The Effect of Dairy on Insulin Sensitivity

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Contents

Abstract

Introduction: Insulin resistance is a condition of impaired sensitivity of tissues for insulin. Insulin sensitivity may be modified by modifying diet, which could include increasing dairy consumption. The literature is divided on dairy's effectiveness at reducing the risk of type 2 diabetes. Dairy may also be linked to cardiovascular health, and two common measures of vascular health are pulse wave velocity and augmentation index which examine the stiffness of arteries.

Objective: To recruit participants at risk of developing type II diabetes as well as healthy participants for a 10-week randomised crossover trial to determine if increased dairy intake improves insulin sensitivity and cardiovascular health.

Methods: 28 Participants underwent a ten-week crossover study and were required to eat a high (4-6 serves/day) and low dairy diet (0-0.5 serves/day) for four weeks each, with a two-week break in-between. A Low Dose Insulin and Glucose Infusion test (LDIGIT), a hyperglycaemic clamp, pulse wave velocity and augmentation index test were performed at the end of each four-week period.

Results: 28 people completed the study. No significant differences in insulin sensitivity were detected (10.2 ml $kg^{-1}min^{-1}$/pmol/Lx10^{-3} in the high dairy diet, and 9.4 x10^{-3} in the low dairy diet for the LDIGIT (*P*=0.7)). The hyperglycaemic clamp test had an insulin sensitivity index of 40.4 x 10^{-3} and 34.2 x 10^{-3} ml $kg^{-1}min^{-1}$/pmol/L for the high and low dairy diets respectively (*P*=0.6).

Augmentation index was 6.7 ± 2.6% for the high dairy diet, and 6.4 ± 2.5% for the low dairy diet. No significant differences were found between diets (*P*=0.9). Pulse wave velocity had a median 6.1 and 6.5 m/s in high and low dairy diets respectively (*P*=0.9).

Average glucose concentrations in the LDIGIT were 5.7 and 5.6 mmol/L in the high and low dairy diets respectively (*P* = 0.9). The LDIGIT steady state glucose (120-150 minutes) had concentrations of 5.4 and 5.3 mmol/L (*P*=0.7).

Fasting insulin LDIGIT values were 10.6 and 11.5 pmol/L in the high and low dairy values (*P*=0.52). Steady state insulin values for the LDIGIT were 44.3 and 44.8 pmol/L in respectively (*P*=0.63). Fasting clamp insulin values were 9.7 and 9.7 pmol/L (*P*= 0.6) respectively. Steady state values were 107.3 and 116.2 pmol/L (*P*=0.5)

Conclusion: No significant differences were detected for insulin sensitivity or cardiovascular health markers. For the hyperglycaemic clamp, in order for the 10% difference observed here to be statistically significant a sample size of 113 would be needed.

Chapter One: Introduction

Type II diabetes mellitus is a serious disease which is characterised by sustained hyperglycaemia (≥7 mmol/L at fasting, ≥11.1mmol/L plasma glucose concentrations after a 2 hour oral glucose tolerance test)(Rodbard, 2008) , due to relative insulin deficiency as well as insulin resistance, a condition of impaired sensitivity of tissues for insulin. Insulin sensitivity is defined as the effectiveness of insulin in reducing blood glucose concentration by directly promoting its uptake into muscle and fat cells (Trout et al., 2007). Type II diabetes prevalence is projected to increase from 225 million cases to 552 million by 2030 (Gijsbers et al., 2016). If left untreated and ignored, this will present a serious impact to almost every nation's health care system, where individuals with type II diabetes will be occupying health professionals' time and hospital beds which could have been of greater use for other patients. One way to prevent this is by improving a tissue's insulin sensitivity, and reducing the risk of developing type II diabetes. One possible way to improve insulin sensitivity is through modifying diet, and a hot topic of discussion is to increase dairy consumption. Increased dairy consumption may improve insulin sensitivity in a weight controlled environment, however the literature is divided on this topic. It is therefore necessary to determine if dairy consumption improves insulin sensitivity in a controlled trial. Cardiovascular health is also closely linked in the metabolic syndrome and is also worth investigating to determine if increased dairy consumption can improve pulse wave velocity and augmentation index.

There are many definitions in diagnosing diabetes, and according to the American Diabetes Association, type II diabetes can be defined as a fasting plasma glucose concentration of 7.0mmol/L or greater following at 8 hour fast, or a 2-hour plasma glucose of 11.1mmol/L during a 75g oral glucose tolerance test. "Prediabetes" is a term used for individuals who possess impaired fasting glucose and/or impaired glucose tolerance (IFG and IGT respectively), defined as a fasting plasma glucose concentration of 5.6mmol/L to 6.9mmol/L, and impaired glucose tolerance as a plasma glucose concentration of 7.8mmol/L to 11.0mmol/L (AmericanDiabetesAssociation, 2016).

The risk factors for both type II diabetes and prediabetes are physical inactivity, having a BMI of greater than, or equal to 25 kg/m^2, and having a first – degree relative with type II

diabetes. Other risk factors include being of a “high – risk” ethnicity, which can be of African, Latino, Asian and Indigenous Australian and American women who gave birth to children with a high birthweight, or diagnosed with gestational diabetes. The presence of hypertension is also a risk factor, and as are a HDL cholesterol level of 0.90mmol/L, and/or a triglyceride level of 2.82mmol/L. Women with polycystic ovary syndrome is also at greater risk of developing prediabetes and type II diabetes. Individuals with other conditions such as severe obesity, or insulin resistance, or a history of cardiovascular disease are also at greater risk of developing type II diabetes. The American Diabetes association recommends that testing for both prediabetes and type II diabetes should commence at age 45 years, and should the results be considered normal, scheduled testing every 3 years is required after the initial visit (AmericanDiabetesAssociation, 2016).

The rise in incidence of type II diabetes will also have significant economic, health and social costs. Type II diabetes alone accounts for billions of dollars spent in treating the chronic disease, responsible for approximately 12% of all health care costs worldwide (Pasin and Comerford, 2015). The rapid increase of Type II diabetes incidence has left researchers to conclude that environmental factors are perhaps to blame, and there may be a lower genetic correlation than previously believed (Candido et al., 2013).

Type II diabetes has been implicated in nearly doubling the risk of experiencing a heart attack or stroke, where Type II diabetes had a hazard ratio of 1.97 when associated with fatal cardiovascular disease (1.72-2.25) (Woodward et al., 2003). It is therefore necessary to identify modifiable risk factors for preventing the development of type II diabetes. While type II diabetes does have genetic ties, the chronic disease is influenced by modifiable lifestyle factors, one of which is dietary behaviour. Pasin et al. (2015) state that this phenomenon is relatively new, and in the last few centuries, there was an increase in processed and refined foods, and a reduction in the food staples that have traditionally sustained humans (Pasin and Comerford, 2015). Diabetes complications can also include retinopathy, neuropathy, hypertension and coronary heart disease (Kitzmiller et al., 2008). Lifestyle factors can also be contributors to type II diabetes development, with a shifting trend to enforcing behavioural changes from later in life to earlier in life (Skau et al., 2016)

The development of type II diabetes can be seen as a domino effect – the presence of obesity can predispose individuals to type II diabetes, which can lead to increased risk of developing other chronic illnesses, as well as several cardiometabolic risk factors, such as hypertension

and dyslipidaemia. Another usually later factor, impaired insulin response, can be modified though changing diet and exercise regimes (Pasin and Comerford, 2015). Dietary modification and weight loss are the primary ways to treat these chronic illnesses, however there is conflict present on what is the best nutritional advice and on prescriptive diets for improving glycaemic control.

Metabolic syndrome is defined as a clustering of interrelated risk factors for the development of cardiovascular disease and type II diabetes. These conditions are more likely to occur together as opposed to developing by chance alone (Alberti et al., 2009). The components of metabolic syndrome include dyslipidaemia – defined as raised triglycerides and lowered high – density lipoprotein cholesterol – raised fasting glucose, hypertension and central obesity. Metabolic syndrome has been identified as having a rising prevalence in today's world, and is largely related to increasing obesity and sedentary lifestyles. As a result of this, metabolic syndrome is now a public health and clinical problem which demands attention.

The established risk factors for type II diabetes are overweight, obesity and physical inactivity, and have been well – known, however there is evidence to suggest that dietary factors play a part in increasing or decreasing an individual's risk of developing the chronic disease. A western diet has been indicated in having an association with type II diabetes, and some studies have associated a positive intake of animal protein and fat, and the rate of type II diabetes development in eastern countries (Aune et al., 2009).

While western diets have indicated the presence of an association with an increased risk of developing type II diabetes, it is unclear as to what components of the diet may increase this risk. Dairy intake has been a controversial point of study in this relationship between diet and type II diabetes risk, where epidemiology data suggest that the intake of dairy has an inverse association with type II diabetes risk.

Theoretically, the high content of calcium, magnesium, vitamin D and whey protein found in dairy could reduce an individual's risk of developing type II diabetes, however, intervention data are still undecided on whether this association is true when comparing dietary intake of dairy alone (Turner et al., 2015). Magnesium intake was found to have no association with type II diabetes risk in individual studies, however there was a significant inverse association observed after performing a meta-analysis (Aune et al., 2013).

Epidemiological data suggest that dairy intake is inversely associated with type II diabetes risk in overweight and obese individuals. Despite this finding, intervention studies are largely

divided on the issue of if dairy intake has any effect on type II diabetes development whatsoever. With this controversy, it is therefore necessary to determine if dairy has an effect on type II diabetes development. This may be achieved through carefully reviewing its components as it has several bioactive components, and through conducting an intervention study focussed on total dairy serve intake and its effects on insulin sensitivity. Epidemiological data suggest that in order to combat the rising cases of prediabetes, type II diabetes and metabolic syndrome, there is a dire need of preventative interventions that are required to be carried out in order to determine how to reduce prevalence as much as possible. It is also advised that health systems around the world start preparing for the increase in cases as soon as possible. By determining if dairy intake can affect type II diabetes risk, there is a possibility to treat type II diabetes in an inexpensive and easily implemented way, thus reducing responsibility on the individual to alter behaviour.

Red meat intake is believed to have an association with the increase in risk of developing type II diabetes, although there may be potential confounders contributing to this association observed (Aune et al., 2009). Whole grain, bran and germ intake has been found to have an inverse association with type II diabetes risk, after adjusting for confounders, as well as BMI, and this association was strongest with whole grain intake (de Munter et al., 2007). High cereal fibre intake is inversely associated with type II diabetes risk, however fruit and vegetable fibre intake found to not be associated with type II diabetes risk. A meta – analysis was able to show that a high cereal fibre intake was inversely associated with type II diabetes risk, whereas there were no significant associations for fruit and vegetable fibre and type II diabetes risk after meta – analysis. Magnesium intake was found to have no association with type II diabetes risk in individual studies, however there was a significant inverse association observed after performing a meta-analysis (Aune et al., 2013).

Chapter Two: Literature review

The following literature review covers epidemiological studies, interventions and meta-analyses focussing on dairy consumption and its relation to insulin sensitivity, type II diabetes and cardiometabolic risk factors in a weight-stable setting. Total dairy serve intake was the main area of focus; however, some studies involving the relationship between specific nutrients and insulin sensitivity and type II diabetes were also included in the review, as they were deemed relevant to the area of focus.

The search strategy used to identify relevant studies for this literature review included no time limit on published date of the studies, and included epidemiological and intervention studies that focused on type II diabetes risk or insulin resistance, and the association of effect of dairy on these conditions. Weight stable intervention studies were included in the literature review. Studies that only examined dairy as a vehicle to test other nutrient effects were not included for analysis, as the study carried out as part of this thesis focused on total dairy itself. No limits were placed on study length or sample size of any studies found using this search strategy. Studies that had a conflict of interests, such as funding from industries which would benefit from the outcomes of the study were not included for analysis.

Insulin resistance and methods for measuring insulin sensitivity

A brief summary of the methods to measure insulin sensitivity to be used in this study will now be given.

Trout et al. (2007) states that insulin resistance is a condition that is a component of several health disorders, the most well-known being type II diabetes. Insulin resistant individuals have an impaired biological response to the normal action of insulin, meaning that they have reduced insulin sensitivity. By measuring an individual's sensitivity to insulin it is possible to identify if an individual is at risk of developing type II diabetes, and to initiate changes to the individual's lifestyle to avoid developing the chronic disease.

There are a few methods that can be used to measure insulin sensitivity, and this review will focus on two main methods: the hyperglycaemic clamp technique and the continuous low dose insulin and glucose infusion test (LDIGIT), two methods that were used as part of the study undertaken in this thesis.

The hyperglycaemic clamp technique (also known as the glucose clamp technique) is a method of measuring insulin sensitivity in a patient who is fasting. The goal of the technique is to raise an individual's plasma glucose concentration acutely and to achieve a fixed plateau, with the goal of maintaining this plateau for two hours (DeFronzo et al., 1979). Acutely raising plasma glucose concentration is achieved by using a priming dose, where a fifteen minute period is used to infuse glucose into an individual intravenously. This is calculated according to the individual's body surface area and is performed to raise the plasma glucose concentration and extravascular glucose compartments to a desired level of hyperglycaemia. After this fifteen minute period, the desired plasma glucose concentration is held using a series of maintenance doses, administered by adjusting the infusion rate of

glucose according to the changes in plasma glucose concentration. This is achieved by sampling blood from the individual, measuring the subsequent plasma glucose concentration and adjusting the infusion rate appropriately to maintain the desired plasma glucose concentration. Blood is sampled at fasting, and then every five minutes until 120 minutes has elapsed (DeFronzo et al., 1979). Glucose and insulin concentration is measured in these samples to determine insulin sensitivity. The purpose of the test is also to assess β-cell sensitivity to glucose, as well as to quantify the amount of glucose that the body metabolises in a hyperglycaemic environment.

The positive aspects of this test are that it is safe and simple to perform, however it is labour intensive and requires either two people or one experienced person in order to perform it successfully and safely. There is a very low risk of hypoglycaemia occurring in the individual, unlike the euglycaemic, hyperinsulinaemic test where hypoglycaemia is a potential threat to the individual (DeFronzo et al., 1979).

The LDIGIT is a method of measuring insulin sensitivity that uses a low dose of glucose and insulin via infusion to measure an individual's sensitivity to insulin. A key difference between the LDIGIT and the hyperglycaemic clamp is that the LDIGIT administers an exogenous insulin dose to the individual, whereas the hyperglycaemic clamp relies on endogenous insulin. The LDIGIT is also 2.5 hours in duration, longer than the 2 hours used in the hyperglycaemic clamp (Piatti et al., 1995).

Insulin doses are 25mU/kg/h, and glucose doses were 4mg/kg/min for each participant. Insulin is injected into the glucose infusion bag, and the mixture of glucose and insulin is infused at an amount appropriate for the individual based on their body weight. Blood samples are taken at baseline, at every five minutes for the first 30 minutes, then once every 20 minutes until the final half hour, where samples are taken at every 5 minutes again. The patient does not experience hyperglycaemia as severe as what is experienced in the hyperglycaemic clamp, and the infusion rate remains constant for the entire 2.5 hours. The LDIGIT is designed to measure insulin sensitivity and according to Piatti et al., insulin secretion in the LDIGIT is typically 3 fold lower when compared to the hyperglycaemic clamp technique (Piatti et al., 1995).

Methods for measuring cardiovascular health

Pulse wave velocity is a measure of arterial stiffness, and is defined as the velocity at which the arterial pulse travels through the circulatory system. It is a highly reproducible measure

and is non-invasive and painless to perform on patients. The velocity at which a pressure wave propagates through the vascular tree and returns to the heart is an indicator of cardiovascular health, where generally the stiffer the arteries in a patient, the quicker the pulse wave velocity. This is a marker for declining cardiovascular health, and can be used to adjust lifestyle factors in a patient to improve cardiovascular health (Wilkinson et al., 1998). The pulse wave velocity measure includes volunteer lie in the supine position, and a blood pressure cuff fitted to the right thigh. Measurements between both pulse sites are made (in this case, femoral and carotid), and these measurements were entered into computer software. A tonometer is placed on the patient's neck to detect carotid pulse. After a suitable pulse was obtained, the cuff automatically inflates, and calculates pulse wave velocity based on the difference between both pulse sites.

Augmentation index is another marker of arterial stiffness and is a ratio calculated from a patient's blood pressure waveform (Frimodt-Moller et al., 2008). It is performed by placing a blood pressure cuff on the patient's arm while resting in a sitting position. Blood pressure measurements are taken and augmentation index is obtained from the calculations.

Wilkinson et al. (1998) reported that it is possible to generate a highly reproducible measure to determine both pulse wave velocity and augmentation index (Wilkinson et al., 1998). This is confirmed by Rodriguez et al. (2016) and Frimodt-Møller et al. (Rodriguez et al., 2016, Frimodt-Moller et al., 2008) using the methods described.

Epidemiology –meta-analyses examining the association between dairy intake and the incidence of type 2 diabetes

Chen et al (2014) conducted an updated meta-analysis with the latest data from the Nurses' Health Study, Nurses' health study II and the Health Professionals Follow Up Study.

Nurses' Health Study, Nurses' Health Study II and Professionals Follow up Study pooled analysis

Chen et al. found that the consumption of yoghurt in higher amounts was associated with a decreased risk of type II diabetes, whereas total dairy intake was not associated with type II diabetes risk in multivariate adjusted models, with a HR of 0.99 for one serving/day increase (95% CI 0.98, 1.01). No interaction between total dairy consumption and age, BMI, vitamin D level, physical activity level and diabetes family history were observed in this meta-analysis (Chen et al., 2014).

Chen et al. found that there were no significant associations between high and low fat dairy types and type II diabetes risk. The dairy subtypes examined in this meta-analysis were adjusted for each other in multivariate models, and in a pooled analysis, it was found that each one serving/day increase of skim milk, cheese and whole milk had an associated 2% (95% CI -1%, 4%), 7% (95% CI 0.75, 0.92) and 22% (95% CI 0.71, 0.86) increase in risk respectively (Chen et al., 2014).

The inverse association noted between ice cream and type II diabetes risk was attenuated when the meta-analysis stopped updating dietary information after the participants in the studies covered had reported hypertension or hypercholesterolaemia during each study run, with a HR of 0.89 (95% CI 0.83, 0.96), although this remained significant. The inverse association between yoghurt intake and risk of type II diabetes remained significant with a HR of 0.86 (95% CI 0.78, 0.94) for one serving/day increments, as 244g/day for yoghurt intake. The remaining dairy types (skim milk, cheese, whole milk and ice cream) were attenuated to null with an HR of 1.01 (95% CI 0.99, 1.03), 1.03 (95% CI 0.99, 1.07) and 1.03 (95% CI 0.99, 1.07) respectively (P=0.05) (Chen et al., 2014).

Meta-analysis of all studies

After adding extra studies into the meta-analysis and updating the results, Chen et al. found that total dairy intake was not significantly associated with the risk of type II diabetes, however yoghurt intake was still significantly associated with a lower risk of type II diabetes (Chen et al., 2014). When classifying studies by follow up length, Chen et al. found that the studies with a shorter term follow up (≤10 years) had a marginally lower risk association with type II diabetes, and yoghurt was inversely associated with type II diabetes in both long and short term studies.

Conversely, in earlier meta-analyses, prior to the updating of the Nurses and Physicians Health Study Tong et al. discovered that low fat dairy consumption (10% reduction in risk per serve) had a greater effect on type II diabetes risk when compared to high fat dairy consumption (no reduction) (Tong et al., 2011). Aune (2013) found a similar effect for low fat dairy and no effect for high fat dairy. Aune also found that cheese (8% reduction per 50g/day) and yogurt (22% reduction per 200g) reduced the risk of developing type II diabetes (Aune et al., 2013).

A recent meta-analysis of 22 cohort studies conducted by Gijsbers et al. found that total dairy intake was inversely associated with type II diabetes risk (RR: 0.97 per 200g/day, 95% CI: 0.95, 1.00, *P*=0.04). Gijsbers et al. found that there was a similar but linear inverse association between low fat dairy and type II diabetes (RR 0.96 per 200g/day, 95% CI 0.92, 1.00, *P*=0.072). Nonlinear inverse associations were found for yoghurt intake in this meta-analysis too, with a RR of 0.86 per 80g/day when compared with no yoghurt intake. Ice cream intake had an inverse association with type II diabetes as well, with a RR of 0.81 (95% CI 0.78, 0.85, *P*<0.001) at 10g/day. No incremental benefits were associate with higher intake of ice cream. This meta-analysis found that other dairy types were not significantly associated with type II diabetes (Gijsbers et al., 2016).

All five meta – analyses examined conclude that dairy consumption has an inverse association with type II diabetes risk. Three of the six state that high fat dairy had a greater inverse association with type II diabetes risk, and three found that low fat dairy consumption had greater inverse associations with type II diabetes risk.

Total Dairy - Cohort studies

The following studies either were not included in the meta – analyses above or provided cross-sectional information from the cohort.

Strujik et al. conducted a cohort study examining the associations between dairy consumption and glucose regulation indices, as well as type II diabetes incidence (which is included in the Chen meta-analysis). The study examined the data of 5953 Danish men and women from the Inter99 study, and utilised a 198-item food frequency questionnaire (Struijk et al., 2013).

The median dairy intake during the study was 204g/day. Low fat dairy with a median intake of 155g/day was the largest contributor to total dairy intake in this study. Median dairy intakes were 17g/day for full fat dairy, 188g/day for milk and milk products, 19g/day for cheese and 59g/day for fermented dairy (Struijk et al., 2013).

Strujik et al. found that there were no significant associations between total dairy intake and type II diabetes incidence in this study, with an OR of 0.95 (95% CI 0.86, 1.06). The consumption of any one dairy subgroup individually were not associated with the incidence of type II diabetes either (Struijk et al., 2013). There were no significant associations found between total dairy intake and any measures of glycaemia at 5 years follow up, which

included fasting plasma glucose, 2 hour plasma glucose or HbA1c, (glycated haemoglobin, which reflects an individual's average blood glucose levels over 3 months). Cheese consumption was inversely associated with 2 hour plasma glucose (β=-0.048, 95% CI= -0.095, -0.001). Fermented dairy also had a similar association with fasting plasma glucose (β=-0.028, 95% CI= -0.048; -0.008). Fermented dairy also had an inverse association with HbA1c after adjusting for waist circumference and other covariates in the study (β=-0.016, 95% CI= -0.030, -0.001) (Struijk et al., 2013).

Intake of total dairy, low-fat dairy, as well as milk and milk products were all positively associated with HOMA2-B, with a 0.8-0.9% increase in HOMA2-B per increase in serving of dairy per day consumed. The association was attenuated to a non-significant finding after adjusting for waist circumference (Struijk et al., 2013).

Fumeron et al. (2011) conducted a cohort study involving participants from the Data from the Epidemiological Study on the Insulin Resistance Syndrome (DESIR), and studied the associations between dairy consumption and the incidence of hyperglycaemia and the metabolic syndrome. 3,435 individuals without type 2 diabetes were examined, and a 23-item food frequency questionnaire was used to assess dairy intake.

Fumeron et al. found that the consumption of dairy products had an inverse relationship with the incidence of metabolic syndrome, regardless of definition. As part of this, the consumption of dairy products also had an inverse relationship with impaired fasting glucose and type II diabetes incidence in the 9 year follow up period (Fumeron et al., 2011). The consumption of cheese in particular was negatively associated with metabolic syndrome incidence, and the consumption of dairy products other than cheese was associated with type II diabetes incidence alone (OR: 0.82, 95% CI: 0.71-0.92, *P*=0.02) (Fumeron et al., 2011). When adjusting for BMI, the association between other types of dairy and type II diabetes was no longer significant.

Fumeron et al. concluded that dietary calcium density, as well as cheese and other dairy product intake was associated with a lower incidence of metabolic syndrome.

Total dairy, high fat dairy milk, cheese, or high fat fermented dairy intake was not associated with developing type II diabetes. Low-fat dairy intake was found to be inversely associated with type II diabetes incidence, with a hazard ratio of 0.81 (95% CI 0.66, 0.98), when comparing the highest tertile of dairy intake with the lowest tertile. Adjusting for potential

confounders attenuated this inverse association to non-significance (HR 0.92, 95% CI: 0.73, 1.17) (O'Connor et al., 2014). A significant inverse association was detected with low fat fermented dairy only, with a HR of 0.76 (95% CI: 0.60, 0.99, *P*=0.049) (O'Connor et al., 2014). Yoghurt intake was inversely associated with developing type II diabetes when the results were adjusted for sex and age. (HR 0.65, 95% CI 0.52, 0.83, *P* = 0.017).

O'Connor et al. also found that participants from the EPIC-Norfolk observational study who used yoghurt in place of snacks were associated with a 47% lower hazard of developing type II diabetes. O' Connor et al. concluded that the highest tertile of low-fat dairy intake (80g/day) was associated with a 24% decreased risk of developing type II diabetes. Vitamin K_2 is found in dairy, and has been associated with reduced risk of developing type II diabetes (O'Connor et al., 2014). It is also found primarily in fermented foods (O'Connor et al., 2014).

Eussen et al. conducted a cohort study to determine the association between dairy consumption, and impaired glucose metabolism and type II diabetes. Individuals aged 40-75 years were recruited for the Maastricht study, and were given 253-item food frequency questionnaires to answer, focusing on dairy items, and split between full-fat, semi-skimmed and skimmed products, as well as fermented and non-fermented products. An oral glucose tolerance test was also performed to determine glucose metabolism.

The study found that after adjusting for age and sex, there were significant inverse associations between total dairy, skimmed, fermented dairy and cheese, as well as yoghurt with impaired glucose metabolism. Eussen et al. adjusted further for BMI, physical activity, smoking status, education and energy, vegetable fruit, meat and fish intake, and found that the associations of total dairy product and cheese intake were no longer statistically significant. In comparing the third tertile of intake to the first, the following OR associations remained significant after adjustment; 0.73 (95% CI 0.55-0.96) for skimmed milk, 0.74 (95% CI 0.54-0.99) for fermented products, and 0.67 (95% CI 0.50-0.90) for yoghurt. In a continuous analysis, the OR per 100g increment in fermented products and per yoghurt serving were statistically significant in the age and sex-adjusted models, and was 0.88 (95% CI 0.80-0.97) in the fully adjusted model (Eussen et al., 2016). Semi-skimmed, full fat and non-fermented products, as well as milk, and curd cheese were not associated with impaired glucose metabolism in any models.

There was a significant inverse association between total dairy products, semi-skimmed products, fermented products and yoghurt and type II diabetes, and full-fat products were

positively associated with type II diabetes in this study. In comparing fully adjusted models of intake, there was an OR of 0.50 (95% CI 0.26-0.93 for total dairy product intake, but and increased OR of 2.01 (95% CI 1.16-3.47) for full-fat dairy products. Fully adjusted continuous models showed an OR of 0.76 (95% CI 0.61-0.95) per 100g increment in total dairy product intake, 0.69 (95% CI 0.50-0.94) per 100g increment in fermented products, and 0.47 (95% CI 0.24-0.89) per serving (150ml) for yoghurt respectively. There were no associations between type II diabetes and non-fermented products, cheese and curd intake in this model (Eussen et al., 2016).

Ericson et al. conducted a cohort study to determine the role of dietary fat in type II diabetes. The population data was obtained from the MDC study, a prospective cohort study in the South of Sweden, with 68,905 eligible participants. In this study, 2680 incident cases of type II diabetes were identified in the follow-up. There were no associations observed between dietary content of total fat and incidence of type II diabetes (P=0.24 (Ericson et al., 2015). There was a significant inverse association between saturated fatty acids and type II diabetes (P=0.01), however this association attenuated after adjusting for the intake of high fat dairy (P=0.61). Ericson et al. also found that there was a significant decreased risk of developing type II diabetes at high intakes of short to medium chain saturated fatty acid chains with 4-10 carbons (P<0.001). In higher intakes of saturated fatty acids with longer chain length, palmitic acid (16:0, P=0.10) and stearic acid (18:0, P=0.36) were not associated with type II diabetes. An interaction between intake of n-3 polyunsaturated fatty acids, sex and type II diabetes was observed. Any further monounsaturated and polyunsaturated fatty acids were not significantly associated type II diabetes in the multivariate analysis (Ericson et al., 2015).

Hodge et al. conducted a prospective case-cohort study, consisting of 3737 adults to determine if fatty acid concentrations in plasma and diet could be used as a predictor of diabetes incidence. Blood samples were collected to analyse plasma fatty acid concentration, and questionnaires were used to ascertain the incidence of type II diabetes cases (Hodge et al., 2007).

The study showed that individuals who developed type II diabetes had higher proportions of 18:0, total saturated fat, 16:1n-7, 20:3n-6, 20:4n-6, total n-3 fatty acids, 20:5n-3, and 22:6n-3. These individuals also had lower proportions of 15:0, total polyunsaturated fat, n-6 fatty acids, 18:2n-6, n-6:n-3, *trans* fats, and conjugated linoleic acid at baseline when compared to individuals who did not develop type II diabetes (Hodge et al., 2007). Individuals who

developed type II diabetes had higher intakes of total fat, total monounsaturated fats, 16:1n-7, 18:1n-9, total polyunsaturated fats, n-6 fats, 18:2n-6, 20:4n-6, n-3 fats, 18:3n-3, and *trans* fats, and also had a lower intake of 15:0 at baseline when compared to individuals who did not develop type II diabetes. In terms of ORs, it was found that in model 1 of dietary fatty acid intake, both 16:0 and 18:0, but not saturated fatty acids were associated with higher risk of developing type II diabetes. 16:1n-7 showed a weaker positive association with diabetes. Positive associations with type II diabetes development were observed for 18:1n-9, monounsaturated fatty acids, 18:2n-6, total n-6 fatty acids, polyunsaturated fatty acids, and 18:3n-3 in model 1, however after adjusting for body size, these findings were no longer significant (Hodge et al., 2007). The ratio of n-6 to n-3 fatty acids showed a positive association that was borderline significant, and did not attenuate after adjusting for body size (Hodge et al., 2007).

Of the six studies included for review, four studies found an inverse association between dairy consumption and type II diabetes risk. Two found no associations between dairy intake and type II diabetes. Of these two studies, the one conducted by Ericson et al. found that there were no associations between dairy fat intake and type II diabetes, and the other conducted by Struijk et al. focused on total dairy intake, finding no associations in type II diabetes risk. Both studies varied in follow up period, the amount of dairy eaten, dietary assessment, the cohorts studied and the factors adjusted for in analysis, so there is no readily available explanation for why these studies arrived at different conclusions when compared to the other cohort studies covered in this literature review.

Total Dairy Cohort studies – metabolic syndrome incidence

Metabolic syndrome is defined as the co-occurrence of several known cardiovascular risk factors, which include insulin resistance, obesity, atherogenic dyslipidaemia and hypertension. The conditions are interrelated and share underlying mediators, mechanisms and pathways(Huang, 2009). As Huang et al. state, metabolic syndrome can be used to identify subgroups of patients who have a high risk of developing cardiovascular disease and type II diabetes (Huang, 2009). It is therefore necessary to cover studies that have analysed the possibility of a relationship between metabolic syndrome and dairy intake.

Lutsey et al. investigated the effect of dietary intake on metabolic syndrome development. The population studied was from the ARIC study, a prospective cohort study aimed to investigate the origins of atherosclerosis in a middle aged population (Lutsey et al., 2008). It

was found that the highest quintile of dairy consumption was associated with a 13% lower risk of developing metabolic syndrome when compared to the lowest quintile of dairy product consumption (HR: 0.87, 95% CI: 0.77-0.98) (Lutsey et al., 2008).

Pereira et al. found that in their cohort study that the association seen between dairy and metabolic syndrome incidence was only applicable to overweight individuals. The prospective cohort study used was the CARDIA study, examining the evolution of cardiovascular disease in young adults (Pereira et al., 2002).

The CARDIA study utilised a food frequency questionnaire that assessed food intake from the 28 days previous, and consisted of 700 items. Increasing dairy intake was associated with a reduction in risk of developing insulin resistance syndrome in this population over 10 years, with an OR of 0.29 (95% CI 0.14-0.58, $P<.001$) for the highest category of dairy intake relative to lowest category of intake. Adjusting for healthy propensity score led to an OR of 0.37 (95% CI 0.18-0.79). The individuals not overweight or obese at baseline had an OR of 0.72 (95% CI 0.39-1.34, $P=0.22$) (Pereira et al., 2002).

Elwood et al. conducted a prospective study using the Caerphilly cohort. In this cohort, 2375 men were selected to examine the association between milk or dairy consumption, and metabolic syndrome incidence (Elwood et al., 2007). Seven day weighed food records were used to assess dairy product intake during the study.

Elwood et al. found that men who consumed one pint of milk or more per day had an adjusted odds ratio of 0.38 for metabolic syndrome incidence (95% CI 0.18, 0.78), when compared to men who drank little to no milk in the same time period. Seven day weighed food records indicated similar associations as well, where the odds of having metabolic syndrome in a quarter of the men who drank the highest amounts milk as 0.43 (95% CI 0.20, 0.95) when comparing with men who had the lowest intake of milk in the same study.

Total dairy consumption yielded similar associations, with Elwood et al. reporting an odds ratio of 0.40 (95% CI 0.20, 0.79) for men in metabolic syndrome incidence who consumed the highest amount of dairy products, when compared to men who consumed the lowest amounts. When this association was redefined to include butter and cream, as well as the existing yoghurt, milk and cheese, the OR was 0.44 (95% CI 0.21, 0.91, $P=0.023$) (Elwood et al., 2007). The consumption of dairy did not lead to a significant reduction in the incidence of type 2 diabetes (0.74 (95% CI 0.26, 2.05), nor did milk (0.57 (95% CI 0.20, 1.63) (Elwood et al., 2007).

Louie et al. conducted a cohort study to determine the associations between dairy fat consumption and the incidence of metabolic syndrome and type II diabetes. A 145-item food frequency questionnaire was used to determine dietary intake, and used every 5 years. Louie et al. found that the participants who had higher quartiles of dairy intake had a significantly lower risk of metabolic syndrome (OR 0.41, 95% CI 0.23-0.71, P=0.004) (Louie et al., 2013). There were no significant associations between baseline total dairy consumption and ten-year incidence of metabolic syndrome in any of the models tested in this study. In the fully adjusted model of analysis, participants who reported low or reduced fat dairy intake were associated with a higher risk of developing metabolic syndrome (P_{trend}= 0.043). In the fully adjusted model, there also no associations between any level of dairy consumption or fat content, and risk of developing type II diabetes (Louie et al., 2013). The analysis was stratified further by BMI status. In the base model obese participants, it was found that the participants in the highest quartile of regular fat dairy intake had a significantly reduced risk of developing type II diabetes (OR 0.39, 95% CI 0.22-0.70). This did not persist after adjusting for confounders, or after excluding the 14% of participants who managed to change BMI category after the 10 year follow up. No significant associations were shown when comparing baseline dairy consumption and 10-year incidence of metabolic syndrome. No significant associations were observed for type II diabetes among obese or non-obese subjects with low or reduced fat dairy intake (Louie et al., 2013).

In the Hoorn study of 2064 men and women median diary consumption was found to be 4.1 servings/day. After adjusting for age and sex, it was found that the consumption of dairy products was inversely associated with systolic and diastolic blood pressure, and triacylglycerol concentrations but not BMI. (Snijder et al., 2007).

After adjusting for potential confounders, the association between dairy consumption and lower diastolic blood pressure remained, and dairy consumption was associated with higher fasting glucose concentrations (Snijder et al., 2007). High fat dairy was significantly

inversely associated with BMI, waist circumference, triacylglycerol and insulin concentrations and positively associated with HDL cholesterol concentration (Snijder et al., 2007).

Yakoob et al. conducted a cohort study to determine the association between dairy fat biomarkers and type II diabetes risk (Yakoob et al., 2016). The study used populations from two prospective studies, and studied blood samples from participants from both studies, if

provided. Total plasma and erythrocyte fatty acid concentration was measured in 3,499 men and women, with 71% of this population being fasted for blood samples.

After adjusting for demographics, metabolic risk factors, lifestyle, dietary habits, and other circulating fatty acids, individuals in the highest quartile compared to the lowest quartile of plasma 15:0 had 44% lower risk of diabetes, with a hazard ratio of 0.56 (95% CI 0.37-0.86, *P*=0.01). Plasma 17:0 had a hazard ratio of 0.57 (95% CI0.39-0.83, *P*<0.01), and t-16:1n-7 was 0.48 (95% CI 0.33-0.70, *P*<0.001). The findings were of similar magnitude, and direction in the two cohorts, without statistically significant effect modification by sex. 14:0 was not found to be associated with diabetes risk (*P*=0.36) (Yakoob et al., 2016).

In continuous evaluation, 15:0 had a pooled HR of 0.62 (95% CI 0.46-0.85), 17:0, with a HR of 0.68 (95% CI 0.50-0.91), and t-16:1n-7, with a HR of 0.54 (95% CI 0.40-0.73), as found in the previous categorical analysis, 14:0 was not found to be associated with diabetes risk (Yakoob et al., 2016).

Liu et al conducted a cohort study to determine the associations between calcium and vitamin D intake on the prevalence of metabolic syndrome in middle-aged and older women in the United States of America. The study found a three to five-fold difference in the median intake of calcium or vitamin D between the highest and lowest quartiles of intake.

The study discovered that the prevalence of metabolic syndrome in this population was lower in women who had the highest intake of calcium, and in women who had the lowest intake of vitamin D. Total calcium intake was significantly associated with the prevalence of metabolic syndrome after adjusting for caloric intake, and intake of calcium from either diet or supplements were also associated with a lower prevalence of metabolic syndrome. After adjusting this further for dietary total fat, cholesterol, protein and glycaemic load, the association did not change. An additional adjustment was also performed on vitamin D, and a significant inverse association was observed (Liu et al., 2005).

Da Silva et al. conducted a cohort study on dairy intake and metabolic risk parameters in 233 healthy French-Canadians. This study found that average dairy consumption was 2.5±1.4 servings of dairy per day, split as 1.6±1.3 low fat servings, and 0.90±0.70 high fat servings. There was a strong association found between low fat dairy, total dairy intake, and plasma glucose concentrations, (r= -0.20, p=0.003, r=-0.21; p=0.001 respectively) (Da Silva et al., 2014). After stratifying based on sex, the fasting glucose concentration findings were still

significant in women (r=-0.24; p=0.007), but did not remain significant in men (r=-0.19, p=0.03) (Da Silva et al., 2014).

Low fat dairy intake was also associated with lower systolic blood pressure in women (r=-0.19, p=0.04), and high fat dairy intake was inversely correlated with diastolic blood pressure in men (r= -0.23, p=0.02), however, total dairy intake was not associated with blood pressure in either sex (Da Silva et al., 2014).

Zong et al. conducted a prospective cohort study to determine the associations between dairy intake, type II diabetes risk and cardiometabolic risk factor changes in 2,091 older Chinese men and women, where dairy consumption was assessed as part of a 74-item food frequency questionnaire. After a 6 year follow-up in this study, it was found that the RR for developing type II diabetes was 0.73 (95% CI 0.58-0.92) in individuals who consumed 0.5-1.0 servings of dairy per day, and an RR of 0.67 (95% CI 0.52-0.88) in individuals who consumed >1 serving of dairy per day, following adjustment for age, sex, region, and residence. These associations were not affected significantly by multivariate adjustments, with an RR of 0.71 for individuals consuming 0.5-1.0 servings of dairy per day (95% CI 0.57-0.89) and 0.65 for individuals consuming >1 serving of dairy per day (95% CI 0.50-0.85) in risk of developing diabetes (Zong et al., 2014). This association did not change after including changes in BMI and waist circumference during follow up, but the associations did attenuate when including changes in glucose concentration over time (Zong et al., 2014).

Kirii et al. conducted a cohort study to determine the associations between calcium, vitamin D and dairy intake in relation to type II diabetes. Food frequency questionnaires were used in this study. There was a statistically significant inverse association with calcium dietary intake and the risk of type II diabetes in women, after adjusting for age and area. The odds ratio comparing highest to lowest quartile of intake was 0.74 (95% CI 0.57-0.96). Despite this finding, the association attenuated after adjusting further for potential confounders. The multivariable odds ratio for calcium intake was 0.76 (95% CI 0.56-1.03, P=0.095) among women, with this OR changing to 0.77 (95% CI 0.56-1.04, P=0.116) among women after excluding calcium supplement users. Vitamin D intake was not associated with type II diabetes risk in men or women, and adjusting for saturated fat intake did not alter these results appreciably (Kirii et al., 2009).

Dairy consumption was significantly inversely associated with type II diabetes risk in women, after adjusting for age and area only, OR=0.65 (95% CI 0.49-0.88, P=0.007) for total

dairy products, 0.79 (95% CI0.64-0.97, *P*=0.02) for milk, 0.94 (95% CI 0.68-1.30 *P*=0.71) for cheese, and 0.72 (95% CI 0.55-0.93, *P*=0.04) for yoghurt. These findings were attenuated after a multivariable analysis. No significant associations between dairy and the risk of type II diabetes was observed in men (Kirii et al., 2009).

Seven of the eight cohort studies in metabolic syndrome incidence in this review found that there were inverse associations between dairy intake and metabolic syndrome incidence. One study by Da Silva et al. finding that dairy fat intake was inversely associated with fasting glucose associations, but found no significant associations with blood pressure and higher fat dairy intake when combining sexes together in their analysis. A significant inverse association for blood pressure and dairy fat intake was detected when splitting the cohort by sex. Zong et al. found that greater consumption of dairy was associated with lower cardiometabolic risk in older individuals. More research is needed in this area to confirm the findings here.

Total Dairy - Cross-sectional studies

A cross-sectional study conducted by Ferland et al. found that there was no significant difference between any tertile of dairy intake and cardiovascular disease risk factors, and diabetes in 543 Inuit. A higher prevalence of Inuit participants with metabolic syndrome was observed in the higher tertile compared with the first tertile (10.3% vs 1.6%; $p < 0.001$) (Ferland et al., 2011).

Tucker at al found in 272 middle-aged women that HOMA was significantly higher in the high dairy intake group (0.41±0.53) when compared to the HOMA in the moderate intake group (0.22±0.55), and the low intake group (0.19±0.58, F=6.90, *P*=0.0006) (Tucker et al., 2015). The association found was attenuated when adjusting for confounders, but it remained statistically significant (p=0.03). Average dairy intake was found to be 1.1±1.0 servings/day, low intake was 0.2±0.2 servings/day, moderate intake was 1.0±0.4servings/day, and high dairy intake was 2.4±0.9 servings/day (Tucker et al., 2015).

Ghotboddin Mohammadi et al. conducted a cross-sectional study on dairy intake with metabolic syndrome prevalence and its components in adolescents, involving 785 adolescents from 10-19 years of age (Ghotboddin Mohammadi et al., 2015). A 168-item food frequency questionnaire was used to assess food intake.

The prevalence of metabolic syndrome in this study was found to be 22.2%, with 19% of girls having metabolic, syndrome, and 25.2% of boys developing the syndrome as well. . There were no significant associations between dairy intake and metabolic syndrome prevalence (0.97 (95% CI 0.56, 1.66).

The study found that the prevalence of insulin resistance syndrome was 23.5% in the population studied, and that the proportions of insulin resistance syndrome decreased along with dairy product consumption P=0.04 for trend) which remained significant after multivariate adjustment (Ghotboddin Mohammadi et al., 2015).

Abreu et al. conducted a cross-sectional study into the effects of dairy product intake on cardiometabolic risk factor clustering in adolescents (Abreu et al., 2014). The data for this study was sourced from a 2008 longitudinal study known as the Azorean Physical Activity and Health Study II, conducted in 6 of the 9 Azorean Islands, involving 494 15-18 year old adolescents.

Abreu et al. found that adolescents who had a higher intake of dairy products were less likely to have cardiometabolic risk factors when compared to those who had a lower dairy or milk intake through the study (Abreu et al., 2014). There were no significant associations found when observing total dairy, yoghurt, and cheese intake for this finding (Abreu et al., 2014).

In the Brazilian longitudinal study of adult health, 10,010 individuals had an oral glucose tolerance test unless diabetes had been diagnosed (Drehmer et al., 2015).

An association was found with total dairy intake and post load insulin, as well as with HOMA-IR (Drehmer et al., 2015). Fermented dairy products such as cheese and yoghurt intake was found to be strongly inversely associated with fasting glucose. One serve of cheese was associated with a –lower fasting glucose of 0.21mg/dl (95% CI -0.046, 0.03) – with yogurt 0.29mg/dl 95% CI -1.03, 0.44) (Drehmer et al., 2015). Participants consuming more than 4 serves of dairy per day were found to have a 29% lower risk of having diabetes discovered on OGTT when compared to participants who consumed less than 1 serve of dairy per day (Drehmer et al., 2015). There was a graded inverse association found between total and full fat dairy product consumption including butter, yogurt and cheese and MetScore, but no association between MetScore and low-fat dairy consumption.

Akter et al. conducted a cross-sectional study to determine the associations between dairy consumption and insulin resistance. The data obtained was from 496 eligible participants, and

it was found that total dairy intake was higher in women than in men, although this was not statistically significant (Akter et al., 2013). Dairy intake was not associated with any insulin resistance markers in this study, namely fasting insulin, glucose and HOMA-IR, when comparing any quartile of dairy intake.

After fully adjusting for any covariates, the intake of full-fat dairy products was inversely associated with fasting plasma insulin 3.71µU/ml (95% CI 3.34-4.12, *P*=0.02)

and HOMA-IR (0.86, 95% CI 0.76-0.96, *P*=0.02)

(*P*=0.02 for both). These results remained unchanged after adjusting for calcium intake. Fasting blood glucose was not associated with full-fat dairy intake (Akter et al., 2013).

Bergholdt et al. conducted a cross-sectional study on the associations between milk intake and the risk of diabetes and overweight or obese status. Dairy intake was self-reported from individuals aged 20-100 years and of Danish descent, selecting from a pool of 97,811 individuals (Bergholdt et al., 2015). No pre-existing conditions, nor the absence of any were included as recruitment criteria in this study. The participants were sourced from the Copenhagen general population study, and required all participants to be of Danish citizenship.

Bergholdt et al. found that there were no consistent observational associations between the risk of type II diabetes and drinking milk when comparing across all quintiles of milk intake to individuals who did not drink milk at all. Individuals drinking 1-3 glasses of milk per week, individuals drinking more than 11 glasses of milk per week, and individuals drinking fat free milk had higher HRs for type II diabetes. When including lipid-lowering therapy, hypertension and BMI in this analysis, the results were found to be similar prior to inclusion. The risk estimates for developing an overweight or obese status were similar to those for type II diabetes, however the 95% CI were narrower. Bergholdt et al. conclude that high milk intake was not associated with a low risk of type II diabetes or becoming overweight or obese via lactase persistence (Bergholdt et al., 2015).

Total dairy consumption in the Horn study of 2064 individuals was not significantly associated with metabolic syndrome incidence in this study, after adjusting for confounders. The OR for the second, third, and fourth quartiles of dairy intake was 0.99 (95% CI 0.74, 1.32), 0.90 (0.67, 1.21) and 1.01 (0.74, 1.39) when comparing against the first quartile of dairy intake. After the 5 components of metabolic syndrome were dichotomised, the

association between dairy consumption and fasting glucose concentrations of ≥6.1 mmol/L was significant when comparing the highest quartile of dairy intake with the lowest quartile of intake, with an OR of 1.16 for the 2nd quartile (95% CI 0.83, 1.62), 1.10 for the 3rd quartile (95% CI 0.78, 1.56), and 1.52 for the 4th quartile (95% CI 1.06, 2.18, *P*=0.040). No other consistent trends were observed between diary consumption and the dichotomous variables (Snijder et al., 2007).

Cross-sectional studies were divided in their findings – in the eight studies covered in the review, three studies found that dairy was associated with an increased risk of type II diabetes and metabolic syndrome prevalence, and only one study found a protective association between fasting glucose concentration and dairy intake. The remaining fours studies found no significant associations between dairy intake and the prevalence of type II diabetes or metabolic syndrome. As shown, there is extensive heterogeneity in these findings. Serving sizes and number of servings consumed per day vary significantly. Additional methods of assessing dietary intake to complement the food frequency questionnaires would be a useful tool in finding a common conclusion to this area of research. It is evident that more cross-sectional studies are required in this area to determine the associations between dairy intake and type II diabetes and metabolic syndrome prevalence in these cohorts.

Cross-sectional studies – Branched chain amino acids

McCormack et al. (2013) state that adults with obesity – regardless of if they suffer from type II diabetes or not – experience hyperaminoacidaemia. The amino acids in particular that are raised in this situation are the branched chain amino acids leucine, isoleucine and valine, which are the same branched chain amino acids found in whey and casein, two types of protein found in dairy products, accounting for 20% and 80% respectively of protein (McCormack et al., 2013).

Increases in BCAA concentration may have an effect on glucose homeostasis, as the oxidation of BCAAs spares glucose utilisation in skeletal muscle. McCormack et al. also state that infusion studies in this area of study have found that at significantly increased BCAA concentrations, insulin signalling can be disrupted at a molecular level, inhibiting glucose transport and phosphorylation, leading to lower rates of glycogen synthesis, potentially leading to amino acid induced decreases in insulin sensitivity. This can lead to an increased risk of developing type II diabetes. Given that these findings were discovered in infusion study designs, it is a priority to determine how BCAA intake in the diet can affect

glucose homeostasis and insulin sensitivity, as individuals would typically consume a diet which would contain a lower concentration of BCAA when compared to amounts found in a BCAA infusion. The amount of BCAA in an individual's diet is of great importance to determine if increased amounts in a diet will have similar effects on insulin sensitivity in an individual.

Wurtz et al. conducted a cohort study examining if branched chain and aromatic amino acids could be used as predictors of insulin resistance in young adults. The participants were sourced from the Cardiovascular Risk in Young Finns Study. A total of 1,809 individuals were eligible for this study. Blood pressure, BMI, smoking status and physical activity were measured in each participant, and blood samples were obtained following a 12 hour fast (Wurtz et al., 2013). Branched chain and aromatic amino acids were associated with HOMA-IR when adjusting for conventional metabolic risk factors. These associations were more pronounced in men (β=0.24) when compared to the associations found in women (β=0.12)

In observing glycaemia, it was found that amino acid intake was associated with baseline figures, however, the magnitude of association was less when comparing to the magnitude for HOMA-IR. This study found that none of the branched chain and aromatic amino acids were predictors of 6 year fasting glucose in the young adults observed (P>0.05), which was in contradiction to the HOMA-IR results obtained. The only finding that was significant was the association between glutamine and 6 year glucose in women (P=0.005) (Wurtz et al., 2013).

McCormack et al. conducted a cross-sectional and longitudinal study into the associations between amino acid concentrations and obesity and future insulin resistance in children and adolescents. The researchers aimed to determine if circulating levels of branched chain amino acids were associated with paediatric obesity, and if it could be used as a predictor of future insulin resistance (McCormack et al., 2013).

The study recruited 69 healthy subjects, aged 8-18 years. In the cross-sectional cohort, 52% of the participants in this cohort were overweight or obese (McCormack et al., 2013). Three day food records were used to assess dietary intake, and the Modifiable Activity Questionnaire was used to assess physical activity.

McCormack et al. found no associations between circulating branched chain amino acid concentration and insulin resistance, expressed as HOMA or WBISI in this cohort. There were no associations between dietary intake of branched chain amino acids and their plasma concentrations after an overnight fast. BCAA concentrations were not related to race,

ethnicity, daily caloric intake, self-reported physical activity or inactivity, type II diabetes family history, Tanner stage or IGF-I level (McCormack et al., 2013). In the longitudinal cohort, none of the participants had impaired fasting glucose, or impaired glucose tolerance at baseline. None of the participants developed diabetes mellitus and five developed impaired fasting glucose and/or impaired glucose tolerance at final measurements. There was a strong association between BCAA concentration at baseline, and HOMA-IR at 18 months (r^2= 0.44, *P*= 0.004). After using a multivariate model adjustment, it was found that absolute HOMA-IR was independently associated with BCAAs measured at baseline, when adjusting for sex, BMI Z-score, and Tanner stage (McCormack et al., 2013). This relationship also remained significant after HOMA-IR was added to the model (*P*=0.01). the relationship between BCAAs at baseline and HOMA-IR at 18 months was also statistically significant after adjusting for a summary measure which reflected the concentrations of all other amino acids at baseline. The baseline concentrations of BCAAs predicted a deterioration in HOMA-IR over time in subjects who previously reported a normal HOMA-IR (less than 2, r^2= 0.40, *P*=0.02, n=13). This effect was not evident when including subjects with an already elevated HOMA-IR (*P*=0.33). Valine and leucine in particular at baseline were significantly associated with HOMA-IR at 18 months (McCormack et al., 2013).

Qin et al. conducted a cross-sectional study in multiple populations, to determine the association between dietary BCAA intake and risk of overweight/obesity among multi-ethnic populations. The cohort studied consisted of 4429 men and women, aged 40-59 years (Qin et al., 2011)

In this cross-sectional study, Qin et al. found that the participants who had the highest intake of BCAAs were most likely to be male, and also had higher total protein, animal protein and fat intake, as well as lower carbohydrate, starch, and total energy intake. BCAA intake was found to be inversely related to BMI and weight status, after adjustment for age, gender, country, employment status, physical activity, smoking status, special diet and intakes of total energy, total carbohydrate, saturated fat and total protein. The OR between weight status and BCAA intake after multivariate adjustment was 0.70 (95% CI 0.57-0.86, *P*<0.01) when comparing the highest quartile of BCAA intake with the lowest quartile of intake (Qin et al., 2011). After removing countries with the lowest prevalence of overweight and obese individuals, the OR changes to 0.75 (95% CI 0.58-0.98, *P*=0.03) when comparing the fourth quartile of intake with the first quartile. This significant inverse association prevailed for western countries in this study, and for the United Kingdom separately. For the United States,

this inverse association remained, but reached non-significance as the trend continued (Qin et al., 2011)

Yamada et al. conducted a cross-sectional study to determine the association between insulin resistance and plasma amino acid profile. A total of 94 Asian men and women were recruited for this study, and were required to provide a fasting blood sample for analysis (Yamada et al., 2015). Several amino acids were found to be positively correlated with HOMA-IR in this study, and in particular, branched chain amino acids were positively associated with insulin resistance, though in males specifically, the association between valine and insulin resistance did not reach statistical significance. Total BCAA was also significantly positively associated with HOMA-IR in both genders (Yamada et al., 2015).

Of the four studies included in this review for BCAAs in cross—sectional studies, three found a positive association between insulin resistance and BCAA intake, and one found an inverse association between BCAA intake and obesity. Given that the three studies that found a positive association focused on insulin resistance as an endpoint, and one study focused on weight status, it can be determined that BCAA intake may be associated with insulin resistance. The three studies vary by follow-up time amount and cohort size, as well as BCAA intake amount, yet all found positive associations with insulin resistance. More cross-sectional studies will need to be conducted to determine if this association between BCAA intake and insulin resistance persists. It is also important to explore the relationship between BCAA concentrations in the blood and type II diabetes risk and determine if there is an association present.

Cross-sectional studies – Fat

Finucane et al. state that intricate cellular mechanisms are what contribute to obesity and type II diabetes, where a critical step is to develop insulin resistance. Insulin resistance that is associated with obesity is the result of networks that connect metabolic and inflammatory processes, in situations where nucleotide binding and oligomerisation domain-like receptor family pyrin domain-containing 3 (NLRP3) inflammasome acts as a key regulatory hub. It us unknown as to what mechanisms are used when different fatty acids regulate NLRP3 inflammasome activity and metabolic dysfunction in adipose tissue.

It is known that saturated fatty acid (SFA) high fat diets enhance the cytokine protein known as interleukin -1β (IL-1β))–mediated adipose inflammation and insulin resistance, however

the mechanism by which different fatty acids can regulate IL-1β, and the effects this cytokine protein has on adipose tissue and insulin sensitivity is an area that requires more focus in research. Finucane et al. state that there is a relationship present between NLRP3 and IL-1β in the setting of peripheral insulin resistance and Type II diabetes development (Finucane et al., 2015). IL-1β release is triggered via a two – step signalling mechanism, where the macrophage is first primed to produce pro-IL-1β via the activation of Tol-like receptor 4 (TLR4). The NLPR3 inflammasome then mediates the maturation of pro-IL-1β to IL-1β through caspase-1-dependent processing. It is also known that saturated fatty acids (SFA) can stimulate the first priming step, with palmitic acid being a trigger SFA for this first step (Finucane et al., 2015).

According to this pathway, SFAs, (such as the SFAs found in dairy products) could help to trigger the production of pro-IL-1β, which matures to IL-1β, and is released by the macrophage, and disrupt insulin signalling, leading to insulin resistance. Finucane et al. investigated the effect of different fatty acids on IL-1β maturation and subsequent secretion in an animal and human model. Using C57BL/6 mice, three diets were given; a SFA high fat diet (HFD), MUFA HFD, or a standard chow.

The human study consisted of 160-184 participants suffering from T2D were examined from the CORDIOPREV study, and categorised on baseline fasting plasma SFA and MUFA concentrations.

The study found that enriching HFDs with MUFA can help to improve insulin sensitivity, reduce adipose IL-1β-mediated inflammation, and lead to promoted adipose hyperplasia, when compared to the SFA enriched HFDs (Finucane et al., 2015). MUFa HFDs did not lead to the priming of IL-1β in this study, and was also coupled with reductions in active IL-1β active protein concentrations, and ATP induced IL-1β secretion. The consumption of oleic acid, the MUFA most abundant in dairy, was found to prevent ATP-induced IL-1β secretion (maturation of pro-IL-1β to IL-1β) in an *in vitro* setting, suggesting that MUFA can impede with NLPR3 inflammasome activation (Vandanmagsar et al., 2011, Finucane et al., 2015).

Rosell et al. conducted a cross-sectional study to determine the associations between the intake of dairy fat, calcium and abdominal obesity. Healthy men were used in this study, aged 63 years and 301 individuals were included for analysis. The participants recruited had no previous diagnoses of cardiovascular disease, and no pharmacological treatment of diabetes,

hypertension, or hypercholesterolaemia. In terms of dietary assessment, 7 day weighed food records were used (Rosell et al., 2004).

Rosell et al. reported that dairy intake was inversely associated with sagittal abdominal obesity only in the URs, and not the non-URs. Similar effects were seen when comparing myristic acid intake, however this association was not as strong as indicated in total dairy fat intake. Inverse correlations can be seen between sagittal abdominal obesity and the energy-adjusted intake of calcium, where this association was found in URs and non-URs. When controlling the relation between sagittal abdominal obesity and dairy fat (g/100g fat) with calcium, the correlations were -0.06 (P=0.28), -0.24 (P=0.025), and 0.04 (P=0.60) in all URs and non-URs respectively (Rosell et al., 2004).

Kratz et al. (2014) conducted a cross-sectional study that compared 17 participants with non-alcoholic fatty liver disease with 15 controls that were BMI and age – matched. It was found that phospholipid 17:0, phospholipid *trans*-16:1n-7, FFA 15:0 and FFA 17:0 were inversely associated with fasting glucose concentrations, after adjusting for BMI, sex and age (Kratz et al., 2014) the effect was attenuated when adjusting for liver: spleen ratio, which was used to determine the amount of liver fat present in each participant. The biomarkers 17:0 and 15:0 were found to be inversely related to AUC glucose after adjusting for age, sex and BMI, in phospholipid and free fatty acid, and it was found that *trans*-16:1, n-7 concentrations in plasma phospholipids were positively associated with systemic insulin sensitivity in both low and high level infusion tests conducted. (Kratz et al., 2014).

None of the 6 biomarkers of fat intake were associated with β-cell function. Dietary differences could not be a viable explanation for these, as dietary components of both the non-alcoholic fatty liver disease and control participants were similar when referring to dietary records.

The increase in dairy intake was associated with lower fasting glucose concentrations, improved glucose tolerance, higher insulin sensitivity, in hepatic and systemic environments, and less liver fat. The study found that the biomarkers of dairy fat intake and glucose tolerance were attenuated when adjusting for liver fat, or insulin sensitivity measures, but β-cell function was not affected by this adjustment. Kratz et al. suggest that the relation between dairy fat intake and glucose tolerance may be mediated through greater insulin sensitivity because of a reduced amount of liver fat in individuals who consume greater dairy fat (Kratz et al., 2014).

Of the six studies examined, four studies concluded that dairy fat intake was inversely associated with type II diabetes risk. One study from this subgroup concluded that dairy fat intake was inversely related to obesity. The remaining two studies found no difference between dairy fat intake and type II diabetes risk.

Of the three studies found for fat intake, one study found an inverse association between dietary MUFA intake on insulin sensitivity, and the remaining two found no significant associations for dairy fat and obesity or β-cell function. The two studies that did not find significant associations examined dairy fat specifically and did not find a significant association with obesity or β-cell function, whereas the remaining study examined MUFA and was not specific to dairy. MUFA may be associated with improved insulin sensitivity, but it is unclear as to whether dairy sources of fat will have the same conclusions made. It is therefore necessary to determine this association by conducting more studies in this area to determine the association between dairy fat and type II diabetes risk.

Total Dairy - Interventions

Drouin-Chartier et al. conducted a randomised crossover study to determine the impact of milk consumption on cardiometabolic risk factors associated with metabolic syndrome in postmenopausal women with abdominal obesity. Twenty-seven women with abdominal obesity were recruited to this study, and subjected to a 4 week run-in period, during which, 1.4 servings per day of 2% fat milk per 2000kcal were given to the participants to consume. The participants were then randomised to one of two treatments; one containing 3.2 servings per day of 2% fat milk, per 2000kcal, and another treatment without milk or other dairy (Drouin-Chartier et al., 2015).

Participants were required to experience each treatment for a 6 week period, undergo a washout period of 6-8 weeks during which they were required to consume 1.3 servings of 2% fat milk per day, and then experience the other treatment for a period of 6 weeks. The participants were required to be weight stable during the study. The investigators also provided participants with meals during the dietary controlled phases of this study, and were instructed to consume all food provided to them, and only to consume what was provided to them during these times. During the week, lunch was consumed under supervision of registered dietitians, and breakfast, dinner and weekend meals were packed and consumed later at home. Checklists were given to account for everything consumed, and deviations to

the diet were reported as they occurred. These measures resulted in a compliance rate of >98% after analysis. Energy and dietary intake was assessed at screening using a food frequency questionnaire, and all participants began the study at a predetermined energy intake value. This value was redefined for individuals who experienced a weight change of more than 1kg. The use of vitamin supplements, and consumption of alcohol was forbidden during experimental periods, and the use of caffeinated beverages was limited to two drinks per day. The diets in each experimental period contained 29% of daily energy from fat, 10% from saturated fat, 55% from carbohydrates and 17% from protein (Drouin-Chartier et al., 2015).

Blood lipids, cholesterol homeostasis, glucose homeostasis, systemic inflammation, blood pressure and endothelial function were measured in this study. It was found that the milk and control diet reduced plasma fasting glucose concentration in a similar magnitude when comparing with diet-specific baseline values (between-diet P=0.22). No significant differences between glucose homeostasis or insulin sensitivity indices between treatments. The study conducted here was deemed to be carefully controlled and the results found in this were consistent with studies conducted in this area as well (Drouin-Chartier et al., 2015, Benatar et al., 2013b). Drouin-Chartier et al. state that previous literature suggests intake of high fat dairy is what is most likely to increase plasma glucose concentrations. The study conducted here led to decreased fasting plasma glucose concentrations, however this was not a significant effect, and not exclusive to the milk group alone (Drouin-Chartier et al., 2015). A similar effect on HOMA insulin sensitivity index was also found. This is in contradiction to other clinical studies in this area, as other studies have found that the consumption of dairy and cheese has been associated with a reduced risk of developing type II diabetes, although Drouin-Chartier et al. suggest that there may be a residual confounding associated with milk and dairy intake with epidemiological studies, and this is perhaps why there are associations with reduced type II diabetes risk (Drouin-Chartier et al., 2015).

Rideout et al. found that consuming up to 4 serves of dairy per day can improve fasting plasma insulin by 9%, and insulin resistance by 11% (HOMA-IR) in overweight and obese individuals compared with consuming 2 serves of dairy per day in 23 volunteers (Rideout et al., 2013). This was in conflict with the results found by Dugan et al., reporting no detectable difference in insulin resistance. Key differences set these studies apart; such as a 6 week study length, as opposed to a 12 month long study, as conducted by Rideout et al.

Dugan et al. examined individuals suffering from metabolic syndrome. Despite the insulin resistance findings, Dugan et al. was able to find a small yet significant decrease in fasting plasma glucose concentration in the dairy group, when compared to the carbohydrate control. The dairy diet did not significantly change the number of metabolic syndrome markers, the diet did change metabolic parameters slightly when comparing by gender. Women experienced lower markers after dairy intake, but this could be attributed to a weight loss of 1.1kg on average (Dugan et al., 2014). Stancliffe et al. conducted a 12 week study investigating dairy's effects on oxidative and inflammatory stress, with insulin sensitivity as a side-measure, finding that a serving of >3.5 serves of dairy had a significant reduction in plasma insulin after 1 week of the intervention period compared with <0.5 servings per day of dairy. The effect continued until the end of the study. This effect was also demonstrated in HOMA (Stancliffe et al., 2011).

Milk and milk proteins were the focus of study in Arnberg et al., where 203 adolescents consumed either 1L of skim milk, whey, casein or water for 12 weeks, and it was confirmed that increasing whey and casein intake led to increased insulin secretion (Arnberg et al., 2012). C-peptide response increased by 14% in the whey group (P= 0.005), and 17% in the casein group (P=0.009), when comparing to the skim milk and water groups, which has been found to be in conflict when compared to the lower C-peptide concentrations observed in longer term studies with this supplementation(Arnberg et al., 2012). When comparing to the plain drinking water group, there were no significant differences in C-peptide concentration, HOMA or plasma insulin concentration for the milk groups observed (Arnberg et al., 2012). HOMA increased by 23% within the whey group (P=0.021) and 32% in the casein group when compared to skim milk and water (P= 0.006).From 0 to 12 weeks, plasma glucose concentration increased only in the whey group (P= 0.017). No differences were observed between the milk group and water group for plasma insulin, HOMA or plasma C-peptide. Consuming 3 serves of dairy per day for a period of 6 weeks was associated with differences in fasting compared with sugar sweetened beverages but not compared to baseline.

Dairy product intake has also been shown to lead to a greater insulin response when followed by consuming a standardised food *ad libitum*, as indicated by Dougkas et al. (2012) who examined the effect of dairy products on appetite and energy intake, with insulin and glucose response as side measures.in a randomised crossover trial lasting four weeks. Participants were expected to consume servings of dairy snacks or water 120min following breakfast.

No differences in plasma amino acid concentration was found in this study between dairy snacks or water consumption. Glucose concentrations were not different across the four treatments, however insulin concentrations were higher after dairy intake compared with the control (Dougkas et al., 2012).

Tanaka et al. conducted a 24 week randomised parallel trial to determine the effects of dairy consumption on waist circumference, blood pressure, fasting blood sugar and lipids. The participants were 200 Japanese men, aged between 20-60 years, and were randomised to either the dairy consumption group, or the control group (Tanaka et al., 2015).

The dairy consumption group were given home deliveries of milk and dairy products for 24 weeks, at no expense to the participant. The amount of dairy consumed was 400g/day in this group. Participants in both groups received dietary counselling focused on weight stability from registered dietitians. The participants in the dairy consumption group were given a choice to consume either 400g/day of milk, or a combination of milk and yoghurt for the 24 week duration. Food records were used throughout the length of the intervention period (Tanaka et al., 2015).

A total of 200 men finished the study, and it was found that after 24 weeks, waist circumference, fasting blood glucose, body weight, body fat percentage, HbA1c, LDL-cholesterol and total cholesterol decreased significantly in both groups. HDL cholesterol was unchanged in the dairy group, but was increased in the control group. In the areas of waist circumference, HDL-cholesterol, weight, body fat percentage, HbA1c and LDL-cholesterol, there was a significantly smaller degree of improvement in the dairy consumption group when compared to the control group (P=0.99) (Tanaka et al., 2015).

A study conducted by Turner et al. required participants to consume two diets for four weeks each as part of a randomised crossover study to determine the effects of red meat and dairy on insulin sensitivity (Turner et al., 2015). One diet required participants to consume red meat, and the other diet required participants to consume dairy. After consuming each diet for a four-week period and undergoing an OGTT, Turner et al. found that participants consuming the red meat diet had similar insulin sensitivity to the control meal consumed prior to starting the study. The same participants showed that their insulin sensitivity was worse after consuming the dairy diet, when compared to the red meat diet (HOMA-IR 1.33±0.8 for red meat, 1.71±0.8 for dairy diet, P<0.01). Fasting insulin was significantly higher in the dairy

diet when compared to the red meat diet (6.64±4.1 mU/L for dairy diet, 5.47±2.4 mU/L for red meat diet, $P<0.01$).

Turner et al. also conducted an acute study on the effect of red meat and dairy on glucose and insulin. Forty-three participants were required to consume two different meals, one as dairy and the other as red meat, as two visits separated by one week. Turner et al found that lean red meat and low fat dairy had similar glycaemic responses. An insulinotropic effect of dairy, as observed in other studies was not evident in the study conducted by Turner et al (Turner et al., 2016).

Of the nine total dairy studies examined, three studies reported an improvement in insulin sensitivity or glucose response. One of these studies reported an improvement on HOMA when participants consumed whey protein, but insulin and C-peptide did not change significantly in this study. Two studies were found to be detrimental to insulin sensitivity, where Dougkas et al. found that increased dairy intake led to an increased insulin response, which can eventually lead to insulin resistance if left unchecked, and Turner et al. found that dairy intake had a lower insulin sensitivity compared to the red meat diet. The remaining four studies found no effect of dairy intake on insulin sensitivity, where Tanaka et al. found that total dairy intake had a significantly lower degree of improvement when compared to the control diet, and Drouin-Chartier et al. found that there may be a potential residual confounding in the epidemiological studies published so far, which may explain the contradiction in study findings between interventions and epidemiological studies. Turner et al. found that there were no differences between dairy and red meat intake in an acute setting. From these intervention studies, it is unclear if increasing dairy intake will have a beneficial effect on insulin sensitivity.

Benatar et al. conducted a randomised parallel trial on the effects of dairy food intake on cardiometabolic risk factors involving 176 healthy volunteers. The volunteers were randomised to one of three possible study arms: increased dairy, reduced dairy or no change for the course of 1 month. The increased dairy group were asked to consume an extra 2-3 servings per day, and to change to high-fat milk, as well as dairy solids, being high in fat (Benatar et al., 2013a). The decreased dairy group were instructed to eliminate all possible sources of dairy, with rice milk or soya alternatives suggested. Fasting blood samples were taken at baseline and at the end of the 1 month period. HOMA was calculated from these fasting blood samples.

Benatar et al. found that there were no clinically or statistically significant changes in blood pressure, heart rate, low or high density lipoprotein, triglycerides, C-reactive protein, glucose, insulin, and insulin resistance in this study after adjusting dairy intake for 1 month (Benatar et al., 2013a).

Total dairy interventions in this area are mixed in terms of their findings – seven found no differences in measures of glycaemia or insulin resistance, two found significant differences and one found that dairy consumption was worse for insulin sensitivity when comparing against a red meat diet. The servings consumed in each study varied, but the studies that have found either no associations or found that dairy was beneficial used similar amounts of dairy. Study types were common across all three study conclusions as well; therefore these aspects of design are not the cause for the heterogeneity observed in this area. The minimum study length for a study to have detected a significant positive effect on insulin resistance was 12 weeks. Studies have ranged from 4 weeks to 12 months, and the shortest studies (4 weeks in Turner et al., 4 weeks in the Dougkas et al. study) were all studies that found no significant differences or found that dairy intake was worse. It may be possible that study length was a factor in this, or at least partially contributes to the results seen here. Due to the heterogeneity present in these results despite similar study designs it is unknown if dairy has or does not have an effect on insulin resistance, and it is necessary to conduct more research in this area.

Total Dairy Interventions - Protein and BCAA

Chiu et al. conducted a study on 158 overweight men and women, examining the effects of a high vs moderate intake of protein on insulin action and lipoprotein concentrations in high and low saturated fat diets. The participants recruited to this study were assigned to follow one of four diets, for a total of four weeks; 1) high protein, 2) high protein, low saturated fat, 3) moderate protein, high saturated fat, or 4) moderated protein, low saturated fat. The participants were monitored to remain weight stable for the duration of the study. After each diet, the participants were subjected to an FSIVGTT (Chiu et al., 2014).

It was found that fasting plasma glucose concentrations increased significantly after consuming the high protein diet, when comparing to the moderate protein diets. A significant interaction between protein and saturated fat intake was observed in this study (P= 0.001), to the point where there was a significant increase in plasma glucose concentrations when

comparing high to moderate protein intake, when saturated fat intake was low (Chiu et al., 2014).

BCAA concentration was found to be positively correlated with fasting plasma insulin and glucose concentrations, and with HOMA-IR score as well (Chiu et al., 2014). BCAA intake was negatively correlated with acute insulin response to glucose and disposition index, but not the metabolic clearance rate of insulin, or insulin sensitivity. Changes in BCAAs were associated inversely with changes in metabolic clearance rate of insulin, and acute insulin response to glucose and disposition index. The study found no significant effects of protein intake on insulin sensitivity in a short-term setting when paired with reduced carbohydrate content in overweight and obese individuals who do not have type II diabetes (Chiu et al., 2014).

Takeshita et al. reported that 12 weeks of participants taking BCAA for 12 weeks led to no differences in glucose metabolism after testing each participant with an OGTT and a hyperinsulinaemic, euglycaemic clamp. Lipid profiles were not significantly different either (Takeshita et al., 2012). After stratifying the results however, BCAA supplementation was found to lead to improved glycaemic control in patients with a lower Matsuda index. . Percentage changes in HbA1c tended to also be correlated with percentage changes in Matsuda index (r= -0.405, *P*=0.69). No other insulin resistance indices were able to significantly predict HbA1c values. As this study found that BCAA supplementation may improve skeletal muscle insulin resistance, the findings in this study suggest that BCAA supplementation may be effective in changing glycaemic control in patients with type II diabetes, characterised by skeletal muscle insulin resistance (Takeshita et al., 2012).

Kalogeropoulou et al. reported that leucine ingestion, couples with glucose ingestion, led to a glucose concentration of 6.3mmol/L, which was lower than that of the 7.3mmol/L after 50 minutes. Leucine ingestion alone did not affect the glucose concentration significantly. Glucose area response was attenuated when ingesting glucose and leucine together, and insulin response after ingesting this combination led to a 72% greater response in insulin concentration when compared to glucose ingestion alone (Kalogeropoulou et al., 2008). After conducting a crossover trial using two types of soy milk and low fat dairy milk, Gardner et al discovered no detectable differences in insulin AUC or glucose at 0, 1 or 2 hours for any of the milks consumed, when comparing for treatments, or when comparing to baseline (Gardner et al., 2007) Breitman et al. study found that the mean fasting glucose of the participants decreased significantly from 113.1± 14.4mg/dl at baseline to 95±15.1mg/dl and 95±9.8mg.dl at 2 and 8 weeks (*P*<0.0001). Mean fasting insulin (16.8±7.6µU/ml to

12.6±16.8μU/ml) and C-peptide levels (3.2±1.1ng/ml to 2.5±1.8ng/ml) also decreased, however this effect was only significant in the C-peptide findings (*P*=0.017) and not insulin (*P*=0.2) (Breitman et al., 2011).

In the five protein interventions covered for this review, two studies found that BCAA led to improved insulin sensitivity and glycaemic control. One study found that BCAA increased insulin significantly, and two studies found no effect of BCAA on insulin sensitivity or glycaemic control. With the heterogeneity present in this study, it is necessary to conduct further research in this area to conclusively determine the effect of BCAA on insulin sensitivity.

Total Dairy Interventions - Fat

Nestel et al. conducted a study to determine whether certain phospholipid species and fatty acids associated with full-fat dairy consumption may also be linked to diminished insulin resistance. A total of 86 middle-age men and women were recruited for this study, and were classified as overweight, with a BMI of >27kg/m^2. The participants also possessed additional metabolic factors found to be consistent with metabolic syndrome. Waist circumference was ≥102cm in men, and ≥88cm in women. The participants each possessed two additional criteria for metabolic syndrome. Four day weighed food records were used to assess dietary intake. Dairy servings in this study are defined as 250ml of milk, 200g of yoghurt, 30g of butter, 40g of cheese, and 50g of ice-cream (Nestel et al., 2014).

Insulin sensitivity and resistance was assessed using a 75g oral glucose tolerance test, with fasting plasma glucose and plasma glucose response, plasma insulin, plasma insulin AUC, HOMA-IR and Matsuda index used to determine insulin sensitivity and resistance.

Nestel et al. found that there were several lipid classes directly associated with the number of dairy servings consumed in this study. Significant positive associations were found for lysophosphatidylcholine, which contains 15:0 and 17:0 (*P*<0.001, which remained significant at *P*<0.01 after adjusting for multiple comparisons) (Nestel et al., 2014). After adjusting for age, sex, waist: hip ratio or BMI and systolic pressure, it was found that lysophosphatidylcholine and lyso-platelet-activating factor were directly associated with Matsuda index (*P*<0.01 when adjusting for waist: hip ratio, *P*<0.02 after adjusting for BMI). Both lysophosphatidylcholine and lyso-platelet-activating factor were associated with HOM-IR (*P*=0.04 for both when adjusting for multiple factors, waist: hip ratio and not BMI), fasting plasma insulin (*P*=0.04 and <0.05 respectively), and $AUC_{0\text{-}120min}$ (*P*<0.01 for both,

corrected for multiple factors and adjusted for waist: hip ratio but not BMI ($P<0.01$ for both, adjustment for BMI led to lesser significance at $P=0.06$ and $P<0.05$, respectively). No other lipids approached significance after correcting for multiple factors (Nestel et al., 2014).

With the exception of 17:0, fatty acids were shown not to be significantly associated with indices of insulin sensitivity and resistance. The fatty acid 17:0 tended to be significantly associated directly with Matsuda index, and was inversely associated with HOMA-IR and fasting insulin. This association was stronger when adjusting for waist: hip ratio than BMI (Nestel et al., 2014).

Despite these findings, Nestel et al. found no associations between full – fat dairy consumption with any indices of insulin resistance or sensitivity. This was consistent across all types of dairy (Nestel et al., 2014).

In terms of fatty acid concentrations, the participants in the high saturated fat diets were found to have higher concentrations of lauric, myristic and pentadecanoic acid, which are enriched in dairy fat.
Nestel et. al investigated the effect of a high dairy fat intake in the form of matured hard cheese or butter as part of a randomised crossover trial, lasting 12 weeks and consisting of 19 participants. Plasma cholesterol, LDL cholesterol, HDL cholesterol, triacylglycerol and glucose measurements were used as endpoints for this study. The participants were expected to consume 40g of dairy fat as part of either matured hard cheese or butter for a period of 4 weeks each. It was found that the consumption of 40g of dairy fat did not lead to a significant difference in plasma glucose concentration in either group (run-in 5.09mmol/L, Butter 5.05mmol/L, Cheese 5.04mmol/L), and no significant differences in plasma insulin concentration in either group (data not reported) (Nestel et al., 2005). The results found by Nestel et al. are not readily explained, and there has been a suggestion that these effects are due to the fermentation, fat globules and cholesterol lowering components in cheese have been implicated (Nestel et al., 2005).

Tholstrup et al. conducted a randomised crossover trial as well, lasting 3 months and 9 weeks, and involving 14 healthy men. Blood lipids, lipoproteins (in fasting and postprandial states), postprandial glucose, and insulin response was examined as the endpoints for this study. The participants were required to consume 3 diets, of which 20% of the total energy would be

derived from dairy fat, in the forms of whole milk, butter or hard cheese for a 3 week period each, and separated by a 1 month washout (Tholstrup et al., 2004).

It was found that total glucose concentration after experiencing all three diets led to a fasting glucose concentration of 4.89±0.08mmol/L after consuming a diet rich in milk, 4.94±0.09mmol/L after a diet rich in cheese, and 4.91±0.07mmol/L after a diet rich in butter (Tholstrup et al., 2004). When performing the postprandial meal tests, it was found that the cheese meal brought about a higher glucose response when compared to the milk meal. An overall increase in insulin concentration was also observed, reaching peak concentration at 30 minutes, and decreasing between 30-60 minutes. When compared to the baseline glucose concentration of 4.84±0.09mmol/L(Tholstrup et al., 2004). Tholstrup et al. indicate that the glycaemic response found in the postprandial cheese meal test could be attributed to a higher amount of readily available lactose in this particular meal, therefore explaining how the higher glucose concentration was seen. Another explanation for this effect is that the texture of foods have been implicated in influencing gastric inhibitory peptide (GIP), as well as glucagon-like peptide-I (GLP-I) in different ways. The consistency of dairy products, namely milk has also been suggested to create the glucose response seen in this study, where the consistency of milk becomes similar to that of cheese in the gut. The coagulation of milk causes casein to precipitate, and Tholstrup et al. indicate that this may explain why the milk meal did not achieve the glycaemic response as expected from the researchers (Tholstrup et al., 2004).

SFA has also been implicated in resulting in reduced skeletal muscle insulin sensitivity, where over provision of SFA can lead to tissue accumulation of lipotoxic fatty acid derivatives, such as diacylglycerol (DAG), and ceramide. This accumulation can lead to the promotion of PKC and PP2A which can impair insulin signalling through serine phosphorylation, or the repression of Atk activation (Nardi et al., 2014).

Through treating the cultures with oleic and linoleic acid, it was found that Atk activation was enhanced greatly, in a dose dependent manner, in concentrations as low as 200μM; within physiological range (Nardi et al., 2014).

Nardi et al. conclude that there is an insulin-sensitising effect of oleic and linoleic acid when culturing the myotubes with the MUFA and a sub-maximal dose of insulin, attributed to the suppression of PP2A action (Nardi et al., 2014).

Fat content of dairy and what amount of fat present in dairy should be consumed each day is also an important area of research, as this area is frequently plagued with conflicting information.

Two interventions in this section of the literature review found no significant effects of fat on insulin sensitivity, and one study conducted by Tholstrup et al. found that all three dairy diets (rich in milk, cheese or butter) led to reduced fasting glucose concentrations but also led to increased insulin concentration in the participants studied. The epidemiological data suggested that dairy fat may have a similar association, and the interventions reviewed appear to agree with this, however it may be of benefit to test this further to confirm this relationship between dairy fat and insulin sensitivity.

Meta-analysis – Micronutrients

Dong et al. conducted a meta-analysis on magnesium intake and the risk of developing type II diabetes. Dong et al. state that magnesium is an important component of many foods, including whole grains, nuts, and green leafy vegetables, and serves as an essential cofactor for enzymes involved in glucose metabolism. Magnesium is found in dairy products, however the amount found in dairy is relatively small when comparing to the aforementioned foods, with a total of 25.4mg found in a 250ml serve of milk, accounting for 6% of the daily magnesium requirements of an average adult (Dong et al., 2011). Dong et al. Used 13 prospective cohort studies focusing on magnesium intake and type II diabetes risk. It was found that in a dose response setting, the summary RR of developing type II diabetes for every 100mg/day of magnesium (4 serves/milk per day) ingested was 0.86 (95% CI 0.82-0.89). In comparing the highest versus the lowest intake of magnesium, the summary RR of developing type II diabetes was 0.78 (95% CI 0.73-0.84). The association was not substantially modified by geographic region, follow-up length in each study, sex, or family history of type II diabetes. The inverse association was more easily observed among participants with a BMI $\geq$25 kg/m^2, with a RR of 0.73 (95% CI 0.66-0.81). There was no association with BMI<25 kg/m^2. When Dong et al. restricted the analysis to just dietary intake of magnesium, the RR was reported as 0.80 (95% CI 0.66-0.81). When restricting the analysis by controlling for cereal fibre, the RR was reported as 0.74 (95% CI 0.68-0.80).

Dong et al. conclude that there was a substantial heterogeneity observed in this meta-analysis, which was to be expected when observed the diverse methods used to collect data in the studies included for analysis. Despite this, a stratified analysis by BMI was shown to have an

absence of heterogeneity, and therefore, this may mean that BMI may be contributing to the overall between-study variation. BMI may also serve as an effect modifier in this association between magnesium intake and type II diabetes risk. Dong et al. state that only three of the 13 studies included found more pronounced associations in overweight participants compared to normal weight participants. While the stratified analysis of Dong et al.'s meta-analysis found an association with BMI, a test for interaction did not lead to a statistically significant finding ($P_{Interaction}$= 0.13) (Dong et al., 2011).

Cohort studies – Micronutrients

Lopez-Ridaura et al. conducted a prospective cohort study to determine the associations between magnesium intake and the risk of developing type II diabetes in men and women. The Nurses' health Study and the Health Professionals' Follow-up study were used as cohorts in this study, including individuals aged 30-75 years. A 61-item food frequency questionnaire was used to assess dietary intake in the nurses' health study. Similar food frequency questionnaires were used in the follow up periods of the nurses' health study and the health professionals' follow-up study. Body weight was self-reported on baseline questionnaires, and this was updated every 2 years. Self-reported weights were highly correlated with the updated weight data. The health professionals' follow up study contained detailed information on leisure time physical activity, and energy expenditure was calculated from this data (Lopez-Ridaura et al., 2004).

A follow up of 18 years in the nurses' health study and 12 years in the health professionals' follow up study was conducted here, and it was found that there was a significant inverse association between magnesium intake and risk of type II diabetes in both cohorts. When comparing the top versus lowest quintile of intake RR was 0.55 (95% CI 0.50-0.61) and 0.56 (95% CI 0.47-0.67) in women and men, respectively. After adjusting for BMI, this association was attenuated in both cohorts. When adjusting for non-dietary covariates instead, this significant association remained. The RRs remained significantly different when adding dietary factors into the multivariate adjustment, however further adjustment for caffeine intake led to the association becoming slightly attenuated. The RRs between extreme quintiles of intake was 0.83 (95% CI 0.73-0.95) and 0.76 (0.61-0.94) in women and men respectively. The adjustment for other minerals, such as calcium, potassium and phosphorus did not change the association estimate for women significantly, with a RR of 0.74 (95% CI

0.63-0.88). A stronger inverse association was observed for men, with a RR of 0.62 (95% CI 0.48-0.81) (Lopez-Ridaura et al., 2004)

He et al. conducted a prospective study on magnesium intake and the incidence of metabolic syndrome among young adults. The cohort was sourced from the Coronary Artery Risk Development in Young Adults (CARDIA) study, which was a multicentre, longitudinal study designed to identify the role of physiological, psychological and lifestyle factors in the cardiovascular disease risk evolution among young adults. A total of 5115 black and white men and women, aged 18-30 years were enrolled in this study (He et al., 2006).

In this prospective study, He et al. included 4637 individuals for analysis (52.8% female), and 608 cases of incident metabolic syndrome were identified in the 15 year follow up period. Sixteen percent of participants included for analysis used magnesium supplements. Magnesium intake was inversely associated with the risk of incident metabolic syndrome. Individuals with the two highest quintiles of magnesium intake had a significantly lower risk of incident metabolic syndrome, with a HR of 0.69 (95% CI 0.52, 0.91, $P< 0.01$) for participants with the highest quintile of magnesium intake compared to the lowest quintile of intake, after adjusting for potential dietary and non-dietary confounders, as well as each metabolic syndrome component at baseline (He et al., 2006).

Magnesium intake was also inversely associated with individual components of metabolic syndrome , with significant associations found between magnesium intake and fasting glucose level, waist circumference, and HDL cholesterol. Inverse associations for blood pressure and triglycerides were attenuated when adjusting for major dietary and lifestyle factors (He et al., 2006). He at al. also adjusted for potassium, calcium and folic acid intake, as these were correlated with magnesium intake in the follow up period. The HR for magnesium intake and metabolic syndrome risk in the highest versus lowest quintile of intake was 0.65 (95% CI 0.47, 0.90, $P<0.01$) for calcium intake, 0.75 (95% CI 0.53, 1.06, $P=0.07$) for potassium, and 0.75 (95% CI, 0.52, 1.07, $P=0.08$) after additional adjustment for calcium and potassium). After adjusting for folic acid intake, the HR was 0.72 (95% CI 0.53, 0.98, $P=0.02$).

As wholegrains are a major source of magnesium intake, He et al. adjusted for wholegrain intake in the subjects studied. The multivariable HRs found when adjusting for wholegrain intake were 1.0, 0.71 (95% CI 0.56, 0.88), 0.77 (95% CI 0.61, 0.96) and 0.80 (95% CI 0.63,

1.01, *P*=0.22). Analysing dietary sources of magnesium alone did not change the HR significantly (He et al., 2006).

In a prospective cohort study, van Dam et al. included participants from the Black Women's Health Study without a history of diabetes to determine the association between dietary calcium, magnesium, major food sources and the risk of developing type II diabetes. The study found that women who had higher intakes of magnesium tended to be older, more highly educated, leaner, more physically active, and non-smokers. Women with a higher calcium intake tended to be more highly educated, more physically active, and non-smokers.

van Dam et al. found that increased calcium intake was associated inversely with type II diabetes risk; however the association was weakened significantly after adjusting for potential confounders. Magnesium intake had a hazard ratio of 0.69 (95% CI 0.59-0.81) for the highest intake of magnesium, compared with the lowest quartile of intake ($P<0.0001$), after multivariate adjustment. Magnesium and calcium intake were mutually adjusted in this study, and following this, the inverse association with magnesium and type II diabetes risk remained, whereas calcium's inverse association did not (van Dam et al., 2006).

Women who used calcium supplements were found to have a lower risk of developing type II diabetes when compared to non-users, with a hazard ratio of 0.82 (95% CI 0.73-0.91), however neither the amount, nor the duration of supplementation was associated with a lower risk of type II diabetes. Excluding non-users of calcium supplements did not change the association (van Dam et al., 2006).

Three studies found that micronutrient supplementation was associated with an inverse risk of type II diabetes.

Cross-sectional studies – Micronutrients

Song et al. conducted a cross-sectional study to determine the associations between magnesium intake, C-reactive protein and the prevalence of metabolic syndrome in middle-aged and older U.S women. The cohort was sourced from the Women's Health Study, with 39,876 female health professionals' ≥45 years of age, free of coronary heart disease, stroke, and cancer at baseline. A total of 98% of the cohort provided detailed diet information, through the use of a 131 semi-quantitative food frequency questionnaire, with a follow-up period of 8.8 years (Song et al., 2005) .

Song et al. found that dietary sources of magnesium accounted for 96% of intake among the women analysed. The median intake was 326mg/day, with a 1.5 fold difference in total magnesium intake between the highest and lowest quintile of intake (422 mg/day versus 252mg/day). There was a significant observed decrease in the prevalent risk of developing metabolic syndrome across all quintiles of magnesium intake. The multivariate adjusted ORs of prevalent metabolic syndrome in this study, from the lowest quintiles to the highest quintiles of intake were 1.00, 0.91 (95% CI 0.78-1.06), 0.84 (95% CI 0.72-0.99), 0.81 (95% CI 0.68-0.96), and 0.73 (95% CI 0.60-0.88, P=0.0008). When adjusting for dietary fibre intake, these associations did not change significantly. When adjusting the definition of obesity by using a BMI cut-off of 30 kg/m^2 or a value of BMI corresponding to the same percentile cut point for BMI, or using a waist circumference of 88cm, the results reported were similar to the original results reported. Results did not noticeably change when restricting magnesium intake to dietary intake only (Song et al., 2005).

Song et al. found that there was a significantly decreased risk of prevalent metabolic syndrome.

Intervention studies - Micronutrients

Guerrero-Romero et al. conducted a 3 month randomised parallel trial to determine the effect of oral magnesium supplementation on altering insulin sensitivity in non-diabetic subjects. 57 participants were involved in this study, and were randomised to receive either 2.5g of $MgCl_2$ as an oral dose, or a placebo.

The study found that there was a strong, yet independent relationship observed between low serum magnesium levels and HOMA-IR at baseline. Individuals who received magnesium experienced reduced fasting serum glucose (from 5.8±0.9 to 5.0±0.6mmol/L in the magnesium group, 5.7±0.4 to 5.6±0.5 mmol/L in the control group, P<0.05), insulin levels and HOMA-IR index. Lipid profiles also improved, with decreased serum triglycerides, total and LDL cholesterol, and increased HDL cholesterol levels, which were not observed in the control group. This study found that magnesium supplementation led to a decrease in fasting insulin concentrations by 32%, (from 103.2±56.4 to 70.2±29.6mmol/L in the magnesium group, 123.0±47.4 to 132.1±75.6mmol/L in control group, P<0.05) and a 43.5% reduction in HOMA-IR index (from 4.6±2.8 to 2.6±1.1, P<0.0001). Control group was 0.62±0.08 to 0.61±0.08, P<0.01). (Guerrero-Romero et al., 2004).

Guerrero-Romero et al. conducted a randomised parallel trial to determine whether oral supplementation of magnesium chloride improves beta cell ability to compensate for variations in insulin sensitivity in non-diabetic individuals experiencing significant hypomagnesaemia.

A total of 97 individuals were randomised to either receive 50ml of 5% $MgCl_2$ solution for 3 months, or an inactive solution for the same amount of time. The study found that serum magnesium levels were significantly increased in the magnesium group as compared to the placebo group. HOMA-β index was decreased significantly in the magnesium group when compared to the placebo group (from 253.2±130.3.to 170.1±40.1 in magnesium group, 271.8±112.6 to 262.7±102.9 in placebo group, $P<0.05$). (Guerrero-Romero and Rodriguez-Moran, 2011). At baseline, 16 individuals had impaired fasting glucose in the magnesium group, and seven in the placebo group. At the end of the trial, one individual in the magnesium group and none in the placebo group managed to reduce fasting plasma glucose levels to 5.6mmol/L. Compensatory insulin secretion response was found to be altered in the magnesium group, whereas the placebo group was unchanged from baseline in this study. It was concluded that supplementation of 5% $MgCl_2$ led to improved beta cell ability to compensate for decreases in insulin sensitivity in non-diabetic individuals with significant hypomagnesaemia.

Mooren et al. conducted a double-blinded randomised parallel trial to determine the effects of oral magnesium supplementation on insulin resistance in non-diabetic participants. A total of 47 participants were randomised to receive either magnesium oral supplements, or a placebo. There were no differences in individual characteristics observed at baseline.

The study found that fasting plasma glucose concentrations were significantly lower in the magnesium group, (4.75±1.04 mmol/L for magnesium, 4.97±0.87 mmol/L for placebo respectively. $P=0.0237$) and fasting serum insulin displayed a trend for improvement in the magnesium group, however this was not significant (100.00±46.38 pmol/L for magnesium, 117.39±58.70 pmol/L for placebo. $P= 0.875$). There were no differences observed from plasma glucose and serum insulin levels between groups after 120 min of the OGTT used to test participants in this study. ISI HOMA and ISI-Matsuda were significantly improved in the magnesium group when compared to the placebo group, (HOMA: 2.974±1.682 for magnesium, 3.713±2.517 for placebo, $P=0.0376$. Matsuda: 4.037±2.040 for magnesium,

3.150±1.288 for placebo, *P*=0.0127) and there was also a trend observed for diastolic blood pressure reduction in the magnesium group (*P*=0.0561).

Mooren et al. found that magnesium supplementation improves insulin resistance in obese, insulin resistant individuals, and two of three insulin sensitivity measures in this study support the hypothesis further. ISI HOMA and ISI Matsuda showed significant improvement in insulin sensitivity, but ISI Gutt did not, and it is suggested that since there was an increase in physical activity observed in the placebo group, a difference in insulin sensitivity for both groups was more difficult to determine. Another suggesting for the results observed with ISI Gutt is that the test may not be sensitive enough to detect a significant difference in the sample size of this study, and hence, a larger sample size would be needed to detect a significant difference. Mooren at al. suggest that magnesium predominantly influences hepatic insulin resistance, rather than peripheral insulin resistance. ISI HOMA and Matsuda mainly reflect hepatic insulin resistance, and fasting plasma glucose (Mooren et al., 2011).

Chacko et al conducted a double blinded, controlled crossover trial to determine the effects of magnesium supplementation on metabolic and inflammatory markers, as well as global genomic and proteomic profiling in overweight adults.

Chacko et al. state that magnesium is an essential mineral that can be found in whole grains, leafy greens, vegetables, legumes, and nuts. It is also present in dairy. Magnesium acts as a cofactor in hundreds of enzymatic activities in the body, and there is evidence to suggest a possible association between increased magnesium intake and a favourable outcome on metabolic and inflammatory disorders. These disorders include insulin resistance, dyslipidaemia, hypertension, type II diabetes, cardiovascular disease and metabolic syndrome.

A total of 26 participants completed the study, and were assigned to either have a placebo, or 500mg magnesium citrate every day for four weeks before crossing over to experience the remaining treatment. Initially, this study was designed as a parallel study, however the investigators decided to adjust the design to a crossover study. The treatments lasted 4 weeks each, with a 4 week washout period in-between treatments.

At baseline, all participants were overweight or obese, and 43% of participants were classified as hypomagnesemic, and after the washout period in between treatments, the same proportion of individuals suffered from hypomagnesaemia. Mean serum magnesium concentration levels returned to baseline in this 4 week period.

Chacko et al. found that the supplementation of 500mg of magnesium per day for four weeks led to a slightly decreased fasting C-peptide concentration (1.5±0.9 for magnesium, 2.0±0.9 for placebo, *P*=0.004). The supplementation of magnesium also appeared to lead to a decreased fasting insulin concentration (4.8±3.7μU/ml for magnesium, 7.4±3.7μU/ml for placebo) *P*=0.25). The results obtained suggest that magnesium may have a role in the regulation of insulin and glucose homeostasis (Chacko et al., 2011).

Paolisso conducted a double blinded, randomised crossover trial to determine the effects of magnesium supplementation on glucose handling in elderly participants. For four weeks, participants were either randomised to the placebo, or to magnesium supplementation.

Twenty-five healthy participants and twelve elderly, non-obese participants were recruited to this study, and randomised to receive either chronic magnesium administration (4.5g/day for 4 weeks) or a placebo for the same amount of time. Each participant was subjected to an intravenous glucose tolerance test, and a euglycaemic glucose clamp at the end of treatment periods.

It was found that elderly subjects with insulin resistance have a lower erythrocyte magnesium concentration, decreased erythrocyte membrane viscosity, improved insulin action, and enhanced total-body and oxidative glucose metabolism. These changes were observed either at baseline, or after magnesium supplementation.

Mechanism of action

Plasma magnesium concentrations is tightly regulated by several factors, with one of these factors being insulin. Insulin is an important modulator of cellular magnesium, with Takaya et al. stating that there are several *in vivo* and *in vitro* studies indicating that insulin modulates the movement of magnesium from extracellular space to intracellular space. Insulin was also identified in the ion transport mechanism in cells, responsible for erythrocytes, platelets and rat uterus cells. This is performed by insulin regulating magnesium ion concentration by stimulating the ATPase pump and erythrocyte magnesium uptake. One of magnesium's functions is to form a complex with ATP, and since magnesium is a necessary cofactor in all ATP transfer reactions, this means that magnesium concentration is necessary in the phosphorylation on the insulin receptor (Takaya et al., 2004).

The first step in having insulin binding to target tissues is to bind to specific receptors on the target tissue itself. The receptors are heterotetrametric glycoproteins, with two α-subunits and two β-subunits, and possess intrinsic tyrosine kinase activity (Takaya et al., 2004). By binding to the receptor, insulin activates tyrosine kinase phosphorylation at the intracellular portion of the receptor, initiating a series of reactions.

Takaya et al. state that a decreased concentration of magnesium is associated with a diminished ability to stimulate glucose uptake in insulin sensitive tissues, such as adipose cells and skeletal muscle tissue. It has been shown in the past that low magnesium concentrations can reduce glucose uptake *in vitro*, it would appear that a reduction in magnesium concentration interferes with insulin signalling as part of glucose transport. Altered magnesium concentration may result in decreased cellular glucose utilisation, which could lead to peripheral insulin resistance through a post receptor mechanism (Takaya et al., 2004).

In individuals with type II diabetes, Takaya et al. state that there is an impaired ability for insulin to stimulate magnesium and glucose uptake, and that type II diabetes may be associated with magnesium depletion, which can contribute to metabolic complications of diabetes, such as vascular disease, osteoporosis and polyneuropathy (Takaya et al., 2004).

While studies who have examined subjects with type II diabetes show that plasma magnesium concentration is inversely correlated with the degree of glycaemic control, it is reported that there is no correlation between intracellular and plasma magnesium concentrations (Takaya et al., 2004).

Of the four studies covered, three studies concluded that magnesium intake in particular may be beneficial for insulin sensitivity, and one suggested that intake of magnesium may be detrimental to insulin sensitivity.

All five studies in this area found that supplementation with magnesium had a beneficial effect on glucose homeostasis and insulin resistance, which was consistent with the epidemiology. Despite these findings, it would be difficult to replicate these results with dairy alone as the amount of magnesium consumed in supplementation would be far greater than any amount achieved through eating dairy products alone.

Conclusions

Of the thirty-two epidemiological studies included in this literature review, seventeen suggest that dairy intake may be inversely associated with type II diabetes, metabolic syndrome, and cardiometabolic risk, six suggest a positive association and the remaining nine suggest no association whatsoever. It is necessary to conduct further research into this area to address the heterogeneity of the results, and to conclusively determine the associations between dairy intake and type II diabetes and metabolic syndrome risk. Only interventions can clearly answer this question.

To summarise the findings of this literature review, there is heterogeneity present in the findings established by the studies covered in this field of interest. There is conflict present as to what dose of dairy, length of time for consumption and the exact effects of dairy on insulin sensitivity in a weight-stable setting. It is therefore necessary to determine the effect of increased dairy consumption in overweight or obese individuals who have impaired glucose tolerance (IGT), and to determine the effects of this increased dairy consumption on insulin sensitivity and cardiovascular health. The selection of normal glucose tolerant (NGT) and IGT individuals in the current study were made so as to boost recruitment rate as difficulties had been encountered in recruiting only overweight and obese individuals with IGT who do not possess type II diabetes.

Evidence tables summarising the studies included in this literature review can be found in the appendix of the thesis.

Chapter Three: Aim, Hypothesis and Methods

Aim

To recruit normal weight or overweight/obese participants with NGT or IGT in a 10 week randomised crossover trial to determine if a high dairy diet improves insulin sensitivity compared with a low dairy diet.

Hypothesis

Increased dairy consumption will lead to a ≥30% increase in insulin sensitivity when compared to insulin sensitivity in the low dairy diet.

Methods

Sample size

By examining the observed SD of 1.99 from all similar studies in this area in 2014, and performing a power calculation, it was determined that a sample size of 60 overweight and obese participants would be sufficient to detect a 30% difference in insulin sensitivity with a power of 80% at 0.05. It was considered that a 30% change in insulin sensitivity with a larger intake of dairy foods would be reflective of the 15% difference seen in epidemiological studies with a more modest difference in dairy intake.

Inclusion criteria

The inclusion criteria are as follows:

- BMI $\geq 18 kg/m^2$
- Weight stable
- Not: pregnant, smoking
- Not suffering from Type II diabetes

  No: active gastrointestinal or eating disorders, adverse responses to dairy products, use of over-the-counter pharmaceuticals to counteract obesity/overweight condition.

Exclusion criteria

The exclusion criteria for this study were as follows:

- Weight change greater than 3kg in the last 3 months
- Pregnant
- Smoking
- Suffering from type II diabetes
- Presence of active gastrointestinal or eating disorders or use of over-the-counter pharmaceuticals to counteract obesity/overweight condition

Recruitment

Volunteers were recruited using flyers posted in local universities, as well as the use of online advertisements. Volunteers who had successfully completed previous studies and met the recruitment criteria of the study were also contacted from an encrypted record and recruited.

Volunteers were screened by measuring height, weight, assessing a 3 day weighed food record, and by assessing tolerance to a 75g oral dose of glucose. This was performed to confirm that the individuals screened did not suffer from type II diabetes. Veins were also checked on both arms in preparation for future test appointments. If the volunteer was determined to be non-diabetic, they were enrolled into the study. Initially we had planned to recruit 60 individuals as part of this intervention; however, difficulties in recruiting overweight or obese individuals who had impaired glucose tolerance led us to expand the recruitment criteria to include individuals with normal glucose tolerance as well. Unfortunately, only 30 people could be recruited in the time available for the Masters, which reduced the power of the study. The course of the study in terms of participant number can be found in figure 1.

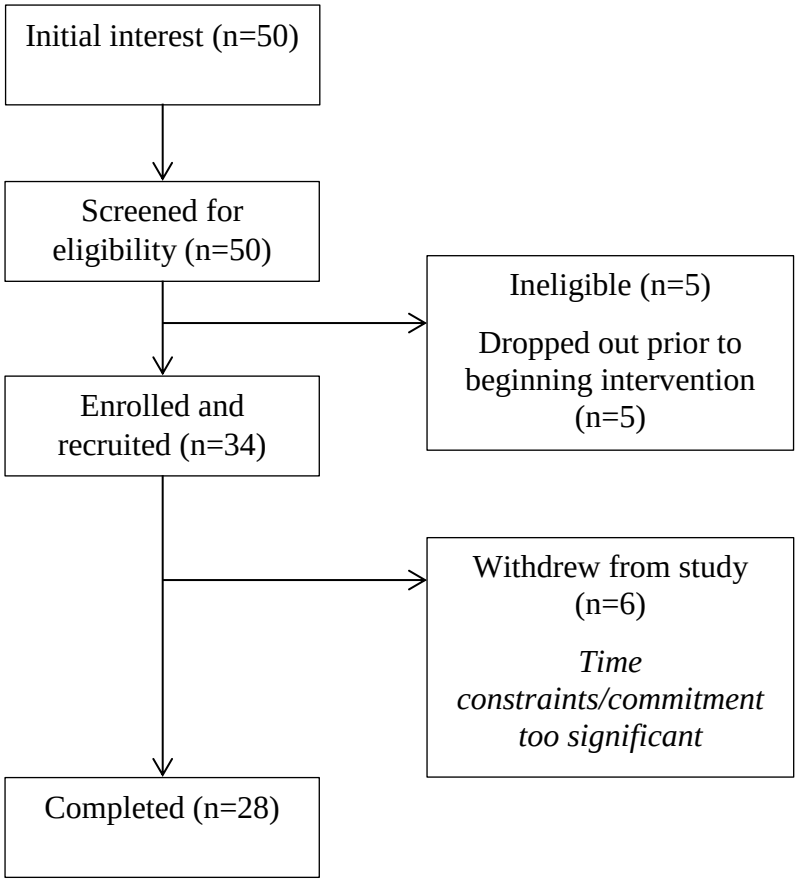


Fig 1: Participant recruitment.

Ethics, registration and consent

Ethics was obtained by the University Of Adelaide Human Ethics Resource Committee (HREC), and the study was registered with ANZCTR. Information on the study was given to each participant in writing and verbally, and consent was obtained from each participant. Consent forms were signed in duplicate, with one copy for the participant to retain along with an information sheet, and the other was kept for records. Any deviations to protocol, changes after obtaining ethics clearance and adverse events during data collection were reported to HREC.

Chapter Four: Study design and Statistics

This was a 10 week randomised crossover intervention study, which consisted of a four week diet, followed by a two week break where participants resumed their usual diets, and then another four week diet. Testing was performed at the end of each four week diet, where a LDIGIT, a hyperglycaemic clamp, and aorto-femoral pulse velocity along with augmentation index was performed on each participant over a two day testing period. There was no testing done at the beginning of the study or at the beginning of each diet. A check-up visit was performed twice, with each check-up taking place two weeks after starting each diet. Participants followed the study requirements and ate the diets as soon as they were recruited to the study. A crossover intervention was chosen as it halved the number of participants required for the intervention.

Diets

The volunteers were instructed to consume two diets for four weeks each, one being a low dairy diet, and the other being a high dairy diet. In the low dairy diet, volunteers were expected to consume a maximum of 0.5 serves of dairy per day. In the high dairy diet, volunteers were expected to consume 4-6 serves of dairy, with at least two of these being yoghurt. There were no restrictions placed on fat or sugar content of the yoghurt or other dairy types consumed. A dairy serve is defined as one of the following:

- 1 cup or 250ml of milk (fresh, UHT, reconstituted powdered milk)
- 40g, equivalent to 2 slices, or 4x3x2cm cube of hard cheese (e.g. cheddar)
- ½ cup or 120g ricotta cheese
- ¾ cup or 200g of yoghurt

Data collection – Dietary assessment

Two weeks into each diet, each volunteer attended a check-up visit, during which they were weighed, and have another 3 day weighed food record examined. The volunteer also has an opportunity to voice any difficulties with the diet, with the researcher offering solutions in response.

Data collection- testing

Baseline height, weight, fasting blood glucose concentration and glucose tolerance was obtained at baseline. All blood glucose data was obtained through the use of a finger prick.

The recruitment period lasted from September 2014 to October 2015, and the intervention lasted 10 weeks for each participant, and the data collection phase lasted from January 2015 to December 2015. A diet lasted 4 weeks each, and a two week period was included between diets as a break for the participants. Participants were exposed to the intervention and tested as soon as they were recruited.

At the end of each diet (4 weeks and 10 weeks into the intervention), each volunteer had a pulse wave velocity and augmentation index assessment performed when fasting. The pulse wave velocity measure included having the volunteer lie in the supine position, and a blood pressure cuff fitted to the right thigh, and has the sternal notch to carotid pulse site, sternal notch to thigh cuff, and thigh cuff to femoral pulse site measured. These measurements were entered into computer software, and a tonometer was placed on the volunteer's neck in an effort to detect the carotid pulse. After a suitable pulse was obtained, the cuff was automatically inflated, and obtained a pulse wave velocity based on the difference between both pulse sites. This measure was performed three times (Wilkinson et al., 1998, Frimodt-Moller et al., 2008, Rodriguez et al., 2016).

Augmentation index was measured by attaching a blood pressure cuff to the arm, and allowing the pump to automatically inflate the cuff while the patient remained seated upright. The results were presented at the end of each measure. Three measures for pulse wave velocity and augmentation index each were performed (Wilkinson et al., 1998, Frimodt-Moller et al., 2008, Rodriguez et al., 2016).

At the end of each diet (4 weeks and 10 weeks into the intervention), volunteers were subjected to a hyperglycaemic clamp, and a LDIGIT. For the hyperglycaemic clamp, the volunteer lies in the supine position and has both arms cannulated. The patient is subjected to a glucose infusion, and samples are obtained every five minutes for 120 minutes. The first 15

minutes of this infusion is a loading dose, which is designed to increase the individual's blood glucose concentration level to 10 mmol/L. After obtaining each blood sample, the blood glucose concentration was measured, and the infusion rate adjusted accordingly in order to maintain the level of 10 mmol/L. Insulin blood samples were stored in serum tubes, and preserved in ice 30 minutes after obtaining the sample. Storage at room temperature was required to ensure complete clotting. . Glucose blood samples were stored in EDTA tubes, and preserved in ice immediately after obtaining the sample.

The LDIGIT consisted of a set infusion rate, and lasted 150 minutes. The infusion also included a small amount of insulin, and the rate is dependent on body weight and the amount of glucose required for each patient. The insulin dose was 25mU/kg body weight/hour, and the glucose dose was 4mg/kg body weight/minute. Blood was sampled at 0, 5, 10, 15, 20, 25, 30, 50, 70, 90, 110, 120, 125, 130, 135, 140, 145, 150 minutes, and the blood glucose concentration measured using a pocket glucometer and over-the-counter pharmacy glucose test strips.

Blood samples for insulin analysis were collected in serum tube samples for both tests for all four tests during the participants' 10 week study runs, and c-peptide response was measured in the first 30 minutes of each test to determine endogenous insulin response to the doses of glucose in both LDIGIT and glucose clamp technique tests.

Sample handling

Insulin and c-peptide blood samples were stored in serum tubes, and preserved in ice 30 minutes after obtaining the sample. Glucose blood samples were stored in EDTA tubes, and preserved in ice immediately after obtaining the sample. Blood samples were pipetted into aliquots immediately after the end of each test, placed into plastic freezer boxes, and stored in -80°C freezers.

Glucose was measured at two occasions for each test: once at the moment of obtaining the blood sample using a pocket glucometer and over-the-counter pharmacy glucose test strips, and once more during analysis, using a Konelab clinical chemistry analyser. Insulin and C-peptide were measured using ELISA kits. Lipids were also measured using a Konelab clinical chemistry analyser at a later date after all participants had completed the study.

At the end of the first diet, these measures were performed, and afterwards, the volunteers were placed on a two week break, during which their conventional diet was consumed. After

the end of this two week period, the volunteers began the second diet. At the end of the second diet, the same test measures were performed again. Volunteers were awarded an honorarium for their participation.

Data entry

Three day weighed food records were collected from each participant on five occasions: at the screening visit, at the halfway point in diet one and two, and at the end of both diets. If the participant reported any home-cooked meals in the three day weighed food record, a recipe containing the ingredients for the dish was also provided by the participant. Food record data was added into the program *Xyris FoodWorks 8,* and the number of dairy serves consumed in each record was also recorded. These records were used to ensure compliance to the protocol. The halfway points at each diet was used as a check-up visit, requiring the participant to arrive at the testing facility in a fasted state. The participant was weighed and a 3-day food record was examined to determine compliance. The participant was given instructions based on their consumed number of dairy serves and changes in body weight.

Statistics

The results of this study were analysed using *IBM SPSS Statistics 24*. The data was tested for normality using the Shapiro-Wilk and Kolmogorov-Smirnov tests. Variables which were determined to be normally distributed were used in a repeated measures ANOVA, with age and BMI included as covariates in the analysis. Yoghurt was also included as a covariate in the ANOVA as the epidemiological data suggested that increased yoghurt intake was consistently inversely related to diabetes risk. The results were reported as means and SEM in a table, and plotted onto column graphs. Variables determined to be not normally distributed from the Shapiro-Wilk and Kolmogorov-Smirnov tests were reported as median and interquartile range. Data was analysed using the Friedman test, or the Wilcoxon signed rank test. These results were plotted into boxplots and are included in the results section. In both variable distribution types, $P < 0.05$ was considered to be significant. Dietary variables were tested with a Spearman's correlation coefficient against ISI separately in each diet phase.

Chapter Five: Results

A total of 28 participants completed the study. Six individuals dropped out of the study due to not being able to comply with the study methodology. A total of 23 participants were defined

as having normal glucose tolerance, and five participants were classified as having impaired glucose tolerance. The five participants who were classified as having impaired glucose tolerance were recruited, randomised into their respective diets and either completed the intervention or were close to their final round of testing. At the same time, the University of Adelaide Human Ethics Committee was contacted in order to amend the ethics application to expand recruitment criteria to include normal weight and normal glucose tolerant individuals due to difficulty in finding individuals who met the previous recruitment criteria and were still willing to participate. As a result, it was much easier to recruit the remaining 23 volunteers but it had no effect on the results.

The average dairy serve intake in this study was 4.4 serves for the high dairy diet, and 0.2 for the low dairy diet. Average yoghurt intake during the high dairy diet was 1.8 serves.

Dietary outcomes

Every dietary variable across baseline, high dairy and low dairy testing periods were not significantly different in this study, with the exception of total fat intake which was higher in the high dairy period (P=0.03), and saturated fat intake, also higher in the high dairy diet (P<0.01). Calcium intake was significantly higher in the high dairy diet ($P<0.001$) and so was energy intake derived from saturated fat intake (P<0.01) (Table 1).

Table 1: Diet Information

Variable	**Baseline**	**High Dairy**	**Low Dairy**	***P*-value**
Energy (kJ)	**8802±572**	**9155±549**	**8216±534**	**0.4**
kJ from Protein (%)	**17.8±0.8**	**20.5±0.9**	**18.9±0.8**	**0.2**
kJ from Fat (%)	**32.0±1.2**	**33.8±1.4**	**32.9±1.2**	**0.2**
kJ from Saturated fat (%)	10.9, 5.1	15.8, 5.3	10.3, 3.4	p<0.001
kJ from Carbohydrate (%)	**44.3±2.0**	**41.9±1.3**	**43.3±1.4**	**0.5**
kJ from Fibre (%)	2.1, 1.08	1.8, 1.03	2.2, 1.2	0.6

Carbohydrate (g)	247.5, 91.3	227.8, 113.4	212.3, 98.5	0.3
Protein (g)	90.8, 41.6	108.0, 48.5	88.2, 47.1	0.07
Total Fat (g)	66.4, 43.4	89.2, 44.8	76.05, 40.5	0.03
Saturated fat (g)	24.4, 18.2	39.0, 19.1	22.1, 15.3	p<0.001
Fibre (g)	24.5, 8.7	21.3, 10.9	22.9, 14.8	0.9
Calcium (mg)	669, 679	1701, 499	487, 580	p<0.001
Participant body weight screening visit/check-up (Kg)	**70.1±3.01**	**69.8 ±2.8**	**70.2 ±2.8**	**0.4**
Participant body weight Test Day (Kg)	**N/A**	**69.9 ± 2.8**	**70.5 ± 2.8**	**0.1**

Presented as Median, IQR

Variables in bold presented as Mean ± SEM

LDIGIT

Glucose Concentration

Average glucose concentrations in the LDIGIT were 5.7 and 5.6 mmol/L (SEM 0.2 and 0.2 respectively, table 2, fig 2) in the high and low dairy diets respectively ($P = 0.9$). The LDIGIT steady state glucose (120-150 minutes) had mean values of 5.4 and 5.3 (SEM 0.3, 0.3 respectively) mmol/L (table 2, fig 2&3), with a significance of 0.7.

Table 2: Outcomes by diet

Variable	**High Dairy**	**Low Dairy**	***P*-value**
Fasting Insulin LDIGIT (pmol/L)	10.6, 15.2	11.5, 15.3	0.5
Insulin Steady State LDIGIT (pmol/L)	44.3, 42.7	48.8, 59.9	0.6
LDIGIT Average Glucose	**5.7±0.2**	**5.6±0.2**	**0.9**

Concentration (mmol/L)			
LDIGIT Steady State Glucose Concentration (mmol/L)	**5.4±0.3**	**5.3±0.3**	**0.7**
Insulin sensitivity index LDIGIT (ml $kg^{-1}min^{-1}$/pmol/L)	10.2 x 10^{-3}, 8.9 x 10^{-3}	9.4 x 10^{-3}, 12.9 x 10^{-3}	0.7
C-Peptide Fasting LDIGIT (ng/ml)	1.2, 0.8	1.04, 0.6	0.09
C-Peptide 5-30 minutes LDIGIT (ng/ml)	2.1, 1.8	2.1, 1.2	0.2
Insulin Fasting Clamp (pmol/L)	9.7, 22.2	9.7, 24.0	0.6
Insulin Steady State Clamp (pmol/L)	107.3, 153.3	116.0, 115.2	0.5
Average glucose concentration Clamp (mmol/L)	**7.9 ± 0.2**	**8.1 ±0.2**	**0.4**
Clamp Steady State Glucose Concentration (mmol/L)	**8.1 ± 0.2**	**8.3 ± 0.2**	**0.4**
Insulin sensitivity index Clamp (ml $kg^{-1}min^{-1}$/pmol/L)	40.4 x 10^{-3}, 54.9 x 10^{-3}	34.2 x 10^{-3}, 62.8 x 10^{-3}	0.6
C-Peptide Fasting Clamp (ng/ml)	1.1, 0.6	1.0, 0.5	0.1
C-Peptide 5-30 minutes Clamp (ng/ml)	2.5, 1.9	2.8, 1.5	0.7
Augmentation Index (AIx)	**6.6±2.6**	**6.3±2.5**	**0.9**
Pulse Wave velocity (m/s)	**6.06±2.2**	**6.4±2.0**	**0.9**

Presented as Median, IQR

Variables in bold presented as Mean ± SEM

Fig 2

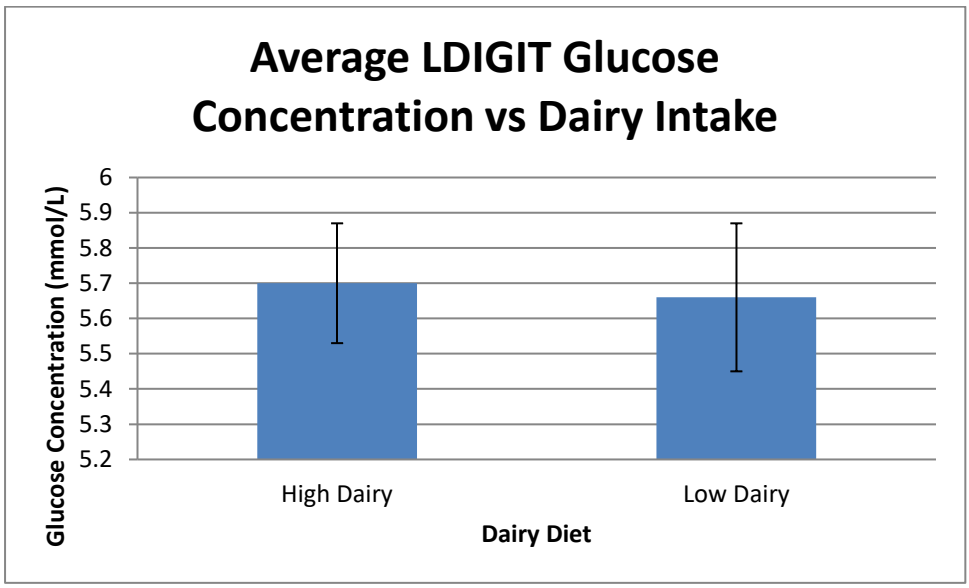
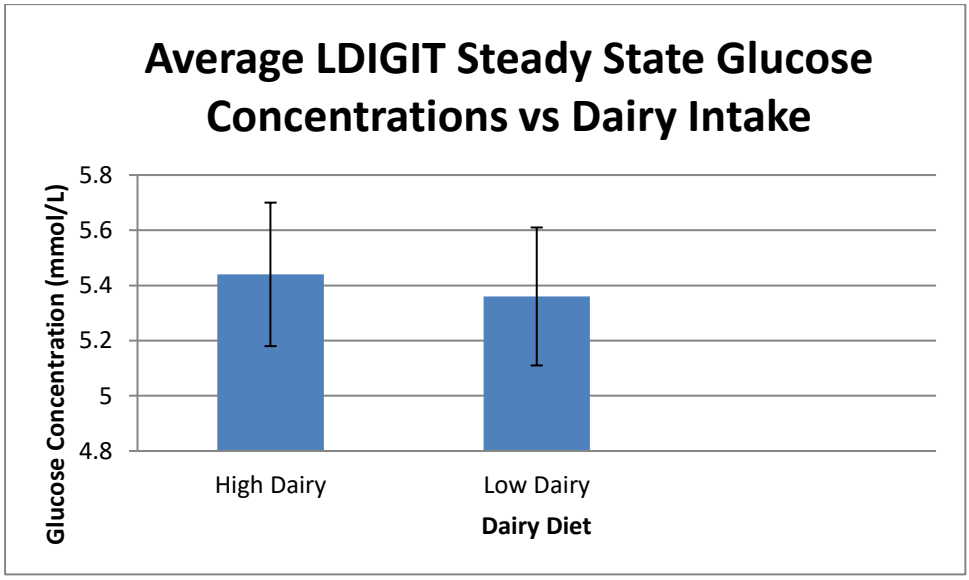


Fig 3



Insulin concentration

Fasting insulin LDIGIT values have a median value of 10.6 and 11.5 pmol/L with an interquartile range (IQR) of 1.8 and 1.2 respectively (table 2, fig 7) in the high and low dairy values (*P=0.5)*. Steady state insulin values for the LDIGIT had a median of 44.3 and 44.8 pmol/L (table 2, fig 6) in the high and low dairy diets respectively (IQR 42.7, 59.9 respectively, *P*= 0.6). No significant differences were detected.

Insulin Sensitivity Index

Insulin sensitivity was not different between diets , with a value of 10.2 ml $kg^{-1}min^{-1}$/pmol/L x 10^{-3} in the high dairy diet, and 9.4 x10^{-3} in the low dairy diet for the LDIGIT (IQR 8.9, 12.9 respectively, *P*= 0.7, table 2, fig 7).

C-peptide

C-peptide response in the LDIGITs were 1.2 and 1.04 ng/ml for the fasting samples (IQR 0.8, 0.5 respectively, *P*= 0.09), and 2.1 and 2.1 ng/ml (IQR 1.8, 1.2 respectively, *P*= 0.2, table 2, fig 9) for the first 30 minutes in the high and low dairy diet.

Fig 4

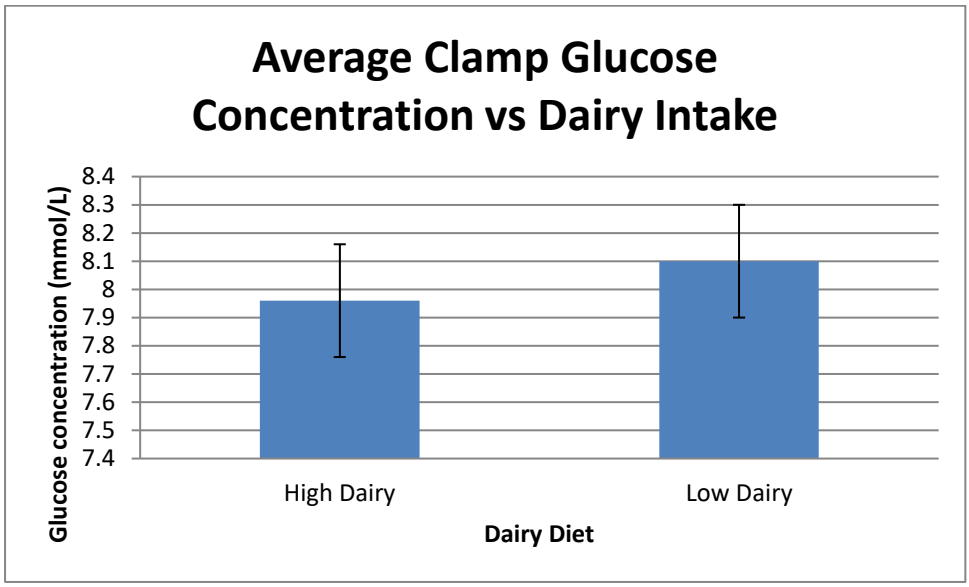
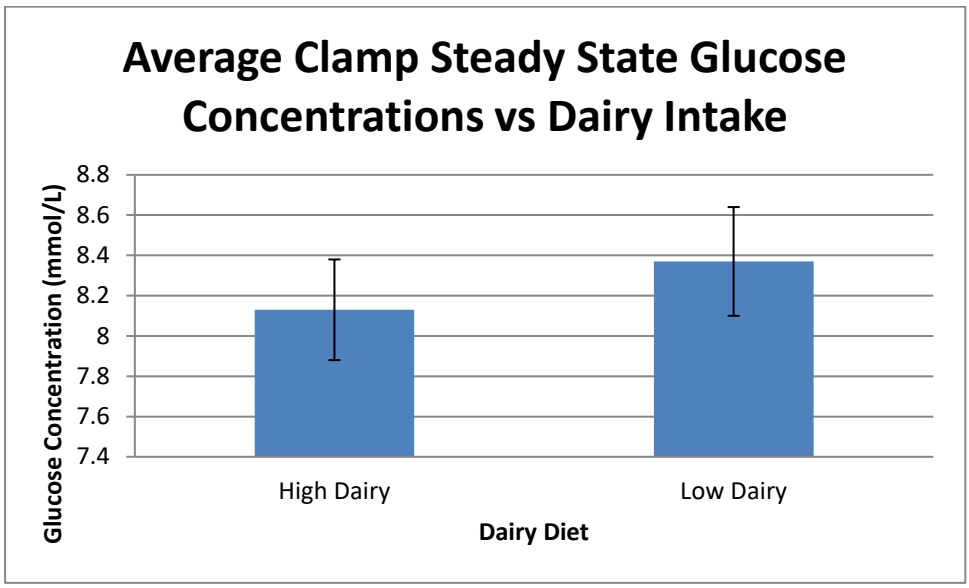


Fig 5.



Hyperglycaemic Clamp

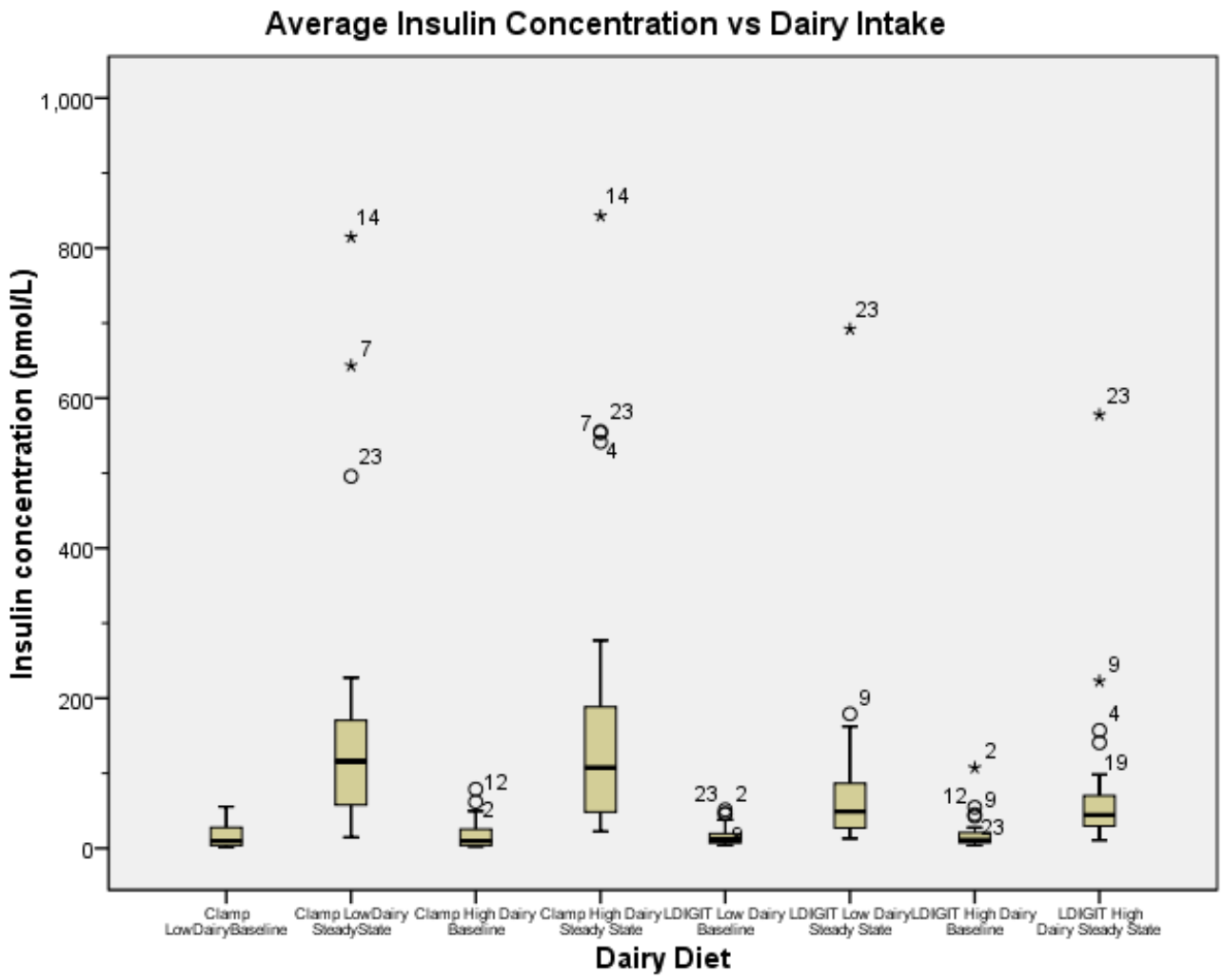
Glucose concentration

Average glucose concentrations across all time points in the hyperglycaemic clamp were 7.9 and 8.1 mmol/L in the high and low dairy diets respectively (SEM 0.2 for both diets, *P* = 0.4, table 2, fig 4). Steady state concentrations had a mean value of 8.1 and 8.3 mmol/L (SEM 0.3, 0.3 respectively, *P*= 0.4, table 2, fig 5).

Insulin concentration

Fasting hyperglycaemic clamp insulin values were 9.7 and 9.7 pmol/L (IQR 22.2, 24.0 respectively, *P*= 0.6, table 2, fig 6) in the high and low dairy diets respectively, and in the steady state the median values were 107.3 and 116.02 pmol/L (IQR 153.3, 115.2 respectively, table 2, fig 6). No significant differences were detected between diets (*P*= 0.5).

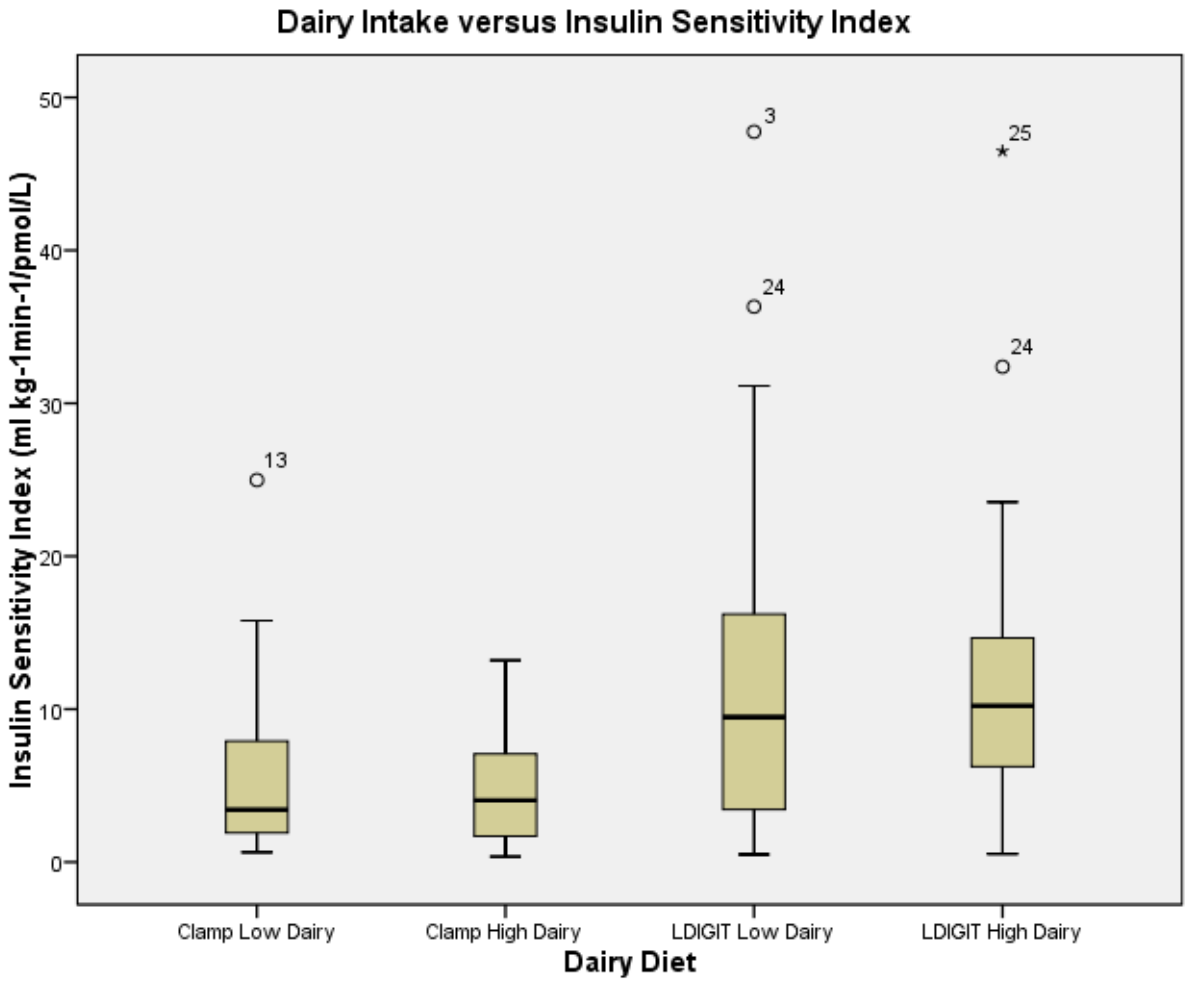
Fig 6



Insulin Sensitivity Index

The hyperglycaemic clamp test had an index of 40.4 x 10^{-3} and 34.2 x 10^{-3} ml $kg^{-1}min^{-1}$/pmol/L for the high and low dairy diets respectively (IQR 54.9 x 10^{-3}, 62.8 x 10^{-3} respectively, table 2, fig 7, *P*=0.6).

Fig 7



C-Peptide

C-peptide concentrations were 1.1 and 1.07 ng/ml (IQR 0.6, 0.5 respectively P=0.1, table 2, fig 8&9) values for fasting, at high and low dairy respectively. Median c-peptide

concentrations over the first 30 minutes were 2.5 and 2.8 ng/ml (IQR 1.9, 1.5 respectively, *P*= 0.7, table 2, fig 8&9) for the high and low dairy diets respectively.

Fig 8

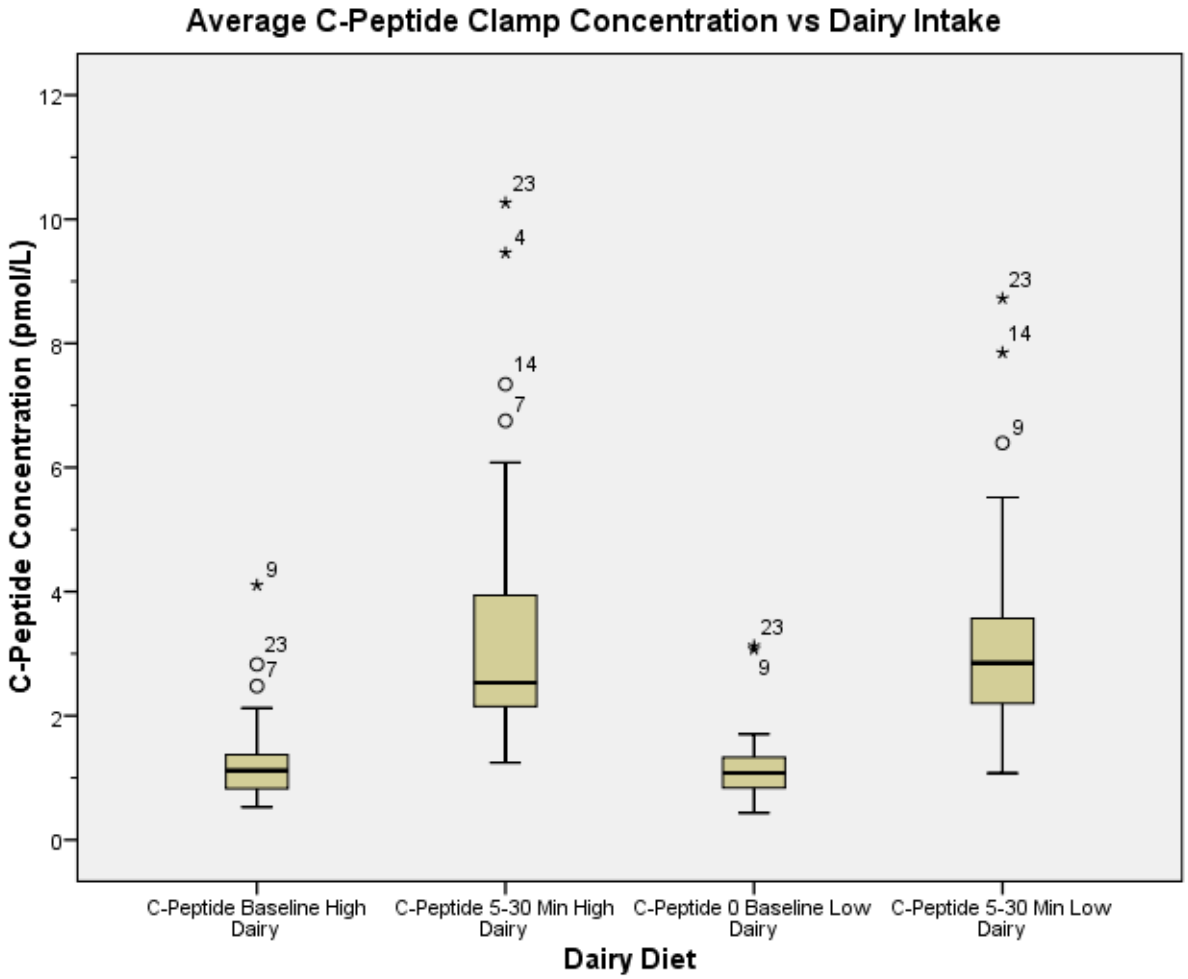
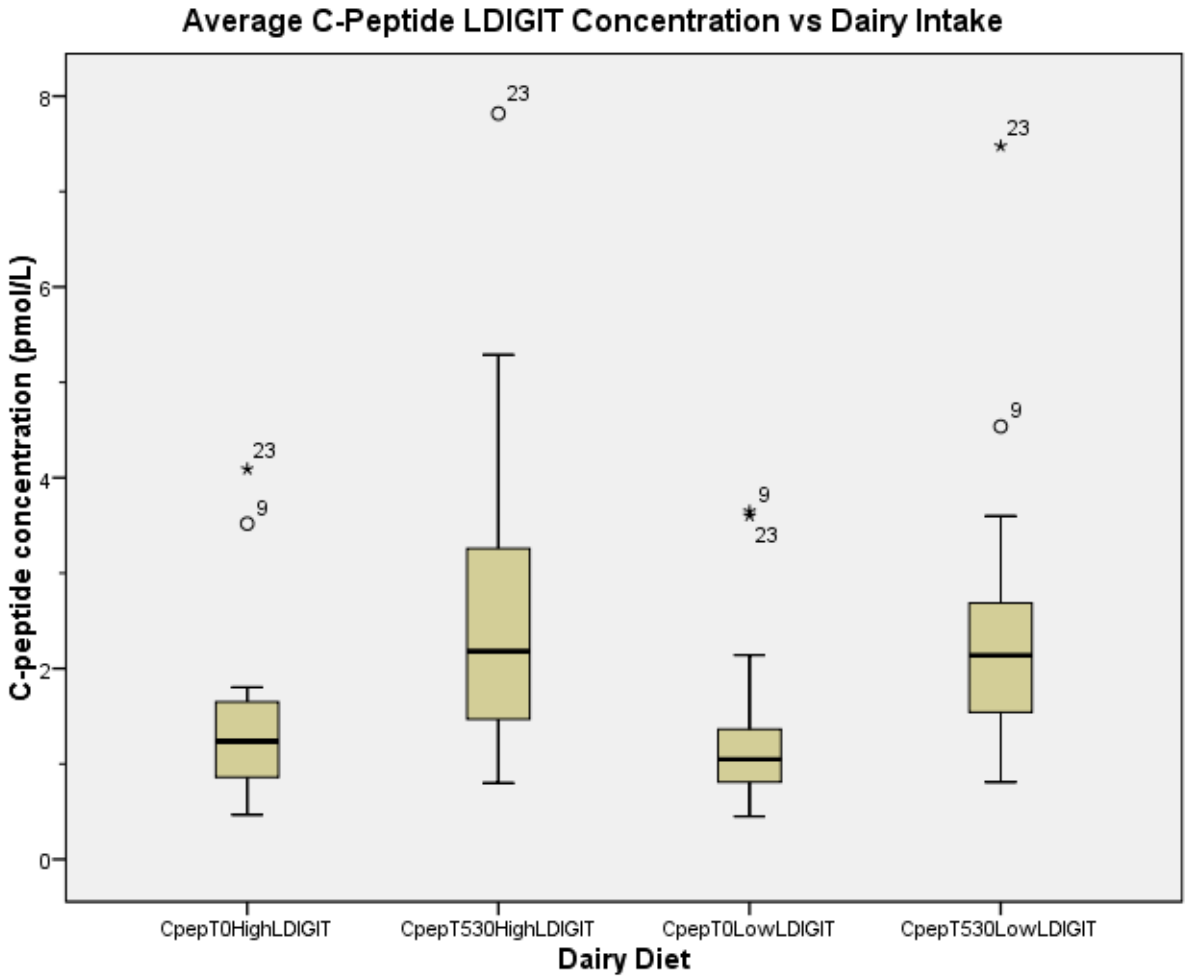


Fig 9



PWV and AIx

Aorto-femoral pulse wave velocity and augmentation index were had median values of 6.1 and 6.5 m/s (table 2, fig 10) in the high and low dairy diets respectively (IQR 2.2, 2.02, *P*=0.9). Average augmentation index was found to be 6.7% and 6.4% in the high and low dairy diets respectively, with a *P* value of 0.9 (SEM 2.6, 2.5 respectively, *P*= 0.9, table 2, fig 11).

Fig 10

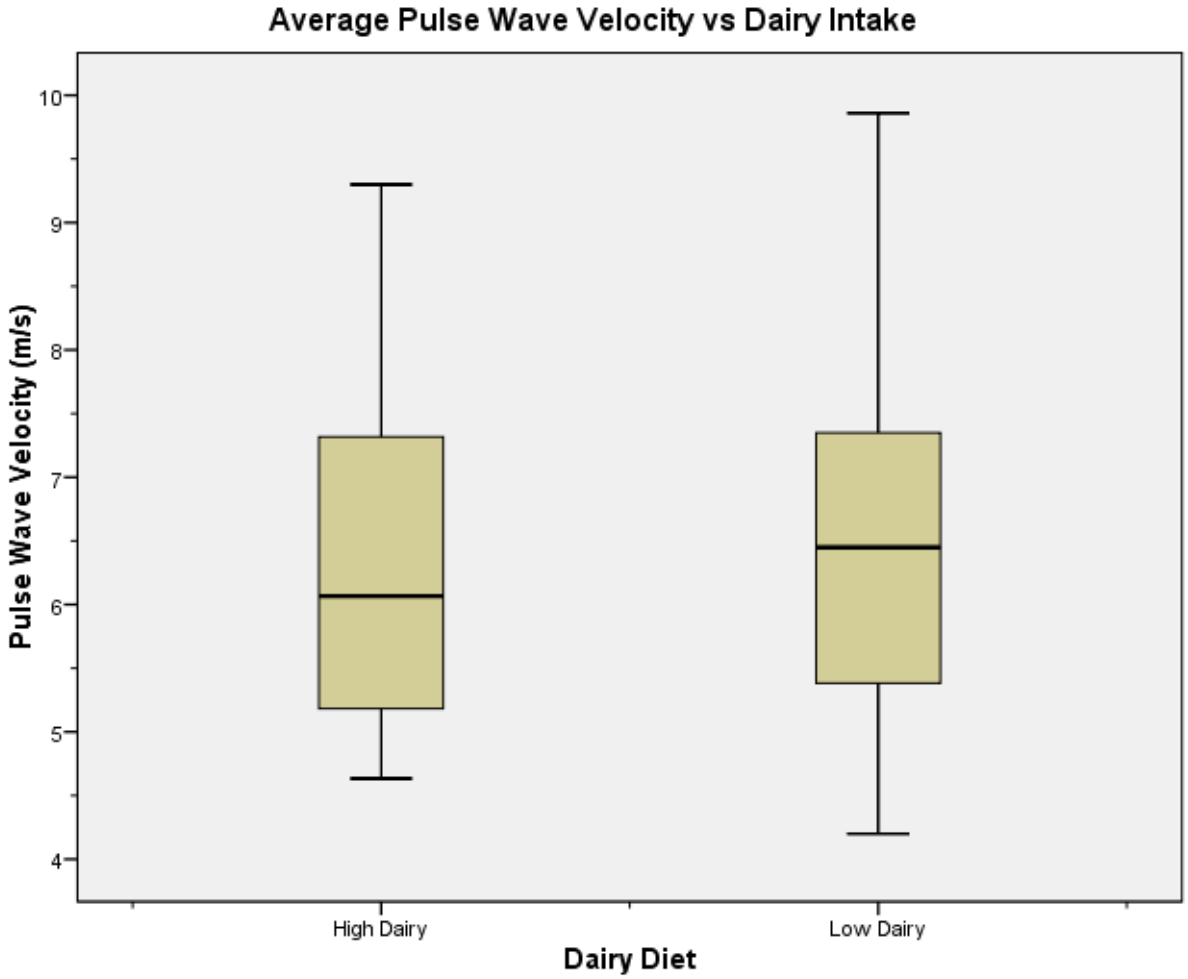
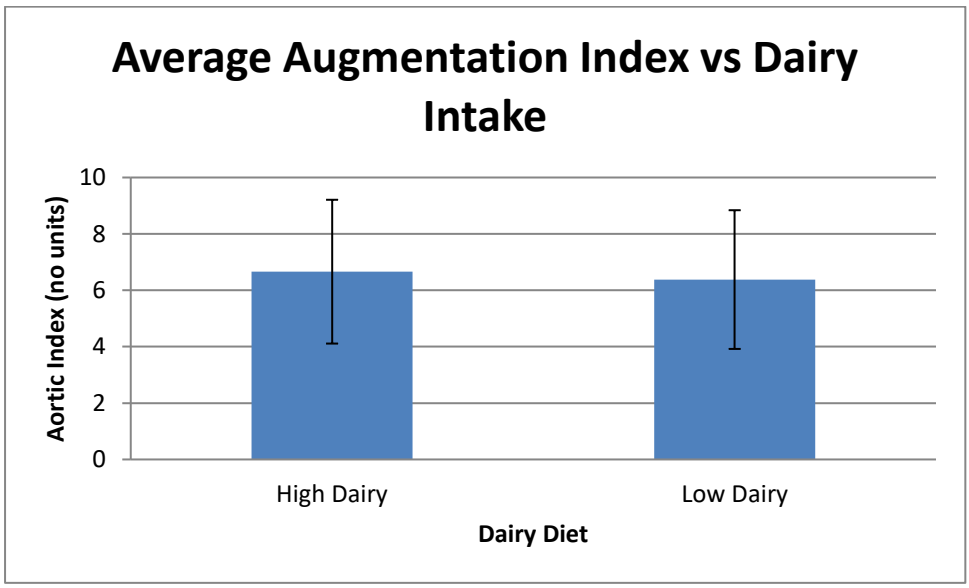

Average Pulse Wave Velocity vs Dairy Intake
Pulse Wave Velocity (m/s)
10
9
8
7
6
5
4
High Dairy
Low Dairy
Dairy Diet

Fig 11



Yoghurt intake

Yoghurt intake was added into the ANOVA as a covariate, and did not significantly influence the outcome.

Table 3: Correlation between ISI and Dietary Variables

Variable	Hyperglycaemic Clamp High Dairy ISI	LDIGIT High Dairy ISI	Hyperglycaemic Clamp Low Dairy ISI	LDIGIT Low Dairy ISI
Energy (kJ)	0.3	0.2	0	0.1
Carbohydrate (g)	0.2	0.4*	0	0.1
Protein (g)	-0.02	0	0	0.1
Fat-total (g)	0.3	0.2	-0.1	0
Fat-saturated (g)	0.2	0.2	-0.1	0
Dietary fiber (g)	0.2	0.1	0	0.2
Calcium	-0.1	0.03	0	0.2
kJ from Protein (%)	-0.3	-0.3	0	0
kJ from Fat (%)	0.2	0.06	-0.1	0
kJ from saturated fat (%)	0.1	0	-0.1	0
kJ from CHO (%)	0	0.1	0.1	0.1
kJ from fiber (%)	0.2	0	0.2	0.3

***P*-value=0.03**

Correlation

Insulin sensitivity was not strongly correlated between the LDIGIT and hyperglycaemic clamp tests (0.5 for high dairy $P < 0.05$, 0.3 for low dairy, $P = 0.05$).

In the high and low dairy diets, there was either a weak correlation or no correlation between each dietary variable and insulin sensitivity derived from the hyperglycaemic clamp and LDIGIT (Table 3). Table 3 was presented in this manner in order to establish the correlation coefficients between each dietary component in the high and low dairy diets, with both methods for testing insulin sensitivity.

Chapter Six: Discussion

Summary of results

The intake of 4-6 serves and 0.5 serves of dairy products per day for four weeks had no significant effects on insulin sensitivity, c-peptide response or pulse wave velocity and augmentation index. This could be interpreted as the intake of dairy products does not affect insulin sensitivity and therefore type II diabetes risk, or cardiovascular health in this dairy amount, length of diet or population amount and type studied. A 30% difference in means for insulin sensitivity was not observed.

There were significant differences in dietary outcomes for this study where fat intake was higher in the high dairy diet. Saturated fat intake was also higher in the high dairy diet. Calcium intake and energy intake derived from saturated fat intake was also higher in the high dairy diet. These results are to be expected, as dairy is abundant in these nutrients. It is therefore expected that these values would be significantly different to the low dairy phase and the baseline visit. Apart from these differences, other nutrients measured did not differ significantly from either diet.

Study comparisons

Of the interventions included in the literature review, just two found a significant improvement in insulin sensitivity, insulin concentrations or glucose concentrations, with these studies ranging from 12 weeks to 12 months. These studies were longer in length when compared to the study conducted here (4 weeks), but the participants consumed similar amounts of dairy compared to ours. Stancliffe et al. required their participants consume 3.5

servings and 0.5 servings in the high and low dairy diet respectively. Rideout et al. required their participants to consume four servings per day and ≤2 servings per day for their participants. The shortest study in these two was conducted by Stancliffe et al., and required their participants to consume less dairy than what our participants consumed. The amount of time that their participants consumed the high dairy diet was three times the length of time our participants consumed high dairy products. There may be a possibility that the length of time that an individual consumes dairy may have an effect on their insulin sensitivity.

These findings were in contrast to a recently completed study conducted by Turner et al. on the effects of a red meat versus a dairy diet on insulin sensitivity. After a four-week period of consuming each diet and undergoing an OGTT, Turner et al. found that participants consuming the red meat diet had similar insulin sensitivity to the control meal consumed prior to starting the study. The same participants showed that their insulin sensitivity was worse after consuming the dairy diet, when compared to the red meat diet, whereas our study found that there were no significant differences observed in insulin sensitivity in either the high or low dairy diet (Turner et al., 2015). Turner et al. had 47 participants complete this study, and the length of each diet was four weeks; the same as the current study. The study design also required participants to be weight stable for the intervention period, as was required in the current study. There is a possibility that dairy may have no effect on insulin sensitivity based on the findings of this study, and the current study may have confirmed these findings (Turner et al., 2015). Turner et al. also found that there were no differences in dairy and red meat intake in an acute setting in a later study (Turner et al., 2016).

These findings are in conflict with the conclusions drawn by epidemiological data in this field of study, which appears to suggest an inverse association between increased dairy and risk of type II diabetes and cardiovascular health complications is present.

The Chen et al. meta-analysis suggested that the intake of total dairy had no significant associations with type II diabetes risk, however it did find that there was a dose-dependent relationship present when examining dairy types individually. The same meta-analysis also found that there was an inverse association between yoghurt intake and type II diabetes risk in a multivariate analysis, however this effect was not observed in the current study. Previously established dietary interventions which included yoghurt did not examine the dairy food by itself and often used it as a vehicle to assess other nutrients on glycaemic

control and type II diabetes risk, and therefore there are no current studies that examine the effect of yoghurt alone on type II diabetes risk (Chen et al., 2014).

Tong et al. and Aune et al. found that low fat dairy consumption had a greater inverse association with type II diabetes risk in their meta analyses (Tong et al., 2011, Aune et al., 2013).

A recent meta-analysis of 22 cohort studies conducted by Gijsbers et al. found that total dairy intake was inversely associated with type II diabetes risk (RR: 0.97 per 200g/day, 95% CI: 0.95, 1.00, P=0.04). Gijsbers et al. found that there was a similar but linear inverse association between low fat dairy and type II diabetes (RR 0.96 per 200g/day, 95% CI 0.92, 1.00, P=0.072). Nonlinear inverse associations were found for yoghurt intake in this meta-analysis too, with a RR of 0.86 per 80g/day when compared with no yoghurt intake. Ice cream intake had an inverse association with type II diabetes as well, with a RR of 0.81 (95% CI 0.78, 0.85, P<0.001) at 10g/day. No incremental benefits were associated with higher intake of ice cream. This meta-analysis found that other dairy types were not significantly associated with type II diabetes (Gijsbers et al., 2016).

One reason to explain this difference in findings between intervention and epidemiological data may be due to epidemiological data not covering aspects of an individual's lifestyle in the surveys used to assess them. Physical activity and mood can affect body weight, and therefore insulin sensitivity in individuals. There is a possibility that these factors may have affected the associations observed in the epidemiological data. There may be a possibility that the associations concluded in these studies are perhaps actually showing the associations of a balanced diet and regular physical activity on insulin sensitivity.

Protein intake has been shown to increase insulin secretion following ingestion. Consuming amino acids leads to a GLP-1 and glucose-dependent-insulinotropic polypeptide activation, which leads to a greater secretion of insulin following ingestion. Whey protein is a source of branched chain amino acids – leucine, valine and isoleucine. Whey protein is responsible for the greatest increase in serum insulin concentration when compared to casein and other sources when consumed with or before a carbohydrate meal. Given that increased insulin secretion could eventually prove detrimental to an individual consuming large amounts of whey protein due to an increased risk of developing insulin resistance (Turner et al., 2015).

A study run by Takeshita et al. was able to find that an increase in total dairy intake, and therefore BCAA oral supplementation was able to lead to improved glycaemic control among

men aged 20-60 years with type II diabetes. The increase in BCAA intake was also able to lead to an improved Matsuda index of insulin sensitivity, concluding that BCAA intake may aid in improving skeletal muscle insulin resistance. In the total number of protein studies examined, 5 of the 9 studies reported the intake of BCAAs as effective in improving insulin sensitivity, glycaemic control or both. Three studies found no interaction between BCAA intake and insulin sensitivity or glycaemic control.

Six micronutrient studies were found to be relevant in analysis, and all six support the idea of micronutrients improving insulin sensitivity. Magnesium is a component of dairy and when taken as an oral supplement, magnesium has been indicated to express a lower fasting serum glucose and insulin concentration, as well as lower HOMA-IR index. Lipid profiles also improve when ingesting magnesium oral supplements. Compensatory insulin secretion response is altered after ingesting magnesium as found by Guerrero-Romero et al. when compared to a placebo (Guerrero-Romero and Rodriguez-Moran, 2011), and Mooren et al. has also found that fasting plasma glucose concentrations were significantly lower after ingesting magnesium (Mooren et al., 2011). Insulin sensitivity as measured by HOMA and Matsuda index was also improved. Chacko et al. report a lower c-peptide response and lower insulin secretion in response to magnesium intake (Chacko et al., 2011). Paolisso et al. report similar improved insulin action, as well as total-body oxidative glucose metabolism (Paolisso et al., 1992).

Dairy fat is another component of dairy that is focused on in epidemiological studies, as well as interventions. It is comprised of approximately 70% saturated fatty acids, and has been indicated to impair insulin sensitivity in healthy individuals. High concentrations of dairy fat are inversely associated with fasting plasma glucose, and has been indicated to show higher systemic and hepatic insulin sensitivity in individuals consuming high amounts of dairy.

Of the six studies examined in fat intake and type on insulin sensitivity, two were found to be positively associated with insulin sensitivity, where Nardi et al. determine that oleic and linoleic acid have an insulin sensitising effect when culturing myotubes in skeletal muscle, showing that MUFAs may be beneficial (Nardi et al., 2014). Jans et al. suggest that PUFAs can increase membrane fluidity, increase the number of insulin receptors, and increase the affinity of insulin to the insulin receptor. MUFAs induced changes in gene expression and skeletal muscle fatty acid partitioning (Jans et al., 2012). One study by Tholstrup et al. found that high fat dairy may be detrimental to insulin sensitivity, showing that consuming high fat

dairy led to a higher glucose and insulin response, which could be attributed to more readily available lactose in some dairy types, and the food texture being a factor which influenced GIP and GLP-I. The remaining three studies in this area found no interaction between fat intake and insulin sensitivity (Jans et al., 2012).

Drouin-Chartier et al. conducted a total dairy intervention involving 27 women with obesity as part of a crossover intervention. The women were required to consume 3.2 serves of 2% fat milk per day, and another diet without milk or other dairy products for six weeks each, after experiencing a run-in period of 4 weeks, requiring participants to consume 1.4 serves of the same 2% fat milk per day (Drouin-Chartier et al., 2015). The investigators also provided the participants with meals for every day of the dietary-controlled periods, and also reported the meals consumed by participants, as well as any deviations to the diet. The study had a compliance rate of 98% as a result, ensuring that this would be a well-controlled environment to assess glucose homeostasis. Despite these measure, Drouin-Chartier et al. found no that that while there was a decreased plasma fasting glucose response observed in the dairy group, the change was not significant, and that the effect was not exclusive to the dairy group. This of course is in direct conflict with the conclusions drawn by the epidemiological studies included here. Drouin-Chartier et al.'s findings were different with the study here, where fasting glucose concentration was higher in the high dairy diet, but this was not a significantly different result. We did not find a significant improvement in insulin sensitivity either, and Drouin-Chartier et al. suggest that there may be a residual confounding effect present in the epidemiological studies performed so far, perhaps explaining the associations with reduced type II diabetes risk (Drouin-Chartier et al., 2015).

Rideout et al. found that 12 months of consuming four serves of dairy per day can improve fasting plasma insulin by 9% and insulin resistance by 11% as measured by HOMA-IR, when compared to 2 serves of dairy per day. This study recruited 23 volunteers and was able to find a significant difference in fasting plasma insulin and insulin resistance by requiring participants to consume both diets for six months each (Rideout et al., 2013). Our study ran for 10 weeks only, and perhaps this may be the key to detecting a difference in diets with the small sample size that we have here. It could prove challenging to have a high compliance rate in this study should it run for 12 months as well, so adding a significant honorarium may be the biggest motivator to ensure a higher rate of compliance among participants here.

Dugan et al. found that no detectable differences in insulin resistance in their study, with a study length of six weeks. Despite this, a small yet significant rise in fasting plasma glucose was detected in the dairy group when compared to the control (Dugan et al., 2014). Stancliffe et al. found that >3.5 serves of dairy per day led to a significant effect on plasma insulin when compared to an intake of 0.5 serves per day for a period of 12 weeks. The effect continued on to the end of the study, and the effect was also seen in HOMA as well (Stancliffe et al., 2011).

Arnberg et al found that consuming 1L of skim milk, whey and casein per day for 12 weeks, where it was confirmed that whey and casein intake increased insulin secretion. Arnberg et al. found no significant differences in c-peptide concentration, HOMA or plasma insulin secretion for the milk groups observed (Arnberg et al., 2012). HOMA was increased in the whey group by 23%, and 32% in the casein group. We would not be able to detect differences such as this in our completed study as these differences were detected using whey and casein supplements, where concentrations of both would be much higher than what would be found in a conventional dairy product. An individual would be required to consume a significantly higher number of serves of dairy in order to match the whey and casein consumed in this study (Arnberg et al., 2012).

Dougkas et al. found no difference in glucose concentrations between the dairy snacks and water phases of their crossover intervention lasting four weeks, however there was a higher insulin concentration observed in the dairy group for this study. Given that this was a side measure, the amount of dairy snacks consumed by the participants was entirely *ad libitum*. It is therefore entirely possible that every participant involved in the study did not eat the same amount of dairy snacks, and could be the main factor behind the results shown. The length of the study could also be a factor that affected the results seen here (Dougkas et al., 2012).

Tanaka et al. found that fasting blood glucose, body weight, body fat percentage, HbA1c, LDL- cholesterol and total cholesterol decreased significantly when consuming either 400g/day of milk or a combination of milk and yoghurt in a sample size of 200 men. The study was 24 weeks long and had almost ten times the number of participants that we had in the current study. At this number it is certain that the Tanaka et al. study was adequately powered in order to observe the discoveries reported, which is a big difference between our study and theirs (Tanaka et al., 2015).

To the best of our knowledge, the studies included in the literature review did not receive funding from industries or organisations which could have possibly introduced a bias toward the findings.

Difficulties

Difficulties were encountered in carrying out this study, such as having an initial difficulty to recruit overweight and/or obese participants with impaired glucose tolerance or impaired fasting glucose. Selecting from an already narrow pool of people for these criteria lead to a slow initial recruitment rate. We made the decision to open the recruitment criteria to also include individuals with normal glucose tolerance, normal body weight and BMI, and this enabled us to recruit participants at a higher rate. Including participants with a normal weight and normal glucose tolerance in this study may have impacted the differences in insulin sensitivity and other markers we measured, as the differences observed in healthy individuals may have been minimal. There is a possibility that recruiting healthy individuals may have had an effect on our results.

Initially we aimed to recruit a total of 60 participants using a SD of 1.99 in the initial sample size calculations, however due to time constraints we had to stop recruiting after only 34 subjects were enrolled. A post hoc calculation confirms that this study was therefore underpowered to reach the aim outlined. We used the log-transformed mean and standard deviation for the hyperglycaemic clamp technique and the LDIGIT, as these results were considered normally distributed after transformation. When using the hyperglycaemic clamp technique, and assuming that the power is 80% at a significance of 0.05, in order for the 10% difference observed here to be considered statistically significant we would need a sample size of 113 individuals, assuming a log-transformed SD of 0.40.

Strengths

A strength of this study was the crossover design, allowing participants to serve as their own controls for the duration of the intervention. The study was also free-living in the sense that participants were allowed to incorporate the required number of dairy serves from each phase into their regular diets and choose fat content of the products consumed, as long as they were able to control energy intake and remain weight stable. A combination of these factors, plus

the analysis of both diet outcomes and the number of dairy serves consumed suggests that compliance was good; however, energy intake was higher in the high dairy diet when compared to the low dairy diet. There is the chance that some may have misreported in their food records and this would prove difficult to control in a free-living setting. There are few ways to control for this, and some studies have resorted to providing the participants with dairy serves as well as to have participants visit the testing centre to consume one meal a day in front of the investigators. In the context of the current study this may have drastically affected recruitment and dropout rate, as most of the participants enrolled were of working age or studying at a tertiary level.

Future improvements

The current study could be improved by increasing the sample size and making it sufficiently-powered. As discussed before, we encountered difficulties in recruitment rate and the time frame to collect data was significantly reduced as well, hence the decision to expand our recruitment criteria to include normal weight and normal glucose tolerant individuals. We had a final number of five individuals in this study that possessed impaired glucose tolerance, and there was no interaction between glycaemic status and ISI. An improvement to this would be to engage a wider range of media and services to recruit our target population, and to expedite recruitment rate. By engaging a wider range of media and services it may be possible to identify individuals who satisfy the recruitment criteria easier and quicker, and it may even be possible to recruit more overweight and obese individuals as originally planned for this study.

Chapter Seven: Conclusion

There were no significant differences detected between high and low dairy diets for insulin sensitivity, or the cardiovascular health markers pulse wave velocity and augmentation index. There is a possibility that increased dairy consumption has no effect on insulin sensitivity, however it is unclear whether dairy has no effect on these endpoints, or if the absence of an effect was due to an underpowered study. Future research that is more robustly designed in this area is required.

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Appendix A – Evidence tables

Cohort studies

Author, year	Study name	Follow up period	Study size, sex, number of cases	Dietary assessment	Endpoints	Exposure	Quantity	RR (95%CI)	Adjustment for confounders
(Struijk et al., 2013)	Inter99 prospective study	5 years	61,301 men and women, 13,016 studied	FFQ, OGTT, blood sampling	T2D, glucose metabolism measures.	Dairy product intake	204g/day median intake	OR 0.95 (0.86) for Type II diabetes No significant association with glycaemia measures, except for cheese and fermented dairy	Gender, age, physical activity, diabetes family history, education level, smoking status, known diabetes.
(Fumeron et al., 2011)	The DESIR study	9 year follow up	3,435 males and females	23-item FFQ	Insulin resistance, metabolic syndrome incidence	Dairy consumption	Not specified	significant inverse association detected with low fat fermented dairy only, HR 0.76 (95% CI: 0.60, 0.99, *P*=0.049) Yoghurt intake adjusted for sex and age. (HR 0.65, 95% CI 0.52, 0.83, *P*= 0.017	Sex, age
(O'Connor et al., 2014)	EPIC-Norfolk Study	11 years	4,000 males and females	7 day food diary	Type II diabetes incidence	Dairy intake (high fat, low fat	80g/day	Low fat dairy: 0.76 (95% CI 0.66, 0.90, *P*=0.049) Yoghurt: 0.72 (95% CI 0.55, 0.95)	Age and sex, anthropometric, dietary and diabetes risk factors.

Author, year	Study name	Follow up period	Study size, sex, number of cases	Dietary assessment	Endpoints	Exposure	Quantity	RR (95%CI)	Adjustment for confounders
(Eussen et al., 2016)	Maastricht Study	6 years	2391 Males and females aged 40-75 years	253-food item FFQ	Impaired glucose metabolism and type II diabetes	Dairy consumption (all fat types)	Total dairy products (100g)	OR: 0.73 (95% CI 0.55-0.96) for skimmed milk, 0.74 (95% CI 0.54-0.99) for fermented products, and 0.67 (95% CI 0.50-0.90) for yoghurt. OR of 0.76 (95% CI 0.61-0.95) per 100g increment of total dairy after full adjustment.	Age, sex BMI, physical activity, smoking status, education, energy intake, vegetable, meat and fish intake
(Ericson et al., 2015)	Malmo diet and Cancer cohort	14 years	26,930 aged 45-74 years, 2860 cases	7 day menu book to record diet, 168-item questionnaire, 45 minute interview.	Type II diabetes incidence	Diet	6.3 and 6.6 portions per day (type II diabetes cases vs non cases)	No associations observed between dietary content of total fat and type II diabetes incidence	Age, sex, diet method version, season, energy intake.
(Hodge et al., 2007)	The Melbourne Collaborative Cohort Study (MCCS)	4 years	3737 adults aged 36-72 years, 292 cases	Self-reported	Type II diabetes incidence	Diet	Not specified	Individuals who developed type II diabetes had higher proportions of 18:0, total saturated fat, 16:1n-7, 20:3n-6, 20:4n-6, total n-3 fatty acids, 20:5n-3, and 22:6n-3	Age, sex, country of birth, physical activity, family history of diabetes, alcohol intake.

Cohort studies - Micronutrients

Author, year	Study name	Follow up period	Study size, sex, number of cases	Dietary assessment	Endpoints	Exposure	Quantity	RR (95%CI)	Adjustment for confounders
(Kirii et al., 2009)	Japan public health center-based prospective study	5 years	59,796 middle aged and older men and women with no history of type II diabetes. 1,114 cases	Food frequency questionnaire	Type II diabetes risk	Calcium, vitamin D and dairy intake	Not specified.	OR 0.65 (95% CI 0.49-0.88, *P*=0.007) for dairy consumption 0.79 (95% CI0.64-0.97, *P*=0.02) for milk 0.94 (95% CI 0.68-1.30 *P*=0.71) for cheese 0.72 (95% CI 0.55-0.93, *P*=0.04) for yoghurt	Sex, , age, area
(van Dam et al., 2006)	Black Women's Health Study	8 years	41,186 African-American Women without a history of diabetes	Validated food frequency questionnaire	Type II diabetes risk	Dietary calcium, magnesium, major food sources and type II diabetes risk	Whole milk, 2% milk, Skim milk, 1% milk, butter milk, yoghurt (8 ounce glass) Milk/cream in	Magnesium intake had a hazard ratio of 0.69 (95% CI 0.59-0.81) (*P*<0.0001), Magnesium and calcium intake were mutually adjusted in this study, and following this, the inverse association with magnesium and type II diabetes risk remained, whereas calcium's inverse association did not	Age, BMI, smoking status, energy intake, strenuous physical activity, alcohol consumption, intake of processed

Author, year	Study name	Follow up period	Study size, sex, number of cases	Dietary assessment	Endpoints	Exposure	Quantity	RR (95%CI)	Adjustment for confounders
							coffee/tea (1 tablespoon) Frozen yoghurt /ice cream (1 scoop) Butter (2 pats) Cheese/cheese spreads not including cottage cheese (2 slices or 2 ounces)		and red meat.
(Lopez-Ridaura et al., 2004)	The Nurses' Health Study & The Health Professional	18 years in Nurses' study and 12 years in health	51, 374 Men and women aged 30-75 years	61 item food frequency questionnaire and other food frequency	Magnesium intake and risk of developing type II diabetes	Magnesium intake	Not specified	Significant inverse association between magnesium intake and risk of type II diabetes in both cohorts. When comparing the top versus lowest quintile of intake RR was 0.55 (95% CI 0.50-0.61) and 0.56 (95%	BMI, calcium, potassium and phosphorous.

Author, year	Study name	Follow up period	Study size, sex, number of cases	Dietary assessment	Endpoints	Exposure	Quantity	RR (95%CI)	Adjustment for confounders
	s' Follow-up Study	professionals' study		questionnaires				CI 0.47-0.67) in women and men, respectively. Association attenuated in both cohorts.	

Cohort studies- Others

Author, year	Study name	Follow up period	Study size, sex, number of cases	Dietary assessment	Endpoints	Exposure	Quantity	RR (95%CI)	Adjustment for confounders
(Zong et al., 2014)	Nutrition and health of Aging Population in China	6 years	2,091 older Chinese men and women	74-item food frequency questionnaire	Dairy intake and cardiometabolic risk	Dairy intake	0.5->1 serves	RR 0.73(95% CI 0.58-0.92) for 0.5-1.0 serves consumed RR 0.67(95% CI 0.52-0.88) for >1 serves consumed	Age, sex, region, residence

Cohort studies- Metabolic Syndrome

Author, year	Study name	Follow up period	Study size, sex, number of cases	Dietary assessment	Endpoints	Exposure	Quantity	RR (95%CI)	Adjustment for confounders
(Pereira et al., 2002)	The Cardia study	1985-1986, 1995-1996, 10 year follow up	Men and women, aged 18-30y	FFQ, 24 hour recall	Cumulative incidence of insulin resistance syndrome (IRS)	Dairy consumption	<10 times per week to ≥35 times per week	OR 0.28, 95% CI 0.14-0.58 for developing insulin resistance when comparing highest versus lowest category of dairy consumption. OR 0.79 for each daily occasion of developing insulin resistance	Age, sex, race, energy intake, study centre.
(Elwood et al., 2007)	Caerphilly prospective study	1979-1983, 20 year	Men, 45-59 years old, 2375 studied	Blood sampling, 7 day weighed food	Presence of metabolic syndrome, T2D	Milk and dairy consumption	1≥ pint milk/day	OR 0.38 (0.18, 0.78) for metabolic	None identified.

Author, year	Study name	Follow up period	Study size, sex, number of cases	Dietary assessment	Endpoints	Exposure	Quantity	RR (95%CI)	Adjustment for confounders
		follow up		records, FFQ.				syndrome presence	
(Yakoob et al., 2016)	The Nurses' Health Study & The Health Professionals' Follow-up Study	15.2±5.6 years	3,333 men and women	Plasma and erythrocyte fatty acid concentration	Presence of type II diabetes	Dairy fat biomarkers and type II diabetes risk	Not specified	Highest quartile of intake compared to the lowest quartile of plasma 15:0 had HR 0.56 (95% CI 0.37-0.86, *P*=0.01). Plasma 17:0 HR: 0.57 (95% CI0.39-0.83, *P*<0.01), and t-16:1n-7 was 0.48 (95% CI 0.33-0.70, *P*<0.001) In continuous evaluation, 15:0 had a pooled HR of 0.62 (95% CI 0.46-0.85), 17:0, with a HR of	Demographics, metabolic risk factors, lifestyle, dietary habits, and other circulating fatty acids

Author, year	Study name	Follow up period	Study size, sex, number of cases	Dietary assessment	Endpoints	Exposure	Quantity	RR (95%CI)	Adjustment for confounders
								0.68 (95% CI 0.50-0.91), and t-16:1n-7, with a HR of 0.54 (95% CI 0.40-0.73), as found in the previous categorical analysis, 14:0 was not associated with diabetes.	
(Liu et al., 2005)	Women's health study	Average of 8.8 years	10,066 Middle-aged and older women	131-item semi-quantitative food frequency questionnaire, 71% provided baseline blood samples	Calcium Vitamin D intake and metabolic syndrome	Dairy intake	Not specified	Calcium quintiles of intake: 1.00, 0.82 (95% CI 0.70-0.97), 0.84 (95% CI 0.71-0.99), 0.70 (95% CI 0.59-0.83), and 0.64 (95% CI 0.54-0.77)	Age, smoking status, exercise, alcohol intake, multivitamin use, parental history of myocardial infarction before 60 years, total fat,

Author, year	Study name	Follow up period	Study size, sex, number of cases	Dietary assessment	Endpoints	Exposure	Quantity	RR (95%CI)	Adjustment for confounders
								Vitamin D intake inversely associated with metabolic syndrome prevalence, but not independent of calcium intake. No appreciably altered by additional adjustment for other dietary factors or vitamin D intake.	cholesterol, protein, glycaemic load and total calcium and vitamin D intake.
(Da Silva et al., 2014)	Population from Rudkowska et al., studying cardiometabo	Not specifie d	233 Healthy French-Canadians	Validated food frequency questionnair e	Dairy product intake and metabolic syndrome	Dairy intake and metabolic syndrome	Not specified	Fasting glucose concentration findings were still significant in women (r=-0.24; p=0.007).	Age, BMI.

Author, year	Study name	Follow up period	Study size, sex, number of cases	Dietary assessment	Endpoints	Exposure	Quantity	RR (95%CI)	Adjustment for confounders
	lic risk factors Stearoyl-CoA Desaturase (SCD) -1 gene polymorphisms and n-3polyunsaturated fatty acid supplementation.				risk parameters	risk parameters		Low fat dairy intake was also associated with lower systolic blood pressure in women (*r*=-0.19, *p*=0.04), high fat dairy intake was inversely correlated with diastolic blood pressure in men (*r*= -0.23, *p*=0.02), total dairy intake was not associated with blood pressure in either sex.	
(He et al., 2006)	The CARDIA study	15 years	5155 black and white men and women,	Interviewer-administered	Physiological, psychological and lifestyle	Magnesium intake	Not specified	Two highest quintiles of magnesium intake HR of 0.69 (95% CI	Major dietary and lifestyle factors, potassium, calcium, folic

Author, year	Study name	Follow up period	Study size, sex, number of cases	Dietary assessment	Endpoints	Exposure	Quantity	RR (95%CI)	Adjustment for confounders
			aged 18-30 years	quantitative FFQ	factors in cardiovascular disease			0.52, 0.91, *P*< 0.01) inversely associated with incident metabolic syndrome.	acid intake, wholegrain intake
(Lutsey et al., 2008)	Atherosclerosis Risk in Communities (ARIC) study	9 years	9514 participants aged 45-64 years, 3782 cases of metabolic syndrome	66-item food frequency questionnaire	Diet and metabolic syndrome risk	Western Diet, including dairy	Not specified	Dairy consumption HR: 0.87, (95% CI: 0.77-0.98)	Demographic factors, smoking, physical activity, energy intake
(Louie et al., 2013)	Blue Mountains Eye Study (BMES)	10 years	3267 participants aged 49+ years	145-item food frequency questionnaire	Metabolic syndrome and type II diabetes incidence	Dairy in diet	250g milk 200g yoghurt 250ml custard, 40g cheese	Participants who had higher quartiles of dairy intake had a significantly lower risk of metabolic syndrome (OR 0.41, 95% CI 0.23-0.71, *P*=0.004), no	Age, sex, smoking status, physical activity, dietary glycaemic load, dietary fibre, total energy intake and family

Author, year	Study name	Follow up period	Study size, sex, number of cases	Dietary assessment	Endpoints	Exposure	Quantity	RR (95%CI)	Adjustment for confounders
								associations between dairy intake and type II diabetes	history of type II diabetes

Cross-sectional studies

Author, year	Study name	Follow up period	Study size, sex, number of cases	Dietary assessment	Endpoints	Exposure	Quantity	RR (95%CI)	Adjustment for confounders
(Ferland et al., 2011)	Not specified	5 years	543 Inuit men and women	Food frequency questionnaire	Body weight, blood pressure, type II diabetes and CVD risk factors	Dairy consumption	Lowest tertile: 120g/day Highest tertile: 290g/day	Higher prevalence of Inuit participants with metabolic syndrome was observed in the higher tertile compared with the first tertile (10.3% vs 1.6%; $p < 0.001$).	Age, sex, energy intake, cigarette smoking, education level, and dietary patterns
(Tucker et al., 2015)	Not specified	Not specified	272 middle-aged men and women	7 day weighed food records	Insulin resistance	Dairy consumption	1 serve = 12g CHO, 8g protein, 5g fat	HOMA was significantly higher in the high dairy intake group (0.41±0.53) when compared to HOMA in moderate	Body fat, physical activity, diet, energy intake,

Author, year	Study name	Follow up period	Study size, sex, number of cases	Dietary assessment	Endpoints	Exposure	Quantity	RR (95%CI)	Adjustment for confounders
								intake group (0.22±0.55), and the low intake group (0.19±0.58, F=6.90, *P*=0.0006)	
(Ghotboddin Mohammadi et al., 2015)	Tehran Lipid and glucose study	12 years	785 adolescents aged 10-19 15.1-22% developed metabolic syndrome	168-item food frequency questionnaire	Metabolic syndrome	Dairy consumption	<311.3->622.8g/day	No significant associations between dairy intake and metabolic syndrome (0.97 (95% CI 0.56, 1.66)	Age, sex, energy intake.
(Abreu et al., 2014)	Azorean Physical activity and Health study II	11.5±20 months	494 15-18 year old adolescents	Semiquantitative food frequency questionnaire	Cardiometabolic risk factors	Dairy consumption	483.25g/day	No significant associations found.	Parental education, pubertal stage, low-energy reporter, energy intake, total fat intake, protein intake, and dietary fibre intake.

Author, year	Study name	Follow up period	Study size, sex, number of cases	Dietary assessment	Endpoints	Exposure	Quantity	RR (95%CI)	Adjustment for confounders
(Drehmer et al., 2015)	The Brazilian Longitudinal Study of Adult Health (ELSA-Brasil)	Described as ”long-term follow-up”	15,105 adults aged 35-74 years	Food frequency questionnaire	Glycaemia and insulinaemia	Dairy consumption	2.69 serves/day	One serve of cheese was associated with a – lower fasting glucose of 0.21mg/dl (95% CI -0.046, 0.03) – with yogurt 0.29mg/dl 95% CI -1.03, 0.44)	Demographic characteristics, family income, menopause, family history of diabetes, smoking status, alcohol intake, physical activity, calorie intake, non-dairy food groups.
(Akter et al., 2013)	Data obtained from health surveys during regular check-ups	No follow up	496 participants aged 20-68 years	Validated brief dietary history questionnaire.	Fasting serum insulin plasma glucose and HOMA	Dairy	≤13.7g/day-≥135.1g/day	HOMA 0.86 (95% CI 0.76-0.96, *P*=0.02) Fasting insulin 3.71µU/ml (95% CI 3.34-4.12, *P*=0.02) Fasting blood glucose not	Age, sex, work place, sedentary work, non-occupational physical activity, smoking status, alcohol consumption, total fibre intake and PUFA/SAFA

Author, year	Study name	Follow up period	Study size, sex, number of cases	Dietary assessment	Endpoints	Exposure	Quantity	RR (95%CI)	Adjustment for confounders
								associated with full-fat dairy intake.	
(Bergholdt et al., 2015)	Copenhagen city heart study	5.5 years	97,811 individuals aged 20-100 years	Self-reported from questionnaires from the study	Diabetes and overweight/obese status	Milk	1-≥11 glasses/week	No consistent observations between risk of type II diabetes and milk consumption	Sex, age, height, population
(Snijder et al., 2007)	The Hoorn study	Not specified	2064 men and women, aged 50-75y	FFQ	Presence of metabolic syndrome	Dairy consumption	4.1 servings/day	Dairy consumption associated with higher fasting glucose concentrations (0.04_0.02 mmol/L per serving), inversely associated with blood pressure	Total energy intake, alcohol intake, antihypertensive medication use, smoking status, physical activity, income, education level.

Branched chain amino acids

Author, year	Study name	Follow up period	Study size, sex, number of cases	Dietary assessment	Endpoints	Exposure	Quantity	RR (95%CI)	Adjustment for confounders
(Wurtz et al., 2013)	Cardiovascular risk in Young Finns study	6 years	1,680 young adults	Self-reported questionnaires	Insulin resistance	Diet	Not specified	Branched chain and aromatic amino acids were associated with HOMA-IR when adjusting for conventional metabolic risk factors. These associations were more pronounced in men (β=0.24) when compared to the associations found in women (β=0.12)	Age, BMI, systolic blood pressure, HDL cholesterol, triglycerides, smoking status, physical activity.
(McCormack et al., 2013)	N/A	Not specified	74 children aged 8 to 18 years	Dietary and exercise questionnaires	Insulin resistance	Elevated concentrations of BCAA	1.75g/kg (75g maximum) oral dose	Strong association observed between BCAA concentration at baseline, and HOMA-IR at 18 months (r^2= 0.44, *P*= 0.004)	Not specified

Author, year	Study name	Follow up period	Study size, sex, number of cases	Dietary assessment	Endpoints	Exposure	Quantity	RR (95%CI)	Adjustment for confounders
(Qin et al., 2011)	INTERMAP	Not specified	4429 men and women, aged 40-59 years	In depth, multiple pass 24-hour food record.	Obesity prevalence	Elevated amino acid intake	Not specified	OR between weight status and BCAA intake after multivariate adjustment was 0.70 (95% CI 0.57-0.86, *P*<0.01) OR changes to 0.75 (95% CI 0.58-0.98, *P*=0.03) after adjusting for countries with lowest prevalence of obese individuals.	Age, gender, country, employment status, physical activity, smoking status, special diet, total energy intake, total available carbohydrate, saturated fat intake, protein intake.
(Yamada et al., 2015)	N/A	N/A	94 men and women	Not specified	Insulin resistance	Amino acid profile	N/A	Several amino acids were found to be positively correlated with HOMA-IR, branched chain amino acids were positively associated with insulin resistance	BMI.

Fat

Author, year	Study name	Follow up period	Study size, sex, number of cases	Dietary assessment	Endpoints	Exposure	Quantity	RR (95%CI)	Adjustment for confounders
(Kratz et al., 2014)	N/A	N/A	17 men and women with non-alcoholic fatty liver disease, and 15 matched controls	Blood samples, OGTT and IVGTT	Associations between biomarkers of fat intake and β-cell function	Intake of *trans*-16:ln-7 or dairy fat in general in association with glucose tolerance and factors determining glucose tolerance	Not specified	No biomarkers of fat intake were associated with β-cell function	BMI, sex, age, liver-spleen ratio

Author, year	Study name	Follow up period	Study size, sex, number of cases	Dietary assessment	Endpoints	Exposure	Quantity	RR (95%CI)	Adjustment for confounders
(Finucane et al., 2015)	CORDIPREV study	N/A	160-184 participants suffering from type II diabetes	Glucose tolerance tests and insulin tolerance tests	Monounsaturated fatty acid (MUFA)-enriched high-fat diets and insulin resistance	Dietary MUFA and habitual dietary MUFA intake on insulin sensitivity	Not specified	MUFA intake can help to improve insulin sensitivity.	Not specified
(Rosell et al., 2004)	Participants who under went health screening study in 1997-1999	Not specified	301 healthy, 63 year old men with differing fasting insulin concentrations	7-day food registration, fatty acid composition in adipose tissue and phospholipids.	Abdominal obesity	Dairy fat, calcium intake	Not specified	Correlations between obesity and dairy fat-0.06 (*P*=0.28), -0.24 (*P*=0.025), and 0.04 (*P*=0.60) Inverse association between dairy fat and obesity not seen in non-under reporters	Energy

Micronutrients

Author, year	Study name	Follow up period	Study size, sex, number of cases	Dietary assessment	Endpoints	Exposure	Quantity	RR (95%CI)	Adjustment for confounders
(Song et al., 2005)	Women's health Study	8.8 years	11,686 women aged ≥45 years, free of cardiovascular disease and cancer	131 item semi-quantitative food frequency questionnaire	Metabolic syndrome prevalence	Magnesium intake	252mg/day to 422mg/day	Significant observed decreased in risk of prevalent metabolic syndrome, multivariate adjusted OR for quintiles of magnesium intake and metabolic syndrome prevalence: 1.00, 0.91 (95% CI 0.78-1.06), 0.84 (95% CI 0.72-0.99), 0.81 (95% CI 0.68-0.96), and 0.73 (95% CI 0.60-0.88, *P*=0.0008).	Age, BMI, smoking, exercise, total calories, alcohol use, multivitamin use, diabetes history, hypertension history, high cholesterol, total fat, cholesterol, folate, glycaemic load.

Interventions – total dairy

Author, year	**Study size, sex, length**	**Endpoints**	**Exposure**	**Result**
(Stancliffe et al., 2011)	40 overweight and obese men and women, 12 weeks Randomised parallel study	Early and sustained effects of dairy in metabolic syndrome subjects.	Adequate (3.5 servings/day) dairy group, low (<0.5 servings/day) dairy.	Significant reduction in plasma insulin and HOMA in the adequate dairy group.
(Arnberg et al., 2012)	203 overweight adolescents, 12 weeks, randomised parallel study	Body weight, waist circumference, HOMA, plasma insulin, C-peptide concentration	1L of skim milk, whey, casein or water.	No significant differences in plasma c-peptide in skim milk or water group. High intake of skim milk, whey and casein increase BAZ, whey and casein increased insulin secretion.
(Drouin-Chartier et al., 2015)	27 women, 24 week randomised crossover	Cardiometabolic risk factors associated with metabolic syndrome	3.2 servings/day of 2% fat milk per 2000kcal No milk or dairy	No significant differences detected.
(Rideout et al., 2013)	23 healthy volunteers, 12 month randomised crossover study	Body weight, body composition, energy expenditure, blood pressure, blood glucose, blood lipid, blood lipoprotein responses, HOMA, plasma insulin and insulin resistance	High dairy (4 servings/day) Low dairy (≤2 servings/day)	Plasma insulin decreased by 9% ($p<0.05$) in the high dairy group compared to low dairy Insulin sensitivity decreased by 11% ($p=0.03$) in high dairy group compared to low dairy

Author, year	Study size, sex, length	Endpoints	Exposure	Result
(Dugan et al., 2014)	37 adults with metabolic syndrome, 16 week randomised crossover study	Anthropometrics, plasma lipids, glucose	Low fat dairy (10oz of 1% milk, 6oz of non – fat yoghurt, and 2 oz of 2% cheese, 3 serves/day) Carbohydrate control intervention (1.5oz granola bar and 12oz of juice)	No significant change in insulin levels in either gender observed (p=0.125 in men, p= 0.748 in women) Small yet significant change in plasma glucose in male participants (p=0.048)
(Dougkas et al., 2012)	40 overweight men, 4 week randomised parallel study	Appetite and subsequent energy intake after a preload.	3 servings of dairy snacks, or water	No differences in glucose, nor insulin measurements between groups.
(Tanaka et al., 2015)	200 Japanese men aged 20-60 years, 24 week randomised parallel	Waist circumference, blood pressure, fasting blood glucose, lipids.	400g/day milk 400g/day milk and yoghurt combination	No changes in fasting glucose concentrations.
(Turner et al., 2015)	47 overweight and obese men and women, 10 week randomised crossover study	Fasting insulin, fasting glucose, insulin sensitivity	Dairy diet high in milk, yoghurt or custard, Red meat diet	Fasting insulin was significantly higher in dairy diet (6.64±4.1 mU/L for dairy diet, 5.47±2.4 mU/L for red meat diet, P<0.01), no change in fasting glucose. Insulin sensitivity was lower in red meat diet when compared to dairy diet (1.33±0.8 for red meat, 1.71±0.8 for dairy diet, P<0.01).

Author, year	Study size, sex, length	Endpoints	Exposure	Result
(Turner et al., 2016)	43 men and women, 2 week randomised crossover study	Insulin and glucose response	2 test meals: 1 meal containing red meat, and orange juice. 1 meal containing skim milk, low-fat yoghurt, cheese and bread. Meals are separated by one week	iAUC glucose significantly higher in dairy meal when compared to red meat meal (2.23±0.49 compared to 0.88±0.57 mmol/L/3h, *P*=0.004). Insulin AUC and iAUC was not different between meals (159.65±20.0mU/L/3h for red meat, 167.49±24.1mU/L/3h for dairy). No differences detected, differences in glucose response attributed to glycaemic load differences in the meals.

Protein

Author, year	Study size, sex, length	Endpoints	Exposure	Result
(Takeshita et al., 2012)	27 participants with chronic hepatitis, 24 week randomised crossover study	BCAA supplementation and glucose tolerance and insulin sensitivity, fatty acid levels, insulin sensitivity.	4.15g/day of BCAA for 12 weeks, then a control diet for another 12 weeks.	BCAA supplementation led to improved glycaemic control in participants with lower Matsuda index.
(Kalogeropoulou et al., 2008)	13 healthy participants, 4 week randomised crossover study	Metabolic effects of ingested individual amino acids. Serum leucine, glucose, insulin, glucagon, and α-amino nitrogen concentrations.	25g glucose or 1 mmol/kg lean body mass leucine or 1 mmol/kg lean body mass leucine plus 25g glucose	Glucose concentration of 6.3mmol/L, which was lower than that of the 7.3mmol/L after 50 minutes. Leucine ingestion improved glucose concentration significantly. A 72% greater response in insulin was observed.
(Gardner et al., 2007)	28 participants aged 30-65 years with LDL-C concentrations of 160-220 mg/dl 24 week randomised crossover trial	Soy and dairy milk on lipids, blood glucose and insulin concentrations.	25g/day of protein from each milk source for 4 weeks each.	No detectable differences in insulin AUC or glucose at 0, 1 or 2 hours for any of the milks consumed
(Breitman et al., 2011)	30 participants undergoing laparoscopic gastric bypass 10 week, randomised controlled parallel study.	Amino acid supplementation on glucose homeostasis and hormonal and inflammatory markers after laparoscopic gastric bypass – weight change, glucose,	24g oral supplement containing leucine metabolite, glutamine and arginine twice a day.	Mean fasting glucose decreased significantly from 113.1± 14.4mg/dl at baseline to 95±15.1mg/dl and 95±9.8mg.dl at 2 and 8 weeks (P<0.0001). Mean fasting insulin decreased (16.8±7.6µU/ml to 12.6±16.8µU/ml), but was not statistically significant.

Author, year	Study size, sex, length	Endpoints	Exposure	Result
		insulin, c-peptide, insulin sensitivity, IL-6, CRP, leptin, IGF-1, ghrelin, incretin.		
(Chiu et al., 2014)	158 overweight men and women, 8 week randomised parallel trial.	High vs moderate protein intake on insulin action and lipoprotein concentrations.	High protein diet High protein & low saturated fat diet Moderate protein & high saturated fat Moderate protein & low saturated fat	BCAA concentration was positively correlated with fasting plasma insulin, glucose concentrations and HOMA-IR. No significant effects of protein intake on insulin sensitivity.

Fat

Author, year	Study size, sex, length	Endpoints	Exposure	Result
(Nestel et al., 2014)	86 overweight middle aged men and women, randomised parallel study	Phospholipid species and fatty acids associated with full-fat dairy consumption and insulin resistance	Assessed dairy intake through food records and insulin sensitivity through OGTT	Nestel et al. found no associations between full – fat dairy consumption with any indices of insulin resistance or sensitivity. This was consistent across all types of dairy
(Nestel et al., 2005)	19 participants, 12 week randomised crossover study	Effect of dairy on plasma cholesterol, LDL and HDL cholesterol, triacylglycerol and glucose measurements.	Consumption of 40g of dairy fat as either matured hard cheese or butter for 4 weeks.	No significant differences in plasma insulin concentrations in either group.
(Tholstrup et al., 2004)	14 healthy men, 3 months, 9 week randomised crossover study	Blood lipids, lipoproteins, postprandial glucose and insulin response	3 diets, 20% of energy intake from dairy fat, as whole milk, butter or hard cheese for 3 weeks each.	All three diets led to a fasting glucose concentration of 4.89±0.08mmol/L after consuming a diet rich in milk, 4.94±0.09mmol/L after a diet rich in cheese, and 4.91±0.07mmol/L after a diet rich in butter. An increase in insulin concentration was observed, reaching peak concentration at 30 minutes, and decreasing between 30-60 minutes.

Micronutrients

Author, year	Study size, sex, length	Endpoints	Exposure	Result
(Guerrero-Romero et al., 2004)	57 non-diabetic men and women, 3 month randomised parallel trial	Effects of oral magnesium supplementation on insulin sensitivity.	2.5g/day of oral $MgCl_2$ or a placebo	Low magnesium levels and HOMA-IR strongly related. Magnesium supplementation led to reduced fasting glucose (from 5.8±0.9 to 5.0±0.6mmol/L in the magnesium group, 5.7±0.4 to 5.6±0.5 mmol/L in the control group, $P<0.05$), insulin (32% reduction, from 103.2±56.4 to 70.2±29.6mmol/L in the magnesium group, 123.0±47.4 to 132.1±75.6mmol/L in control group, $P<0.05$), HOMA-IR (43.5% reduction from 4.6±2.8 to 2.6±1.1, $P<0.0001$). Control group was 0.62±0.08 to 0.61±0.08, $P<0.01$).
(Guerrero-Romero and Rodriguez-Moran, 2011)	97 non-diabetic men and women suffering from hypomagnesaemia, 3 month parallel trial	Effect of Magnesium chloride supplementation on β-cell ability to compensate for variations in insulin sensitivity	50ml of 5% $MgCl_2$ solution for 2 months, or inactive solution.	HOMA- β decreased significantly in magnesium group (from 253.2±130.3.to 170.1±40.1 in magnesium group, 271.8±112.6 to 262.7±102.9 in placebo group, $P<0.05$). Compensatory insulin secretion response was altered in the magnesium group. Placebo group was unchanged.
(Mooren et al., 2011)	47 normomagnesaemic overweight, insulin resistant participants and healthy participants, 6 month double-blinded randomised parallel trial	The effect of magnesium supplementation on glucose homeostasis and insulin sensitivity in normomagnesaemic overweight, insulin	Magnesium-aspartate-hydrochloride (365mg/day) or a placebo taken orally	Fasting plasma glucose significantly lower in magnesium group (4.75±1.04 mmol/L for magnesium, 4.97±0.87 mmol/L for placebo respectively. $P=0.0237$) fasting insulin displayed trend for improvement in magnesium group (100.00±46.38 pmol/L for magnesium, 117.39±58.70 pmol/L for

Author, year	Study size, sex, length	Endpoints	Exposure	Result
		resistant participants and healthy participants.		placebo. *P*= 0.875), but was not statistically significant. ISI HOMA and ISI Matsuda significantly improved in magnesium group HOMA: 2.974±1.682 for magnesium, 3.713±2.517 for placebo, *P*=0.0376 Matsuda: 4.037±2.040 for magnesium, 3.150±1.288 for placebo. *P*=0.0127
(Chacko et al., 2011)	26 participants, 12 week double-blinded controlled crossover.	The effect of magnesium supplementation on metabolic and inflammatory markers	Placebo, and 500mg magnesium citrate supplement for four weeks each, separated by a four-week washout in between.	Slightly decreased C-peptide concentration after magnesium supplementation (1.5±0.9 for magnesium, 2.0±0.9 for placebo, *P*=0.004). Decreased fasting insulin concentration after magnesium supplementation. (4.8±3.7µU/ml for magnesium, 7.4±3.7µU/ml for placebo) *P*=0.25)
(Paolisso et al., 1992)	27 healthy and elderly, non-obese participants, 8 week double-blinded randomised crossover trial	The effect of magnesium supplementation on glucose handling in elderly patients	4.5gday magnesium or a placebo for four weeks each	Improved insulin action, enhanced total-body and oxidative glucose metabolism in magnesium supplemented arm.

Others

Author, year	**Study size, sex, length**	**Endpoints**	**Exposure**	**Result**
(Benatar et al., 2013a)	180 healthy men and women, 1 month	Effects of changing dairy intake on cardio-metabolic risk factors	Increased dairy (extra 2-3 servings per day), reduced dairy (0 servings/day), and no change.	No statistically significant changes in insulin sensitivity