# The effects of self-directed low carbohydrate diets on metabolic biomarker profiles and disease risk among New Zealand adults – the LOCA study

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# Abstract

***Background:*** Low carbohydrate (LCHO) diets are dietary trends often adopted for fast weight loss. Concerns regarding their safety and effects on cardiovascular disease (CVD), kidney disease and diabetes risk have been raised.

***Aim***: To investigate the associations between dietary intake and metabolic and inflammation biomarkers of self-reported LCHO diet consumers (men and women aged 20 to 45 years) in Auckland, New Zealand.

***Methods:*** This cross-sectional study recruited men and women aged 20 to 45 years following an LCHO diet for a minimum of 4-months. Four-day weighed food record, anthropometric measurements, and fasting venous blood samples were collected from participants. Participants were divided into three groups: very low carbohydrate (VLCHO) (<50g), LCHO (50-100g) and moderately low carbohydrate (MLCHO) (>100 - <150g) carbohydrate groups. Dietary intake, metabolic biomarkers and anthropometric measurements were examined in those three intake groups.

***Results:*** A total of 74 men and women participated in the LOCA study with a mean age of 35 years. The median intake of carbohydrates in this group was 14 [11.4, 26.7]% of total energy (%TE), while fat intake was 58.1% [49.1-66.0] and protein intake was 24.4% [22.9, 25.9]. Based on their carbohydrate intakes, participants in the VLCHO, LCHO and MLCHO groups, experienced elevated total cholesterol (94.7%, 89.5% and 88.9%, respectively), LDLC (94.6%, 100% and 88.9%, respectively) and HDLC (92.1%, 94.7% and 100%, respectively) concentrations. The majority of the participants experienced low estimated glomerular filtration rates (VLCHO: 89.5%, LCHO: 89.5%, and MLCHO: 88.9%). Carbohydrate intakes (grams and %TE) negatively correlated with total cholesterol (TC) (r= -0.353, *P*= 0.003 and r= -0.403, *P*= 0.001), and low-density lipoprotein cholesterol (LDLC) (r= -0.329, *P*= 0.007 and r= -0.335, *P*= 0.006). Total cholesterol concentrations were significantly associated with carbohydrate, total fat and saturated fat (SFA) intakes as %TE. Only total fat intake (%TE) significantly associated with LDLC concentrations

***Conclusions:*** Our findings suggest LCHO diets followers predominantly replaced carbohydrate with protein and fat. High fat and SFA intakes (%TE) due to carbohydrate restrictions were

accompanied by high TC, LDLC and HDLC concentrations. It is crucial to weigh the benefits and harms of LCHO diets on CVD risk.

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# Abbreviation List

%TE – Percentage of total energy

AMDR – Acceptable Macronutrient Distribution Range

BF% – Body fat percentage

BG – Blood Glucose

BMI – Body Mass Index

BP – Blood Pressure

BW – Body Weight

CI – Confidence Interval

CVD – Cardiovascular Disease

DBP – Diastolic Blood Pressure

eGFR – estimated Glomerular Filtration Rate

g – Grams

G. mean – Geometric mean

GFR – glomerular filtration rate

Hb – Haemoglobin

HbA1c – Glycated Haemoglobin

HC – High carbohydrate

HDLC – High-Density Lipoprotein Cholesterol

HNRU – Human Nutrition Research Unit

IQR – Interquartile Range

Kcal – Kilocalorie

LCHO – Low Carbohydrate

LDLC – Low-Density Lipoprotein Cholesterol

LF – Low Fat

mg – Milligrams

MLCHO – Moderately Low Carbohydrate

mmol – Millimole

MUFA – monounsaturated Fatty Acids

NNS – National Nutrition Survey

NZ – New Zealand

PUFA – Polyunsaturated Fatty Acids

RCT – Randomised Controlled Trials

SBP – systolic blood pressure

SD – Standard deviation

SFA – Saturated Fatty Acid

T2DM – Type 2 Diabetes Mellitus

TC – Total Cholesterol

TE – Total Energy

TG – Triglycerides

VLCHO – Very Low Carbohydrate

# Chapter One

# Introduction

## 1.1 Background

The life years lived with disability are greatly attributed to the incidence of lifestyle diseases (Ministry of Health, 2016; World Health Organisation, 2005). Although some lifestyle diseases are influenced by heredity, others like obesity, Type 2 diabetes mellitus (T2DM) (Cameron et al., 2003), cardiovascular disease (CVD) (Mertens, Markey, Geleijnse, Givens, & Lovegrove, 2017), and renal function (Foster et al., 2015), are greatly influenced by modifiable lifestyle choices like dietary habits and physical activity levels (Ministry of Health, 2016).

New Zealand (NZ) is currently ranked as the third most obese nation globally, with 30.9% of NZ adults suffering from obesity (Ministry of Health, 2019c). This is an approximate 2% increase since 2011/12 (Ministry of Health, 2019c). Obesity is associated with increased risk of CVD and T2DM. CVD contributes to 33% of annual deaths in NZ (Ministry of Health, 2019b), where one in twenty NZ adults suffer from CVD (Ministry of Health, 2019a). Approximately 6% of the NZ population suffers from diabetes, with 90-95% of all cases suffering from T2DM (Ministry of Health, 2015). With this increased incidence of obesity and chronic diseases in mind, awareness of the effects of such conditions on health and wellbeing is rising among the wider public. This increase in awareness alongside the need for change to improve body image and to ameliorate familial and social relationships provide motivation for behavioural change, which can lead to changes in dietary patterns and behaviours (Thomas, Hyde, Karunaratne, Kausman, & Komesaroff, 2008).

One of the current dietary trends, often implemented for weight loss, is low carbohydrate (LCHO) diets (Clarke & Best, 2017; Dyson, Beatty, & Matthews, 2007; Jallinoja, Niva, Helakorpi, & Kahma, 2014). Carbohydrates are one of the three macronutrients obtained from food alongside protein and fat. They are primarily a source of energy, but also play a role in growth, immune function, satiety, blood glucose and insulin regulation, gut microbiome composition as well as bowel health (Cummings & Stephen, 2007). The term carbohydrate includes simple and complex carbohydrates. Simple carbohydrates include monosaccharides (e.g. glucose and fructose) and disaccharides (e.g. sucrose). Simple carbohydrates are found in raw or brown sugar, fruit juice concentrates, or sugar-sweetened beverages. Simple carbohydrates are

easily digestible and therefore, can be absorbed rapidly into the blood, causing a rapid increase in blood glucose (Aller, Abete, Astrup, Alfredo, & van Baak, 2011). Complex carbohydrates include polysaccharides like starches and dietary fibre which are found in foods like whole-grain bread (Cummings & Stephen, 2007; Holesh & Martin, 2019). Unlike simple carbohydrates, complex carbohydrates require a longer time to digest and absorb, which results in a gradual and steady increase in blood glucose levels (Aller et al., 2011). Additionally, the slow digestion of complex carbohydrates results in greater satiety and promote a favourable intestinal environment for the growth of beneficial bacterial strains by providing sources of energy and nutrition (Singh et al., 2017).

A healthy diet that includes appropriate amounts of the different macronutrients can protect against chronic diseases of lifestyle like obesity, CVD, hypertension and T2DM (World Health Organization, 2018). The Acceptable Macronutrient Distribution Range (AMDR) utilises scientific evidence to provide recommendations of macronutrient intakes that elicit the least risk of developing chronic diseases. The AMDR is expressed as a percentage of total energy (%TE) intake, therefore, providing an individualised range of macronutrient requirement (NHMRC, 2006). The current recommended AMDR for carbohydrates, proteins and fats are 45-65%TE, 15-25%TE and 20-35%TE, respectively (Laffel, 1999; Ministry of Health, 2011; NHMRC, 2006).

Diets that are LCHO can be defined as being devised to restrict the amount of energy obtained from carbohydrates. LCHO diets compensate the restricted macronutrient (carbohydrate) by increasing the remaining macronutrients (either fat or protein intakes, or both) (Paoli, Rubini, Volek, & Grimaldi, 2013; Wylie-Rosett, Aebersold, Conlon, Isasi, & Ostrovsky, 2013). However, a consistent definition classifying the level of ingested carbohydrate to define LCHO diets is lacking (Wylie-Rosett et al., 2013). This lack of consistency greatly influences the trends and behaviours of LCHO diet followers, through dietary patterns such as the Ketogenic diet, Palaeolithic diet and Atkins Diet. The Ketogenic diet, for example, is characterised by decreasing daily carbohydrate intake to that lower than 50g (<20%TE) per day and alternatively increasing consumption of fat and protein (Paoli et al., 2013). The Palaeolithic diet, however, restricts carbohydrate to about 23%TE by eliminating grains, legumes, dairy products and starchy vegetable intakes (Pastore, Brooks, & Carbone, 2015). Despite this lack of consistency, LCHO diets can be classified as very low (<50g/day) (Brouns, 2018; Feinman et al., 2015; Harvey, Schofield, Williden, & McQuillan, 2018), low (50-100g/day) (Bilsborough & Crowe, 2003), and

moderately low (>100 – <150g/day) (Brouns, 2018), based on grams of carbohydrate ingested daily.

Low carbohydrate diets are beneficial for achieving weight loss among overweight and obese individuals (Boaz & Raz, 2015; Boden, Sargrad, Homko, Mozzoli, & Stein, 2005; Clifton, Condo, & Keogh, 2014; Goday et al., 2016; Naude et al., 2014). However, when compared to low fat (LF) or balanced diets, LCHO diets had similar effects on weight, T2DM and CVD risk factors (Boaz & Raz, 2015; Clifton et al., 2014; Naude et al., 2014). In contrast, a meta-analysis by Mansoor et al. (2016) found that LCHO diets resulted in a greater weight loss in comparison to LF diets. They also resulted in mixed cardiovascular risk factors with a considerable increase in high-density lipoprotein cholesterol (HDLC) and low-density lipoprotein cholesterol (LDLC).

Although many studies show the benefits of LCHO diets on weight loss and T2DM management (Foster et al., 2010; Gardner et al., 2018; Tay et al., 2018), some studies have found that inappropriate macronutrient intakes, outside of the AMDR, are associated with disease and increased risk of mortality (Lagiou et al., 2007; Noto, Goto, Tsujimoto, & Noda, 2013; Seidelmann et al., 2018). Lagiou et al. (2007) have found that individuals with low total carbohydrate intakes and high protein intakes had a 1.3 times higher risk of total mortality. This increase in mortality as a result of altered macronutrient intake is consistent with the results of the meta-analysis conducted by Noto et al. (2013). Both Noto et al. (2013) and Lagiou et al. (2007) concluded that LCHO diets provide no cardiovascular protection.

Consuming LCHO diets can easily result in inadequate fibre intake as carbohydrate- and fibre-rich foods are often avoided (Noto et al., 2013). Furthermore, the increase of replacement nutrients can lead to increased risk for total and especially cardiovascular mortality due to higher animal protein, cholesterol and saturated fat (SFA) intakes (Noto et al., 2013; Seidelmann et al., 2018). In fact, research shows that diets abundant in dietary fibre and polyunsaturated fats (PUFA) are associated with a decreased risk of metabolic syndrome among those with high cardiovascular risk (Cabello-Saavedra et al., 2010). In contrast, carbohydrate intakes above the AMDR (> 60%TE) were associated with metabolic syndrome in adults (Carnethon et al., 2004; Park et al., 2003). Therefore, both high and low carbohydrate intakes were associated with increased mortality. A meta-analysis by Seidelmann et al. (2018), found a U-shaped association between the %TE from carbohydrate and mortality with the smallest risk of mortality identified with a carbohydrate intake of 50 – 55%TE. The OmniHeart trial, on the other hand, concluded that a mild restriction of carbohydrate intake to about 48%TE, and a slight increase in either

protein or unsaturated fat intake to 25%TE and 31%TE respectively, were associated with decreased risk of CVD, lower blood pressure and improved lipid levels (Appel et al., 2005). Furthermore, Seidelmann et al. (2018) found that in the case of carbohydrate restriction, the source of the replacing macronutrients played a crucial role in determining the risk of mortality. Dietary patterns favouring animal protein and fat sources were associated with a higher risk of mortality and those favouring plant-based protein, and fat sources were associated with a lower risk of mortality.

### *1.1.1 The Theoretical Background of LCHO diets*

One of the many theories behind carbohydrate restriction is the carbohydrate-insulin model. This model describes a proportional relationship between insulin secretion and carbohydrate intakes, where increased carbohydrate intake causes an increase in insulin release (Hall, 2017). Increased insulin release, in return, encourages the storage of circulating fatty acids and inhibits further release of fatty acids from adipose tissue. Decreased circulating fatty acids due to increased circulating insulin leads to reduced energy expenditure, increased fat storage in adipocytes and increased hunger, therefore creating a positive energy balance further contributing to increased body weight and obesity (Hall, 2017).

Another theory suggests that nutritional ketosis is linked to appetite suppression (Gibson et al., 2015; Sumithran et al., 2013). Ketosis is the increase in ketone body formation by the liver to provide an alternative source of energy when glucose supply is insufficient (Gibson et al., 2015). Increased ketone body concentrations appear to suppress orexigenic signals such as suppressing the increase in ghrelin (Sumithran et al., 2013). Additionally, they exert anorexigenic effects such as increasing cholecystokinin release (Paoli, Bosco, Camporesi, & Mangar, 2015). Ghrelin is a hormone released as a result of diet-induced weight loss to induce weight re-gain and return the body to a state of equilibrium by signalling the hunger centres in the brain (Sumithran et al., 2013). In contrast, cholecystokinin, a peptide produced by the duodenum and jejunum, acts on the vagus nerve resulting in decreased food intake, as well as meal size and duration (Paoli et al., 2015).

### *1.1.2 Current Trends of Carbohydrate Restriction*

Nutritional analyses from various countries such as China (Zhao et al., 2018), The United States (Makarem, Scott, Quatromoni, Jacques, & Parekh, 2014; Vadiveloo, Scott, Quatromoni, Jacques, & Parekh, 2014) and NZ (Jayasinghe et al., 2017; Ministry of Health, 2011; Sam et al., 2020;

Schrijvers, McNaughton, Beck, & Kruger, 2016) have found that carbohydrate intakes declined over the years. The China Health and Nutrition Survey prospective study has shown that Chinese females decreased carbohydrate intake from 62.8 to 51.6%TE over 24 years from 1991 to 2015. This decrease in carbohydrate intake was accompanied by a ten %TE increase in total fat intake with protein intake remaining stable (12.4 to 12.6%TE) (Zhao et al., 2018). Within this sample, women aged 50 – 64 years had a greater increase in fat intake compared to women aged 18 – 49 years (20g compared to 11.4g, respectively). These observed changes were interestingly accompanied by an overall decline in total energy intake of 446.0kcal per day, with a greater reduction observed in the 18-49-year-old age group compared to the 50-64-year-old group (446.0kcal per day compared to 334.7kcal per day, respectively) (Zhao et al., 2018).

Similarly, American adults from the Framingham Heart Study population showed altered macronutrient intake, where a decline in carbohydrate intake from 50.1 to 46.0%TE was observed from 1991 to 2008 (Makarem et al., 2014; Vadiveloo et al., 2014). Despite the decrease in the overall carbohydrate contribution to energy intake, fibre intake significantly increased by 1.2g per day (Makarem et al., 2014). This decrease in carbohydrate intake was accompanied by an increase in total fat intake from both animal and plant sources, from 27.3% to 29.8% (Vadiveloo et al., 2014). The increase in fat intake was mostly attributed to MUFA sources, followed by SFA and PUFA. (Vadiveloo et al., 2014). A slight increase of 1.2% in protein intake was also observed (increasing from 16.8% to 18.0%) (Makarem et al., 2014; Vadiveloo et al., 2014).

In NZ, carbohydrate intake comprises only 46.6%TE, a finding which remained consistent across both the 1997/98 and 2008/09 National Nutrition Surveys (NNS) (Ministry of Health, 2011). Although this carbohydrate intake is within the recommended AMDR range, it is sitting at the lower end of the range. Alongside the NNS, a study by Sam et al. (2020) has shown that the daily carbohydrate consumption among NZ adults aged 30-59 years was 48.6%TE while their total fat intake was 32%TE. Studies among NZ women aged 16-45 years have also shown LCHO intakes: Schrijvers et al. (2016) reported 42.6%TE with increasing fat to 34.5%TE, and Jayasinghe et al. (2017) reported 42%TE with increasing fat intake to 37%TE.

Interestingly, LCHO diet trends indicate that followers often self-direct carbohydrate intakes (Jallinoja et al., 2014). Jallinoja et al. (2014) found that many individuals strictly controlled carbohydrate intakes while others did not actively avoid carbohydrates, or sugars and consumed foods that are ‘forbidden’ in such diets. Many individuals who identified themselves as LCHO

diet followers, however, had higher carbohydrate intakes than what is considered as LCHO, thus indicating limited food and nutrition knowledge (Jallinoja et al., 2014).

### *1.1.3 The Relevance of Research to NZ*

Low carbohydrate diets have become a popular trend for weight loss (Boaz & Raz, 2015; Clifton et al., 2014; Goday et al., 2016; Naude et al., 2014). Although beneficial in improving body weight and BMI, the literature suggests that LCHO diets may exert harmful effects on chronic diseases like CVD due to a negative influence on some elements of the blood lipid profile (Mansoor et al., 2016). Additionally, the increase in social media presence and influence resulting in individuals to seek quick-fix strategies for weight loss, one of which being LCHO diets. With many different types of LCHO diets and social media trends and messages, it is unclear which messages are implemented and the effect of such messages on individuals' health. Consequently, it is essential to understand the different dietary practices of the wider population and the impact they may have on health.

To our knowledge, the LOCA study is the first study to examine the current dietary practices of LCHO diet followers among NZ adults and the effect of such practices on their metabolic biomarkers of health. The present study aimed to achieve an understanding of LCHO trends among New Zealanders and to provide a snapshot of dietary practices associated with increased risk of poor metabolic biomarker profiles accompanying high risk of developing chronic diseases such as CVD and T2DM. Achieving an understanding of the various practices of LCHO diet followers will not only aid in increasing knowledge of self-directed LCHO diets among NZ adults. It will also increase the awareness whether such trends are contributing to or minimising the risk of developing chronic conditions like obesity, CVD and T2DM.

## 1.2 Aims and Objectives

### *1.2.1 Aim*

To investigate metabolic and inflammation biomarkers and associations with dietary intake of self-reported low carbohydrate diet consumers (men and women aged 20 to 45 years) in Auckland, NZ.

### *1.2.2 Objectives*

- To investigate the biomarker profiles and associations with metabolic disease risk (diabetes, CVD, obesity and kidney disease risks) of self-reported low carbohydrate consumers in relation to gender and different levels of low-carbohydrate intakes.
- To explore the association between all biomarkers and
    - Energy and Nutrient intakes,
    - Low carbohydrate diet duration,
    - Replacement nutrients.

## 1.3 Structure of the Thesis

The present thesis is presented in four chapters. The first chapter introduces LCHO diets and chronic diseases and provides the purpose of the present study and its relevance to the NZ population. The second chapter presents a review of the literature on LCHO diets, as well as benefits, risks and effects of such diets on metabolic biomarkers of diseases of lifestyle. The third chapter presents the research manuscript which provides a brief introduction on LCHO diets and chronic diseases, outlines and justifies study design and procedures, outlines the findings of the study, and discusses and compares results of the LOCA study with comparable studies in the literature. This chapter is structured according to the British Journal of Nutrition's guidelines and requirements. However, for the purposes of this thesis, line numbers were excluded to keep consistent formatting throughout the chapters. Additionally, only one reference list was created to combine all references from all four chapters; this list is found at the end of this thesis. The fourth chapter summarises and concludes the research. This chapter provides an overview of the main findings and new knowledge acquired, outlines the strengths and limitations of the project and suggests recommendations for future research.

## 1.4 Contributors to the Research

A summary of the researchers' contributions and roles are outlined in Table 1.1.

***Table 1.1****. Researcher's contribution towards the LOCA study*

| Researchers | Contributions to Thesis |
|---|---|
| **Linda Rassam** *Student* | Recruited participants, collected data, performed phlebotomy and processed blood samples, completed data entry, conducted statistical analyses, interpreted and discussed results, author of thesis. |
| **Associate Professor Rozanne Kruger** *Main Supervisor* | Academic supervisor designed the LOCA study, developed methodology protocols, assisted with statistical analysis and interpretation of results, reviewed and approved thesis. |
| **Dr Marilize Richter** *Co-Supervisor* | Academic supervisor; designed the LOCA study, completed the ethics application, established methodology protocols, assisted with statistical analysis and interpretation of results, reviewed and approved thesis. |
| **Tayla Knightbridge-Eager and Viola Lasardo** | Involved in participant recruitment, data collection, data entry |
| **Owen Mugridge** | Provided phlebotomy training, as well as training for processing of blood samples, facilitated participants testing, assisted in data collection. |
| **Viola Lasardo, and Tania George** | Facilitated participant testing, assisted with data collection. |
| **Viola Lasardo, and Nico Bejcek** | Data entry. |
| **PC Tong** | Facilitated participant testing and organised equipment for data collection. |

# Chapter Two

# Literature Review

The World Health Organization (2014) defines obesity as the excessive and abnormal accumulation of adipose tissue in relation to lean muscle tissue (World Health Organization, 2014). This accumulation of fat results in impaired physiological functions characterised by insulin resistance and alterations in concentrations of hormone, pro-inflammatory substances, cytokines, and non-esterified fatty acid concentrations (Al-Goblan, Al-Alfi, & Khan, 2014). Such impairments in physiological functions result in an increased risk of developing chronic diseases. Overweight is characterised by a body mass index (BMI) of 25-29.9kg/$m^2$ (World Health Organisation, 2018), while obesity is characterised by a BMI equal to or greater than 30kg/$m^2$ (Pi-Sunyer, 2002; World Health Organization, 2018).

Alongside BMI, body fat percentage (BF%) is also used to identify obesity among men and women; however, cut off values vary throughout the literature. The American Council on Exercise (2009) defines BF% as equal to or greater than 32% and 25% for women and men, respectively, as the cut-off points for obesity identification. López-Jiménez and Cortés-Bergoderi (2011) consider BF% of 20-25% and 30-35% as excessive for men and women, respectively. Oliveros et al. (2014), however, suggest BF% cut-off points based on age. Men and women aged 20-39 years with BF% >19% and >32%; 40-59 years with BF% >21% and 33% and 60-79 years with BF% > 24% and 35%, respectively, are considered excessive and therefore obese (Oliveros et al., 2014).

More than 1.9 billion adults worldwide are overweight, and 650 million of those are obese (World Health Organisation, 2018). From 1980 to 2013, the percentage of overweight and obese adults increased from 28.8% to 36.9% in men and 29.8% to 38.0% in women (Ng et al., 2014). Overweight and obesity are significant risk factors for type 2 diabetes mellitus (T2DM), cardiovascular disease (CVD), cancers and kidney disease (Kovesdy, Furth, & Zoccali, 2017). Increased awareness of the impact of overweight and obesity on health (Thomas et al., 2008) has resulted in increased alterations of macronutrient (carbohydrates, protein and fat) contributions to energy intake. Such modifications take place to achieve rapid weight loss as well as manage biomarkers of chronic diseases (Paoli et al., 2013; Wylie-Rosett et al., 2013). Low carbohydrate (LCHO) diets are a popular dietary pattern used for weight loss (Clarke & Best, 2017; Dyson et

al., 2007; Jallinoja et al., 2014) that involve restricting energy intake from carbohydrates and replacing it with fat or protein or both (Paoli et al., 2013; Wylie-Rosett et al., 2013).

## 2.1 Low Carbohydrate Diets and Metabolic Biomarkers of Health

### *2.1.1 Low Carbohydrate Diets and Weight loss*

Obesity is often the result of poor lifestyle choices (e.g. inappropriate dietary choices and physical inactivity) leading to energy disequilibrium where energy intake is higher than energy expended as resting metabolic rate and physical activity (Pi-Sunyer, 2002). Obesity is a strong predictor of T2DM and CVD (Casanueva et al., 2010). The modification of lifestyle factors, including dietary intake (e.g. improving food choices and portion sizes) and physical activity (e.g. increasing aerobic and resistance exercise), can reduce the risk of overweight and obesity (Wadden, Webb, Moran, & Bailer, 2012). Such lifestyle changes may result in a shift in energy balance, where portion control and appropriate food choices can result in a decline in energy intake. Increased physical activity, however, can lead to increased energy expenditure (Wadden et al., 2012). For weight loss to take place, a shift in energy balance is necessary, where energy expenditure must exceed energy intake (Hill, Wyatt, & Peters, 2012). Dietary modification in conjunction with increased physical activity can allow for this shift in energy balance and can, therefore, initiate weight loss (Hill et al., 2012; Wadden et al., 2012). Dietary approaches may include energy-restricted diets, as well as modified macronutrient content of diets such as the Mediterranean diet, DASH (dietary approach to stop hypertension) diet, low-fat (LF) diets and low carbohydrate diets (LCHO) (Ministry of Health, 2017).

Low carbohydrate diets have shown beneficial effects in reducing body weight among overweight and obese individuals using varying levels of carbohydrate restrictions and durations. For example, carbohydrate restriction of <20g per day for six months resulted in a significant weight loss of about 12kg (Hussain et al., 2012; Yancy, Olsen, Guyton, Bakst, & Westman, 2004), and a carbohydrate restriction of 40g per day for 12 months resulted in a significant 5.3kg loss (Bazzano et al., 2014). Elhayany et al. (2010) in their study, restricted carbohydrate to 35%TE for 12 months, resulting in an 8.9kg decline in weight among overweight participants with T2DM. Diets using slight restrictions in carbohydrate content have demonstrated similar results to very restrictive carbohydrate diet (Shai et al., 2008). Shai et al. (2008) demonstrated that LCHO with moderate restriction (40%TE carbohydrates) showed similar reductions in weight to the Mediterranean diet (50%TE CHO), which in this case was superior to LF diets.

The success of LCHO diets in reducing body weight can be attributed to a spontaneous decline in energy intake, thus resulting in an energy deficit (Boden et al., 2005; Dansinger, Gleason, Griffith, Selker, & Schaefer, 2005; Foster et al., 2010; Lindström et al., 2006; Meckling, O'Sullivan, & Saari, 2004; Sato et al., 2017). This decline in energy intake may be a result of altered macronutrient composition, and types of foods consumed as increased fat and protein intakes induce early satiety, which can result in decreased energy intake (Westerterp-Plantenga, Lemmens, & Westerterp, 2012). Protein rich foods have shown to sustain satiety despite the deficit in energy intake (Westerterp-Plantenga et al., 2012). Additionally, increased protein intake has shown to spare fat-free mass, which sustains basal energy expenditure (Westerterp-Plantenga et al., 2012). A protein intake of 20-30%TE alongside energy deficit has shown to improve fat-free mass to fat mass ratio (Westerterp-Plantenga et al., 2012).

Low carbohydrate-high fat diets have shown to preserve gut hormone peptide YY secretion (Hu et al., 2016), a hormone with the role of preserving satiety (Karra, Chandarana, & Batterham, 2009). Maintaining Peptide YY secretion provides appropriate physiological conditions (e.g. reduced hunger and subsequent food intake) that favour weight loss (Hu et al., 2016). Additionally, very LCHO diets are known to cause ketogenesis by restricting daily carbohydrate intake to <10%TE or <20g/day (Volek et al., 2004). Ketogenesis is the process by which ketone bodies or ketones are formed as a result of starvation, fasting and nutrient deprivation. Ketones are an alternative fuel source for the brain and are formed as a result of fat metabolism in the liver through beta-oxidation. Fat metabolism takes place when the liver's glycogen stores are depleted (Puchalska & Crawford, 2017). Alongside its role as an alternative energy source, ketones play a role in reducing appetite (Gibson et al., 2015; Sumithran et al., 2013; Volek et al., 2004), by exerting orexigenic signals by suppressing an increase in ghrelin (Sumithran et al., 2013), and increasing cholecystokinin release resulting in appetite suppression (Paoli, Bosco, Camporesi, & Mangar, 2015; Sumithran et al., 2013).

In comparison to LF energy-restricted diets ($P<0.05$), LCHO diets have been shown to cause greater weight loss when followed for less than six months (Davis et al., 2009; Gardner et al., 2018; Nordmann et al., 2006; Stern et al., 2004). However, when those diets were followed for a duration of one year or more, the difference in weight loss disappeared, and LCHO diets caused similar reductions in weight to LF diets ($P>0.05$) (Bradley et al., 2009; Dansinger et al., 2005; Davis et al., 2009; Foster et al., 2010; Gardner et al., 2018; Meckling et al., 2004; Nordmann et al., 2006; Sato et al., 2017; Stern et al., 2004). The initial more significant decline in body weight

in LCHO despite isocaloric conditions is due to body water loss alongside fat loss, which takes place as glycogen depletion secondary to limited carbohydrate intake (Boden et al., 2005; Brehm, Seeley, Daniels, & D'Alessio, 2003).

The comparable long-term effect of LCHO and LF diets on weight can be attributed to the spontaneous decline in energy intake in LCHO diet resulting in similar energy intakes in both LCHO and LF- energy-restricted diets (Gardner et al., 2018; Nordmann et al., 2006; Stern et al., 2004). Elhayany et al. (2010) demonstrated this, where they compared three isocaloric diets (LCHO-, Mediterranean- and American diabetes association (ADA) diets) and found that all three diets resulted in similar weight loss (*P*-value= 0.557) of 8.9kg, 7.4 and 7.6kg respectively, over a one-year dietary intervention. Furthermore, the decline in weight is primarily the result of the experienced energy deficit (Boden et al., 2005; Lindström et al., 2006). Boden et al. (2005) compared actual weight loss experienced by their participants to a predicted weight loss. Predicted weight loss was determined using the observed spontaneous decline in energy intake among LCHO followers. The calculated predicted weight loss of 1.6kg was similar to the actual weight loss of 1.65kg in the LCHO group. This, therefore, supports that weight loss is influenced by changes in dietary energy density, the most significant predictor for weight loss (Lindström et al., 2006). Meta-analyses conducted by Bueno et al. (2013) and Mansoor et al. (2016) found a more substantial decline in body weight of 0.91kg over ≥12months and 2.17kg over a duration greater than six months among obese individuals following LCHO diet, compared to those following LF diet, respectively. This may have been due to greater caloric restrictions; however, energy intakes were not provided in either meta-analysis. Weight loss can result in a decline in body fat as well as lean mass (Brehm et al., 2003; Meckling et al., 2004). Low carbohydrate diets with a 50-70g daily intake from carbohydrates have shown to cause a similar decline in BF% to LF, energy-restricted diets (Meckling et al., 2004). However, Brehm et al. (2003) have found that a carbohydrate restriction of 15.4%TE resulted in a 2.7kg greater reduction of body fat mass than LF diet after a six-month intervention among 53 obese women ($P < 0.01$). This greater decline in fat mass took place despite a comparable reduction in energy intake throughout the intervention period. These findings were consistent with those from Bazzano et al. (2014), Volek et al. (2004) and Hashimoto et al. (2016). The differences in fat mass changes between LCHO and LF diets is due to LCHO followers maintaining greater resting energy expenditure compared to LF diet followers (Bazzano et al., 2014; Ebbeling et al., 2012; Volek et al., 2004). The physiological basis for the difference in resting energy expenditure is not fully understood (Ebbeling et al., 2012). However, it is thought to be related to increased demand for protein turnover (Kao et al.,

2011; Veldhorst, Westerterp-Plantenga, & Westerterp, 2009), gluconeogenesis, and increased thermic effect of protein (Pesta & Samuel, 2014; Veldhorst et al., 2009). Carbohydrate intakes (both %TE and in gram) were found to be positively correlated with visceral adiposity even after adjusting for age and energy intake (Sasakabe, Haimoto, Umegaki, & Wakai, 2015).

Furthermore, lean muscle mass is a metabolically active tissue that allows the body to perform activities of everyday living. The loss of muscle mass causes lowered resting metabolic rate, increased risk of injury and falls, fatigue as well as increased fat mass secondary to decline in metabolic rates (Willoughby, Hewlings, & Kalman, 2018). Low carbohydrate diet intervention (daily carbohydrate intake of 50-70g) has shown to cause a greater decline of 0.9kg in lean muscle mass from baseline than LF diets (Meckling et al., 2004). Similarly, Brehm et al. (2004) found that the LCHO diet (daily carbohydrate intake <15.4%TE) group experienced a 2kg decline in lean mass compared to a 0.7kg reduction in the LF diet group, which indicates that LF diets may be more effective in preserving muscle mass (Brehm et al., 2003; Meckling et al., 2004). Additionally, ketogenic LCHO diets with carbohydrate intakes <5%TE and non-ketogenic with carbohydrate intakes less than 40%TE, showed similar changes in fat mass from the baseline, in a six-week intervention (Johnston et al., 2006).

### *2.1.2 Low Carbohydrate Diets and Diabetes Markers*

Diabetes Mellitus is a metabolic disease characterized by abnormal insulin secretion, action or both (American Diabetes Association, 2010). Diabetes can develop as a result of an autoimmune disease, seen in type 1 diabetes, or impaired insulin secretion and action secondary to insulin resistance, as seen in T2DM (American Diabetes Association, 2010). T2DM is characterised by insulin resistance, reduced insulin production, pancreatic β-islet failure as well as impaired blood glucose control, hyperglycaemia and lipolysis (Janghorbani & Amini, 2011; Olokoba, Obateru, & Olokoba, 2012). Although T2DM can transpire due to hereditary risk factors, it is primarily a disease of lifestyle (Hu et al., 2001). Overweight, obesity (Guh et al., 2009; Hu et al., 2001), poor dietary choices and physical inactivity (Hu et al., 2001) are all associated with the development of T2DM, with adiposity being the strongest predictor (Guh et al., 2009; Hu et al., 2001).

Dietary carbohydrates are digested and broken down into glucose. Following a meal containing carbohydrate, glucose is absorbed into the bloodstream causing an increase in blood glucose concentrations which then results in the release of insulin from β pancreatic islets (Rorsman & Braun, 2013; Wilcox, 2005). In T2DM, metabolic tissue's sensitivity to insulin is reduced,

resulting in reduced glucose uptake. As a result, blood glucose levels do not return to normal levels and remain elevated. Since blood glucose is still at a high concentration, glycation of haemoglobin is increased and therefore resulting in increased glycated haemoglobin (HbA1c) concentrations. The rate of glucose absorption from the gastrointestinal tract influences the rate of blood glucose increase (or spike) and glycaemic control, thus also impacting HbA1c levels (Sherwani, Khan, Ekhzaimy, Masood, & Sakharkar, 2016). A positive correlation is, therefore, observed between total carbohydrate intake and HbA1c levels (Haimoto, Watanabe, Komeda, & Wakai, 2018). Healthy HbA1c concentrations are ≤40 mmol/mol (5.8%). Slightly elevated concentrations ranging from 41 mmol/mol (5.9%) to 49 mmol/mol (6.6%) are considered as pre-diabetic levels, whereas concentrations ≥50 mmol/mol (6.7%) are used to diagnose diabetes (New Zealand Guidelines Group, 2011).

Lifestyle modifications such as increased physical activity, dietary change and achieving a healthy weight (Nield et al., 2007), have shown benefits in decreasing the risk of T2DM (Steyn et al., 2004; Tay et al., 2014) and its management (Nielsen & Joensson, 2008; Tay et al., 2018). Fasting blood glucose (FBG), and HbA1c are metabolic biomarkers used in the diagnosis and management of T2DM and are greatly influenced by dietary choices (New Zealand Guidelines Group, 2011; Sherwani et al., 2016). Glycated haemoglobin provides an insight into blood glucose control over two to three months (half-life of a red blood cell) due to the influence of blood glucose concentrations and compromised glucose metabolism. Hyperglycaemia results in increased HbA1c formation, while hypoglycaemia results in decreased HbA1c formation. The occurrence of such glycaemic conditions is dependent on glucose and haemoglobin interactions and the non-enzymatic reaction between those elements (Sherwani et al., 2016).

Many studies have examined the effects of restricting carbohydrate intake on glycaemic control, HbA1c levels, FBG and fasting insulin levels when compared to conventional dietary approaches such as energy restriction (Hussain et al., 2012; Westman, Yancy, Mavropoulos, Marquart, & McDuffie, 2008). Moderately low carbohydrate (<44%TE/day) (Elhayany et al., 2010; Esposito et al., 2009) and very low carbohydrate (<50g/day) (Boden et al., 2005; Goday et al., 2016; Hussain et al., 2012; Meng et al., 2017; Saslow et al., 2014, 2017; Tay et al., 2014; Westman et al., 2008) diets have demonstrated beneficial effects on HbA1c levels; the lower the carbohydrate levels, the greater the decline in HbA1c levels. Both Hussain et al. (2012) and Westman et al. (2008) reported similar reductions of 1.3% and 1.5% in HbA1c levels respectively after restricting carbohydrates to 20g per day for 24 weeks in overweight and obese individuals with

T2DM. Interestingly, a similar result was observed in other studies, despite a different macronutrient composition (Hussain et al., 2012; Westman et al., 2008). This indicated that HbA1c is highly dependent on the amount of carbohydrate consumed. Although carbohydrate restriction has shown to lower HbA1c levels, some studies have found that carbohydrate restriction did not result in a significant reduction in FBG among diabetic individuals (Meng et al., 2017; Tay et al., 2014).

Moderate carbohydrate restriction (CHO 44%TE) following a Mediterranean diet was found to significantly lower HbA1c (-1.2% in the first year and -0.9% over four years) and fasting blood glucose levels when compared to LF diets among overweight and obese individuals with T2DM (Esposito et al., 2009). Further restricting the carbohydrate content of a Mediterranean diet to 35%TE, resulted in a greater decline in HbA1c levels of 2.0% than the traditional Mediterranean diet (CHO 50%TE) (Elhayany et al., 2010). Similarly, a greater decrease in fasting glucose levels was associated with higher carbohydrate restriction of 35%TE (-4.29mmol/L) (Elhayany et al., 2010) compared to carbohydrates restriction of 44%TE (-2.3mmol/L) (Esposito et al., 2009).

A meta-analysis by Snorgaard et al. (2017) found that although the LCHO diet was superior to the high carbohydrate diet in reducing HbA1c levels, this effect diminished following the first year of intervention, with both diets having comparable results in decreasing HbA1c levels. These findings were consistent with those from randomised controlled trials (Davis et al., 2009; Esposito et al., 2009; Nielsen & Joensson, 2008; Sato et al., 2017, 2016; Tay, Luscombe-Marsh, et al., 2015; Tay et al., 2018). Esposito et al. (2009) found that the greatest change in HbA1c and FBG concentrations occurred in the first year of intervention, with a significantly lower decrease over the remainder of their four year intervention (Esposito, Maiorino, Petrizzo, Bellastella, & Giugliano, 2014). Tay, Luscombe-Marsh, et al. (2015) and Tay et al. (2018) have demonstrated that LCHO diets (CHO <50g) caused a comparable effect on HbA1c to traditional weight loss diets (LF, high carbohydrate diets, carbohydrates 53%TE). Furthermore, LCHO also produced a larger decline in fasting glucose than in the high carbohydrate group; however, when compared to LCHO, the difference was not significant.

Comparing the effects of LCHO diets in diabetic versus non-diabetic overweight individuals (managing T2DM with either diet or metformin), diabetic individuals experienced a 0.3% greater drop in HbA1c compared to a 0.1% reduction in the non-diabetic individuals (Dyson et al., 2007). Unlike the non-diabetic group, the change in HbA1c for the diabetic group was considered clinically significant; however, statistically insignificant. Westman et al. (2008) found that LCHO

diets (CHO <20g) resulted in 95.2% of participants reducing or eliminating diabetes medication compared to 62% of participants following low glycaemic- energy-restricted diet (500kcal deficit).

Pre-diabetes is a transitional stage in which glycaemic parameters are higher than normal, however, below the threshold for diabetes. During this state, the diabetes biomarkers, HbA1c and fasting glucose are elevated (Bansal, 2015; Rett & Gottwald-Hostalek, 2019). In obese pre-diabetic individuals, mildly LCHO diets (CHO 40%TE) were found to cause the remission of prediabetes (Stentz et al., 2016). Consuming 40%TE from carbohydrate, 30%TE from protein and 30%TE from fat, resulted in improved glycaemic control as well as induced the remission of pre-diabetes. Stentz et al. (2016) demonstrated that all participants in the LCHO group experienced a remission of pre-diabetes, compared to only a third of the high carbohydrate (55%TE from CHO, 15%TE from protein and 30%TE from fat) group.

It is important to note that weight loss greatly influences glycaemic control and, therefore, T2DM management (Petersen et al., 2005). Insulin resistance accompanying T2DM results in decreased mobilisation of the glucose transporter (GLUT4) to the cellular surface by SNARE proteins in response to insulin (Tokarz, MacDonald, & Klip, 2018). This decrease in GLUT4 mobilisation results in reduced glucose uptake from the blood leading to hyperglycaemia (Sherwani et al., 2016). A weight loss of 8% has shown to normalise FBG among T2DM patients with insulin resistance. Petersen et al. (2005) found that the improvements in insulin responsiveness are related to normalised insulin response in hepatic glucose production. Campos et al. (2010) found that changes in peripheral glucose uptake are correlated with the extent of weight loss ($r=0.68$, $P=0.02$). Tay et al. (2014) determined that the reduction in HbA1c was not related to dietary intervention. This evidence, therefore, supports the theories that weight loss plays a significant role in addition to decreased carbohydrate intakes in improving FBG, insulin resistance and peripheral glucose uptake in diabetic individuals which in return translates into beneficial changes in diabetes biomarkers.

### *2.1.3 Low Carbohydrate Diets and Cardiovascular Disease Markers*

Cardiovascular disease is the number one cause of mortality worldwide contributing to 31% of all global deaths in 2016 (World Health Organisation, 2017). Cardiovascular disease encompasses all disorders of the heart and the blood vessels such as hypertension, coronary heart disease, and cerebrovascular disease. The most common disorder accompanying CVD is the blockage of

blood vessels causing heart attacks or strokes (World Health Organisation, 2017). The build-up of lipid deposits most commonly causes these blockages on the inner lining of the blood vessels as a result of hyperlipidaemia (Omenn, Beresford, & Motulsky, 1998). Although hyperlipidaemia can be caused by genetic factors, it is greatly influenced by lifestyle choices, including dietary intake (Blesso & Fernandez, 2018; Cohen, 2008).

Hyperlipidaemia causes alterations in blood lipid profile, biomarkers for assessing CVD risk (Blesso & Fernandez, 2018). The blood lipid profile includes the following: total cholesterol (TC), high density lipoprotein cholesterol (HDLC), low density lipoprotein cholesterol (LDLC), triglycerides (TG) and TC:HDLC ratio (Blesso & Fernandez, 2018). A desirable blood lipid profile involves maintaining TC < 4mmol/L, LDLC < 2mmol/L, and HDLC > 1mmol/L, to reduce the risk of developing atherosclerotic plaques ("Canterbury District Health Board," 2020; Daniels, Killinger, Michal, Wright, & Jiang, 2009). Elevated serum TC, LDLC and TG alongside low serum HDLC are risk factors for CVD (Bayturan et al., 2010; Cooney et al., 2009; MacMahon et al., 2007) due to the nature of those lipoproteins. LDLC transport lipids from the liver and deposit it in blood vessels and other tissues. HDLC, however, transports fats away from blood vessels and organs and to the liver to be metabolised (Daniels et al., 2009; Heart Foundation, 2019).

The elements of the blood lipid profile are highly influenced by the amount and type of dietary fat consumed (MacMahon et al., 2007). Increased saturated fatty acid (SFA) intakes result in increased TC and LDLC concentrations, thus contributing to an elevated CVD risk. Decreasing SFA intake may reduce CVD risk whereby replacing one per-cent of energy from SFA with polyunsaturated fatty acid (PUFA) resulted in a significant decrease in TC by 0.064mmol/L and LDLC by 0.055mmol/L (Mensink & World Health Organisation, 2016). Additionally, Li et al. (2015) found that replacing 5% of SFA by PUFA can decrease the risk of developing CVD by as much as 25%. Based on such evidence, the NZ Heart Foundation currently recommends limiting SFA intake to less than 8% of total energy intake (The Heart Foundation, 2020).

Very low carbohydrate diets (<50g carbohydrates per day) have consistently shown to significantly decrease TG and increase HDLC (Bazzano et al., 2014; Dashti et al., 2006, 2007; Hussain et al., 2012; Westman, Yancy, Olsen, Dudley, & Guyton, 2006; Yancy et al., 2004). Favourable changes in HDLC and TG in LCHO diets can be attributed to the alteration in dietary composition (Zinn, McPhee, et al., 2017). Increased carbohydrate intakes and particularly simple and refined carbohydrates result in elevated TG concentrations (Hudgins, 2000; Reizlaff C'arolvn

et al., 1995; Sacks et al., 2014). A study by Frayn & Kingman (1995) suggests that the greater the change in carbohydrate intake, the greater the change in serum TG concentrations (Frayn & Kingman, 1995). Therefore, the significant reduction in carbohydrate intakes accompanying LCHO diets is expected to cause a substantial decline in serum TG concentrations. Additionally, in a one-year longitudinal analysis, Ma et al. (2006) demonstrated that glycaemic load (describes glycaemic index and carbohydrate intake) had a positive relationship with TC and LDLC and an inverse relationship with HDLC. This suggests that low glycaemic load diets are associated with lowering CVD risk (Halton et al., 2006) by increasing HDLC and reducing TC and LDLC concentrations.

Although LCHO diets have shown beneficial effects on TG and HDLC concentrations, they have demonstrated diverse effects on TC and LDLC concentrations. Some studies have shown that LCHO diets exert no influence on TC and LDLC (Bazzano et al., 2014; Santos, Esteves, Da Costa Pereira, Yancy, & Nunes, 2012; Sharman et al., 2002; Westman et al., 2006). Other studies, however, have shown to decrease (Dashti et al., 2006, 2007; Hussain et al., 2012; Paoli, Cenci, & Grimaldi, 2011) or increase (Bueno et al., 2013; Noakes et al., 2006) TC and LDLC concentrations. Among both hypercholesterolaemic and normocholesterolemic individuals, LCHO diets resulted in significant declines in TC, LDLC, and TG, and increasing HDLC concentrations compared to baseline (Dashti et al., 2006). When comparing hypercholesterolaemic to normocholesterolemic individuals, LCHO diet exerted comparable effects on the blood lipid profile. TC concentrations were an exception to this, where hypercholesterolaemic individuals experienced a significantly greater decline (Dashti et al., 2006).

Studies comparing the effects of LCHO diet to LF diet among obese and hyperlipidaemic individuals have found that LCHO diets are superior, causing a substantial decrease in TG concentrations and increases in HDLC concentrations, however, causing comparable changes in TC and LDLC (Bazzano et al., 2014; Westman et al., 2006; Yancy et al., 2004). In contrast, Keogh et al. (2008) and Phillips et al. (2008) have shown that LF diets showed superiority in decreasing TC and LDLC compared to LCHO diets. Although cholesterol is synthesised in the body, ingesting excessive dietary fat and cholesterol enters the bloodstream and can contribute to elevated blood cholesterol concentrations (Cohen, 2008).

In obese individuals with hyperglycaemia, LCHO diets have shown to not only decrease TG and increase HDLC but also to significantly reduce TC compared to normoglycemic individuals

(Dashti et al., 2007). The relationship between CVD and T2DM is well known, where insulin resistance results in increased free fatty acid release to the blood (Ginsberg, 2000). Increased free fatty acid concentrations alongside increased adequate glycogen stores promote TG formation, thus promoting LDLC formation and particularly very low-density lipoproteins (Sears & Perry, 2015). Dashti et al. (2007) achieved a significant reduction in blood glucose concentrations in hyperglycaemic individuals. Hussain et al. (2017) found HbA1c to be positively correlated to TC, TG, and LDLC concentrations and to be a predictor of hypercholesterolaemia. Furthermore, in obese but otherwise healthy individuals, LCHO diets resulted in a greater decrease in TG and TC:HDLC ratio, and a greater increase in HDLC compared to LF diets (Bazzano et al., 2014).

In obese adults, RCTs have found that LCHO does not significantly influence TC and LDLC from baseline concentrations (Bazzano et al., 2014; Meckling et al., 2004; Sharman et al., 2002). They instead cause a significant increase in HDLC and a decrease in TG concentrations (Meckling et al., 2004; Nordmann et al., 2006; Sharman et al., 2002). Meckling et al. (2004) found that LCHO diets had significantly higher TC, LDLC and HDLC compared to LF. Triglycerides showed similar decreases in both LCHO and LF diets (Meckling et al., 2004). Additionally, the effects of LCHO on blood lipid profile can be related to changes in body weight (James et al., 2003; Patalay, Lofgren, Freake, Koo, & Fernandez, 2005), carbohydrates intakes and lastly fat intakes (Frayn & Kingman, 1995; Zinn, McPhee, et al., 2017).

Meta-analysis by Mansoor et al. (2016) demonstrated that despite the significant weight losses, LCHO diets resulted in a greater increase in LDLC compared to traditional LF weight loss diets. Additionally, some RCT's demonstrated that LDLC concentrations either maintained similar concentrations to pre-diet or increased concentrations even with weight loss (Bueno et al., 2013; Noakes et al., 2006; Santos et al., 2012; Sharman et al., 2002; Westman et al., 2006). Petersen et al. (2005) have found a reduction in body weight by 8 % resulted in a 10% decrease in plasma cholesterol concentrations in individuals suffering from T2DM (Petersen et al., 2005). In addition, weight loss has shown to decrease serum TG (Patalay et al., 2005) and LDLC concentrations (James et al., 2003; Patalay et al., 2005). This decrease is thought to be related to an increase in the activity of lipoprotein lipase, an enzyme responsible for the breakdown of TG (Patalay et al., 2005). Since weight loss is known to reduce TG and LDLC concentrations, the clear effect of the nutrient profile of LCHO diets may be minimised as weight loss is often seen when following LCHO.

Although LCHO shows beneficial effects in improving elements of the blood lipid profile and achieving significant weight loss, those benefits must be weighed against the possible harmful effects of increased LDLC concentrations. Despite the increase in HDLC concentrations and weight loss, increased HDLC concentrations cannot be translated into decreased CVD risk. This finding is consistent with other meta-analyses (Bueno et al., 2013; Nordmann et al., 2006; Schwingshackl & Hoffmann, 2014).

### *2.1.4 Low Carbohydrate Diets and Kidney Function*

Chronic kidney disease (CKD) is defined as the gradual decline in kidney functions characterised by a decrease in glomerular filtration rate (GFR) for a duration of three months or greater (Martin, Armstrong, & Rodriguez, 2005; Webster, Nagler, Morton, & Masson, 2017). CKD is highly influenced by lifestyle choices as well as chronic diseases (Dunkler et al., 2015; Stengel, Tarver–Carr, Powe, Eberhardt, & Brancati, 2003), with high blood pressure and diabetes being the two leading causes for CKD (Webster et al., 2017). Obesity is also associated with increased risk of CKD (Stengel et al., 2003) and nephrosclerosis (Vupputuri & Sandler, 2003). The decline in renal function, accompanying CKD is often identified by decreased estimated GFR (eGFR) indicating a decrease in the kidney's ability to excrete metabolic waste and toxins (Martin et al., 2005; Webster et al., 2017).

The effects of LCHO diets on renal function have been exhaustively examined as carbohydrate intake is replaced with protein, fat or both (Paoli, Rubini, Volek, & Grimaldi, 2013; Wylie-Rosett, Aebersold, Conlon, Isasi, & Ostrovsky, 2013). High protein intakes are thought to influence kidney function by causing an initial increase in eGFR (Cirillo et al., 2014; Juraschek, Appel, Anderson, & Miller III, 2013; Oyabu et al., 2016; Schwingshackl & Hoffmann, 2014), serum urea (Cirillo et al., 2014; Friedman et al., 2012; Schwingshackl & Hoffmann, 2014) and creatinine clearance (Friedman et al., 2012) among overweight and obese adults with no pre-existing renal disease. High protein diets have shown to cause an initial increase in eGFR (Cirillo et al., 2014; Juraschek et al., 2013; Oyabu et al., 2016; Schwingshackl & Hoffmann, 2014), a physiological response as the body attempts to maintain a constant serum creatinine concentration through increasing its excretion via urine. This increase in eGFR is thought to result in glomerular hyperfiltration (Toubro et al., 1999). Increased eGFR is also believed to be the result of increased intraglomerular pressure, as demonstrated in animal studies (Schrijvers, Rasch, Tilton, & Flyvbjerg, 2002).

Increased serum urea and creatinine with high protein diets is related to the increased intake of protein and therefore increased protein metabolism. Although serum creatinine concentrations reflect muscle mass, high protein intakes can also elevate serum creatinine. The cooking process of animal protein results in the conversion of creatine to creatinine, creatinine is then absorbed into the bloodstream (Gowda et al., 2010; Hosten, 1990). Similarly, serum urea is also influenced by protein intake. Urea is the by-product of protein breakdown, where protein metabolism results in nitrogen formation, which is then converted to urea in the urea cycle (Hosten, 1990). The urinary excretion of both urea and creatinine primarily depends on GFR, where decreased GFR leads to increased concentrations of urea and creatinine. Therefore, urea and creatinine are often used in conjunction with eGFR to assess CKD (Gowda et al., 2010; Hosten, 1990).

A study by Friedman et al. (2012) found LCHO initially caused minor reductions in serum creatinine which only took place in the first three months of the dietary intervention. Friedman also found an increase in creatinine clearance which took place up to 12 months from initiation of dietary intervention. At two years of intervention, Tay, Thompson, et al. (2015) found that LCHO caused an increase in serum creatinine as well as a decrease in eGFR and creatinine clearance. Changes in those biomarkers took place along with a significant weight loss from baseline (Friedman et al., 2012; Tay, Thompson, et al., 2015).

When comparing LCHO diets to LF and traditional weight loss diets, LCHO has shown to cause a similar decline in eGFR levels (Brinkworth, Buckley, Noakes, & Clifton, 2010; Westman et al., 2008; Yancy et al., 2004). Brinkworth et al. (2010), however, found that the decrease in GFR levels was not correlated with dietary intakes ($P$=0.86). They, however, found that weight loss ($r$=0.37, $P$=0.01) and decreased body surface area ($r$=0.38, $P$=0.001) showed significant positive correlations with eGFR. Brinkworth et al. (2010) concluded that weight loss achieved with LCHO diets in obese, but otherwise healthy individuals did not adversely affect renal function. Those findings were consistent with Westman et al. (2008) and Yancy et al. (2004). Obesity is associated with a significant increase in blood pressure, elevated eGFR (Chagnac et al., 2003), intraglomerular pressure and albumin excretion rate (Kovesdy et al., 2017). Achieving a modest weight loss has shown to significantly decrease GFR, renal plasma flow and blood pressure (Chagnac et al., 2003).

In individuals with no pre-existing kidney disease, high protein intakes were not associated with altered eGFR. However, among those with pre-existing renal disease, protein intake was significantly correlated with eGFR (Knight, Stampfer, Hankinson, Spiegelman, & Curhan, 2003).

Knight et al. (2003) found that non-dairy animal protein may accelerate the decline in renal function among women with mild renal insufficiency (Knight et al., 2003).

Interestingly, Cirillo et al. (2014) found that in the short-term (cross-sectionally), increased protein intake was positively correlated with eGFR levels, where 1g increase in protein intake resulted in 4.7ml/min.1.73m$^2$ increase in eGFR. Long-term (longitudinally); however, increased protein intake was negatively correlated with eGFR levels, where 1g increase in protein intake resulted in 4.1ml/min.1.73m$^2$ decrease in eGFR (Cirillo et al., 2014). The short-term increase in eGFR suggests a hyperfiltration as a result of increased intake (Toubro et al., 1999).

## 2.2 Summary

Overweight and obesity are the main risk factors of developing T2DM, and CVD as well as influencing the risk of CKD. Carbohydrate restriction has shown to decrease total energy intake and therefore result in weight loss. Weight loss greatly affects metabolic biomarkers of CVD, T2DM and renal functions. In some studies, the change in body weight was the main factor in improving those biomarkers. Low carbohydrate diets have shown to cause a significant decline in body weight from baseline, improve diabetes biomarkers, and exert some beneficial effects on blood lipid profile. When compared to traditional weight loss diets, long term adoption of LCHO has shown to result in similar effects on weight loss, improving HbA1c and fasting glucose.

# Chapter Three

# Research Manuscript

# The effects of self-directed low carbohydrate diets on metabolic biomarker profiles and disease risk among New Zealand adults– the LOCA study

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**A shortened version of the title:** Low carbohydrate diet trends and disease risk

## Abstract

Low carbohydrate (LCHO) diets have become popular due to their effectiveness for weight loss; however, there are concerns regarding their safety and impact on cardiovascular disease (CVD), kidney disease and diabetes risk. This study aimed to investigate metabolic and inflammation biomarkers and associations with dietary intake of self-reported LCHO diet consumers in NZ. This cross-sectional study recruited men and women aged 20-45 years following an LCHO diet for at least four months. Participants completed a health and demographic questionnaire, a 4-day weighed dietary record and provided anthropometric measurements and blood samples. Participants were divided into three groups based on carbohydrate intake to very low carbohydrate (VLCHO) (<50g), LCHO (50-100g) and moderately low carbohydrate (MLCHO) (>100 -<150). Seventy-four individuals with a mean age of 35 years participated in this study. Their median macronutrient intakes were 14 [11.4, 26.7]% of total energy (%TE) from carbohydrates, 58.1 [49.1-66.0]%TE from fat and 24.4 [22.9, 25.9]%TE from protein. Participants in the VLCHO, LCHO and MLCHO groups, respectively, had elevated cholesterol (94.7%, 89.5% and 88.9%), LDLC (94.6%, 100% and 88.9%) and HDLC (92.1%, 94.7% and 100%) concentrations. Carbohydrate intakes (grams and %TE) negatively correlated with TC (r= -0.353, *P*= 0.003 and r= -0.403, *P*= 0.001), and LDLC (r= -0.329, *P*= 0.007 and r= -0.335, *P*= 0.006). Our findings suggest that LCHO diets followers predominantly replaced carbohydrate with fat. High fat and SFA intakes (%TE) due to carbohydrate restriction were accompanied by high cholesterol, LDLC and HDLC concentrations. It is important to weigh the benefits and harms of LCHO diets on CVD risk.

## 3.1 Introduction

In light of the escalating rate of obesity and its associated comorbidities, there has been unprecedented demand from the public for safe and effective weight loss strategies (Thomas et al., 2008). Globally, 1.9 billion (39%) adults are overweight, and 13% are obese (World Health Organisation, 2018). In NZ, around 31% of adults are obese (Ministry of Health, 2019c). Low carbohydrate (LCHO) diets have become an increasingly popular dietary trend for fast and effective weight loss (Clarke & Best, 2017; Dyson et al., 2007; Jallinoja et al., 2014). Low carbohydrate diets are characterised by restricting energy intake from carbohydrate sources and replacing it by increasing either fat or protein sources of energy, or both, in the diet (A Paoli et al., 2013; Wylie-Rosett et al., 2013).

Low carbohydrate diets encompass a wide array of carbohydrate restrictions and applications. Both a clear definition and consistent categorisation of LCHO diets are lacking (Wylie-Rosett et al., 2013). Most often, dietary intake of carbohydrate below the Acceptable Macronutrient Distribution Range (AMDR) recommendations of 45-65%TE (Laffel, 1999; Ministry of Health, 2011; NHMRC, 2006) is considered as LCHO. This lack of consistency results in self-interpreted LCHO diets, where some individuals strictly monitor carbohydrate intakes, while others do not (Jallinoja et al., 2014). Nevertheless, some studies have classified carbohydrate intakes as very low carbohydrate (VLCHO) (<50g/day) (Brouns, 2018; Feinman et al., 2015; Harvey et al., 2018), LCHO (50 – 100g/ day) (Bilsborough & Crowe, 2003), and moderately low carbohydrate (MLCHO) (100-150g /day) (Brouns, 2018).

Low carbohydrate diets have shown benefits in fast weight loss (Boaz & Raz, 2015; Boden et al., 2005; Clifton et al., 2014; Goday et al., 2016; Naude et al., 2014), with some studies demonstrating superiority over the traditional low fat (LF) weight loss diets (Mansoor et al., 2016; Nordmann et al., 2006). Low carbohydrate diets have also shown improvements in some risk factors for T2DM and CVD (Mansoor et al., 2016). In contrast, other studies have found that improvements in weight and risk factors of T2DM and CVD were similar to traditional weight loss diets (Boaz & Raz, 2015; Clifton et al., 2014; Naude et al., 2014; Seidelmann et al., 2018). Despite the benefits of LCHO diets, their effect in altering macronutrient intakes have shown to increase the risk of total mortality (Lagiou et al., 2007; Noto et al., 2013); additionally, increased animal protein and fat result in an increased risk of mortality compared to plant source of those nutrients (Noto et al., 2013; Seidelmann et al., 2018). Furthermore, the success of weight loss diets in reducing body weight and body mass index (BMI) is attributed to individuals ability to

adhere to dietary interventions rather than altering macronutrient intake (Alhassan, Kim, Bersamin, King, & Gardner, 2008; Dansinger et al., 2005).

A spontaneous decline in carbohydrate intakes has been observed globally (Jayasinghe et al., 2017; Makarem et al., 2014; Ministry of Health, 2011; J. Schrijvers et al., 2016; Vadiveloo et al., 2014; Zhao et al., 2018). In New Zealand, the National Nutrition Survey (NNS) 2008/09 demonstrated that carbohydrate intakes comprise only 45-47%TE, an intake in the lower range of the AMDR recommendation (Ministry of Health, 2011). A study by Sam et al. (2020) has found that NZ adults aged 30-59 years had carbohydrate intakes of 48.6%TE and a total fat intake of 32%TE. Other studies among NZ women aged 16-45 years have shown low carbohydrate intakes (below AMDR) for example, Schrijvers et al. (2016) reported carbohydrate intake of 42.6%TE with high fat intakes of 34.5%TE, and Jayasinghe et al. (2017) found carbohydrate intake of 42%TE with high fat intakes of 37%TE.

In NZ, few studies have examined the various effects of LCHO diets. However, none of those studies examine current practices of LCHO diet followers and the impact of those practices on metabolic biomarkers for T2DM and CVD. The LOCA (LOw CArbohydrate) study is the first study aimed to investigate the dietary practices of self-reported low carbohydrate diet followers (men and women) aged 20 to 45 years in NZ. It also aimed to explore the associations between dietary intake and metabolic and inflammation biomarkers of self-reported low carbohydrate diet consumers in NZ.

## 3.2 Materials and Methods

### *3.2.1 Design*

The LOCA study is a cross-sectional study investigating the different trends of self-reported low carbohydrate diets in New Zealand and their effects on lifestyle, dietary intake and practices as well as selected metabolic biomarkers. This sub-study aimed to investigate metabolic and inflammation biomarkers and associations with dietary intake of self-reported low carbohydrate diet consumers (men and women aged 20 to 45 years) in Auckland, NZ. It more specifically investigated the biomarker profiles associated with metabolic disease risk (T2DM, CVD, obesity and kidney disease risks) of self-reported low carbohydrate consumers in relation to gender and different levels of low-carbohydrate intakes and explored the association between all biomarkers and energy and nutrient intakes, low carbohydrate diet duration, and replacement nutrients.

### *3.2.2 Participants*

The LOCA study recruited a total of 74 participants which provided a power of 69.9%. The study examined nutrient intakes, anthropometric and biomarker measurements of three groups to determine a large effect size *f* of 0.4 at a significance level of $P = 0.0167$ (using G*Power 3.1.9.4) (Faul, Erdfelder, Buchner, & Lang, 2009). *Inclusion criteria:*

- Adults, all genders, aged between 20 and 45 years.
- Following a low carbohydrate diet for at least four months.

*Exclusion criteria:*

- Suffering from chronic conditions such as cancer, kidney, and liver disease
- Taking medication that influences the blood biomarkers assessed in this study such as medications that alters blood cholesterol and lipids, blood glucose and blood pressure
- Women who are pregnant or breastfeeding
- Individuals who have had bariatric surgery.

### *3.2.3 Study Procedures*

The LOCA study was conducted according to the guidelines laid down in the Declaration of Helsinki (World Medical Association, 2008) between October 2018 and June 2019. Permission and ethical approval to conduct the present study was obtained from the Massey University Human Ethics Committee (MUHEC): Southern A Committee, application SOA 18/22, on July 19th, 2018. Written informed consent was obtained from all participants.

#### 3.2.3.1 Screening and Recruitment

The study took place in the Human Nutrition Research Unit (HNRU) at the Auckland campus of Massey University, therefore recruited participants were mainly those living in Auckland City. Participants were approached through social media as well as through posters distributed to local gymnasiums. Those interested were directed to the LOCA study's website for online information

sheets and consent forms. Participants were able to register their interest by completing an online screening questionnaire.

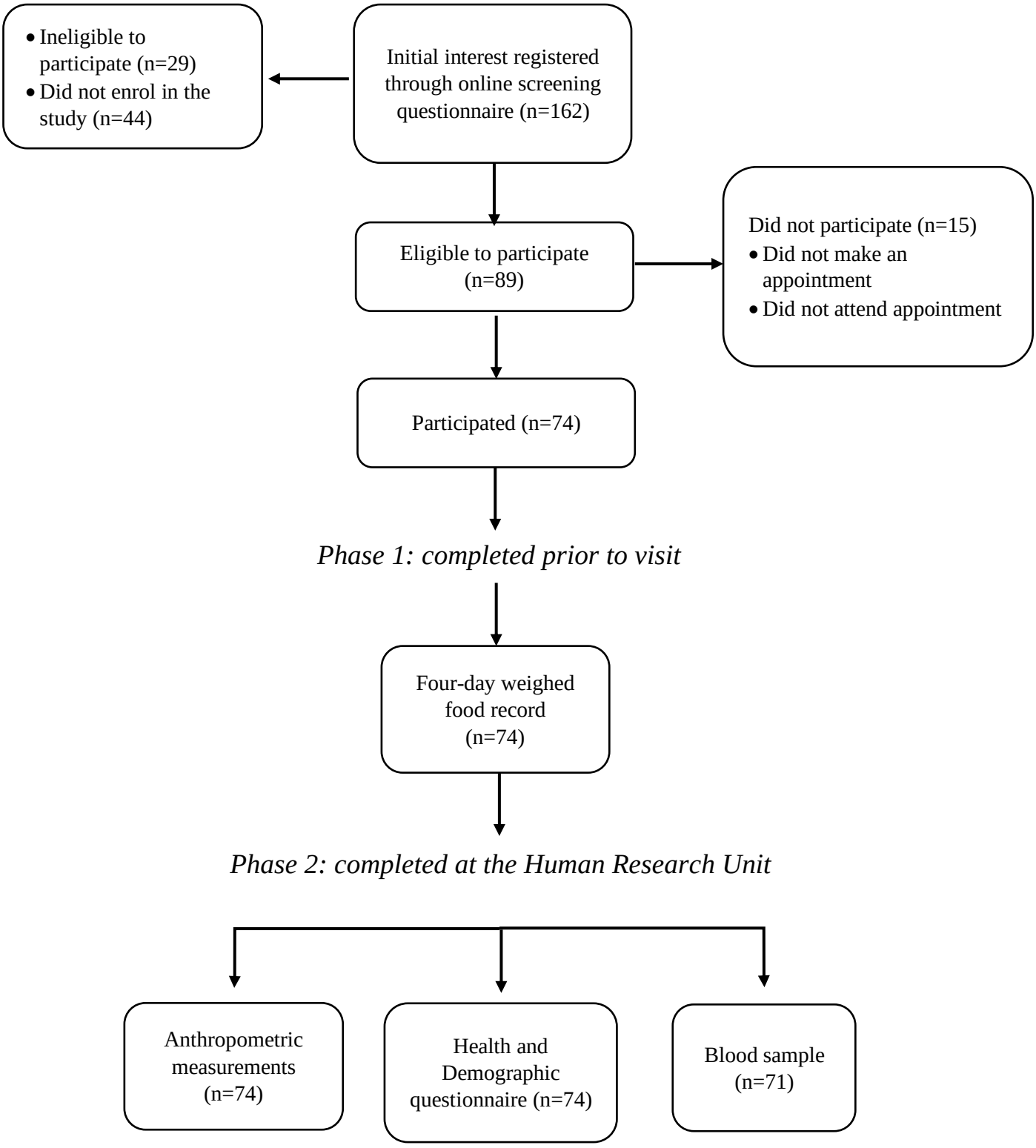



***Figure 3.1.*** *Flow diagram of the present sub-study of the LOCA study, participants, procedure and measures.*

### 3.2.3.2 Data Collection

Phase two took place at the HNRU, where participants completed a Health and Demographic questionnaire online questionnaire. Anthropometric and blood pressure measurements were also taken during this part of the study alongside fasted blood and urine samples.

#### 3.2.3.2.1 Anthropometric Measurements

Anthropometric measurements were obtained by trained researchers using the International Society for the Advancement of Kinanthropometry (ISAK) protocols (Marfell-Jones, Stewart, & Ridder, 2012). Height was measured using a stadiometer (Marfell-Jones et al., 2012; Ministry of Health, 2008). Both weight and body composition (body fat percentage (BF%)) were determined using bioelectrical impedance analysis (BIA) (InBody230, Biospace Co. Ltd, Seoul).

Waist and hip circumferences were measured using a Lufkin tape. Height, hip and waist circumferences were repeated twice to ensure the accuracy of the measurements. If the first two measurements varied by more than 1%, a third measurement was taken following the ISAK protocol (Marfell-Jones et al., 2012).

#### 3.2.3.2.2 Metabolic Biomarkers

Venous blood samples were collected by a trained phlebotomist using ethylenediaminetetraacetic acid (EDTA), fluoride and heparin vacutainers after an overnight fast. Prior to processing, the blood sampled in the EDTA vacutainers were used to determine blood ketone and HbA1c levels using FreeStyle Optium Neo Monitor (Abbott Diabetes Care Inc., 1360 South Loop Road, Alameda, CA 94502, United States) was used to measure blood ketone concentrations. Additionally, Cobas b 101 (Roche Diagnostics International Ltd CH-6343 Rotkreuz Switzerland) was used to measure HbA1c levels, where 2 μL of blood is added to the HbA1c disc which is then processed by the Cobas. The vacutainers were then centrifuged for 15 minutes at 3500rpm at 4°C. Plasma was sampled into Eppendorfs and stored at -80$^{o}$C, following Standard Operating Procedures.

Plasma lipid, glucose and insulin, and serum C-reactive protein, creatinine and urea analyses were from the blood samples, and the analysis was conducted by Canterbury Health Laboratories, Christchurch, New Zealand (IANZ ISO 15 189). Serum Insulin was measured using the electrochemiluminescence immunoassay “ECLIA” method (Roche Diagnostics, Mannheim, Germany) on the Cobas e411 analyser (Hitachi High Technologies Corporation, Tokyo,

Japan).The homeostasis model assessment (HOMA) was used to calculate insulin resistance (IR), based on fasting insulin and glucose concentrations using the following equation: Fasting plasma insulin [microU/L] x Fasting plasma glucose [nmol/L]/22.5 (Wallace, Levy, & Matthews, 2004). Estimated glomerular filtration rate (eGFR) was calculated using the MDRD equation: [175 × (serum creatinine [mg/dl])$^{-1.154}$ × (age [years])$^{-0.203}$ × (0.742 if female)] (Levey et al., 2006; National Kidney Foundation, 2019).

Blood pressure was measured using the Omron digital blood pressure monitor, where three blood pressure measurements were taken. The averaged blood pressure measurement was used in data analysis.

#### 3.2.3.2.3 Weighed Food Record

Participants completed a WFR, where all foods, beverages and supplements consumed were recorded across four non-consecutive days, including three weekdays and one weekend day. Trained researchers reviewed the food records and conducted a short discussion with participants to clarify any unclear components (e.g. portion sizes, unfamiliar foods or brands), where needed. The WFR were then entered and analysed using Foodworks 9 software (Food Works Professional, Xyris software, Queensland, Australia) (Xyris, 2019). This software allows access to multiple databases for dietary analysis. In the present study, the New Zealand Food Composition Database (NZ FOODfiles 2016) was preferred. In the case where foods were unavailable in the New Zealand Composition database, Australian databases including FSANZ (Food Standards Australia New Zealand), AusFoods, and AusBrands were used. Some dietary assumptions were made when foods were not found on the databases provided by FoodWorks 9, for example, if foods were not available on FoodWorks, the most suitable alternative was used (e.g. “Ice cream, vanilla, low fat” replaced “Halo Top Ice Cream”). Additionally, recipes that were not available on FoodWorks (e.g. ‘fat bomb’, homemade ‘seed crackers’), were manually entered into the software. Many participants used supplements (e.g. multivitamins, creatine) and Chinese herbal medicines, however, these were not included in this analysis. However, supplements that were used within a meal or as part of a recipe (e.g. psyllium husk added to homemade bread, or protein powder used in a smoothie) were included as part of the dietary analysis.

#### 3.2.3.2.4 Questionnaires

In this sub-study, the Health and Demographics Questionnaire was used to identify participant group's characteristics such as age, gender, level of education, ethnicity, working pattern, income level, allergies and medical conditions as well as dietary restrictions (Appendix A). The survey was administered using the SurveyMonkey online system.

### *3.2.4 Statistical Analysis*

All statistical analyses were performed using IBM SPSS statistics package (IBM corp., Armonk, NY, USA). The Kolmogorov-Smirnov and Shapiro-Wilk tests and box plots were used to examine the data for normality. In contrast, Levene's test was used to test the homogeneity of variance of the data. Non-normally distributed data were log-transformed using the natural log and were then retested for normality. Normally distributed variables were expressed as mean and standard deviation. Log transformed variables were used if the variable achieved normality after transformation and presented as geometric mean (95% confidence interval). Non-normally distributed variables were expressed as median and [25th - 75th percentiles]. Categorical variables were expressed as proportions or n (%). Furthermore, independent t-test (parametric) and Mann-Whitney U test (non-parametric) were used to determine the differences in anthropometric, metabolic biomarker and dietary intakes between men and women.

Participants were grouped based on carbohydrate intakes (g) into three groups; VLCHO (<50g/day) (Brouns, 2018; Feinman et al., 2015; Harvey et al., 2018), LCHO (50-100g/day) (Bilsborough & Crowe, 2003), and MLCHO (>100 - <150g/day) (Brouns, 2018). Individuals with carbohydrate intakes greater than 150g per day were excluded from the ANCOVA analysis (n=5) because their CHO intake did not classify as low. Anthropometric, metabolic biomarkers and macronutrient intake variables were presented as adjusted means and standard deviations after controlling for age, gender and income using ANCOVA. A P-value less than 0.05 was considered statistically significant (Field, 2013). Post-hoc Bonferroni correction was used following significant ANCOVA tests to identify groups that were statistically different (*P*-value <0.0167). Subsequently, Pearson's and Spearman's correlations were performed to calculate r value for effect sizes ranging from small (r = 0.1), medium (r = 0.3) to large (r = 0.5) (Field, 2013). Chi-Square test was performed to examine the relationship between carbohydrate groups and metabolic biomarkers. Additionally, linear regression analyses were performed using the

enter method to identify the associations between carbohydrate intakes and metabolic biomarkers (while controlling for age, gender and income and duration of following LCHO diet).

## 3.3 Results

A total of 162 men and women registered interest in participating in the LOCA study, with only 74 participants completing both phases one and two of data collection (Figure 3.1). The majority of the 74 participants were women (73%), and only 20 men (27%) participated. Their mean age was 35 (SD: 7) years. The majority of participants were of New Zealand European ethnicity (70.3%), with tertiary education (79.7%) and had a regular working pattern (73.1%) (Table 3.1).

***Table 3.1.*** *Demographic characteristics of the LOCA study population*

| **Variables** | **n (%)** |
|---|---|
| Age (years) | |
| Mean | 35 |
| Standard deviation | 7 |
| Range | 20 – 45 |
| Gender | |
| Male | 20 (27%) |
| Female | 54 (73%) |
| Highest level of education | |
| Tertiary Education | 59 (79.7%) |
| Secondary School or other | 15 (20.3%) |
| Ethnicity | |
| New Zealand European | 52 (70.3%) |
| Maori | 1 (1.4%) |
| European | 7 (9.5%) |
| Asian | 8 (10.8%) |
| Other | 6 (8.1%) |
| Current working Pattern | |
| Regular Working Pattern | 49 (73.1) |
| Irregular Working Pattern | 18 (26.9) |

| Variables | n (%) |
|---|---|
| Total Monthly Income | |
| Less than $3000 | 11 (17.2%) |
| $3001-$8000 | 30 (46.9%) |
| Greater than $8000 | 23 (35.9%) |
| Alcohol Intake | |
| Never or very rarely | 34 (45.9%) |
| One drink per week | 14 (18.9%) |
| More than one drink per week | 26 (35.1%) |
| Dietary restrictions | |
| No | 70 (95.9%) |
| Yes | 3 (4.1%) |
| Allergies | |
| Do not suffer from allergies | 56 (75.7%) |
| Suffer from allergies | 18 (24.3%) |
| Smoking Status | |
| Not Currently Smoking | 69 (93.2%) |
| Currently Smoking | 5 (6.8%) |
| Supplement Intake | |
| No | 30 (41.1) |
| Yes | 43 (58.9) |

Dietary data analysis of all participants revealed that participants had a total energy intake of 1780 (SD: 565)kcal per day as well as low intakes of carbohydrates of 65.9g (95% CI: 52.1, 79.7) and 14.0%TE (95% CI: 11.4, 16.7), and fibre of 18.5g (95% CI: 15.6, 21.4). In contrast, they had high intakes of protein 24.4%TE (95% CI: 22.9, 25.9), total fat 58.1%TE [IQR: 9.1 – 66.0], SFA 22.0%TE (SD: 7.17) and dietary cholesterol 453g [IQR: 312-666] (Table 3.2A).

The study population had a mean body weight of 75.1 ±14.7kg, BF% of 27.7 ± 9.8% and a median BMI of 25.5 [IQR: 25.5, 26.5]kg/$m^2$ (Table 3.2B). They also had elevated TC and LDLC as well as low eGFR with all other biomarkers within normal ranges. The BMI did not differ between men and women (P=0.673), whilst the BMI of all participants was slightly above normal, placing this group just in the overweight range (Table 3.2B). The study population had low eGFR, and high TC, LDLC and HDLC concentrations as well as high TC:HDLC ratio. The

majority of the metabolic biomarkers were within reference ranges, excluding TC, LDLC and eGFR (Table 3.2B). TC and LDLC concentrations were comparable between men and women. Interestingly, high HDLC concentrations were seen among men and women, with men having significantly lower concentrations than women (1.47 ± 0.29mmol/L and 1.77 ± 0.49mmol/L, p= 0.027). Men had significantly higher eGFR levels compared to women ( 82.9 ± 12.3ml/min/1.73m$^2$ versus 73.5 ± 9.60ml/min/1.73m$^2$, p= 0.001).

***Table 3.2.A.*** *Dietary analysis of the LOCA study's total participants and the characteristics of men and women.*

| Variables | Reference range | Total (n=74) | | Men (n=20) | | Women (n=54) | | *P-value** |
|---|---|---|---|---|---|---|---|---|
| | | Mean† G. mean§ Median¥ | SD†, 95% CI§, 25th –75th%¥ | Mean† G. mean§ Median¥ | SD†, 95% CI§, 25th –75th%¥ | Mean† G. mean§ Median¥ | SD†, 95% CI§, 25th –75th%¥ | |
| ***Dietary intake*** | | | | | | | | |
| Energy (kcal) † | | 1780 | 565 | 2197 | 625 | 1626 | 458 | <0.001* |
| Protein (g)§ | | 104 | 95.8, 111 | 125 | 107, 144 | 95.6 | 88.0, 103 | 0.001* |
| Total fat (g) † | | 115 | 46.2 | 144 | 54.2 | 104 | 38.2 | 0.001* |
| SFA (g) † | | 44.7 | 21.2 | 57.6 | 27.3 | 39.9 | 16.2 | 0.012* |
| PUFA (g)§ | | 15.9 | 13.9, 17.9 | 20.1 | 15.0, 25.3 | 14.3 | 12.5 – 16.2 | 0.038* |
| MUFA (g) † | | 43.8 | 18.7 | 52.8 | 21.9 | 40.5 | 16.5 | 0.011* |
| Dietary cholesterol (mg) ¥ | <300mg/day | 453 | 312 – 666 | 564 | 364 – 925 | 445 | 299 – 595 | 0.049* |
| Carbohydrates (g) § | | 65.9 | 52.1, 79.7 | 80.8 | 47.3, 114 | 60.4 | 45.5, 75.2 | 0.192 |
| Fibre (g) § | 25-30g/day | 18.5 | 15.6, 21.4 | 21.7 | 13.1, 30.3 | 17.3 | 14.8, 19.9 | 0.320 |
| Starch (g) § | | 28.4 | 20.0, 36.8 | 37.7 | 13.9, 61.4 | 24.9 | 17.0, 32.9 | 0.183 |
| Sugar (g) § | | 36.7 | 30.0, 43.3 | 42.1 | 27.6, 56.6 | 34.6 | 27.0, 42.3 | 0.328 |
| Protein (%TE) § | 15 – 25%TE/day | 24.4§ | 22.9, 25.9§ | 24.2† | 8.58† | 24.5† | 5.57† | 0.839 |
| Total fat (%TE) | 20 – 35%TE/day | 58.1¥ | 49.1 – 66.0¥ | 56.8† | 12.9† | 56.6† | 12.3† | 0.940 |
| PUFA (%TE) | | 7.93§ | 7.30, 8.56§ | 8.15† | 3.29† | 7.85† | 2.49† | 0.820 |

| Variables | Reference range | Total (n=74) | | Men (n=20) | | Women (n=54) | | *P-value** |
|---|---|---|---|---|---|---|---|---|
| | | Mean† G. mean§ Median¥ | SD†, 95% CI§, 25th –75th%¥ | Mean† G. mean§ Median¥ | SD†, 95% CI§, 25th –75th%¥ | Mean† G. mean§ Median¥ | SD†, 95% CI§, 25th –75th%¥ | |
| MUFA (%TE) † | | 23.0 | 5.60 | 21.2 | 5.54 | 22.3 | 5.64 | 0.453 |
| SFA (%TE) † | <8%TE/day | 22.0 | 7.17 | 22.7 | 8.52 | 21.8 | 6.67 | 0.632 |
| Carbohydrate (%TE) § | 45 – 65%TE/day | 14.0 | 11.4, 16.7 | 13.9 | 8.50, 19.4 | 14.1 | 10.9, 17.2 | 0.192 |
| LCHO duration (months) ¥ | | 9.00 | 5.00 -18.0 | 9.00 | 5.00 – 22.0 | 9.00 | 5.00 – 18.0 | 0.961 |

%TE, percentage of total energy; G.mean, geometric mean; Kcal, kilocalorie; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SD, standard deviation, SFA, saturated fatty acids;

*Values of the significance difference between men and women (P<0.05; statistics between groups: parametric variables – independent t-test; non-parametric variables – Mann – Whitney U test). † Variable described as mean. ‡ Variable described as standard deviation. § Variable described as 95th confidence intervals. ¥ Variable described as a median. ₮ Variable described as 25th – 75th percentile.

***Table 3.2.B.*** *Anthropometric and metabolic characteristics of the LOCA study's total participants and the characteristics of men and women.*

| Variables | Reference range | Total (n=74) | | Men (n=20) | | Women (n=54) | | *P-value** |
|---|---|---|---|---|---|---|---|---|
| | | Mean[†] G. mean[§] Median[¥] | SD[†], 95% CI[§], 25th –75th%[¥] | Mean[†] G. mean[§] Median[¥] | SD[†], 95% CI[§], 25th –75th%[¥] | Mean[†] G. mean[§] Median[¥] | SD[†], 95% CI[§], 25th –75th%[¥] | |
| ***Anthropometry*** | | | | | | | | |
| Weight (kg) [†] | | 75.1 | 14.7 | 84.0 | 7.70 | 76.2 | 9.90 | <0.001* |
| Body Fat Percentage (%)[†] | | 27.7 | 9.8 | 18.7 | 7.10 | 31.1 | 8.50 | <0.001* |
| BMI ($kg/m^2$)[§] | 18.5-24.9 $kg/m^2$ | 25.5 | 24.5, 26.5 | 25.7 | 24.3, 27.2 | 25.4 | 24.2, 26.7 | 0.673 |
| Waist circumference (cm) | Men ≤102cm Women ≤88cm | 78.3[§] | 76.0, 80.6[§] | 82.8 | 79.5 – 89.6 | 73.8 | 68.5 – 81.8 | 0.001* |
| Waist: Hip ratio[¥] | Men ≤0.90 Women ≤0.80 | 0.73 | 0.7 – 0.81 | 0.82 | 0.79 – 0.87 | 0.71 | 0.69 – 0.76 | <0.001* |
| ***Metabolic Biomarkers*** | | | | | | | | |
| Systolic Blood pressure (mmHg) [†] | 110-130mmHg | 117 | 12.0 | 124 | 11.00 | 114 | 11.0 | <0.001* |
| Diastolic Blood pressure (mmHg) [†] | 70-80mmHg | 75.0 | 10.0 | 76.0 | 9.00 | 75.0 | 10.0 | 0.589 |
| HbA1c (mmol/mol) [†a] | ≤40mmol/mol | 32.0 | 3.00 | 32.0 | 3.00 | 32.0 | 3.00 | 0.498 |
| Fasting glucose (mmol/L) [†b] | ≤6mmol/L | 5.30 | 0.50 | 5.40 | 0.50 | 5.30 | 0.50 | 0.363 |
| Insulin (pmol/mol) [§b] | 10-80pmol/L | 52.4 | 47.1, 57.7 | 48.0 | 39.0 – 66.0 | 46.0 | 37.0 – 70.0 | 0.887 |
| HOMA-IR [§b] | 0.5-1.4 | 1.14 | 1.05, 1.23 | 1.12 | 0.97, 1.26 | 1.15 | 1.04, 1.26 | 0.730 |

| Variables | Reference range | Total (n=74) | | Men (n=20) | | Women (n=54) | | *P-value** |
|---|---|---|---|---|---|---|---|---|
| | | Mean† G. mean§ Median¥ | SD†, 95% CI§, 25th –75th%¥ | Mean† G. mean§ Median¥ | SD†, 95% CI§, 25th –75th%¥ | Mean† G. mean§ Median¥ | SD†, 95% CI§, 25th –75th%¥ | |
| Ketones (mmol/L) ¥ | <0.6mmol/L | 0.30 | 0.20 – 1.10 | 0.40 | 0.2 – 1.6 | 0.30 | 0.20 – 0.80 | 0.603 |
| Urea (mmol/L) †b | 3.2-7.7mmol/L | 5.20 | 1.30 | 5.70 | 1.50 | 5.00 | 1.20 | 0.048* |
| Creatinine (umol/L) †b | 45-90umol/L | 83.0 | 11.0 | 93.0 | 11.0 | 79.0 | 9.00 | <0.001* |
| eGFR (ml/min/1.73m$^2$) †b | >90ml/min/1.73m$^2$ | 76.1 | 11.2 | 82.9 | 12.3 | 73.5 | 9.59 | 0.001* |
| Total cholesterol (mmol/L) ¥b | <4.0mmol/L | 5.20 | 4.50 – 6.10 | 5.20 | 4.60 – 5.40 | 5.20 | 4.50 – 6.40 | 0.275 |
| HDLC (mmol/L) †b | >1.0mmol/L | 1.69 | 0.46 | 1.47 | 0.29 | 1.77 | 0.49 | 0.027* |
| LDLC (mmol/L) ¥c | <2.0mmol/L | 3.1 | 2.60 – 3.60 | 3.10 | 2.20 – 3.30 | 3.10 | 2.60 – 4.30 | 0.282 |
| Triglycerides (mmol/mol)§b | <1.7mmol/L | 1.02 | 0.82, 1.21 | 1.36 | 0.69, 2.04 | 0.88 | 0.81, 0.95 | 0.151 |
| TC:HDLC ratio§b | <4.0 | 3.46 | 3.22, 3.71 | 3.64 | 3.10, 4.17 | 3.40 | 3.12, 3.68 | 0.245 |

BMI, Body mass index; cm, centimetre; CI, confidence interval; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; G.mean, geometric mean; HbA1c, Glycated haemoglobin; HDL, High density lipoprotein; HOMA-IR, Homeostatic Model Assessment of Insulin Resistance; LDL, low density lipoprotein; SBP, systolic blood pressure; SD, standard deviation, SFA, saturated fatty acids; TC:HDL ratio, Total cholesterol to high density lipoprotein ratio; TG, triglycerides.

a Missing value= 2. b Missing values= 3. c Missing values= 4.

*Values of the significance difference between men and women (P<0.05; statistics between groups: parametric variables – independent t-test; non-parametric variables – Mann – Whitney U test). † Variable described as mean. ‡ Variable described as standard deviation. § Variable described as 95th confidence intervals. ¥ Variable described as a median. ₹ Variable described as 25th – 75th percentile.

Owing to the broad range of carbohydrate intakes within the LOCA population ranging from as low as 6.8g (2.0%TE) per day to as high as 301g (57.2%TE) per day, participants were grouped based on intakes to VLCHO, LCHO and MLCHO. Five participants were not included in those groups due to high intakes of carbohydrates >150g per day. Exploring the different levels of carbohydrate intake revealed that the VLCHO had the lowest energy intake, which was significantly lower than the MLCHO group ($p$=0.001). As carbohydrate intakes increased, energy and fibre intakes increased, while total fat and SFA intakes decreased (Table 3.3A). Protein intakes remained similar within the three groups ($p$=0.808). SFA (%TE) ($p$=0.001 and $p$<0.001) and total fat (%TE) ($p$=0.002 and $p$<0.001) intakes were significantly higher in VLCHO than LCHO and MLCHO intake groups, respectively. Post Hoc tests for intakes of carbohydrates (both grams and %TE), starch and sugars between groups showed significantly lower intakes between the VLCHO and LCHO and MLCHO diet groups ($p$<0.001 for all variables) (Table 3.3A).

There were no apparent differences in weight, BF%, BMI and most biomarkers between the three carbohydrate intake level groups. Total cholesterol (*P=0.048),* and LDLC (*P=0.044)* differed significantly between the three groups. Both TC ($P$=0.011) and LDLC ($P$=0.006) were significantly higher in the VLCHO group compared to the MLCHO group. However, after controlling for age, gender and income using ANCOVA, differences in TC and LDLC were no longer significant. Additionally, ketone concentrations were significantly higher in the VLCHO group compared to the LCHO group ($P$=0.001) (Table 3.3B).

Most participants were of healthy weight in all carbohydrate groups. The VLCHO was the only group to have underweight participants, with only 5% being underweight. Similar percentages of overweight and obese participants were seen in VLCHO (20.5% overweight and 20.5% obese) and MLCHO (20% overweight and 20% obese) groups. The LCHO group, however, had the highest number of overweight participants (40%) and the lowest number of obese participants (10%) (Figure 3.2 A). Although the majority of all participants had insulin concentrations within normal parameters, some participants in the VLCHO (18.4%) and LCHO (5.3%) groups had elevated insulin concentrations (Figure 3.2. B). The VLCHO group had the most abnormal urea levels compared to the remaining carbohydrate groups, where 2.6% of participants had low urea concentrations, and 5.1% had elevated urea concentrations (Figure 3.2 C). Many participants in the LCHO group had elevated creatinine concentrations (36.8%) followed by the MLCHO (33.3%) and the VLCHO (13.2%) groups (Figure 3.2. D). Interestingly, the majority of

participants in the VLCHO (89.5%), LCHO (89.5%) and MLCHO (88.9%) groups had reduced eGFR levels (Figure 3.2. E). Blood lipid profile analysis revealed that the majority of the participants had elevated TC and LDLC concentrations across all three carbohydrate intake groups. However, the LCHO group had the highest percentage of normocholesterolemic participants (10.5%), followed by 10.1% in the MLCHO group and 5.3% in the VLCHO group. The MLCHO group had most participants with normal LDLC concentrations of 10.1%, followed by VLCHO group with only 5.4%. Interestingly, none of the participants in the LCHO group had normal LDLC concentrations (Figure 3.2. F and G). Furthermore, the majority of the participants had high, but within the normal range, HDLC concentrations across all groups (Figure 3.2 H). About a third of participants (34.2%) in the VLCHO group had elevated TC:HDLC concentrations, followed by 15.8% in the LCHO group, and 11.1% in the MLCHO group (Figure 3.2 J).

***Table 3.3.A.*** *Dietary intake of the LOCA study participants by carbohydrate intake levels.*

| Variables | Reference range | Carbohydrate Intake | | | | | | *P* – value** |
|---|---|---|---|---|---|---|---|---|
| | | Very low carbohydrate intake (<50g/day) (n=39) | | Low carbohydrate intake (50 – 100g/day) (n=20) | | Moderately low carbohydrate intake (>100 – <150g/day) (n=10) | | |
| | | Mean* | 95$^{th}$ CI | Mean* | 95$^{th}$ CI | Mean* | 95$^{th}$ CI | |
| ***Dietary intake*** | | | | | | | | |
| Energy (kcal) | | 1578 [d] | 1427, 1730 | 1876 | 1663, 2088 | 2182 [d] | 1884, 2480 | 0.001** *r*=0.38 |
| Protein (g) | | 96.6 [d] | 88.1, 106 | 108 | 95.6, 120 | 137 [d] | 119, 154 | <0.001** *r*=0.40 |
| Total fat (g) | | 115 | 101, 128 | 120 | 100, 139 | 118 | 90.1, 146 | 0.909 |
| Dietary Cholesterol (mg) | <300mg/day | 562 | 462, 662 | 498 | 358, 638 | 689 | 492, 886 | 0.294 |
| SFA (g) | | 47.0 | 40.7, 53.3 | 43.6 | 34.8, 52.5 | 42.1 | 29.7, 54.6 | 0.718 |
| PUFA (g) | | 14.2 | 11.7, 16.7 | 18.1 | 14.6, 21.7 | 16.6 | 11.7, 21.6 | 0.189 |
| MUFA (g) | | 82.8 | 37.0, 48.7 | 46.9 | 38.7, 55.1 | 47.4 | 35.9, 58.9 | 0.640 |
| Carbohydrate (g) | | 27.3 [d] | 22.8, 31.8 | 69.4 [d] | 63.1, 75.7 | 125[d] | 116, 134 | <0.001** *r*=0.91 |
| Starch (g) | | 8.33 [d] | 4.22, 12.4 | 30.0 [d] | 24.3, 35.8 | 55.2 [d] | 47.1, 63.3 | <0.001** *r*=0.79 |
| Sugar (g) | | 18.3 [d] | 14.8, 21.7 | 38.3 [d] | 33.4, 43.1 | 68.7 [d] | 61.9, 75.5 | <0.001** *r*=0.83 |

| Variables | Reference range | Carbohydrate Intake | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Very low carbohydrate intake (<50g/day) (n=39) | | Low carbohydrate intake (50 – 100g/day) (n=20) | | Moderately low carbohydrate intake (>100 – <150g/day) (n=10) | | *P* – value** |
| | | Mean* | 95th CI | Mean* | 95th CI | Mean* | 95th CI | |
| Fibre (g) | 25 – 30g/day | 13.2 [d] | 10.1, 16.3 | 20.8 | 16.4, 25.1 | 24.6[d] | 18.5, 30.7 | 0.001** *r*=0.43 |
| Carbohydrate (%TE) | 45 – 65%TE/day | 6.85 [d] | 5.40, 8.30 | 15.3 [d] | 13.3, 17.4 | 24.7 [d] | 21.8, 27.5 | <0.001** *r*=0.79 |
| Protein (%TE) | 15 – 25%TE/day | 25.4 | 23.4, 27.3 | 24.4 | 21.7, 27.2 | 25.8 | 21.9, 29.7 | 0.808 |
| Total fat (%TE) | 25 – 35%TE/day | 63.36 [d, e] | 60.4, 66.3 | 54.3 [e] | 50.2, 58.4 | 44.8 [d] | 39.0, 50.6 | <0.001** *r*=0.59 |
| Saturated fat (%TE) | <8%TE/day | 25.7 [d, e] | 23.9, 27.6 | 19.7 [e] | 17.1, 22.3 | 16.0[d] | 12.3, 19.6 | <0.001** *r*=0.54 |
| PUFA (%TE) | | 8.11 | 7.31, 8.92 | 8.46 | 7.33, 9.59 | 6.36 | 4.77, 7.95 | 0.090 |
| MUFA (%TE) | | 24.3[d] | 22.7, 25.8 | 21.6 | 19.5, 23.8 | 18.4[d] | 15.3, 21.4 | <0.003** r= 0.41 |
| Duration of following LCHO (months)†*** | | 9 | 5 - 18 | 10 | 5 - 25 | 11 | 4 - 37 | 0.672 |

%TE, percentage of total energy; cm, centimetre; CI, confidence interval; Kcal, kilocalorie; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids.

a Missing values= 2. b Missing values= 3. c Missing values= 4. d-e values with similar letters indicate values are significantly different

*Mean adjusted for age, gender, and income - determined using ANCOVA.

¥ Variable described as a median and 25th-75th percentile.

| Variables | Reference range | Carbohydrate Intake | | | | | | *P* – value** |
|---|---|---|---|---|---|---|---|---|
| | | Very low carbohydrate intake (<50g/day) (n=39) | | Low carbohydrate intake (50 – 100g/day) (n=20) | | Moderately low carbohydrate intake (>100 – <150g/day) (n=10) | | |
| | | Mean* | 95$^{th}$ CI | Mean* | 95$^{th}$ CI | Mean* | 95$^{th}$ CI | |

** Values of the significant difference between carbohydrate intake groups: very low CHO, low CHO, and moderate carbohydrate intake groups (P<0.05; controlling for covariates (age, gender and income) – ANCOVA).

*** Variables were not controlled for age, gender or income.

***Table 3.3.B.*** *Anthropometric measurements and metabolic biomarker levels of the LOCA study participants by carbohydrate intake levels.*

| Variables | Reference range | Carbohydrate Intake | | | | | | *P* – value** |
|---|---|---|---|---|---|---|---|---|
| | | Very low carbohydrate intake (<50g/day) (n=39) | | Low carbohydrate intake (50 – 100g/day) (n=20) | | Moderately low carbohydrate intake (>100 – <150g/day) (n=10) | | |
| | | Mean* | 95$^{th}$ CI | Mean* | 95$^{th}$ CI | Mean* | 95$^{th}$ CI | |
| ***Anthropometry*** | | | | | | | | |
| Weight (kg) | | 74.0 | 70.6, 79.5 | 74.7 | 68.5, 80.9 | 79.1 | 67.3, 84.8 | 0.969 |
| Body Fat percentage (%) | | 28.6 | 26.0, 31.3 | 27.6 | 23.9, 31.4 | 25.6 | 20.3, 30.8 | 0.582 |
| BMI (kg/m$^2$) | | 25.7 | 24.3, 27.1 | 25.2 | 23.3, 27.2 | 25.9 | 23.1, 28.6 | 0.899 |
| Waist Circumference (cm) | | 78.6 | 75.5, 81.8 | 77.2 | 72.7, 81.6 | 79.8 | 73.5, 86.0 | 0.774 |
| ***Biochemistry*** | | | | | | | | |
| SBP (mmHg) | 110-130mmHg | 118 | 114, 122 | 116 | 111, 121 | 110 | 103, 118 | 0.171 |
| DBP (mmHg) | 70-80mmHg | 76.8 | 73.6, 79.9 | 74.2 | 69.8, 78.6 | 69.8 | 63.6, 76.0 | 0.132 |
| HbA1c (mmol/mol)[a] | ≤40mmol/mol | 31.8 | 30.8, 32.8 | 32.1 | 30.6, 33.6 | 31.6 | 23.6, 33.7 | 0.915 |
| Glucose (mmol/L)[b] | ≤6mmol/L | 5.36 | 5.21, 5.50 | 5.43 | 5.23, 5.64 | 5.02 | 4.73, 5.32 | 0.068 |
| Insulin (pmol/L)[b] | 10-80pmol/L | 52.3 | 45.0, 59.6 | 52.7 | 42.3, 63.1 | 40.8 | 25.8, 55.8 | 0.356 |
| HOMA-IR [b] | 0.5-1.4 | 2.12 | 1.79, 2.44 | 2.14 | 1.68, 2.60 | 1.51 | 0.838, 2.17 | 0.234 |

| Variables | Reference range | Carbohydrate Intake | | | | | | *P* – value** |
|---|---|---|---|---|---|---|---|---|
| | | Very low carbohydrate intake (<50g/day) (n=39) | | Low carbohydrate intake (50 – 100g/day) (n=20) | | Moderately low carbohydrate intake (>100 – <150g/day) (n=10) | | |
| | | Mean* | 95th CI | Mean* | 95th CI | Mean* | 95th CI | |
| Urea (mmol/L)[b] | 3.2-7.7mmol/L | 5.31 | 4.92, 5.70 | 5.31 | 4.76, 5.87 | 5.52 | 4.72, 6.32 | 0.893 |
| eGFR (mL/min/1.73 $m^2$) [b] | >90ml/min/1.73$m^2$ | 77.0 | 73.8, 80.2 | 74.1 | 69.6, 78.7 | 72.8 | 66.2, 79.3 | 0.399 |
| Creatinine (umol/L) [b] | 45-90umol/L | 81.6 | 78.5, 84.7 | 84.5 | 80.1, 88.9 | 85.1 | 78.8, 91.5 | 0.430 |
| Total cholesterol (mmol/L)[b] | <4.0mmol/L | 6.04 | 5.45, 6.62 | 5.61 | 4.78, 6.45 | 4.77 | 3.57, 5.80 | 0.171 |
| *Total cholesterol (mmol/L) ¥ *** [b]* | | *5.4* [d] | *5.0 - 6.4* | *5.3* | *4.4 – 6.4* | *5.0* [d] | *4.7 – 5.1* | *0.048*** |
| Triglycerides (mmol/L)[b] | <1.7mmol/L | 1.19 | 0.925, 1.45 | 0.841 | 0.470, 1.21 | 0.724 | 0.188, 1.26 | 0.168 |
| LDLC (mmol/L) [c] | <2.0mmol/L | 3.78[d] | 3.26, 4.30 | 3.55 | 2.82, 4.28 | 2.72 [d] | 1.67, 3.78 | 0.210 |
| *LDLC (mmol/L) ¥*** [c]* | | *3.30* | *2.90 - 4.30* | *3.3* | *2.5 – 4.3* | *2.7* | *2.7 – 2.9* | *0.044*** |
| HDLC (mmol/L)[b] | >1.0mmol/L | 1.70 | 1.55, 1.84 | 1.69 | 1.48, 1.90 | 1.70 | 1.40, 2.01 | 0.997 |
| TC:HDLC ratio[c] | <4.0 | 3.70 | 3.37, 4.03 | 3.39 | 2.92, 3.86 | 2.90 | 2.20, 3.56 | 0.092 |
| Ketones (mmol/L)[b] | <0.6mmol/L | 0.976 [d] | 0.744, 1.21 | 0.210 [d] | -0.120, 0.540 | 0.674 | 0.224, 1.12 | 0.002** |

BMI, Body mass index; cm, centimetre; CI, confidence interval; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; HbA1c, Glycated haemoglobin; HDL, High density lipoprotein; HOMA-IR, Homeostatic Model Assessment of Insulin Resistance; LDL, low density lipoprotein; SBP, systolic blood pressure; TC:HDL ratio, Total cholesterol to high density lipoprotein ratio; TG, triglycerides.

a Missing values= 2. b Missing values= 3. c Missing values= 4. d-e values with similar letters indicate values are significantly different

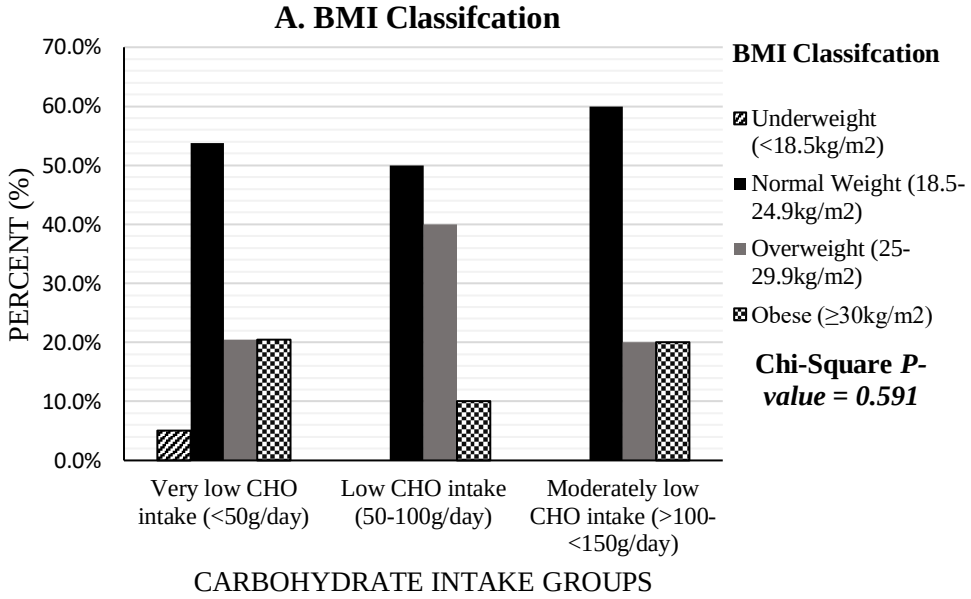
*Mean adjusted for age, gender, and income - determined using ANCOVA.

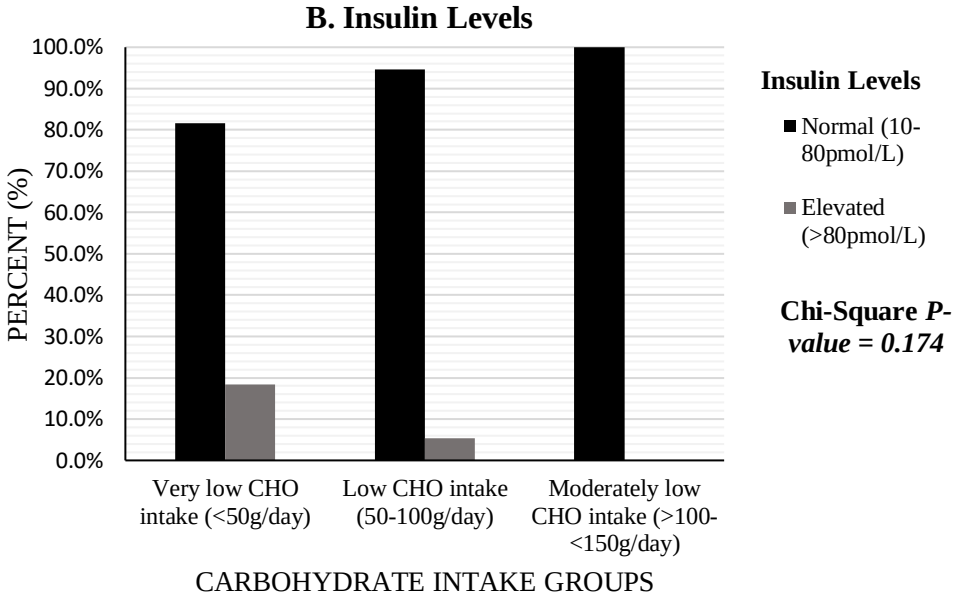
| Variables | Reference range | Carbohydrate Intake | | | | | | *P* – value** |
|---|---|---|---|---|---|---|---|---|
| | | Very low carbohydrate intake (<50g/day) (n=39) | | Low carbohydrate intake (50 – 100g/day) (n=20) | | Moderately low carbohydrate intake (>100 – <150g/day) (n=10) | | |
| | | Mean* | 95$^{th}$ CI | Mean* | 95$^{th}$ CI | Mean* | 95$^{th}$ CI | |

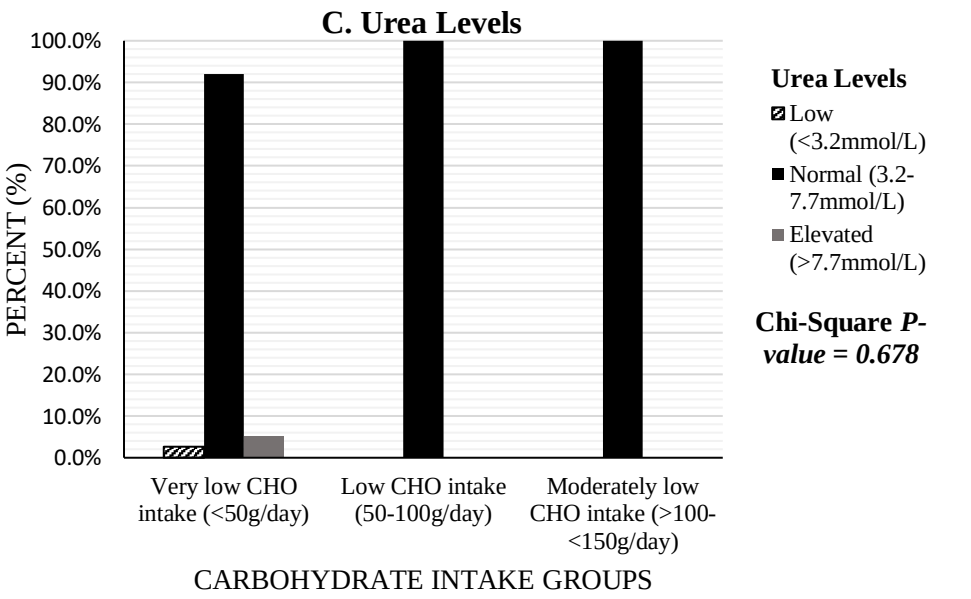
¥ Variable described as a median and 25th-75th percentile.

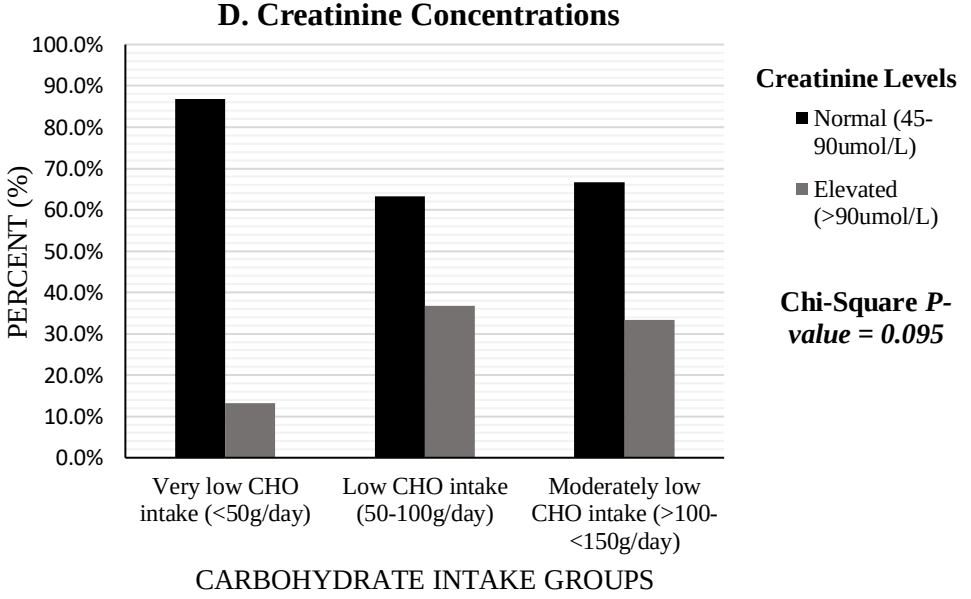
** Values of the significant difference between carbohydrate intake groups: very low CHO, low CHO, and moderate carbohydrate intake groups (P<0.05; controlling for covariates (age, gender and income) – ANCOVA).

*** Variables were not controlled for age, gender or income.

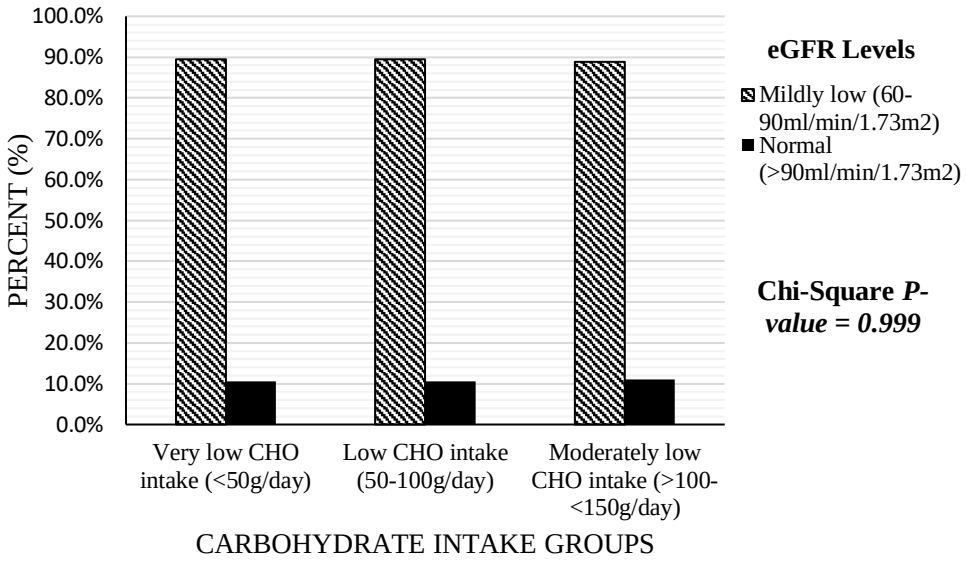

A. BMI Classifcation
70.0%
60.0%
50.0%
40.0%
30.0%
20.0%
10.0%
0.0%
PERCENT (%)
Very low CHO intake (<50g/day)
Low CHO intake (50-100g/day)
Moderately low CHO intake (>100-<150g/day)
CARBOHYDRATE INTAKE GROUPS
BMI Classifcation
Underweight (<18.5kg/m2)
Normal Weight (18.5-24.9kg/m2)
Overweight (25-29.9kg/m2)
Obese (≥30kg/m2)
Chi-Square P-value = 0.591


B. Insulin Levels
100.0%
90.0%
80.0%
70.0%
60.0%
50.0%
40.0%
30.0%
20.0%
10.0%
0.0%
PERCENT (%)
Very low CHO intake (<50g/day)
Low CHO intake (50-100g/day)
Moderately low CHO intake (>100-<150g/day)
CARBOHYDRATE INTAKE GROUPS
Insulin Levels
Normal (10-80pmol/L)
Elevated (>80pmol/L)
Chi-Square P-value = 0.174

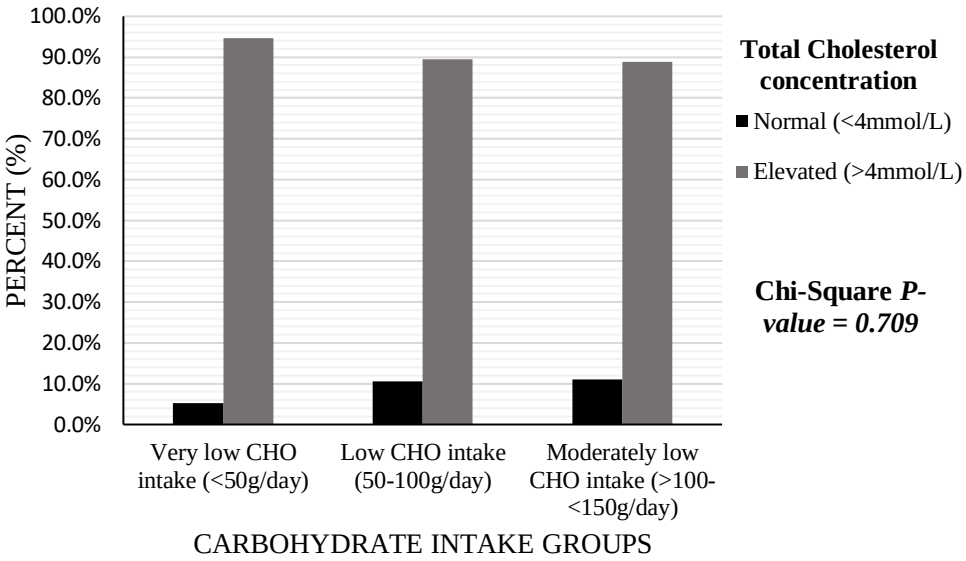

C. Urea Levels
100.0%
90.0%
80.0%
70.0%
60.0%
50.0%
40.0%
30.0%
20.0%
10.0%
0.0%
PERCENT (%)
Very low CHO intake (<50g/day)
Low CHO intake (50-100g/day)
Moderately low CHO intake (>100-<150g/day)
CARBOHYDRATE INTAKE GROUPS
Urea Levels
Low (<3.2mmol/L)
Normal (3.2-7.7mmol/L)
Elevated (>7.7mmol/L)
Chi-Square P-value = 0.678


D. Creatinine Concentrations
100.0%
90.0%
80.0%
70.0%
60.0%
50.0%
40.0%
30.0%
20.0%
10.0%
0.0%
PERCENT (%)
Very low CHO intake (<50g/day)
Low CHO intake (50-100g/day)
Moderately low CHO intake (>100-<150g/day)
CARBOHYDRATE INTAKE GROUPS
Creatinine Levels
Normal (45-90umol/L)
Elevated (>90umol/L)
Chi-Square P-value = 0.095

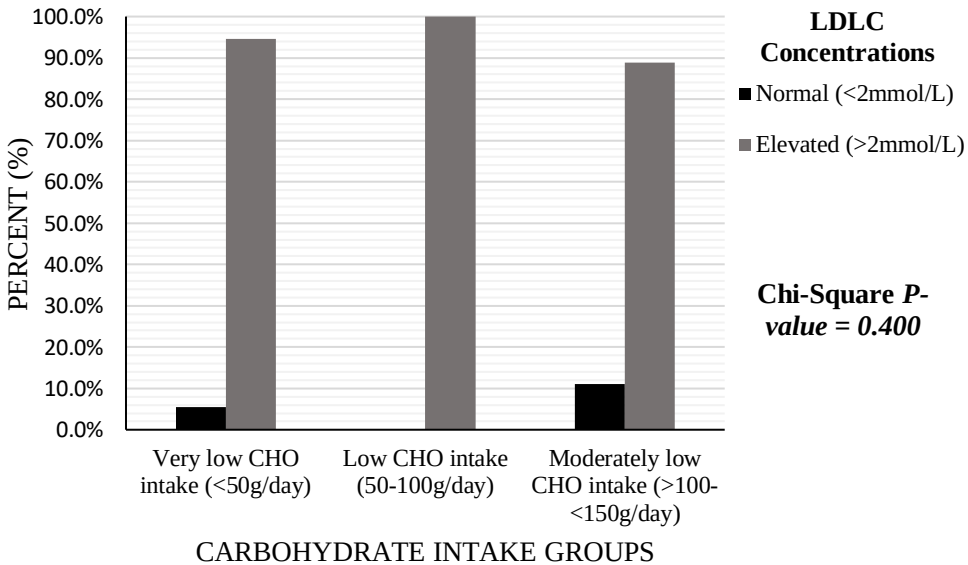
**E. Estimated Glomerular Filtration Rate**



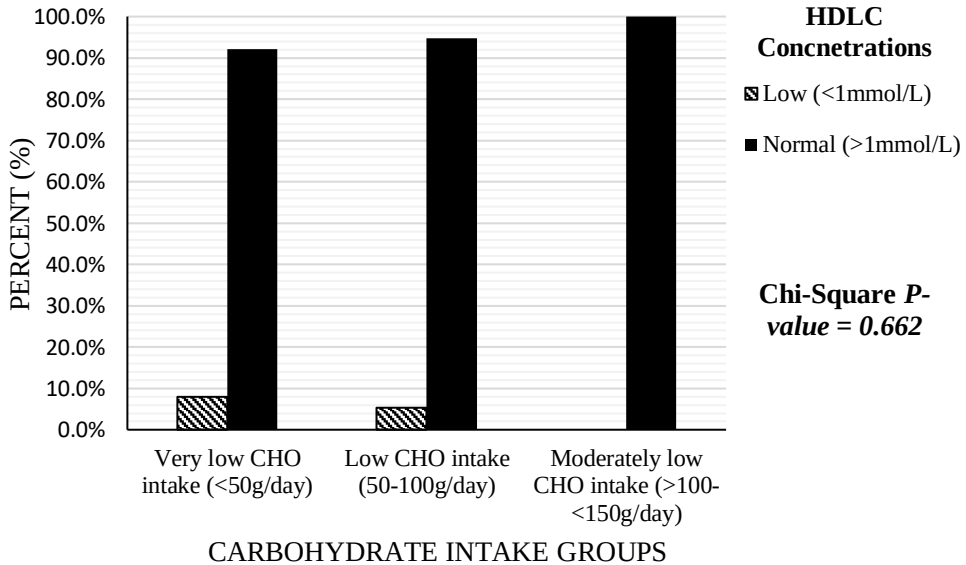

**F. Total Cholesterol Concentration**




**G. LDLC Levels**




**H. HDLC Concentration**



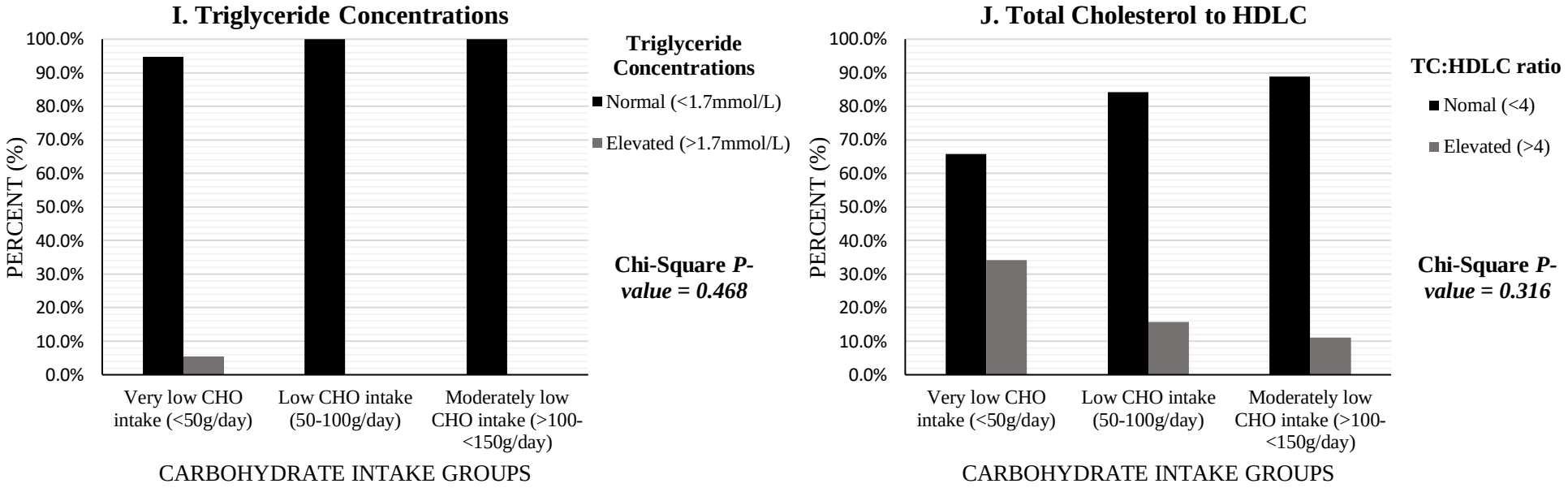



***Figure 3.2*** *The contribution (percentage) of participants for the ranges of biomarker levels within each of the three carbohydrate intake groups.*

**Table 3.4**. Correlations of anthropometry and biomarkers with carbohydrate intake (in grams and as a percentage of total energy intake) and duration of following LCHO diet.

| Variables | CHO intake (g) | | | CHO intake (%TE) | | | CHO (%TE)** | | | LCHO duration (months) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | r | *P* | N | r | *P* | N | r | *P* | N | r | *P* | N |
| ***Anthropometry*** | | | | | | | | | | | | |
| Weight (kg) | 0.052 | 0.666 | 74 | 0.017 | 0.891 | 74 | 0.021 | 0.865 | 74 | -0.175 | 0.145 | 74 |
| Body fat (%) | -0.105 | 0.385 | 74 | 0.025 | 0.836 | 74 | 0.027 | 0.823 | 74 | -0.067 | 0.582 | 74 |
| BMI (kg/m2) | -0.079 | 0.515 | 74 | -0.073 | 0.546 | 74 | -0.069 | 0.569 | 74 | -0.256 | 0.031* | 74 |
| ***Biomarkers*** | | | | | | | | | | | | |
| SBP (mmHg) | -0.092 | 0.456 | 74 | -0.084 | 0.498 | 74 | -0.086 | 0.491 | 74 | 0.118 | 0.325 | 74 |
| DBP (mmHg) | -0.090 | 0.465 | 74 | -0.077 | 0.531 | 74 | -0.072 | 0.564 | 74 | 0.005 | 0.968 | 74 |
| HbA1c (mmol/mol) | 0.156 | 0.204 | 72 | 0.132 | 0.283 | 72 | 0.123 | 0.324 | 72 | 0.021 | 0.866 | 72 |
| Glucose (mmol/L) | -0.086 | 0.483 | 71 | -0.040 | 0.743 | 71 | -0.059 | 0.639 | 71 | 0.207 | 0.090 | 71 |
| Insulin (pmol/L) | 0.123 | 0.319 | 71 | 0.180 | 0.142 | 71 | 0.178 | 0.153 | 71 | 0.010 | 0.933 | 71 |
| HOMA-IR | 0.081 | 0.510 | 71 | 0.152 | 0.215 | 71 | 0.150 | 0.231 | 71 | 0.035 | 0.779 | 71 |

| Variables | CHO intake (g) | | | CHO intake (%TE) | | | CHO (%TE)** | | | LCHO duration (months) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | r | *P* | N | r | *P* | N | r | *P* | N | r | *P* | N |
| Urea (mmol/L) | -0.171 | 0.163 | 71 | -0.152 | 0.216 | 71 | -0.153 | 0.221 | 71 | 0.030 | 0.809 | 71 |
| Creatinine (umol/L) | 0.046 | 0.709 | 71 | 0.067 | 0.589 | 71 | 0.081 | 0.520 | 71 | 0.102 | 0.409 | 71 |
| eGFR (mL/min/1.73m$^2$) | -0.055 | 0.656 | 71 | -0.089 | 0.472 | 71 | -0.100 | 0.422 | 71 | -0.086 | 0.487 | 71 |
| TC (mmol/L) | -0.353 | 0.003* | 71 | -0.403 | 0.001* | 71 | -0.419 | <0.001* | 71 | 0.092 | 0.455 | 71 |
| HDLC (mmol/L) | -0.058 | 0.639 | 71 | -0.141 | 0.257 | 71 | -0.141 | 0.258 | 71 | 0.148 | 0.230 | 71 |
| TG (mmol/L) | -0.235 | 0.055 | 71 | -0.174 | 0.158 | 71 | -0.172 | 0.168 | 71 | -0.017 | 0.888 | 71 |
| LDCL (mmol/L) | -0.329 | 0.007* | 70 | -0.335 | 0.006* | 70 | -0.344 | 0.005* | 70 | 0.044 | 0.722 | 70 |
| TC:HDLC ratio | -0.207 | 0.092 | 70 | -0.129 | 0.299 | 70 | -0.127 | 0.311 | 70 | -0.086 | 0.488 | 70 |
| Ketones (mmol/L) | -0.404 | 0.001* | 72 | -0.421 | <0.001* | 72 | -0.420 | <0.001* | 72 | -0.32 | 0.792 | 72 |

BMI, Body mass index; HbA1c, Glycated haemoglobin; HDL, High density lipoprotein; LDL, low density lipoprotein; TG, triglycerides; TC:HDL ratio, Total cholesterol to high density lipoprotein ratio.

All correlations were controlled for age, gender and income using partial parametric and non-parametric correlations

*Statistically significant correlations (*P*-value <0.05)

** Variable controlled for age, gender, income and duration of following an LCHO diet

Carbohydrate intake in grams and as a %TE correlated negatively with total cholesterol (r= -0.353, P-value= 0.003 and (r= -0.403, P-value= 0.001, respectively,), LDLC (r= -0.329, P-value= 0.007, and r= -0.335, P-value= 0.006, respectively) and ketones (r= -0.404, P-value= 0.001 and r= -0.421, P-value= 0.001, respectively). These correlations stayed significant after adjusting for age, gender, duration and income (Table 3.4). Furthermore, the duration of following LCHO diet correlated negatively with BMI (r= -0.256, P-value= 0.031) (Table 3.4).

Carbohydrate intake (%TE), gender, age, income and length of LCHO diet accounted for 16.4% of the variance for total cholesterol (model 1, Table 3.5), where for every 1% decrease in carbohydrate intake, TC increased by 0.041mmol/L. For similar models, however, replacing carbohydrates (%TE) with fat (%TE) and SFA (%TE) accounted for 19% and 17.9% of the variance of TC concentrations, respectively (models 3 and 4, Table 3.5). For every 1% increase in total fat and SFA intakes, TC increased by 0.045mmol/L and 0.073mmol/L, respectively. Furthermore, carbohydrate intakes, total fat and SFA intakes also accounted for LDLC concentrations (Table 3.5). For every 1% increase in carbohydrate intake, LDLC decreases by 0.032mmol/L. Additionally, for every 1% increase in total fat and SFA resulted in 0.034 and 0.050mmol/L increase in LDLC concentrations.

Furthermore, a linear regression analysis has confirmed that the duration of following an LCHO diet as a significant predictor of BMI, rather than the macronutrient intakes of carbohydrates, protein, fats and SFA (Appendix B1). Additionally, the duration of LCHO diet and fibre intake (%TE) (model 5) accounted for 16.5% of BMI, where for every gram increase in fibre intake, BMI decreased by 0.07kg/$m^2$. Age and gender were the main predictors of eGFR levels. Macronutrients and duration of LCHO diet do not significantly affect eGFR levels (Appendix B2).

***Table 3.5.*** *Linear regression for macronutrient intakes (%TE) correlated to total cholesterol.*

| | ***Models for Total Cholesterol*** | B | Std Error | Std'ised β | P-value | 95% CI |
|---|---|---|---|---|---|---|
| 1 | (Constant) | 4.169 | 1.280 | | 0.002 | 1.612, 6.726 |
| | Age | 0.046 | 0.029 | 0.190 | 0.116 | -0.012, 0.105 |
| | Gender | 0.399 | 0.465 | 0.099 | 0.394 | -0.529, 1.326 |
| | Income | -0.048 | 0.061 | -0.092 | 0.434 | -0.169, 0.074 |
| | LCHO diet duration | -0.004 | 0.012 | -0.040 | 0.731 | -0.028, 0.020 |
| | Carbohydrate (%TE) | -0.041 | 0.019 | -0.264 | 0.030 | -0.079, -0.004 |
| | | | F(5, 70)= 2.551, $R^2$ =0.164, *P*-value= 0.036 | | | |
| 2 | (Constant) | 3.475 | 1.517 | | 0.025 | 0.445, 6.506 |
| | Age | 0.063 | 0.029 | 0.256 | 0.036 | 0.004, 0.121 |
| | Gender | 0.350 | 0.481 | 0.087 | 0.470 | -0.611, 1.310 |
| | Income | -0.081 | 0.063 | -0.156 | 0.203 | -0.207, 0.045 |
| | LCHO diet duration | -0.005 | 0.013 | -0.044 | 0.712 | -0.030, 0.020 |
| | Protein (%TE) | -0.010 | 0.034 | -0.034 | 0.777 | -0.077, 0.057 |
| | | | F (5, 70)= 1.480, $R^2$ =0.102, *P*-value= 0.209 | | | |
| 3 | (Constant) | 0.999 | 1.453 | | 0.494 | -1.903, 3.901 |
| | Age | 0.046 | 0.028 | 0.188 | 0.112 | -0.011, 0.103 |
| | Gender | 0.415 | 0.457 | 0.103 | 0.368 | -0.499, 1.328 |
| | Income | -0.053 | 0.059 | -0.102 | 0.372 | -0.171, 0.065 |
| | LCHO diet duration | -0.002 | 0.012 | -0.019 | 0.866 | -0.026, 0.022 |
| | Fat (%TE) | 0.045 | 0.017 | 0.310 | 0.009 | 0.011, 0.079 |
| | | | F(5, 70)= 3.054, $R^2$ =0.190, *P*-value= 0.015 | | | |
| 4 | (Constant) | 1.759 | 1.337 | | 0.193 | -0.912, 4.429 |
| | Age (years) | 0.047 | 0.029 | 0.194 | 0.103 | -0.010, 0.105 |
| | Gender | 0.473 | 0.463 | 0.118 | 0.310 | -0.451, 1.397 |
| | Income | -0.048 | 0.060 | -0.093 | 0.425 | -0.168, 0.072 |
| | LCHO diet duration | -0.001 | 0.012 | -0.005 | 0.966 | -0.025, 0.024 |
| | SFA (%TE) | 0.073 | 0.030 | 0.292 | 0.016 | 0.014, 0.132 |
| | | | F(5, 70)= 2.827, $R^2$ =0.179, *P*-value= 0.023 | | | |
| 5 | (Constant) | 4.824 | 1.639 | | 0.005 | 1.550, 8.098 |
| | Age (years) | 0.061 | 0.029 | 0.251 | 0.037 | 0.004, 0.119 |
| | Gender | 0.336 | 0.473 | 0.084 | 0.480 | -0.609, 1.282 |
| | Income | -0.061 | 0.062 | -0.117 | 0.329 | -0.184, 0.062 |
| | LCHO diet duration | -0.004 | 0.012 | -0.037 | 0.755 | -0.028, 0.021 |
| | Fibre (g) | -0.566 | 0.384 | -0.174 | 0.146 | -1.334, 0.201 |
| | | | F(5, 70)= 1.945, $R^2$ =0.130, *P*-value= 0.099 | | | |

***Table 3.6.*** *Linear regression for macronutrient intakes (%TE) correlated to LDLC.*

| | ***Models for LDLC*** | B | Std Error | Std'ised β | P-value | 95% CI |
|---|---|---|---|---|---|---|
| 1 | (Constant) | 2.200 | 1.128 | | 0.056 | -0.054, 4.454 |
| | Age | 0.037 | 0.026 | 0.175 | 0.158 | -0.015, 0.089 |
| | Gender | 0.382 | 0.418 | 0.109 | 0.364 | -0.453, 1.218 |
| | Income | -0.039 | 0.054 | -0.087 | 0.471 | -0.146, 0.068 |
| | LCHO diet duration | -0.004 | 0.011 | -0.047 | 0.688 | -0.025, 0.017 |
| | Carbohydrate (%TE) | -0.032 | 0.017 | -0.233 | 0.060 | -0.065, 0.001 |
| | | | F(5, 69)= 2.083, $R^2$ =0.140, *P*-value= 0.079 | | | |
| 2 | (Constant) | 1.415 | 1.324 | | 0.289 | -1.230, 4.059 |
| | Age | 0.049 | 0.026 | 0.231 | 0.063 | -0.003, 0.101 |
| | Gender | 0.358 | 0.430 | 0.102 | 0.408 | -0.500, 1.216 |
| | Income | -0.060 | 0.055 | -0.134 | 0.282 | -0.170, 0.050 |
| | LCHO diet duration | -0.005 | 0.011 | -0.057 | 0.642 | -0.027, 0.017 |
| | Protein (%TE) | 0.003 | 0.029 | 0.011 | 0.932 | -0.056, 0.061 |
| | | | F (5, 69)= 1.278, $R^2$ =0.091, *P*-value= 0.284 | | | |
| 3 | (Constant) | -0.210 | 1.283 | | 0.871 | -2.772, 2.353 |
| | Age | 0.037 | 0.025 | 0.173 | 0.154 | -0.014, 0.087 |
| | Gender | 0.394 | 0.413 | 0.112 | 0.344 | -0.432, 1.219 |
| | Income | -0.043 | 0.052 | -0.096 | 0.415 | -0.147, 0.062 |
| | LCHO diet duration | -0.003 | 0.010 | -0.029 | 0.802 | -0.024, 0.018 |
| | Fat (%TE) | 0.034 | 0.015 | 0.272 | 0.025 | 0.004, 0.064 |
| | | | F(5, 69)= 2.437, $R^2$ =0.160, *P*-value= 0.044 | | | |
| 4 | (Constant) | 0.480 | 1.187 | | 0.687 | -1.891, 2.850 |
| | Age (years) | 0.039 | 0.026 | 0.186 | 0.129 | -0.012, 0.091 |
| | Gender | 0.418 | 0.419 | 0.119 | 0.322 | -0.419, 1.256 |
| | Income | -0.042 | 0.053 | -0.093 | 0.438 | -0.148, 0.065 |
| | LCHO diet duration | -0.002 | 0.011 | 0-.020 | 0.866 | -0.023, 0.020 |
| | SFA (%TE) | 0.050 | 0.026 | 0.230 | 0.062 | -0.003, 0.103 |
| | | | F(5, 69)= 2.071, $R^2$ =0.139, P-value= 0.081 | | | |
| 5 | (Constant) | 3.077 | 1.427 | | 0.035 | 0.226, 5.929 |
| | Age (years) | 0.048 | 0.025 | 0.227 | 0.062 | -0.002, 0.099 |
| | Gender | 0.331 | 0.521 | 0.094 | 0.435 | -0.510, 1.171 |
| | Income | -0.045 | 0.053 | -0.100 | 0.407 | -0.151, 0.062 |
| | LCHO diet duration | -0.004 | 0.011 | -0.041 | 0.728 | -0.025, 0.018 |
| | Fibre (g) | -0.566 | 0.334 | -0.201 | 0.095 | -1.234, 0.102 |
| | | | F(5, 69)= 1.907, $R^2$ =0.130, P-value= 0.105 | | | |

## 3.4 Discussion

The dietary intake of the LOCA study population has shown substantial differences to the intakes observed in the wider NZ population, as demonstrated in the 2008/09 NNS. Participants in this study consumed a substantially greater %TE of fat (58.1%), SFA (22%) and protein (42.4%) compared with the 2008/09 NNS reported intakes of 33.7%, 13.1% and 16.5%, respectively (Ministry of Health, 2011). (Ministry of Health, 2011). However, fibre intakes were lower in this study compared with the 2008 NNS (18.5g versus 20.3g/day, respectively) (Ministry of Health, 2011).

The self-directed low carbohydrate (LCHO) diet followers participating in the LOCA study demonstrated a variation in carbohydrate intakes. Carbohydrate intakes ranged from as low as 6.8g (2.0%TE) per day to as high as 301g (57.2%TE) per day. Due to such variations, participants were grouped based on their carbohydrate intake; however, five participants who identified themselves as LCHO diet followers and consumed more than 150g of carbohydrates per day, which is not considered as low intake, and they were excluded from the carbohydrate intake group analyses. Studies have shown that spontaneous decline in carbohydrate intakes is accompanied by increased fat intake while maintaining a similar protein intake (Makarem et al., 2014; Vadiveloo et al., 2014, Zhao et al., 2018). For example, Zhao et al. (2018) found that fat intake increased from 24.8-35.6%TE, and carbohydrate decreased 62.8-51.6%TE, while protein intake, did not change, among Chinese females aged 18-49 years. Although this sub-study of the LOCA study did not examine the food choices of individuals, Jallinoja et al. (2014) have found that LCHO diet followers in the Finnish population consumed carbohydrate-containing foods less frequently than non-LCHO diet followers.

LCHO diet followers are known to actively avoid carbohydrates, sugars and refined grain products and replace those foods with meat and animal fats. This observation is consistent with that of the LOCA study, where carbohydrates were replaced by fat and protein. Furthermore, protein intakes were similar within the carbohydrate intake groups; however, fat intakes varied. This suggests that fat is the predominant replacement macronutrient to carbohydrates, a finding consistent with the observed spontaneous decline in carbohydrates. The lack of consistency in using the term ‘low carbohydrate diet’ provides a large room for self-interpretation of levels of carbohydrate restriction among the general public. Despite the level of restriction, our study has demonstrated that the majority of self-directed LCHO diet followers experienced some abnormal

biomarker concentrations. This discussion examines the causes and implications of abnormal biomarker levels seen among LCHO diet followers.

### *3.4.1 Carbohydrate Restriction and Cardiovascular Risk Factors.*

In the LOCA population, carbohydrate restriction was accompanied by high total fat and SFA intakes, and in turn with hypercholesterolaemia among the majority (91%) of the participants. This is consistent with reported evidence that increased dietary SFA intakes results in increased TC, HDLC and LDLC concentrations (German & Dillard, 2004; Noakes et al., 2006). Similarly, Noakes et al. (2006) have shown that LCHO-ketogenic diets with LCHO intake ≤50g per day and high fat intakes (61%TE) led to greater increases in TC and LDLC concentrations compared to LF diets over 12 months; a finding consistent with the meta-analysis by Bueno et al. (2013). LCHO diet followers in this study had high intakes of total fat and particularly SFA which may have resulted in high LDLC concentrations as excess SFA intakes inhibit LDLC receptor activity and increase apolipoprotein B (apo B) production. This, therefore, results in high LDLC levels due to a decline in LDL-receptor mediated clearance (Siri-Tarino, Sun, Hu, & Krauss, 2010). Increased LDLC concentrations are known to increase CVD risk (MacMahon et al., 2007) due to its atherosclerotic effects and its nature in depositing fat in blood vessels and tissue (Daniels et al., 2009; Heart Foundation, 2019). Furthermore, increased serum LDLC and apo B concentrations can lead to an increase in the likeliness of the oxidation and glycation of those elements. The oxidation and glycation of those molecules activate endothelial cells' expression of monocytes and increases the adhesion of immune cells. The inflammatory pathways caused by the aforementioned immune cells, lead to increased oxidative stress which causes further oxidation of LDLC, endothelial activation and increased risk of atherosclerosis and the build-up of fatty plaques (Harris et al., 2009). Thus, it is crucial to weigh the benefits (improved TG and HDLC concentrations), and the harms (elevated LDLC) that LCHO diets exert on blood lipid profile as observed in the literature and the present study and the effects such diets impose on CVD risk.

In the present study, participants in the VLCHO intake group (<50g) had the highest TC and LDLC concentrations; this elevation was accompanied by the highest SFA (%TE) compared to LCHO and MLCHO intake groups. Additionally, a significant negative correlation between carbohydrate intakes (grams and %TE) and both TC and LDLC concentrations were observed when controlling for age, gender and income. A finding, consistent with the cross-sectional results of Ma et al. (2006) reporting that over one year, the type of carbohydrates and glycaemic

load were negatively correlated with TC and LDLC concentrations. The LOCA study regression models have revealed that one %TE increase in fat intake to replace carbohydrates caused a 0.045mmol/L and 0.034mmol/L increase in TC and LDLC, respectively. A similar effect was evident with increased SFA intakes; for every 1%TE increase in SFA to replace CHO resulted in 0.073mmol/L and 0.05mmol/L increase in TC and LDLC, respectively.

The three different carbohydrate intake level groups of this study all presented with normal TG concentrations and high HDLC concentrations. Both these findings are consistent with those of RCTs and meta-analyses (Bazzano et al., 2014; Santos et al., 2012). In the present study, all participants in the LCHO and MLCHO groups and the majority of those in the VLCHO group had normal TG concentrations. Additionally, the majority of the participants had normal HDLC concentrations, and only 7.9% and 5.3%, respectively in the VLCHO and LCHO groups had low concentrations. LCHO diets have shown to decrease TG and increase HDLC concentrations (Bazzano et al., 2014; Boaz & Raz, 2015; Naude et al., 2014; Santos et al., 2012). Both the quantity and quality of carbohydrate consumed influence TG and HDLC concentrations (Zinn, McPhee, et al., 2017). Simple carbohydrates with high glycaemic load have shown to increase TG and decrease HDLC (Ma et al., 2006). Additionally, high total carbohydrate intake has shown to increase TG (Hudgins, 2000; Reizlaff C'arolvn et al., 1995; Sacks et al., 2014) and decrease HDLC (Ma et al., 2006). The effect of high carbohydrate intakes on high TG concentrations may be related to carbohydrate-induced hypertriglyceridemia, which may be due to increased fatty acid synthesis (Hudgins et al., 2000). Increased free fatty acid concentrations alongside increased adequate glycogen stores promote TG formation (Sears & Perry, 2015).

Furthermore, HDLC concentrations are not only influenced by carbohydrate intakes but also by fat intakes, where increased SFA has been shown to increase HDLC concentrations (German & Dillard, 2004). The increase in HDLC concentrations was demonstrated in animal models to mimic the effects in a human situation. High fat and SFA intakes resulted in both increased transport rate and decreased catabolic rate of HDLC cholesterol esters and APO A-I. This suggests that as dietary fat intake increases, a decline in HDLC degradation takes place, resulting in increased HDLC concentrations (Hayek et al., 1993). The beneficial influence of LCHO on serum HDLC and TG concentrations is associated with decreased CVD risk (Bayturan et al., 2010; Cooney et al., 2009; MacMahon et al., 2007).

### *3.4.2 Carbohydrate Restriction and Kidney Function.*

Men and women in the present study had protein intakes of 24.16%TE and 24.5%TE, respectively. Those intakes are higher than the NZ population reported in the 2008 NNS (16.4%TE for men and 16.5%TE for women, recommended intake 25%TE) (Ministry of Health, 2011). All three carbohydrate intake groups in the LOCA study had high mean intakes of energy from protein. The high protein intakes within those groups were accompanied by reduced eGFR levels among the majority of the participants (VLCHO: 89.5%, LCHO: 89.5%, and MLCHO: 88.9%) as well as some participants having elevated serum creatinine concentrations (VLCHO: 13.2%, LCHO: 36.8% and MLCHO: 33.3%). Decreased eGFR indicates a mild reduction in kidney function, and together with high protein intakes may indicate that glomerular hyperfiltration (Toubro et al., 1999) and increased intraglomerular pressure may have taken place with the initial increase in protein intake; a response observed in animal studies (Schrijvers et al., 2002). Hyperfiltration is an adaptive mechanism resulting in increased eGFR, which is thought to take place as the body's attempt to maintain serum creatinine concentrations – an initial response to increased protein intakes (Toubro et al., 1999). However, prolonged hyperfiltration and high intraglomerular pressure can lead to kidney damage (Sasson & Cherney, 2012). Cirillo et al. (2014) demonstrated that short-term increased protein intake was positively correlated with eGFR concentrations, where 1g increase in protein intake resulted in 4.7ml/min.1.73m2 increase in eGFR. Long-term increase in protein intake, however, was negatively correlated with a decrease in eGFR, where every 1g increase in protein intake, decreased eGFR by 4.1ml/min.1.73m2 (Cirillo et al., 2014). Reductions in eGFR can lead to elevated serum creatinine, as creatinine clearance is highly dependent on renal function (Gowda et al., 2010; Hosten, 1990). Elevated serum creatinine levels alongside mild reductions in kidney function were observed among participants in the VLCHO, LCHO, and MLCHO groups, both of which may be effects of a long-term increase in protein intake (Tay, Thompson, et al., 2015). Although the effects of high protein intakes on kidney function have been well examined, in the present study, the linear regression analysis failed to link macronutrient intakes to changes in eGFR. We instead have found that only age and gender were associated with eGFR. This finding may be due to the small sample size of the present study. Furthermore, the target population of the present study is a high protein intake population, thus limiting the spread of the results causing difficulty in determining correlations between protein intakes and eGFR values.

### *3.4.3 Strengths and Limitations*

To our knowledge, the LOCA study is the first to shed light on practices of self-reported low carbohydrate diet followers in Auckland, New Zealand. It also contributes to understanding the effects of self-prescribed LCHO diets on biomarkers associated with metabolic disease. Additionally, this study provides associations between those biomarkers and nutrient and energy intakes, LCHO diet duration, and replacement nutrients. Nevertheless, the limitations of this study must be considered when interpreting the results. The first limitation is the cross-sectional nature of the cross-sectional study design, which illustrates associations between dietary intake and anthropometric and metabolic biomarkers. The limitation of this study design is that causality cannot be established. Secondly, the LOCA study originally intended to recruit a sample size of 207 (power of 99.8%), however, due to time pressure and a low response rate from the target population, researchers had to lower participant numbers, which impacted on the analysis strategy. Given the study's sample size and study design, the findings of this study cannot be generalised to the wider population of LCHO diet followers and those aged 45 years and older. Lastly, the assumptions of the regression analysis were not fully met, as TC, LDL, and duration of LCHO diet were not normally distributed, even following a log transformation.

## 3.5 Conclusion

In conclusion, self-directed LCHO diet followers had high protein and fat intakes, with fat being the primary replacement macronutrient. High energy intakes from fats were accompanied by high SFA intakes. Carbohydrate restriction and high fat intakes have shown negative associations with TC and LDLC concentrations; however, it has shown beneficial effects on HDLC and TG concentrations. This effect of LCHO diets was related to increased total fat and particularly SFA intake. Such observations among LCHO diet followers raises concerns on the impact of such dietary practices on CVD risk. Furthermore, elevated serum creatinine and mild decline in kidney function were also observed with the current practices of the LOCA study population. Further investigations into the practices of self-directed LCHO diet followers, while encompassing a larger group of the NZ population, may contribute to a greater understanding of the effect of such practices on biomarkers and risk of chronic disease.

# Chapter Four

# Conclusions and Recommendations

## 4.1. Overview and Conclusions

The constant rise in the global incidence of overweight and obesity has resulted in increased awareness of the effects of increased adiposity and its comorbidities on health and wellbeing. In return, this increase in awareness has resulted in a constant search for effective and fast weight loss strategies by the general public, with the desire to improve body image and strengthen social relationships. Such desires can result in individuals adopting various types of diets, one of which being low carbohydrate (LCHO) diets. LCHO diets have increased in popularity due to their effectiveness in causing quick weight loss. LCHO diets involve adjusting macronutrient intakes to decrease energy intake from carbohydrate and increase energy intakes from fat and protein to maintain current energy intake. However, both consistent definition and categorisation of LCHO diets are lacking, thus resulting in individualised interpretations of LCHO diets. Such self-directed LCHO diets and the ill-advised alteration of macronutrient intakes raises concerns on the safety of these diets, particularly their effect on cardiovascular and kidney disease risk. Although a consistent definition is lacking, Brouns, (2018), Feinman et al. (2015) and Harvey et al. (2018) have categorised carbohydrate intakes below 50g per day as very low. Bilsborough & Crowe, (2003) have categorised carbohydrate intakes between 50 to 100g per day as low, while Brouns, (2018) has categorised carbohydrate intakes between 100 to 150g per day as moderately low. Carbohydrate intakes above 150g were considered as normal intakes.

In New Zealand (NZ), there is limited research on LCHO diets and their effects on metabolic biomarkers of disease profile. Additionally, there is no research examining the current practices of LCHO diet followers and the impact of such practices on kidney function nor on cardiovascular disease (CVD) and type 2 diabetes mellitus (T2DM) risk. The LOCA (LOw CArbohydrate) study aimed to investigate the practices of NZ men and women aged 20 to 45 years who are following a self-directed LCHO diet and the associations between such dietary practices and metabolic and inflammation biomarkers. To fulfil the aim of this study, a non-consecutive 4-day food record (3 weekdays and one weekend), as well as anthropometric measurements and fasting venous blood samples, were collected from the LOCA study participants.

The first objective of the present study was "to investigate the biomarker profiles and associations with metabolic disease risk (diabetes, CVD, obesity and kidney disease risks) of self-reported low carbohydrate consumers in relation to gender and different levels of low-carbohydrate intakes". Dietary data, metabolic biomarkers and anthropometric data were stratified by gender to examine the difference in dietary intakes as well as biomarker levels among men and women using independent t-test and Mann-Whitney U test (Tables 3.2). Exploring the dietary intake of the target population showed low carbohydrate intakes and very low fibre intakes, both of which are expected observations of LCHO diets. Alongside LCHO intakes, high protein, fat and saturated fat intakes were observed. High intakes of protein and SFA can suggest high intakes of animal protein, as they can be high in SFA alongside added SFA from fat sources (not examined in this sub-study).

Interestingly, men and women had similar restrictions to carbohydrate intakes, a rather unexpected finding, which may indicate that the majority of the participants follow similar types of LCHO diets (mainly very low to low carbohydrate intakes). Men had significantly higher intakes of protein (g), fat (g) and SFA (g), thus contributing to significantly higher energy intake (Table 3.2A). However, the contributions of those macronutrients to total energy intakes were similar between men and women. Furthermore, both men and women had elevated BMI, placing them in the overweight category. Significant differences between men and women for body fat percentage, waist to hip ratio and waist circumference were expected due to the physical and physiological variations in the human body between men and women.

Elevated TC and LDLC concentrations were observed with no significant differences between men and women; this finding may be related to similar intakes of fat and SFA (%TE). High HDLC concentrations were observed with significantly lower concentrations found in men than women ($P$=0.027). This is an unexpected observation given the absence of any differences in fat intakes (%TE) between genders. This finding may suggest that HDLC concentrations are not only influenced by food intake but also by gender. Such differences may contribute to the observation that men are at higher risk of developing CVD than women. High HDLC concentrations are known to reduce the risk of CVD due to their nature of transporting fat away from blood vessels and therefore, decreasing the formation of atherosclerotic plaques (Daniels et al., 2009; Heart Foundation, 2019). The higher concentrations of HDLC in women may be due to the hormonal effects of oestrogen in premenopausal women, where oestrogen is directly related to HDLC concentrations (Krauss, Lindgren, Wingerd, Bradley, & Ramcharan, 1979), thus, protecting

women from CVD. This observation, alongside similar LDLC concentrations between men and women, may suggest that women in the LOCA study are more protected against CVD than men. Furthermore, high HDLC and normal triglyceride concentrations were expected due to the LCHO intakes in the present populations, as abnormal concentrations (low HDLC and high triglycerides) are related to high carbohydrate intakes.

Participants were grouped based on carbohydrate intakes in very low carbohydrate (VLCHO), low carbohydrate (LCHO) and moderately low carbohydrate (MLCHO) groups. Dietary, anthropometric and metabolic biomarker data were examined across the three carbohydrate intake groups after adjusting for age, gender, and income using ANCOVA (Tables 3.3). The VLCHO had the lowest carbohydrate (g and %TE), fibre (g) and protein (g) intakes; however, total fat (g) intake was similar to that of the remaining carbohydrate intake groups. As carbohydrate intakes increased in the different groups, protein intakes also increased, thus contributing to the observed increase in energy intakes. The increase in energy from protein and carbohydrate intakes resulted in a decline in %TE from total fat; the VLCHO had the highest percentage contribution from fat and the MLCHO the lowest. The differences in energy intakes may be related to both the quantity and type of food consumed. For example, the VLCHO group may consume a lower volume of food or foods that are less energy-dense. The MLCHO group, however, may consume more food or more energy-dense food, which may explain the observed differences in nutrient (g) and energy intakes. Furthermore, the difference in the %TE for the macronutrients between the three carbohydrate groups are highly influenced by the differences in energy intake. Total cholesterol and LDLC concentrations were significantly higher in the VLCHO group compared to the MLCHO group, before controlling for age, gender and income. However, differences were no longer significant after controlling for those variables. Although the relationship between fat and blood lipid profiles have already been established, age, gender and income play substantial roles, thus when eliminating the effect of those variables, the difference between the three carbohydrate intake groups disappeared. Therefore, in this instance, the differences in those biomarkers are primarily related to age, gender and income rather than dietary intake. Furthermore, grams of fat intake between the groups were similar, which can suggest that those variables had a more substantial impact than dietary intake. However, the LOCA population size is quite small, which may be the reason for such observations.

The second objective of this study was “to explore the association between all biomarkers and energy and nutrient intakes, low carbohydrate diet duration,  and replacement nutrients”. To

explore the associations between carbohydrate intake and biomarker profiles of disease, partial correlations were performed between anthropometric and metabolic biomarkers and carbohydrate intake (%TE and grams) and duration of following LCHO diets. Partial correlations were performed to control for age, gender and income as well as the duration of following an LCHO diet (Table 3.4). Significant negative correlations between carbohydrate intakes and TC and LDLC concentrations were observed. This may not be due to the carbohydrate as a macronutrient specifically, but rather due to the replacement macronutrients consumed, with fat exerting the most effect. The increase in fat intakes as a result of carbohydrate restriction may contribute to increased risk of CVD. A significant negative correlation between BMI and the duration of following an LCHO diet further suggests that adherence to the dietary change is the primary influencer on BMI rather than the macronutrient distribution. The long-term reduction in energy intakes as a result of adhering to the diet can result in weight loss, reduction in BF% and therefore changes in BMI. This is not necessarily related to LCHO diets and carbohydrate restriction but more to reductions in energy intakes which can be achieved with many weight loss diets, including low-fat diets.

Linear regression models were used to determine the effects of carbohydrates and replacement nutrients (protein, total fat, saturated fat and fibre) on anthropometric and metabolic biomarkers, while controlling for age, gender, income and duration of following an LCHO diet. Carbohydrate intakes have shown a negative relationship with total cholesterol concentration and LDLC concentrations, while total fat and saturated fat had a positive relationship with TC and LDLC concentrations. This suggests that the combination of decreasing carbohydrate intake and increasing total fat and SFA intakes may contribute to the elevated concentrations of TC and LDLC observed alongside age and gender. Additionally, eGFR levels were influenced by age and gender rather than macronutrient intakes and the duration of following an LCHO diet.

In conclusion, thoroughly exploring those objectives has provided an understanding of the associations of dietary practices of self-directed LCHO diet followers in Auckland, NZ, and anthropometric and metabolic biomarkers. Furthermore, throughout the literature, LCHO diets have shown significant effects on weight loss as a result of their effect on energy intake. However, despite this beneficial effect, LCHO diets can also cause harmful effects in increasing TC and LDLC concentrations, thus worsening the risk of CVD.

## 4.2. Strengths and Limitations

There are several strengths to this study. The LOCA study is the first study to shed light on practices of self-directed low carbohydrate diet followers in Auckland city, New Zealand. The study utilised specific inclusion criteria, thus, allowing to capture a specific and unique group of low carbohydrate followers which included healthy men and women aged 20 to 45 years who were following an LCHO diet for a minimum of four months.

The study also utilised a variety of anthropometric measurements like BMI, waist and hip circumferences and their ratios, as well as BF%. Furthermore, a variety of laboratory measurements such as blood lipid profile, insulin, HbA1c, ketone levels, and urea were used to assess the effects of restricting CHO on metabolic biomarkers. Additionally, the participants were provided with detailed instructions and specific days for the four-day food records to capture their specific dietary intake on their individualised definition of a low carbohydrate diet. Participants were also interviewed to discuss and resolve any unclear information in their food records.

To date, the low carbohydrate research in NZ includes randomised controlled trials investigating the various effects of LCHO diets on weight loss, body composition, and metabolic biomarkers (Harvey et al., 2019; Krebs et al., 2013, 2016; McAuley et al., 2006; Zinn, McPhee, et al., 2017). Another study investigated the effects of LCHO diets and medium-chain triglyceride supplementation on the duration to nutritional ketosis (Harvey et al., 2018). The remaining studies conducted in NZ investigated the effects of LCHO diets on Parkinson's disease (M. C. L. Phillips, Murtagh, Gilbertson, Asztely, & Lynch, 2018), lastly, a pilot case study examining the benefits of ketogenic diets on performance and body composition among NZ endurance athletes (Zinn, Wood, Williden, Chatterton, & Maunder, 2017). However, none of those studies examined the current practices of LCHO diet followers or the effect of self-directed practices on metabolic biomarker profiles of disease.

Considering these strengths, the study also had several limitations. Firstly, because of the cross-sectional nature of the study design, these findings are limited to exploring associations and strengths rather than causation. Secondly, the recruitment of less participants than originally intended (N=74 vs N=207). This resulted in a low statistical power of 69.9% compared to the initial intended power of 99.8%. The small sample size may not provide a true reflection of the dietary practices of LCHO followers or the effects of those diets on anthropometric and metabolic biomarkers on a population level. Additionally, data collection was limited to Auckland city. It,

therefore, did not reflect the larger New Zealand population nor its multicultural nature, especially since the majority of the participants identified themselves as New Zealand European.

## 4.3. Recommendations

*Key learning and recommendations for future research:*

- To include a larger sample size of LCHO diet followers from different ethnic backgrounds.
- To include low carbohydrate diet followers from across NZ, however, such an approach requires stations across NZ for the completion of the anthropometric measurements and the collection of blood samples, as well as larger funding for blood sampling and analysis.
- To investigate the dietary supplements consumed by the target population and the influence of those supplements on the metabolic biomarkers observed in this study.
- To examine the associations between kidney function and gender, protein intake and hydration status over time.
- To investigate the practices of self-directed LCHO diet followers across NZ that includes a large sample size with individuals from different ethnic backgrounds. Additionally, to investigate the associations between dietary practices and patterns of LCHO diet followers and the risk of chronic disease.
- To investigate practices of low carbohydrate diet followers aged greater than 45 years and the effects of those dietary practices on biomarkers of metabolic disease.
- To examine the practices of self-directed LCHO diet followers and its effect on metabolic biomarker profiles as well as the association of physical activity and metabolic biomarker profiles.
- To conduct a longitudinal study assessing the progression and change in the practices of LCHO diet followers and the risk of adiposity and chronic disease among New Zealanders.
- To assess the nutrition knowledge of LCHO diet followers in NZ on the effects of LCHO diets on health.
- To assess the reversibility of the observed effects of LCHO diets and the timeframe required to normalise and improve the concentrations of the metabolic biomarkers.

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# Appendix

***Appendix A.*** *Health and Demographics Questionnaire of the LOCA study.*

## The LOCA Study Health and Demographics Questionnaire

1 Please enter your study ID (if you are not sure, please ask the researcher)

2 Please enter your first name

3 Please enter your gender

- ◯ Male
- ◯ Female

4 When did your last menstrual period start?

Date / Time

Date

DD/MM/YYYY

5 Are you pregnant?

- ◯ Yes
- ◯ No

6 What is your first language?

- ◯ English
- ◯ Other (please specify)

7 What is the highest level of education you have received?

- ◯ Primary School
- ◯ Secondary School (College, e.g. school certificate / bursary / NCEA Level 1-3)
- ◯ Trade Certificate or Diploma
- ◯ University or other Tertiary Education (e.g. Post Graduate Diploma and Certificate, Bachelor's Degree, Masters Degree, PhD

Other (please specify)

8 To which ethnic group do you belong? Choose whichever applies to you, you make choose more than one.

- ☐ New Zealand European
- ☐ Maori
- ☐ Samoan
- ☐ Cook Island Maori
- ☐ Other (please specify)
- ☐ Tongan
- ☐ Niuean
- ☐ Chinese
- ☐ Indian

9 If you chose more than one ethnicity, please state the ethnicity you identify with first and foremost.

10 Which country were you born in?

- ☐ New Zealand
- ☐ Australia
- ☐ People's Republic of China
- ☐ Scotland
- ☐ Other (please specify)
- ☐ South Africa
- ☐ Cook Islands
- ☐ Samoa

11 If you live in New Zealand, but were not born here, when did you first arrive to live in New Zealand?

Date/Month/Year

Date

DD/MM/YYYY

12 What is your date of birth?

Date/Month/Year

Date

DD/MM/YYYY

13 What is your marital status?

- [ ] Single
- [ ] Partner / de facto / married
- [ ] Other

14 What is your living arrangement?

- ◯ Live alone
- ◯ Live with family
- ◯ Flatting
- ◯ Live with a partner

15 What is your occupation? (Tick as many as applicable)

- [ ] Retired
- [ ] Stay at home parent/caregiver
- [ ] Unemployed
- [ ] Paid employment full time / part time (please explain)
- [ ] Student
- [ ] Disability Allowance
- [ ] Beneficiary

16 In your current paid employment: (If more than one, please use your primary employment)

What is your occupation (e.g. nurse, accountant, teacher)

Please enter the main activity of the company/organisation

17 In your current paid employment do you work:

- ◯ Full time
- ◯ Part time

18 In your current paid employment, how many hours do you usually work each day?

19 In your current paid employment, how many hours do you usually work each week?

20 What is your usual work pattern in your current paid work?
Please tick the box that best applies

- ☐ Daytime with no shifts
- ☐ Permanent nights
- ☐ Rotating shifts with nights
- ☐ Irregular or variable
- ☐ Rotating shifts without nights
- ☐ Other (please specify)

21 If you work night shifts, how many do you work in a usual week?

22 In your previous paid employment:

What was your occupation

Please enter the main activity of the company/organisation

23 In your previous paid employment, please enter the period you worked (dd/mm/yyyy)

From

To

24 What would the total monthly income be that the household received from all sources before tax has been taken out?

- ☐ Loss
- ☐ Zero
- ☐ 0-$400
- ☐ $401-$800
- ☐ $801-$1250
- ☐ $1250-$1500
- ☐ $1501-$2500
- ☐ $2501-$3000
- ☐ $3001-$5000
- ☐ $5001-$7000
- ☐ $7001-$8000
- ☐ $8001 or more
- ☐ I don't want to answer

25 Do you follow any dietary restrictions for cultural or religious reasons?

- ◯ No
- ◯ Yes (please explain)

26 At what speed do you eat your meals?

- ◯ Quickly
- ◯ At a moderate pace
- ◯ Slowly

27 Do you smoke cigarettes?

- ◯ Non-smoker
- ◯ Former smoker
- ◯ Current (approximately how many cigarettes per day?)

28 Do you drink alcohol?

- ☐ Never or very rarely
- ☐ One drink per day
- ☐ One drink per week
- ☐ More than one drink per day
- ☐ More than one drink per week

29 Do you have any diagnosed allergies?

- ◯ No
- ◯ Yes (please specify)

30 Are you taking any form of medication, including traditional or homeopathic medicine and contraception?

- ◯ Yes
- ◯ No

31 If yes, please specify the condition, medication, dosage and frequency below.

| | |
|---|---|
| Condition 1 | |
| Medication 1 | |
| Dosage 1 | |
| Frequency 1 | |
| Condition 2 | |
| Medication 2 | |
| Dosage 2 | |
| Frequency 2 | |
| (continue here if further medication) | |

32 Are you taking any form of supplements, including tablets or drinks?

- ◯ Yes
- ◯ No

33 If yes, please tell us the name, brand, dosage and frequency of the supplements you are taking below.

| | |
|---|---|
| Supplement 1 | |
| Brand 1 | |
| Dosage 1 | |
| Frequency 1 | |
| Supplement 2 | |
| Brand 2 | |
| Dosage 2 | |
| Frequeny 2 | |
| (continue here if further supplements) | |

34 Please tell us how you found out about this study.
Did you find out from:

- ☐ A friend?
- ☐ An email list?
- ☐ Social media?
- ☐ Flyer on noticeboard?
- ☐ Other (please specify)

## ***Appendix B.*** *Supplementary Results*

***Appendix B1****. Linear regression for macronutrient intakes (%TE) correlated to BMI*

| | ***Models for BMI*** | B | Std Error | Std'ised β | P-value | 95% CI |
|---|---|---|---|---|---|---|
| 1 | (Constant) | 3.389 | 0.113 | | <0.001 | 3.164, 3.614 |
| | Age | -0.001 | 0.003 | -0.066 | 0.586 | -0.006, 0.004 |
| | Gender | -0.020 | 0.041 | -0.058 | 0.621 | -0.103, 0.062 |
| | Income | -0.009 | 0.005 | -0.186 | 0.120 | -0.019, 0.002 |
| | LCHO diet duration | -0.002 | 0.001 | -0.242 | 0.039 | -0.004, 0.000 |
| | Carbohydrate (%TE) | -0.001 | 0.002 | -0.069 | 0.569 | -0.004,0.002 |
| | | | F(5, 73)= 1.697, $R^2$ =0.111, *P*-value= 0.147 | | | |
| 2 | (Constant) | 3.271 | 0.127 | | <0.001 | 3.017, 3.525 |
| | Age | -0.001 | 0.002 | -0.048 | 0.679 | -0.006, 0.004 |
| | Gender | -0.022 | 0.041 | -0.061 | 0.597 | -0.103, 0.060 |
| | Income | -0.008 | 0.005 | -0.167 | 0.159 | -0.018, 0.003 |
| | LCHO diet duration | -0.002 | 0.001 | -0.262 | 0.025 | -0.005, 0.000 |
| | Protein (%TE) | 0.004 | 0.003 | 0.157 | 0.184 | -0.002, 0.010 |
| | | | F (5, 73)= 2.026, R2 =0.130, P-value= 0.086 | | | |
| 3 | (Constant) | 3.360 | 0.130 | | <0.001 | 3.101, 3.620 |
| | Age | -0.001 | 0.003 | -0.050 | 0.676 | -0.006, 0.004 |
| | Gender | -0.022 | 0.041 | -0.061 | 0.603 | -0.104, 0.061 |
| | Income | -0.009 | 0.005 | -0.199 | 0.094 | -0.020, 0.002 |
| | LCHO diet duration | -0.002 | 0.001 | -0.243 | 0.040 | -0.004, 0.000 |
| | Fat (%TE) | 0.000 | 0.002 | 0.011 | 0.928 | -0.003, 0.003 |
| | | | F(5, 73)= 1.626, $R^2$ =0.107 P-value= 0.165 | | | |
| 4 | (Constant) | 3.316 | 0.118 | | <0.001 | 3.080, 3.552 |
| | Age (years) | -0.002 | 0.003 | -0.073 | 0.540 | -0.007, 0.003 |
| | Gender | -0.017 | 0.041 | -0.049 | 0.678 | -0.100, 0.065 |
| | Income | -0.008 | 0.005 | -0.179 | 0.131 | -0.019, 0.003 |
| | LCHO diet duration | -0.002 | 0.001 | -0.228 | 0.053 | -0.004, 0.000 |
| | SFA (%TE) | 0.003 | 0.003 | 0.115 | 0.340 | -0.003, 0.008 |
| | | | F(5, 73)= 1.830, $R^2$ =0.119, *P*-value= 0.119 | | | |
| 5 | (Constant) | 3.568 | 0.138 | | <0.001 | 3.292, 3.843 |
| | Age (years) | -0.001 | 0.002 | -0.062 | 0.584 | -0.006, 0.003 |
| | Gender | -0.024 | 0.040 | -0.068 | 0.548 | -0.104, 0.056 |
| | Income | -0.007 | 0.005 | -0.156 | 0.177 | -0.018, 0.003 |
| | LCHO diet duration | -0.002 | 0.001 | -0.225 | 0.048 | -0.004, 0.000 |
| | Fibre (g) | -0.070 | 0.032 | -0.246 | 0.033 | -0.134, -0.006 |
| | | | F(5, 73)= 2.682, $R^2$ =0.165, P-value= 0.029 | | | |

***Appendix B2**. Linear regression for macronutrient intakes (%TE) correlated to eGFR*

| | ***Models for eGFR*** | B | Std Error | Std'ised β | P-value | 95% CI |
|---|---|---|---|---|---|---|
| 1 | (Constant) | 106.361 | 7.496 | | <0.001 | 91.391, 121.331 |
| | Age | -0.446 | 0.171 | -0.297 | 0.011 | -0.788, -0.105 |
| | Gender | -8.146 | 2.720 | -0.330 | 0.004 | -13.578, -2.715 |
| | Income | 0.309 | 0.356 | 0.097 | 0.390 | -0.403, 1.020 |
| | LCHO diet duration | -0.050 | 0.070 | -0.077 | 0.481 | -0.190, 0.091 |
| | Carbohydrate (%TE) | -0.076 | 0.110 | -0.079 | 0.489 | -0.295, 0.143 |
| | | | F(5, 70)= 4.171, R2 =0.243, P-value= 0.002 | | | |
| 2 | (Constant) | 107.203 | 8.585 | | <0.001 | 90.057, 124.348 |
| | Age | -0.417 | 0.166 | -0.278 | 0.014 | -0.748, -0.087 |
| | Gender | -8.243 | 2.721 | -0.334 | 0.004 | -13.677, -2.808 |
| | Income | 0.214 | 0.357 | 0.067 | 0.551 | -0.499, 0.926 |
| | LCHO diet duration | -0.047 | 0.071 | -0.072 | 0.512 | -0.189, 0.095 |
| | Protein (%TE) | -0.100 | 0.190 | -0.059 | 0.598 | -0.480, 0.279 |
| | | | F (5, 70)= 4.118, R2 =0.241, P-value= 0.003 | | | |
| 3 | (Constant) | 99.696 | 8.609 | | <0.001 | 82.502, 116.890, |
| | Age | -0.454 | 0.169 | -0.302 | 0.009 | -0.791, -0.116 |
| | Gender | -8.093 | 2.710 | -0.328 | 0.004 | -13.505, -2.682 |
| | Income | 0.308 | 0.350 | 0.097 | 0.383 | -0.392, 1.008 |
| | LCHO diet duration | -0.045 | 0.070 | -0.069 | 0.527 | -0.185, 0.096 |
| | Fat (%TE) | 0.100 | 0.100 | 0.111 | 0.322 | -0.100, 0.300 |
| | | | F(5, 70)= 4.306, R2 =0.249, P-value= 0.002 | | | |
| 4 | (Constant) | 104.109 | 7.926 | | <0.001 | 88.279, 119.939 |
| | Age (years) | -0.422 | 0.170 | -0.281 | 0.016 | -0.761, -0.083 |
| | Gender | -8.191 | 2.742 | -0.332 | 0.004 | -13.667, -2.715 |
| | Income | 0.265 | 0.356 | 0.083 | 0.459 | -0.446, 0.975 |
| | LCHO diet duration | -0.050 | 0.071 | -0.077 | 0.487 | -0.193, 0.093 |
| | SFA (%TE) | 0.026 | 0.175 | 0.017 | 0.881 | -0.323, 0.376 |
| | | | F(5, 70)= 4.050, R2=0.238, P-value= 0.003 | | | |
| 5 | (Constant) | 101.562 | 9.425 | | <0.001 | 82.739, 120.386 |
| | Age (years) | -0.414 | 0.166 | -0.275 | 0.015 | -0.745, 0.083 |
| | Gender | -8.207 | 2.722 | -0.332 | 0.004 | -13.644, 2.771 |
| | Income | 0.223 | 0.354 | 0.070 | 0.532 | -0.485, 0.930 |
| | LCHO diet duration | -0.054 | 0.071 | -0.083 | 0.447 | -0.195, 0.087 |
| | Fibre (g) | 1.094 | 2.210 | 0.055 | 0.622 | -3.321, 5.508 |
| | | | F(5, 70)= 4.109, $R^2$ =0.240, *P*-value= 0.003 | | | |

***Appendix C.*** *British Journal of Nutrition Manuscript preparation guidelines.*

**DETAILED MANUSCRIPT PREPARATION INSTRUCTIONS**

Language

Papers submitted for publication must be written in English and should be as concise as possible. We recommend that authors for whom English is not their first language have their manuscript checked by someone whose first language is English before submission, to ensure that submissions are judged at peer review exclusively on academic merit. Please see the Author Language Services section below for more information.

Spelling should generally be that of the *Concise Oxford Dictionary* (1995), 9th ed. Oxford: Clarendon Press. Authors are advised to consult a current issue in order to make themselves familiar with BJN as to typographical and other conventions, layout of tables etc. Sufficient information should be given to permit repetition of the published work by any competent reader of BJN.

Published examples of BJN article types can be found below:

Research Article

Review Article

Horizons Article

Letter to the Editor

Authorship

The Journal conforms to the International Committee of Medical Journal Editors (ICMJE) definition of authorship, as described by P.C. Calder (Br J Nutr (2009) 101, 775). Authorship credit should be based on:

Substantial contributions to conception and design, data acquisition, analysis and/or interpretation; and

Drafting the article or revising it critically for important intellectual content; and

Final approval of the version to be published; and

Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

The contribution of individuals who were involved in the study but do not meet these criteria should be described in the Acknowledgments section.

Ethical standards

The required standards for reporting studies involving humans and experimental animals are detailed in an Editorial by G.C. Burdge (*Br J Nutr* (2014) **112**).

Experiments involving human subjects

The notice of contributors is drawn to the guidelines in the World Medical Association (2000) Declaration of Helsinki: ethical principles for medical research involving human subjects, with notes of clarification of 2002 and 2004 (https://www.wma.net/policies-post/wma-declaration-of-helsinki-ethical-principles-for-medical-research-involving-human-subjects/), the *Guidelines on the Practice of Ethics Committees Involved in Medical Research Involving Human Subjects* (3rd ed., 1996; London: The Royal College of Physicians) and the Guidelines for the ethical conduct of medical research involving children, revised in 2000 by the Royal College of Paediatrics and Child Health: Ethics Advisory Committee (*Arch Dis Child* (2000) **82**, 177–182). Articles reporting randomised trials must conform to the standards set by the Consolidated Standards of Reporting Trials (CONSORT) consortium. A completed CONSORT Checklist (Consolidated Standards of Reporting Trials (CONSORT) consortium) must accompany manuscripts reporting randomised controlled trials. Submissions that do not include this information will not be considered for review until a completed CONSORT Checklist has been submitted and approved.

*Required disclosures:* A paper describing any experimental work on human subjects must include the following statement in the Experimental Methods section: "This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects/patients were approved by the [insert name of the ethics committee; a specific ethics number MUST be inserted]. Written [or Verbal] informed consent was obtained from all subjects/patients. [Where verbal consent was obtained this must be followed by a statement such as: Verbal consent was witnessed and formally recorded]." For clinical trials, the trial registry name, registration identification number, and the URL for the registry should be included.

**PLEASE NOTE:** As a condition for publication, all randomised controlled trials that involve human subjects submitted to BJN for review must be registered in a public trials registry. A clinical trial is defined by the ICMJE (in accordance with the definition of the World Health Organisation) as any research project that prospectively assigns human participants or groups of

humans to one or more health-related interventions to evaluate the effects on health outcomes. Registration information must be provided at the time of submission, including the trial registry name, registration identification number, and the URL for the registry.

Experiments involving the use of other vertebrate animals

Papers that report studies involving vertebrate animals must conform to the 'ARRIVE Guidelines for Reporting Animal Research' detailed in Kilkenny et al. (*J Pharmacol Pharmacother* (2010) **1**, 94-99) and summarised at www.nc3rs.org.uk. Authors MUST ensure that their manuscript conforms to the checklist that is available from the nc3Rs website (the completed check list should be uploaded as a separate document during submission of the manuscript). The attention of authors is drawn particularly to the ARRIVE guidelines point 3b ('Explain how and why the animal species and model being used can address the scientific objectives and, where appropriate, the study's relevance to human biology', point 9c ('Welfare-related assessments and interventions that were carried out prior to, during, or after the experiment') and point 17a ('Give details of all important adverse events in each experimental group'). The Editors will not accept papers reporting work carried out involving procedures that cause or are considered likely to cause distress or suffering which would confound the outcomes of the experiments, or experiments that have not been reviewed and approved by an animal experimentation ethics committee or regulatory organisation.

*Required disclosures:* Where a paper reports studies involving vertebrate animals, authors must state in the Experimental Methods section the institutional and national guidelines for the care and use of animals that were followed and that all experimental procedures involving animals were approved by the [insert name of the ethics committee or other approving body; wherever possible authors should also insert a specific ethics/approval number].

Manuscript Format

The requirements of BJN are in accordance with the Uniform Requirements for Manuscripts Submitted to Biomedical Journals produced by the ICMJE.

Typescripts should be prepared with 1.5 line spacing and wide margins (2 cm), the preferred font being Times New Roman size 12. At the ends of lines, words should not be hyphenated unless hyphens are to be printed. **Line numbering and page numbering are required.**

**MANUSCRIPTS SHOULD BE ORGANISED AS FOLLOWS:**

Cover letter

Papers should be accompanied by a cover letter including a brief summary of the work and a short explanation of the novelty of the study and how it advances nutritional science. As part of the online submission process, authors are asked to affirm that the submission represents original work that has not been published previously, and that it is not currently being considered by another journal. The text for the cover letter should be entered in the appropriate box as part of the online submission process.

Title Page

The title page should include:

The title of the article;

Authors' names;

Name and address of department(s) and institution(s) to which the work should be attributed for each author;

Name, mailing address, email address, telephone and fax numbers of the author responsible for correspondence about the manuscript;

A shortened version of the title, not exceeding 45 characters (including letters and spaces) in length;

At least four keywords or phrases (each containing up to three words).

Authors' names should be given without titles or degrees and one forename may be given in full. Identify each author's institution by a superscript number (e.g. A.B. Smith[1]) and list the institutions underneath and after the final author.

Abstract

Each paper must open with an unstructured abstract of **not more than 250 words**. The abstract should be a single paragraph of continuous text without subheadings outlining the aims of the work, the experimental approach taken, the principal results (including effect size and the results of statistical analysis) and the conclusions and their relevance to nutritional science.

Introduction

It is not necessary to introduce a paper with a full account of the relevant literature, but the introduction should indicate briefly the nature of the question asked and the reasons for asking it. It should be **no longer than two manuscript pages**.

Experimental methods

The methods section must include a subsection that describes the methods used for statistical analysis (see the section on statistical analysis in the Appendix) and the sample size must be justified by the results of appropriate calculations and related to the study outcomes.

*Justification of sample size:* All manuscripts that report primary research must contain a statistical justification of sample size that is stated explicitly in the Statistics sub-section of the Methods. Manuscripts that do not contain this information will be returned to the authors for correction before peer review. The amended versions will be treated as new submissions. The information required must include, but not be restricted to, the following:-

Hypothesised effect size with appropriate justification.

A statement regarding statistical power (typically 80%) and the two-sided significance level (typically 0.05).

An explanation of how the statistical power was calculated.

If sample size is determined by the feasibility of recruitment minimally detectable effect sizes should be provided instead of power analysis.

The only exceptions are:-

Meta-analyses.

Exploratory or secondary analysis of observational studies based on large sample sizes

For studies involving humans subjects or experimental animals, the Methods section must include a subsection that reports the appropriate ethical approvals for the study (see Ethical Standards above).

All analytical procedures must be accompanied by a statement of within and between assay precision.

*Diets:* The nutrient composition of diets used in studies published in BJN must be described in detail, preferably in a table(s). Experimentally relevant differences in composition between diets are essential. For instance, studies of fat nutrition should always include fatty acid compositions of all diets.

*PCR analysis:* Where experiments involve measurement of mRNA including microarray analysis, for analysis of individual genes, mRNA should be measured by quantitative RTPCR. A

statement about the quality and integrity of the RNA must be provided together with the results of eletrophoretic analysis of the purity of the PCR products. Unless published elsewhere, full details of the oligonuceoltide primers and of the PCR protocol must be stated either in the text or in Supplementary Material. The stability of reference genes used for normalisation of PCR data must be reported for the experimental conditions described. Where possible, analysis of mRNA levels should be accompanied by assessment of either protein levels or activities.

*Microarray analysis:* Studies involving microarray analysis of mRNA must conform to the "Minimum Information about a Microarray Experiment" (MIAME) guidelines including deposition of the raw data in an appropriate repository (the Access Code must be state din the Methods). All microarray experiments must be accompanied by appropriate validation by quantitative RTPCR.

Results

These should be given as concisely as possible, using figures or tables as appropriate. Data must not be duplicated in tables and figures.

Discussion

While it is generally desirable that the presentation of the results and the discussion of their significance should be presented separately, there may be occasions when combining these sections may be beneficial. Authors may also find that additional or alternative sections such as 'conclusions' may be useful. The discussion should be **no longer than five manuscript pages**.

Acknowledgments

Here you may acknowledge individuals or organizations that provided advice and/or support (non-financial). Formal financial support and funding should be listed in the following section.

Financial Support

Please provide details of the sources of financial support for all authors, including grant numbers. For example, "This work was supported by the Medical research Council (grant number XXXXXXX)". Multiple grant numbers should be separated by a comma and space, and where research was funded by more than one agency the different agencies should be separated by a semi-colon, with "and" before the final funder. Grants held by different authors should be identified as belonging to individual authors by the authors' initials. For example, "This work was supported by the Wellcome Trust (A.B., grant numbers XXXX, YYYY), (C.D., grant number

ZZZZ); the Natural Environment Research Council (E.F., grant number FFFF); and the National Institutes of Health (A.B., grant number GGGG), (E.F., grant number HHHH)".

This disclosure is particularly important in the case of research that is supported by industry. Support from industry not only includes direct financial support for the study but also support in kind such as provision of medications, equipment, kits or reagents without charge or at reduced cost and provision of services such as statistical analysis; all such support must be disclosed here and if no such support was received this must be stated. Where no specific funding has been provided for research, please provide the following statement: "This research received no specific grant from any funding agency, commercial or not-for-profit sectors."

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Conflict of Interest

Please provide details of all known financial, professional and personal relationships with the potential to bias the work. Where no known conflicts of interest exist, please include the following statement: "None."

For more information on what constitutes a conflict of interest, please see the International Committee of Medical Journal Editors (ICMJE) guidelines.

Authorship

Please provide a very brief description of the contribution of each author to the research. Their roles in formulating the research question(s), designing the study, carrying it out, analysing the data and writing the article should be made plain.

References

References should be numbered consecutively in the order in which they first appear in the text using superscript Arabic numerals in parentheses, e.g. 'The conceptual difficulty of this approach has recently been highlighted[1,2]'. If a reference is cited more than once, the same number should be used each time. References cited only in tables and figure legends should be numbered in

sequence from the last number used in the text and in the order of mention of the individual tables and figures in the text.

Names and initials of authors of unpublished work should be given in the text as 'unpublished results' and not included in the References. References that have been published online only but not yet in an issue should include the online publication date and the Digital Object Identifier (doi) reference, as per the example below.

At the end of the paper, on a page(s) separate from the text, references should be listed in numerical order using the Vancouver system. When an article has more than three authors only the names of the first three authors should be given followed by '*et al.*' The issue number should be omitted if there is continuous pagination throughout a volume. Titles of journals should appear in their abbreviated form using the NCBI LinkOut page. References to books and monographs should include the town of publication and the number of the edition to which reference is made. References to material available on websites should follow a similar style, with the full URL included at the end of the reference, as well as the date of the version cited and the date of access.

Examples of correct forms of references are given below.

*Journal articles*

Rebello SA, Koh H, Chen C *et al.* (2014) Amount, type, and sources of carbohydrates in relation to ischemic heart disease mortality in a Chinese population: a prospective cohort study. *Am J Clin Nutr* **100**, 53-64.

Villar J, Ismail LC, Victora CG *et al.* (2014) International standards for newborn weight, length, and head circumference by gestational age and sex: the Newborn Cross-Sectional Study of the INTERGROWTH-21st Project.*Lancet* **384**, 857-868.

Alonso VR & Guarner F (2013) Linking the gut microbiota to human health. *Br J Nutr* **109**, Suppl. 2, S21–S26.

Bauserman M, Lokangaka A, Gado J et al. A cluster-randomized trial determining the efficacy of caterpillar cereal as a locally available and sustainable complementary food to prevent stunting and anaemia. *Public Health Nutr*. Published online: 29 January 2015. doi: 10.1017/S1368980014003334.

*Books and monographs*

Bradbury J (2002) Dietary intervention in edentulous patients. PhD Thesis, University of Newcastle.

Ailhaud G & Hauner H (2004) Development of white adipose tissue. In *Handbook of Obesity. Etiology and Pathophysiology*, 2nd ed., pp. 481–514 [GA Bray and C Bouchard, editors]. New York: Marcel Dekker.

Bruinsma J (editor) (2003) *World Agriculture towards 2015/2030: An FAO Perspective*. London: Earthscan Publications.

World Health Organization (2003) *Diet, Nutrition and the Prevention of Chronic Diseases*. Joint WHO/FAO Expert Consultation. WHO Technical Report Series no. 916. Geneva: WHO.

Keiding L (1997) *Astma, Allergi og Anden Overfølsomhed i Danmark – Og Udviklingen 1987–199I (Asthma, Allergy and Other Hypersensitivities in Denmark, 1987–1991)*. Copenhagen, Denmark: Dansk Institut for Klinisk Epidemiologi.

*Sources from the internet*

Nationmaster (2005) HIV AIDS – Adult prevalence rate. http://www.nationmaster.com/graph-T/hea_hiv_aid_ad... (accessed June 2013).

For authors that use Endnote, you can find the style guide for BJN here.

Figures

Figures should be supplied as separate electronic files. Figure legends should be grouped in a section at the end of the manuscript text. Each figure should be clearly marked with its number and separate panels within figures should be clearly marked (a), (b), (c) etc. so that they are easily identifiable when the article and figure files are merged for review. Each figure, with its legend, should be comprehensible without reference to the text and should include definitions of abbreviations. The nature of the information displayed in the figures (e.g. mean (SEM)) and the statistical test used must be stated.

We recommend that only TIFF, EPS or PDF formats are used for electronic artwork. Other non-preferred but usable formats are JPG, PPT and GIF files and images created in Microsoft Word. Note that these non-preferred formats are generally NOT suitable for conversion to print

reproduction. For further information about how to prepare your figures, including sizing and resolution requirements, please see our artwork guide.

In curves presenting experimental results the determined points should be clearly shown, the symbols used being, in order of preference, ○, ●, Δ, ▲, □, ■, ×, +. Curves and symbols should not extend beyond the experimental points. Scale-marks on the axes should be on the inner side of each axis and should extend beyond the last experimental point. Ensure that lines and symbols used in graphs and shading used in histograms are large enough to be easily identified when the figure size is reduced to fit the printed page. Statistically significant effects should be indicated with symbols or letters.

Colour figures will be published online free of charge, and there is a fee of £350 per figure for colour figures in the printed version. If you request colour figures in the printed version, you will be contacted by CCC-Rightslink who are acting on our behalf to collect colour charges. Please follow their instructions in order to avoid any delay in the publication of your article.

Images submitted with a manuscript should be minimally processed; some image processing is acceptable (and may be unavoidable), but the final image must accurately represent the original data. Grouping or cropping of images must be identified in the legend and indicated by clear demarcation. Please refer to the Office of Research Integrity guidelines on image processing in scientific publication. Authors should provide sufficient detail of image-gathering procedures and process manipulation in the Methods sections to enable the accuracy of image presentation to be assessed. Authors should retain their original data, as Editors may request them for comparison during manuscript review.

Tables

Tables should be placed in the main manuscript file at the end of the document, not within the main text. Please do**not** supply tables as images (e.g. in TIFF or JPG format). Be sure that each table is cited in the text. Tables should carry headings describing their content and should be comprehensible without reference to the text.

The dimensions of the values, e.g. mg/kg, should be given at the top of each column. Separate columns should be used for measures of variance (SD, SE etc.), the ± sign should not be used. The number of decimal places used should be standardized; for whole numbers 1.0, 2.0 etc. should be used. Shortened forms of the words weight (wt) height (ht) and experiment (Expt) may

be used to save space in tables, but only Expt (when referring to a specified experiment, e.g. Expt 1) is acceptable in the heading.

Footnotes are given in the following order: (1) abbreviations, (2) superscript letters, (3) symbols. Abbreviations are given in the format: RS, resistant starch. Abbreviations in tables must be defined in footnotes in the order that they appear in the table (reading from left to right across the table, then down each column). Symbols for footnotes should be used in the sequence: *†‡§||¶, then ** etc. (omit * or †, or both, from the sequence if they are used to indicate levels of significance).

For indicating statistical significance, superscript letters or symbols may be used. Superscript letters are useful where comparisons are within a row or column and the level of significance is uniform, e.g. '[a,b,c]Mean values within a column with unlike superscript letters were significantly different (*P*<0•05)'. Symbols are useful for indicating significant differences between rows or columns, especially where different levels of significance are found, e.g. 'Mean values were significantly different from those of the control group: **P*<0•05, ***P*<0•01, ****P*<0•001'. The symbols used for *P* values in the tables must be consistent.

Supplementary material

Additional data (e.g. data sets, large tables) relevant to the paper can be submitted for publication online only, where they are made available via a link from the paper. The paper should stand alone without these data. Supplementary Material must be cited in a relevant place in the text of the paper.

Although Supplementary Material is peer reviewed, it is not checked, copyedited or typeset after acceptance and it is loaded onto the journal's website exactly as supplied. You should check your Supplementary Material carefully to ensure that it adheres to journal styles. Corrections cannot be made to the Supplementary Material after acceptance of the manuscript. Please bear this in mind when deciding what content to include as Supplementary Material.