronounced effect on health outcomes, however, the behavioural and practicality of consuming such amounts needs to be examined. A dose-response study may be useful to determine the optimal dose required to exert beneficial effects.
Nuts are known for their numerous health benefits, especially their cardio-protective effects. However, studies have reported that the prevalence of nut consumption in the general population is relatively low (12, 131, 133-135). It is therefore important to find ways to incorporate nuts into the diet. Here, we have bread, a staple in the New Zealand diet, enriched with hazelnuts. Before undertaking a long-term study, we thought it important to determine the acute effects of this nut-enriched bread on PGR and satiety.

The major finding of the present study was that finely sliced nut bread, semi-defatted nut flour bread and the combination bread significantly improved postprandial glycaemic response in healthy individuals compared to a white control bread. Nut-bread consumption also significantly improved diet quality by increasing MUFA, dietary fibre, and vitamin E intakes. This study supports the findings of other studies which suggest that nuts can be incorporated into the diet as a means of improving glycaemic responses, while also improving diet quality. A unique finding of this study is that the improvements in diet quality seen with the regular consumption of nuts is not compromised when nuts are added to bread, a major staple in the New Zealand diet.

While, the addition of 30 g of hazelnuts reduced the GR of the resulting breads, there were no differences observed in satiety between treatment groups. This was somewhat surprising given the increase in satiety observed in previous studies. However this may have been due to the short-term nature of our satiety test (only 3 hours) compared to previous studies over several days.

Interestingly, although the nut-enriched breads were higher in energy compared to the white control bread, there were no statistically significant differences in overall energy intake. This would indicate that participants might have compensated for the additional energy from the nuts over the 24-hour period. This compensatory effect has been observed previously where participants who incorporated nuts into their diet daily compensated for the majority of these calories by displacement of other foods in the diet (88, 104). Moreover, consumption of the test breads did not cause any gastrointestinal discomfort. Therefore, incorporation of hazelnuts into breads seems to be an effective strategy to increase nut intake, which may in turn be an effective method to contribute to a reduction in risk factors for CVD.
7.0 REFERENCES


105. Brennan AM, Sweeney LL, Liu X, Mantzoros CS. Walnut consumption increases satiation but has no effect on insulin resistance or the metabolic profile over a 4-day period. Obesity. 2010;18(6):1176-82.


8.0 APPENDICES
APPENDIX A

Ethical Approval
Dear Dr Brown,

I am writing to let you know that, at its recent meeting, the Ethics Committee considered your proposal entitled “The nut bread study”.

As a result of that consideration, the current status of your proposal is: - Approved

For your future reference, the Ethics Committee’s reference code for this project is: - H14/004.

The comments and views expressed by the Ethics Committee concerning your proposal are as follows:-

While approving the application, the Committee would be grateful if you would respond to the following:

The Committee would be grateful if you could review the Information Sheet to ensure it is more “participant friendly”. Please use simplified language and avoid academic terms. It is best practice to address your participants directly.

Regarding the intended compensation for participants’ time, the Committee notes that this appears to be taxable income. Please ensure participants are aware of this.

Regarding the Consent Form, please delete item 11 as this is not necessary for finger prick samples.

Please provide the Committee with copies of the updated documents, if changes have been necessary.

27 January 2014
Approval is for up to three years from the date of this letter. If this project has not been completed within three years from the date of this letter, re-approval must be requested. If the nature, consent, location, procedures or personnel of your approved application change, please advise me in writing.

Yours sincerely,

Mr Gary Witte  
**Manager, Academic Committees**  
Tel: 479 8256  
Email: gary.witte@otago.ac.nz

cc. Emeritus Professor L J Holloway  Head  Department of Human Nutrition
APPENDIX B

Participant information sheet
Participant Information Sheet

Study title: The nut bread study

Principal investigator:
Rachel Brown
Department of Human Nutrition
Position: Senior Lecturer

Contact phone number:
479 5839

Introduction

Thank you for showing an interest in this project. Please read this information sheet carefully. Take time to consider and, if you wish, talk with relatives or friends, before deciding whether or not to participate.

If you decide to participate we thank you. If you decide not to take part there will be no disadvantage to you and we thank you for considering our request.

What is the aim of this research project?

Nuts are rich sources of good fats, vitamins, minerals as well as other healthy substances. The regular consumption of nuts is associated with a reduction in heart disease risk. To obtain health benefits, nuts must be consumed regularly and in sufficient amounts. The National Heart Foundation of New Zealand recommends the daily consumption of 30 g of nuts as a means to reduce cardiovascular disease. However only a small proportion of the New Zealand population regularly consume nuts. Strategies to increase nut consumption are required. One such strategy is to incorporate nuts into a common staple in New Zealand such as bread. There are several important considerations for the formulation of such a bread, including the effects on health, fullness and acceptability.

Therefore the aim of this study will be to compare the effects of consuming three different forms of hazelnut-enriched bread (sliced, defatted nut meal and a combination of the two), with control bread without nuts, on glucose concentrations, satiety (fullness ratings), and acceptance.

Who is funding this project?

This project is funded by a University of Otago Research Grant.
Who are we seeking to participate in the project?

We are seeking healthy males and females aged 18-65 years who regularly consume at least 3 slices of bread per day.

If you are in one or more of the categories listed below you will not be able to participate in the project:

- People with diabetes mellitus
- Disorders of carbohydrate metabolism
- Intolerances or allergies to the test products i.e. hazelnuts and bread
- Smokers
- Pregnant and lactating women

If you participate, what will you be asked to do?

Should you agree to take part in this project, you will be asked to complete a recruitment questionnaire. If you are eligible for the study you will be asked to attend 10 clinic visits at the University of Otago over a 2 month period. These visits will consist of 8 glycaemic index testing sessions and 2 tasting sessions. The glycaemic index testing visits will take approximately 2 ½ hours each. The tasting sessions will take around 45 minutes. This means the study will require about 22 hours of your time in total over the 2 month period.

You will be asked to attend a tasting session at the sensory laboratory in the Department of Food Science at the beginning and end of the study period. You will be asked to taste different types of bread and rate your liking of each bread on a scale. The entire testing session should take approximately 45 minutes.

You will be required to attend 8 glycaemic index sessions where we will measure your blood glucose (sugar) levels after eating 4 different breads, measured in duplicate. Prior to each clinic visit you will be asked to fast for at least 12 hours. This means you will not be able to eat or drink (except for water) for 12 hours before the test. When you arrive at the clinic we will measure your body weight. On only the first and final visits of the study, our study nurse will collect a 10 ml (about 2 teaspoons) fasting sample of blood taken from a vein in your arm to measure blood cholesterol. Two fasting fingerprick blood samples will then be taken using a disposable lancet. You will then be asked to consume one of the four breads (around 3 slices) with 300 ml of water within a 15 minute period. We will take a further 7 fingerprick blood samples at 15 minute intervals over the following 2 hours. Only a small drop of blood will be collected at each time point. Between blood draws you are free to work on your laptop, watch TV, read and walk to the toilet if required. During each session, following consumption of the bread we will ask you to complete a sensory and a gastrointestinal symptoms questionnaire. At the end of blood collection you will be provided with breakfast.
At the end of the glycaemic index testing session we will provide you with pre-packaged daily portions of bread to eat over the following 6 days. The portion on the first day will be the same portion size that you indicate you usually eat for breakfast in the recruitment questionnaire. You will eat this bread for breakfast in place of your usual bread. Before and after you eat the bread you will complete a hunger/fullness questionnaire. You will also record your dietary intake for the day indicated on the diet record sheet provided. We will provide you with kitchen scales so that you can record the weights of your food and drinks. For the following 5 days, you will eat 120 g of the bread provided. This can be eaten at any time of the day. When you first eat the bread on these days we will ask you to complete a ballot where you will rate your ‘desire to consume’ and ‘overall liking’ for the bread. Each Sunday during the trial period, we will ask you to complete a diet record of all food and drink consumed. We will also provide you will a daily tick sheet where you will describe how you ate the bread each day. After you have consumed the bread for 6 days you will return the to the glycaemic index clinic where we will take the second glycaemic index measure of the bread.

The breads will be tested in random order. You will repeat the testing outline above until you have completed all 4 breads. There will be a one week rest period between consuming the different breads. At the end of the study you will complete an exit questionnaire where you will be asked information on lifestyle changes during the study.

You will receive $320 for the compensation of the costs associated with travel and parking, at the end of the study.

**Is there any risk of discomfort or harm from participation?**

- Finger pricks can cause minor discomfort. There may be some slight bruising around the site. If bruising does occur it should disappear within one day. You will be limited to one glycaemic index test per week. Giving blood from a vein (venepuncture) can be associated with short-term pain and bruising may occur. On the two (only) occasions when you give blood from the vein in your arm your blood samples will be drawn by an experienced registered nurse to minimise any discomfort. Giving blood from a vein will occur only at the first and last appointments – that is 10 weeks apart.

- On rare occasions you may feel unwell during or after testing. We have a bed for you to rest on and our research nurse will monitor the situation. If you feel unwell during or after testing we will provide you with a ride home.
What specimens, data or information will be collected, and how will they be used?

We will be collecting fingerprick blood samples during the glycaemic index testing sessions. These will be measured immediately using a Glucose Analyser. The sample will be disposed of into biohazard containers using standard disposal methods.

The blood taken from your arm will be collected into a tube (Vacutainer). The tube will be inverted and stored in a chilly bin containing chilled ice-pads. The blood will be processed in the laboratory within two hours of being taken. Once the plasma and red blood cells have been separated, the sample will be stored in a freezer at −80°C until all the samples can be analysed at the same time for blood cholesterol, triglycerides (another blood fat) and lipoproteins (compounds that carry the cholesterol around your body).

The data collected will be securely stored in such a way that only the investigators will be able to gain access to it. At the end of the project, any personal information will be destroyed immediately except that, as required by the University's research policy, any raw data on which the results of the project depend will be retained in secure storage for ten years, after which it will be destroyed.

The results of the project may be published and will be available in the University of Otago Library (Dunedin, New Zealand) but every attempt will be made to preserve your anonymity. Any data will in no way be linked to any specific participant.

What about anonymity and confidentiality?

We will be collecting personal information regarding your sex, age, weight, height and general health. The purpose of collecting this information is so that we are able to describe the overall characteristics of the population. Only the investigators will have access to personal information and even then only ID numbers will identify individuals.

If you agree to participate, can you withdraw later?

You may withdraw from participation in the project at any time and without any disadvantage to yourself.
Any questions?

If you have any questions now or in the future, please feel free to contact either:

<table>
<thead>
<tr>
<th>Name</th>
<th>Position</th>
<th>Department of Human Nutrition</th>
<th>Contact phone number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asika Devi</td>
<td>Masters Student</td>
<td></td>
<td>0220276039</td>
</tr>
<tr>
<td>Alex Chisholm</td>
<td>Senior Research Fellow</td>
<td></td>
<td>479 7514</td>
</tr>
<tr>
<td>Rachel Brown</td>
<td>Senior Lecturer</td>
<td></td>
<td>479 5839</td>
</tr>
</tbody>
</table>

This study has been approved by the University of Otago Human Ethics Committee (Health). If you have any concerns about the ethical conduct of the research you may contact the Committee through the Human Ethics Committee Administrator (phone +64 3 479 8256 or email gary.witte@otago.ac.nz). Any issues you raise will be treated in confidence and investigated and you will be informed of the outcome.
APPENDIX C

Consent form
The Nut Bread Study

Principal Investigator: Dr Rachel Brown (rachel.brown@otago.ac.nz & 479 5839)

CONSENT FORM FOR PARTICIPANTS

Following signature and return to the research team this form will be stored in a secure place for ten years.

Name of participant:…………………………………………..

1. I have read the Information Sheet concerning this study and understand the aims of this research project.
2. I have had sufficient time to talk with other people of my choice about participating in the study.
3. I confirm that I meet the criteria for participation which are explained in the Information Sheet.
4. All my questions about the project have been answered to my satisfaction, and I understand that I am free to request further information at any stage.
5. I know that my participation in the project is entirely voluntary, and that I am free to withdraw from the project at any time without disadvantage.
6. I know that as a participant I will be asked to complete a recruitment questionnaire on demographic and health information. I will be asked to attend 8 glycaemic index clinic visits where I will provide 8 fingerprick blood samples per visit. On the first and final visits (only) of the study I will provide a 10 ml (about 2 teaspoon) sample of blood taken from a vein in my arm. I will also be asked to complete a sensory and gastrointestinal symptoms questionnaire at this time. I will then consume one of the 8 breads for 6 days. On day 2 (after the first glycaemic index test) I will complete a hunger/fullness questionnaire and record my food and drink intake for the
day. For the remaining 5 days I will record my ‘desire to consume’ and ‘overall liking’ of the breads. Also I will record my food and beverage intake on one other day. I will complete a tick list each day outlining how I consumed the bread. In addition I will attend a tasting session at the beginning and end of study where I will rate my ‘overall liking’ of bread samples. At the end of the study I will complete an exit questionnaire where I will be asked information on lifestyle changes during the study.

7. I understand the nature and size of the risks of discomfort or harm which are explained in the Information Sheet.

8. I know that when the project is completed all personal identifying information will be removed from the paper records and electronic files which represent the data from the project, and that these will be placed in secure storage and kept for at least ten years.

9. I understand that the results of the project may be published and be available in the University of Otago Library, but that either (i) I agree that any personal identifying information will remain confidential between myself and the researchers during the study, and will not appear in any spoken or written report of the study ☐

10. I know that I will be offered $320 as remuneration for completion of this study to reimburse me for my costs associated with travel and parking, and that no commercial use will be made of the data.

11. I understand that the blood samples collected from venepuncture will be disposed immediately using standard disposal methods. Please circle the appropriate response below:
I consent to my samples being disposed of using standard disposal methods at the end of the study  
**YES / NO**

I wish to have my samples disposed with appropriate karakia at the end of the study  
**YES / NO**

Signature of participant: 

[Signature]

Date: 

[Date]

Signature and name of witness: 

[Signature]

Date: 

[Date]
APPENDIX D

Trial registration
Dear Rachel Brown,

Re: Is nut-enriched bread an acceptable and effective vehicle for improving satiety and blood glucose response?

Thank you for submitting the above trial for inclusion in the Australian New Zealand Clinical Trials Registry (ANZCTR).

Your trial has now been successfully registered and allocated the ACTRN: ACTRN12614000213640


Date submitted: 13/02/2014 3:18:09 PM

Date registered: 27/02/2014 4:50:56 PM

Registered by: Rachel Brown

If you have already obtained Ethics approval for your trial, could you please send the ANZCTR a copy of at least one Ethics Committee approval letter? A copy of the letter can be sent to info@actr.org.au (by email) OR (61 2) 9565 1863, attention to ANZCTR (by fax).

Please be reminded that the quality and accuracy of the trial information submitted for registration is the responsibility of the trial's Primary Sponsor or their representative (the Registrant).

The ANZCTR allows you to update trial data, but please note that the original data lodged at the time of trial registration and the tracked history of any changes made will remain publicly available.

The ANZCTR is recognised as an ICMJE acceptable registry (http://www.icmje.org/faq.pdf) and a Primary Registry in the WHO registry network (http://www.who.int/ictrp/network/primary/en/index.html).

If you have any enquiries please send a message to info@actr.org.au or telephone +61 2 9562 5333.

Kind regards,

ANZCTR Staff

T: +61 2 9562 5333
F: +61 2 9565 1863
E: info@actr.org.au
W: www.ANZCTR.org.au
APPENDIX E

Recruitment poster
The Nut Bread Study

We are looking for volunteers to participate in a 10 week study to find out the effects of eating different types of nut bread on blood sugar levels, fullness ratings and consumer acceptance.

You will be given 4 different breads to eat over a 2 month period. The time commitment for the study will be about 22 hours.

Participants will receive $320 on completion of the study to compensate for their costs.

If you are aged 18 to 65 years and currently eat at least 3 slices of bread per day, have no allergies or intolerances to nuts or bread, do not have diabetes, are not pregnant or lactating, are a non-smoker and would like more information please contact:

Contact Details:

Asika Devi, Department of Human Nutrition, University of Otago.

Email: devas590@student.otago.ac.nz

Txt or call 0220276039

This project has been reviewed and approved by the University of Otago Human Ethics Committee, (Health). Reference: 14/004

| The nut bread study | Asika Devi | 02 20276039 | The nut bread study | Asika Devi | 02 20276039 | The nut bread study | Asika Devi | 02 20276039 | The nut bread study | Asika Devi | 02 20276039 | The nut bread study | Asika Devi | 02 20276039 | The nut bread study | Asika Devi | 02 20276039 | The nut bread study | Asika Devi | 02 20276039 | The nut bread study | Asika Devi | 02 20276039 | The nut bread study | Asika Devi | 02 20276039 | The nut bread study | Asika Devi | 02 20276039 | The nut bread study | Asika Devi | 02 20276039 |
APPENDIX F

Recruitment questionnaire
Recruitment Questionnaire

Demographics and Contact Details

Name: _____________________________________________________

Gender: _____________________________________

Date of Birth: __________________________________________

Address: _________________________________________________________________

Contact no.: ___________________________ (Home)

______________________________ (Work)

______________________________ (Mobile)

Email address: ________________________________

Which is the best method to contact you for appointments and study-related issues?

☐ Mail (per post)

☐ Phone

☐ Home

☐ Work

☐ Mobile

☐ Text

☐ Email
General Information

1. Which ethnic group do you belong to? Mark the space(s), which apply to you.
   - NZ European
   - Māori
   - Samoan
   - Cook Island Maori
   - Tongan
   - Niuean
   - Chinese
   - Other, please specified: __________________________

2. Are you pregnant or lactating? Yes / No

3. Are you a smoker? Yes / No
   If yes, approximately how many cigarettes do you smoke per day? _____per day

4. Will you be in Dunedin for the next 14 weeks? Yes / No

Usual bread consumption

5a. How many slices of bread would you usually eat per day?
    ___________slices

5b. What type of sliced bread would you usually consume?
   - thin
   - thick
   - other, please specify____________________________

6a. When you eat bread for breakfast, how many slices would you usually eat?
    ___________slices
6b. What type of sliced bread would you usually consume at breakfast?

- thin
- thick
- other, please specify_______________________

**Physical Activity**

7. In the last two months, how often did you do vigorous physical activities? This refers to activities that take hard physical effort and make you breathe much harder than normal, e.g. aerobics, running, fast bicycling, fast swimming or heavy lifting.

- Never
- Less than once a month
- 1-3 times per month
- Once per week
- 2 – 4 times per week
- 5 – 6 times per week
- Once per day

8. In the last two months, how often did you do moderate physical activities? This refers to activities that take moderate physical effort and make you breathe somewhat harder than normal, e.g. bicycling at a regular pace, swimming at a regular pace, doubles tennis or carrying light loads.

- Never
- Less than once a month
- 1-3 times per month
- Once per week
- 2 – 4 times per week
- 5 – 6 times per week
- Once per day

**Medical History**

9. Do you have any of the following?

- Asthma          Yes / No
- Dentures or a partial plate Yes / No
Sinus trouble Yes / No

10. Do you have any food allergies? Yes / No
   If yes, please specify foods: ____________________________

11. Do you have any known illnesses? Yes / No
   If yes, please specify illness: ____________________________

12. Do you take any medications? Yes / No
   If yes, please indicate which medication(s) and how long you have taken them.
   ___________________________________________________________________
   ___________________________________________________________________
   ___________________________________________________________________

Thank you very much for taking time to complete this questionnaire! 😊
APPENDIX G

Glycaemic response protocol
Thank you for showing an interest in this project.

What is the aim of this project?
The aim of this project is to determine the postprandial glycaemic responses to three different forms of hazelnut-enriched bread (sliced, defatted nut meal and a combination of the two) with control bread without nuts.

What will participation involve?
You are required to attend the Glycaemic Index clinic where your glucose response will be measured after eating the breads.

Glycaemic index testing will be held at 6.30am in GO.7, (Ground floor, Science 2 building) Department of Human Nutrition, University of Otago, (see map attached) in the week starting 17th March 2014.

You are required to come to the clinic once a week only on your allocated day (Tuesday, or Wednesday or Thursday) by 6.30am.

There are four breads to be tested. The breads will be tested in a random order. There will be a one-week rest period between consuming the different breads.
The GI tests will be held on the following dates:

<table>
<thead>
<tr>
<th>Glycaemic index session</th>
<th>TUESDAY</th>
<th>WEDNESDAY</th>
<th>THURSDAY</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>18th March</td>
<td>19th March</td>
<td>20th March</td>
</tr>
<tr>
<td>2</td>
<td>25th March</td>
<td>26th March</td>
<td>27th March</td>
</tr>
<tr>
<td>3</td>
<td>1st April</td>
<td>2nd April</td>
<td>3rd April</td>
</tr>
<tr>
<td>4</td>
<td>8th April</td>
<td>9th April</td>
<td>10th April</td>
</tr>
<tr>
<td>5</td>
<td>15th April</td>
<td>16th April</td>
<td>17th April</td>
</tr>
<tr>
<td>6</td>
<td>29th April</td>
<td>30th April</td>
<td>1st May</td>
</tr>
<tr>
<td>7</td>
<td>6th May</td>
<td>7th May</td>
<td>8th May</td>
</tr>
<tr>
<td>8</td>
<td>13th May</td>
<td>14th May</td>
<td>15th May</td>
</tr>
</tbody>
</table>

Please note there will be no GI tests held in the following period:

- 18-27th April Easter/mid-semester break

Prior to each glycaemic index clinic:

- Please fast overnight for at least 12 hours. This means you will not be able to eat or drink (except for water) for 12 hours before the test. **DO NOT EAT BREAKFAST ON THE TEST DAY.**
- Please avoid alcohol the evening prior to testing.
- Avoid exercise for 10 hours prior to testing.
- If possible, travel to testing clinic by vehicle on each of the test days.
- If you walk or cycle, please arrive 10 minutes early and rest before you start the test.
- We require you to have similar sedentary behavior patterns on the days prior to testing.

When you arrive at the clinic, we will measure your body weight and height. Please record this on the participant data sheet provided. Also, on the first and last days of the study, the study nurse will collect a 10 ml (about 2 teaspoons) fasting sample of blood taken from a vein in your arm to measure blood cholesterol.

You will then be shown to your seat.
Your booth space should have a tray; containing the bread, 1x ballpoint pen, 1x glass of water, 1x paper towel, 1x participant data sheet, and 1x sensory and 1x gastrointestinal questionnaires. Please let the clinic assistants know if any of these are missing from your tray.

During each session we will collect 2 fasting blood samples (base-line blood sample) by pricking your finger with a disposable lancet. (Please note if you have followed instructions and have not snacked before coming to the clinic, your fasting blood glucose concentration between finger prick 1 and finger prick 2 blood samples should be within 0.5mmol/L). We will need to re-test if the two measurements are further apart. Record your baseline blood glucose result into your participant data sheet.

Once settled in, please advise the clinic assistant that you are ready to start. Please do not start eating until you have had a baseline finger prick taken.

Once baseline finger prick are done, you may then start eating your bread. Please write down the bread code on the participant data sheet. Eat as you normally would but preferably do not wolf it down. For slow eaters we want you to finish within 10 minutes.

Over the following two hours, we will take 7 additional finger prick blood samples at 15, 30, 45, 60, 75, 90, and 120 minutes.

Please record your blood glucose concentrations in your participant data sheet.

Between blood-draws you are free to work on your laptop, watch TV, read and walk to the toilet if required.

During each session, following consumption of the bread we will ask you to complete a sensory and a gastrointestinal symptoms questionnaire.

At the end of blood collection, you will be provided with breakfast, which you can have on the go or in the union court dining room if you like.

At the end of the glycaemic index testing session, we will provide you with pre-packaged daily portions of bread to eat over the following 6 days.

Please make arrangement with Asika and Janet for picking the bread packages and also a kitchen food scale that you will need to help complete your diet records. Please note that you need to return the food scales at the end of the study (in May).
APPENDIX H

Test bread nutrient analysis
**Nutrition Laboratory**

**TN13-657**

<table>
<thead>
<tr>
<th>TO: Alex Chisholm</th>
<th>AT: University of Otago</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SAMPLES: Bread</strong></td>
<td><strong>DATE:</strong> 18/12/13</td>
</tr>
</tbody>
</table>

**TRIAL #: TN13-657**

**Analysis Report, Amended Carbohydrate & GE values**

*DATE SAMPLES RECEIVED: 21/11/2013*

Number of pages in this report: 2

Results are on an as received basis

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Ash %</th>
<th>Moisture %</th>
<th>CP %</th>
<th>Fat %</th>
<th>TDF %</th>
<th>Carb %</th>
<th>GE (calc)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bread A</td>
<td>0.9</td>
<td>44.0</td>
<td>8.3</td>
<td>1.2</td>
<td>2.9</td>
<td>42.7</td>
<td>912</td>
</tr>
<tr>
<td>Bread B</td>
<td>1.2</td>
<td>36.5</td>
<td>9.3</td>
<td>14.8</td>
<td>9.4</td>
<td>28.9</td>
<td>1196</td>
</tr>
<tr>
<td>Bread C</td>
<td>1.0</td>
<td>39.4</td>
<td>8.8</td>
<td>9.2</td>
<td>7.8</td>
<td>33.8</td>
<td>1064</td>
</tr>
<tr>
<td>Bread D</td>
<td>1.8</td>
<td>37.9</td>
<td>13.8</td>
<td>6.0</td>
<td>7.3</td>
<td>33.1</td>
<td>1019</td>
</tr>
<tr>
<td>Bread E</td>
<td>1.3</td>
<td>41.5</td>
<td>10.7</td>
<td>3.4</td>
<td>4.9</td>
<td>38.2</td>
<td>958</td>
</tr>
<tr>
<td>Bread F</td>
<td>1.5</td>
<td>36.9</td>
<td>11.3</td>
<td>10.6</td>
<td>8.2</td>
<td>31.5</td>
<td>1119</td>
</tr>
<tr>
<td>Bread G</td>
<td>1.2</td>
<td>40.0</td>
<td>9.9</td>
<td>6.0</td>
<td>6.0</td>
<td>36.9</td>
<td>1019</td>
</tr>
<tr>
<td>Flour</td>
<td>4.7</td>
<td>8.9</td>
<td>32.8</td>
<td>20.0</td>
<td>31.8</td>
<td>1.8</td>
<td>1328</td>
</tr>
</tbody>
</table>

**IANZ Key Technical Person**

Karl Dale

Leiza Turnbull
Methodology
Protein (CP): Leco, total combustion method. AOAC 968.06
Fat: Acid hydrolysis/Mojonnier extraction. AOAC 954.02
Moisture: Convection oven 105 °C, AOAC 930.15, 925.10
Ash: Furnace 550 °C, AOAC 942.05.
Dietary Fibre: Enzymatic-gravimetric method. AOAC 991.43
*Carbohydrate (Carb): By difference
*Energy: By calculation

*Tests marked with an asterix are currently outside the scope of the Nutrition Laboratory’s accreditation Please don’t hesitate to contact me if you have any questions.

Institute of Food, Nutrition and Human Health
Kunenga Private Bag 11222, Palmerston North 4442, New Zealand
Purehuroa T 64 6 3504336 F 64 6 3505557 http://ifnhh.massey.ac.nz
Nutrition Laboratory
TN13-657

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This report may not be reproduced except in full.

*Samples will be discarded one month from date of this report unless otherwise requested by client.*

Fliss Jackson
Manager, Nutrition Laboratory
Institute of Food, Nutrition & Human Health
Palmerston North
DDI 06 350 5869
Email: F.S.Jackson@massey.ac.nz
APPENDIX I

Gastrointestinal symptom reporting questionnaire
(a) Nut-Bread Symptom Reporting Questionnaire

Baseline (Pre-Bread) Time: ________________

Please think about the symptoms you have had in the last 15 minutes and mark on the scale provided the severity of your symptoms.

Please rate your overall rating by placing a vertical mark ( | ) on the line.

1. Belching
   [-----------------------------]
   No Problem   Very Severe Problem

2. Stomach bloating
   [-----------------------------]
   No Problem   Very Severe Problem

3. Stomach cramping
   [-----------------------------]
   No Problem   Very Severe Problem

4. Flatulence
   [-----------------------------]
   No Problem   Very Severe Problem

5. Diarrhoea
   [-----------------------------]
   No Problem   Very Severe Problem
6. Nausea

<table>
<thead>
<tr>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>No Problem</td>
</tr>
</tbody>
</table>

7. Stomach ache

<table>
<thead>
<tr>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>No Problem</td>
</tr>
</tbody>
</table>
Immediately Post-Bread Ingestion  

Time: ____________________________

Please think about the symptoms you have had in the last 15 minutes and mark on the scale provided the severity of your symptoms.

Please rate your overall rating by placing a vertical mark ( | ) on the line.

1. **Belching**
   
   [--------------------------------------------]
   
   | No Problem | Very Severe Problem |

2. **Stomach bloating**
   
   [--------------------------------------------]
   
   | No Problem | Very Severe Problem |

3. **Stomach cramping**
   
   [--------------------------------------------]
   
   | No Problem | Very Severe Problem |

4. **Flatulence**
   
   [--------------------------------------------]
   
   | No Problem | Very Severe Problem |

5. **Diarrhoea**
   
   [--------------------------------------------]
   
   | No Problem | Very Severe Problem |

6. **Nausea**
   
   [--------------------------------------------]
   
   | No Problem | Very Severe Problem |
7. Stomach ache

<table>
<thead>
<tr>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>No Problem</td>
</tr>
</tbody>
</table>


Post-Bread Ingestion – 15 minutes

Time: __________

Please think about the symptoms you have had in the last 15 minutes and mark on the scale provided the severity of your symptoms.

Please rate your overall rating by placing a vertical mark ( | ) on the line.

1. Belching

[-----------------------------]
No Problem | Very Severe Problem

2. Stomach bloating.

[-----------------------------]
No Problem | Very Severe Problem

3. Stomach cramping

[-----------------------------]
No Problem | Very Severe Problem

4. Flatulence

[-----------------------------]
No Problem | Very Severe Problem

5. Diarrhoea

[-----------------------------]
No Problem | Very Severe Problem

6. Nausea

[-----------------------------]
No Problem | Very Severe Problem
7. Stomach ache

<table>
<thead>
<tr>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>No Problem</td>
</tr>
</tbody>
</table>
Post-Bread Ingestion – 30 minutes

Please think about the symptoms you have had in the last 15 minutes and mark on the scale provided the severity of your symptoms.

Please rate your overall rating by placing a vertical mark ( | ) on the line.

1. Belching.
   [-------------------------------]
   No Problem                      Very Severe Problem

2. Stomach bloating
   [-------------------------------]
   No Problem                      Very Severe Problem

3. Stomach cramping
   [-------------------------------]
   No Problem                      Very Severe Problem

4. Flatulence
   [-------------------------------]
   No Problem                      Very Severe Problem

5. Diarrhoea
   [-------------------------------]
   No Problem                      Very Severe Problem
6. **Nausea**  

| No Problem | Very Severe Problem |

7. **Stomach ache**  

| No Problem | Very Severe Problem |
Post-Bread Ingestion – 45 minutes

Please think about the symptoms you have had in the last 15 minutes and mark on the scale provided the severity of your symptoms.

Please rate your **overall rating** by placing a vertical mark ( | ) on the line.

1. Belching.
   ![Scale](image1)
   
   No Problem | Very Severe Problem

2. Stomach bloating
   ![Scale](image2)
   
   No Problem | Very Severe Problem

3. Stomach cramping
   ![Scale](image3)
   
   No Problem | Very Severe Problem

4. Flatulence
   ![Scale](image4)
   
   No Problem | Very Severe Problem

5. Diarrhoea
   ![Scale](image5)
   
   No Problem | Very Severe Problem

6. Nausea
   ![Scale](image6)
   
   No Problem | Very Severe Problem
7. Stomach ache

<table>
<thead>
<tr>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>No Problem</td>
</tr>
<tr>
<td>Very Severe Problem</td>
</tr>
</tbody>
</table>
Post-Bread Ingestion – 60 minutes  

Time: ____________

Please think about the symptoms you have had in the last 15 minutes and mark on the scale provided the severity of your symptoms.

Please rate your overall rating by placing a vertical mark ( | ) on the line.

1. Belching
   |-----------------------------------------------|
   No Problem                                Very Severe Problem

2. Stomach bloating
   |-----------------------------------------------|
   No Problem                                Very Severe Problem

3. Stomach cramping
   |-----------------------------------------------|
   No Problem                                Very Severe Problem

4. Flatulence
   |-----------------------------------------------|
   No Problem                                Very Severe Problem

5. Diarrhoea
   |-----------------------------------------------|
   No Problem                                Very Severe Problem
6. Nausea

| No Problem | Very Severe Problem |

7. Stomach ache

| No Problem | Very Severe Problem |
Post-Bread Ingestion – 90 minutes

Please think about the symptoms you have had in the last 30 minutes and mark on the scale provided the severity of your symptoms.

Please rate your overall rating by placing a vertical mark ( | ) on the line.

1. Belching
   ![Vertical mark]
   No Problem | Very Severe Problem

2. Stomach bloating
   ![Vertical mark]
   No Problem | Very Severe Problem

3. Stomach cramping
   ![Vertical mark]
   No Problem | Very Severe Problem

4. Flatulence
   ![Vertical mark]
   No Problem | Very Severe Problem

5. Diarrhoea
   ![Vertical mark]
   No Problem | Very Severe Problem
6. **Nausea**

<table>
<thead>
<tr>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>No Problem</td>
</tr>
</tbody>
</table>

7. **Stomach ache**

<table>
<thead>
<tr>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>No Problem</td>
</tr>
</tbody>
</table>
Post-Bread Ingestion – 120 minutes

Please think about the symptoms you have had in the last 30 minutes and mark on the scale provided the severity of your symptoms.

Please rate your overall rating by placing a vertical mark ( | ) on the line.

1. Belching
   [-------------------------------------]
   No Problem                      Very Severe Problem

2. Stomach bloating
   [-------------------------------------]
   No Problem                      Very Severe Problem

3. Stomach cramping
   [-------------------------------------]
   No Problem                      Very Severe Problem

4. Flatulence
   [-------------------------------------]
   No Problem                      Very Severe Problem

5. Diarrhoea
   [-------------------------------------]
   No Problem                      Very Severe Problem
6. Nausea

<table>
<thead>
<tr>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>No Problem</td>
</tr>
</tbody>
</table>

7. Stomach ache

<table>
<thead>
<tr>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>No Problem</td>
</tr>
</tbody>
</table>
APPENDIX J

Appetite rating questionnaire
DAY 2
Appetite-Rating Questionnaire

Baseline Pre-Bread

Time: __________

Study ID: __________ Date: __________ Bread Code: __________

Please complete the following five questions immediately BEFORE you consume the bread and rate your overall rating by placing a vertical mark ( | ) on the line.

1. How hungry do you feel right now?

|---------------------------------|
Not at all hungry                Extremely hungry

2. How strong is your desire to eat right now?

|---------------------------------|
Strong desire not to eat         Strong desire to eat

3. How much food could you eat right now?

|---------------------------------|
Nothing at all                   The most that I have ever eaten

4. How full do you feel right now?

|---------------------------------|
Not at all full                  Extremely full

5. Do you have any preoccupation with thoughts of food right now?

|---------------------------------|
No thoughts of food               Very preoccupied, difficult to concentrate
Immediately Post-Bread Ingestion

Time: __________

Please complete the following five questions IMMEDIATELY AFTER you consume the bread.

1. How hungry do you feel right now?

[------------------------]------------------------
Not at all hungry          Extremely hungry

2. How strong is your desire to eat right now?

[------------------------]------------------------
Strong desire not to eat   Strong desire to eat

3. How much food could you eat right now?

[------------------------]------------------------
Nothing at all             The most that I have ever eaten

4. How full do you feel right now?

[------------------------]------------------------
Not at all full             Extremely full

5. Do you have any preoccupation with thoughts of food right now?

[------------------------]------------------------
No thoughts of food         Very preoccupied, difficult to concentrate
Post-Bread Ingestion: 1 Hour

Please complete the following five questions 1 HOUR AFTER you finish eating the bread.

1. How hungry do you feel right now?

Not at all hungry                           Extremely hungry

2. How strong is your desire to eat right now?

Strong desire not to eat                        Strong desire to eat

3. How much food could you eat right now?

Nothing at all                                     The most that I have ever eaten

4. How full do you feel right now?

Not at all full                                             Extremely full

5. Do you have any preoccupation with thoughts of food right now?

No thoughts of food                             Very preoccupied, difficult to concentrate
Post-Bread Ingestion: 2 Hours

Time: __________

Please complete the following five questions 2 HOURS AFTER finish eating the bread.

1. How hungry do you feel right now?
   
   [-----------------------------|-----------------------------]
   Not at all hungry            Extremely hungry

2. How strong is your desire to eat right now?
   
   [-----------------------------|-----------------------------]
   Strong desire not to eat     Strong desire to eat

3. How much food could you eat right now?
   
   [-----------------------------|-----------------------------]
   Nothing at all               The most that I have ever eaten

4. How full do you feel right now?
   
   [-----------------------------|-----------------------------]
   Not at all full              Extremely full

5. Do you have any preoccupation with thoughts of food right now?
   
   [-----------------------------|-----------------------------]
   No thoughts of food          Very preoccupied, difficult to concentrate
Post-Bread Ingestion: 3 Hours

Please complete the following five questions 3 HOURS AFTER you finish eating the bread.

1. How hungry do you feel right now?

I---------------------------------------------------------------I
Not at all hungry                                                                              Extremely hungry

2. How strong is your desire to eat right now?

I---------------------------------------------------------------I
Strong desire not to eat                                                                      Strong desire to eat

3. How much food could you eat right now?

I---------------------------------------------------------------I
Nothing at all                                                                                The most that I have ever eaten

4. How full do you feel right now?

I---------------------------------------------------------------I
Not at all full                                                                              Extremely full

5. Do you have any preoccupation with thoughts of food right now?

I---------------------------------------------------------------I
No thoughts of food                                                                         Very preoccupied, difficult to concentrate

Thank you so much for contributing to this research. We appreciate the time you are giving. 😊
APPENDIX K

Diet record instructions
The Nut Bread Study Food Diary Instructions

Please read through these instructions before starting your food diary.

We would like you to:

• Write down everything you eat and drink, (when you eat and drink it). Please do not rely on your memory at the end of the day.
• Weigh your food and drink using the scales provided.

To get a more detailed picture of your diet, we would like you to:

1. Record everything you eat and drink. Please try not to change what you usually have just because you are keeping a record!

2. Please record your food and drink intake on **Day 2** (Wednesday) and **Day 6** (Sunday).

Thank you for your help and co-operation.
How to fill out your Diet Record:

- Record the amount and description of ALL foods and drinks consumed — all meals and all snacks.
- Begin each new day on its labelled page, (in this case on day 2 (Wednesday) and day 6(Sunday)) and please fill in all the information at the top of the page (the date and day of the week)
- Use a new line for each food or drink. You can use more than one line for a food or drink, but please start each new food or drink on a separate line.
- Also please remember to include any additions to foods, (for example, tomato sauce, salad dressing, gravy).

<table>
<thead>
<tr>
<th>Time of day</th>
<th>Name, brand and cooking method of food or drink</th>
<th>Weight of plate or mug</th>
<th>Weight of food or drink + plate or mug</th>
<th>Weight of leftover + plate/mug</th>
<th>Amount eaten</th>
</tr>
</thead>
<tbody>
<tr>
<td>Please write down the time you had something to eat or drink, including am or pm.</td>
<td><strong>Name</strong>: Describe the food or drink. <strong>Brand</strong>: Name the brand. <strong>Cooking method</strong>: If the food was cooked write down how it was cooked (roasted, steamed, and fried). If the food was coated in something or you added things like sauce or butter please record this. If a recipe was used to make a dish please write “see recipe” and write out the recipe on the page labelled “Recipes”.</td>
<td>Weigh an empty plate or mug using the scales provided, and write down the weight in this column.</td>
<td>1. Place the first food or drink on the plate/mug on the scales. 2. Write down the weight. 3. If you add several foods to the same plate you will need to write down the weight of each food as you add it.</td>
<td>Place the same plate or mug with leftovers on the scales and write down the total weight of the food or drink and the plate or mug, after you have eaten your meal. Please estimate how much of each food was left over (for example, 1 tablespoon mince, half the potato). “Leftovers” are everything that you didn’t eat so please try and scrape everything you didn’t eat back on to the plate and weigh.</td>
<td></td>
</tr>
</tbody>
</table>
How to estimate amounts of food when you can’t weigh them

Please record an estimated amount in the “Name, brand and cooking method of food or drink” column.

- **HOUSEHOLD MEASURES** — Household measures like cups, tablespoons, and teaspoons can be useful. Please tell us whether you used a heaped or level amount.

- **WEIGHTS MARKED ON PACKAGES** — Use the weight marked on canned or packet foods e.g., half of a 220g can of baked beans, one 60g pottle of yoghurt.

- **RULER** — Foods such as cheese, cakes, meat can be measured using a ruler, e.g., slice of luncheon sausage 8cm x 4cm x 1mm (remember to give length, width and depth!).

- **BREAD** — Tell us the brand, number and the size of the slices e.g., sandwich, medium or toast slice.

- **FRUIT** — Tell us whether the piece of fruit is small, medium or large.

Writing Recipes

Please write down:

1. Name of the recipe(s)
2. Amount of each ingredient (for example, 3 medium carrots, 500g lean beef mince etc.)
3. Any water added.
4. The portion of the whole recipe that you ate (for example one quarter (1/4) of the dish was eaten at dinner)

EXAMPLE of a recipe

Name of recipe: Home-made mince recipe

300g of premium lean beef mince (browned in 1 tablespoon light olive oil)

1 small onion, diced 1 small carrot, diced
1 clove garlic, minced ¼ cup of beef stock (Campbells)
2 tbsp (tablespoons) tomato sauce (Watties) 60g diced potatoes
40g diced kumara ¼ cup of frozen mixed vegetables (Watties)
¼ cup water 1 heaped teaspoon white flour

Cooking method: Mince was stewed in a small pot with lid on.

One quarter (1/4) of this dish was eaten by me.
APPENDIX L

Diet record template
## DIET RECORD

### DAY 2

<table>
<thead>
<tr>
<th>Time of day</th>
<th>Name, brand and cooking method of food or drink</th>
<th>Weight of plate or mug</th>
<th>Weight of food or drink + plate or mug</th>
<th>Weight of leftover + plate/mug</th>
<th>Amount eaten</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time of day</td>
<td>Name, brand and cooking method of food or drink</td>
<td>Weight of plate or mug</td>
<td>Weight of food or drink + plate or mug</td>
<td>Weight of leftover + plate/mug</td>
<td>Amount eaten</td>
</tr>
<tr>
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<td></td>
<td></td>
</tr>
<tr>
<td>Time of day</td>
<td>Name, brand and cooking method of food or drink</td>
<td>Weight of plate or mug</td>
<td>Weight of leftover + plate/mug</td>
<td>Weight of food or drink + plate or mug</td>
<td>Amount eaten</td>
</tr>
<tr>
<td>------------</td>
<td>-----------------------------------------------</td>
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<td>-------------</td>
</tr>
</tbody>
</table>

Thank you so much for contributing to this research. We appreciate the time you are giving.
RECIPE DAY 2

Please write down:

1. Name of the recipe(s)

2. Amount of each ingredient (for example, 3 medium carrots, 500g lean beef mince, 1 onion, etc.)

3. Record the amount of water added.

4. The portion of the whole recipe that you ate (for example, 1/4 of the dish was eaten at dinner, etc.)
APPENDIX M

Post-study questionnaire
Study Exit Questionnaire

1. How did you find the amount of bread we provided during the 5 days after each GI and satiety test?
   - [ ] Too much
   - [ ] Just right
   - [ ] Too little
   - [ ] Other, please specify ____________________________

2. Were there any changes in your lifestyle during the study, e.g. diet, exercise?
   ___________________________________________________________
   ___________________________________________________________
   ___________________________________________________________

3. Did you start taking any medication or supplements during the study? Were there any changes in terms of dosage or frequency of your regular medication and/or supplements?
   ___________________________________________________________
   ___________________________________________________________
   ___________________________________________________________

4. Which of the following statements most accurately describes how you consumed the bread provided during the study period:
   - [ ] I usually ate the bread provided in place of the bread I would normally have eaten
   - [ ] I continued to eat as I normally would and usually ate the bread as an extra food
   - [ ] Other, please specify

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5. In the **last two months**, how often did you do *vigorous physical activities*? This refers to activities that take hard physical effort and make you breathe much harder than normal, e.g. aerobics, running, fast bicycling, fast swimming or heavy lifting.

- Never
- Less than once a month
- 1-3 times per month
- Once per week
- 2 – 4 times per week
- 5 – 6 times per week
- Once per day

6. In the **last two months**, how often did you do *moderate physical activities*? This refers to activities that take moderate physical effort and make you breathe somewhat harder than normal, e.g. bicycling at a regular pace, swimming at a regular pace, doubles tennis or carrying light loads.

- Never
- Less than once a month
- 1-3 times per month
- Once per week
- 2 – 4 times per week
- 5 – 6 times per week
- Once per day

*We appreciate your time and effort.* 😊