Preliminary investigations for studying the effects of low carbohydrate high fat diets on gluconeogenesis in type 2 diabetes patients

Christopher Webster

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Supervisor:

Dr James Smith

University of Cape Town

Co-supervisor:

Prof Timothy Noakes

University of Cape Town

Contents

Declaration

I, Christopher Webster, know the meaning of plagiarism and declare that all the work in this thesis, both in concept and execution, save for that which is properly acknowledged, is my own.

Signature: ……Signed by candidate

Date: 28 March 2020

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Abstract

Type 2 diabetes (T2D) is currently one of the major health challenges across the globe. Lifestyle changes are a key component of T2D management and there is growing interest in low carbohydrate high fat (LCHF) diets as a potential dietary strategy to improve glycaemic control, reduce T2D medication requirements, and improve body weight and lipid profiles. However, carbohydrate restriction is controversial. Results from observational studies generally do not support the food choices associated with carbohydrate restriction while results from short-term randomised controlled trials (RCTs) are more likely to show significant benefits of LCHF diets. Additionally, both study designs have limitations and opinion on LCHF diets is polarised due to ambiguities in how to interpret the available data. Chapter 1 of this thesis reviews the impact of prospective cohort studies, randomised controlled trials, and dietary policies on current opinions towards LCHF diets for the management of T2D. Uncertainty over the safety of LCHF diets remains a concern and additional observational studies and short-term RCTs of the same quality as existing research are unlikely to add any further clarity. For this reason, research focused on understanding the underlying mechanisms of carbohydrate restricted diets may be an alternative approach to alleviate or validate some of the concerns being expressed about LCHF diets. One such mechanism is the dysregulation of glucose production via gluconeogenesis, which is a key pathology of T2D but which has been incompletely studied. Indeed, the effects of LCHF eating on gluconeogenesis in T2D patients has not yet been studied, nor has gluconeogenesis been investigated in the context of T2D remission. This is an area of interest for future research and the aim of this thesis was to conduct preliminary studies to prepare the groundwork for such studies. There is large heterogeneity in the low carbohydrate diets that have been prescribed in controlled trials and the composition and characteristics of the LCHF diets that patients are finding effective in the real world is unknown. Study 1 (Chapter 2 of this thesis) aimed to better understand the LCHF diet by investigating the diet, diabetes status, and personal experiences of T2D patients who had self-selected and followed an LCHF diet of their own accord. This study was a multi-method investigation which consisted of quantitative assessments of diet and diabetes status, as well as in-depth interviews which were analysed using qualitative methods. Results from this study will be used to inform design and protocol decisions in future controlled trial studies. Study 2 (Chapter 3 of this thesis) piloted the use of stable isotope tracers for the quantification of endogenous glucose production and gluconeogenesis in the early post-absorptive state (5 hours after a meal). For methodological reasons, prior investigations have usually measured gluconeogenesis after an overnight fast and therefore, little is known about the effects of dietary composition on gluconeogenesis within the early post-absorptive state. Study 2 quantifies

gluconeogenesis 5 hours after a meal and the validity of the data is discussed. Finally, Chapter 4 outlines future perspectives for research based on findings from Chapter 2 and Chapter 3.

Chapter 1

Overview of thesis and narrative literature review

Overview of thesis

Type 2 diabetes (T2D) is a global epidemic that has become one of the major health challenges around the world. There is currently increasing scientific interest in carbohydrate restricted diets for the management of T2D, as recent studies have demonstrated potential for this dietary strategy to reverse hyperglycaemia, improve body weight and lipid profiles, reduce medication requirements, and induce remission in a relatively high proportion of patients. However, carbohydrate restriction is highly controversial and opinion on this dietary strategy ranges from recommending it as first-line treatment for T2D to condemning it as actively harmful to patients. There is also large heterogeneity in how "low carbohydrate" diets have been interpreted, formulated, and implemented in intervention studies. It is therefore unclear which aspects of these interventions are critical in determining positive outcomes in persons with T2D.

A large number of randomised controlled trials have investigated the effects of a carbohydrate restricted diet on body weight, markers of glycaemic control, and markers of cardiovascular disease risk in T2D patients. However, less is known about how carbohydrate restriction affects the underlying physiology of glucose homeostasis. A particularly striking feature of severe dietary carbohydrate restriction is that T2D patients can experience improvements in blood glucose control within 3 to 5 days of starting the diet. The dysregulation of glucose production via gluconeogenesis is a key pathology which maintains hyperglycaemia in T2D patients but this component of the condition is poorly understood because the measurement of hepatic gluconeogenesis poses significant methodological challenges. Additionally, gluconeogenesis has yet to be studied in the context of T2D remission produced by a carbohydrate restricted diet. This thesis aims to lay a firm foundation for studying the effects of carbohydrate restriction on gluconeogenesis by: 1) understanding the nature of the controversy surrounding carbohydrate restriction; 2) investigating the diet and lifestyle characteristics associated with carbohydrate restriction which may be more likely to produce successful outcomes in future intervention studies; and 3) validating the use of a

modern method (the average deuterium enrichment method) for the quantification of gluconeogenesis in response to a meal.

Chapter 1 is a literature review which investigates the nature of the controversy surrounding carbohydrate restriction. It addresses three aspects of the literature which are particularly pertinent to current medical and scientific opinion on carbohydrate restriction and T2D: 1) the interpretation of observational studies; 2) the interpretation of controlled trials; and 3) dietary policies. **Chapter 2** is a descriptive study of T2D patients who had self-selected and successfully followed a carbohydrate restricted diet for at least 6 months. This study investigated the diets, diabetes status, and personal experiences of these patients to provide insight into the characteristics of a low carbohydrate diet that is being sustained in the real-world. Participants were followed up for 15 months later to assess T2D remission. The study also contains a qualitative component which provides insight into those aspects of carbohydrate restriction which are important from the perspective of the patient and which are more likely to produce successful outcomes. **Chapter 3** consists of the validation of an experimental protocol to quantify gluconeogenesis within 5 hours of a meal using stable isotope tracers. For methodological reasons, prior research has predominantly quantified gluconeogenesis after an overnight fast. However, it would be advantageous to measure gluconeogenesis shortly after a meal to understand the effect of dietary interventions on glucose homeostasis. Lastly, **Chapter 4** summarises the findings of this thesis and outlines the future perspectives of this work.

Associations, controlled trials, and dietary policy: A narrative literature review of carbohydrate restricted diets

Type 2 diabetes mellitus (T2D) is a complex disease that is believed to result from a myriad of genetic, environmental, and lifestyle factors that interact over many decades. The disease is characterized by elevated blood glucose concentrations and is strongly associated with obesity and cardiovascular disease [1]. The primary goals of standard treatment are to reduce weight in overweight or obese patients and to reduce hyperglycaemia to within an individualised target range, usually HbA1c below 7%, via lifestyle changes and glucose lowering medications [2,3]. T2D is often described as a chronic progressive disease, in which glycaemic control and health are expected to decline progressively even in persons treated with standard therapy [3,4]. Over the past few decades, dietary guidelines for T2D have typically recommended calorie restriction for weight loss, reduced consumption of total fat, saturated fat, red and processed meats, and simple sugars, and the increased consumption of wholegrains, complex carbohydrates, fruits, and vegetables [5,6].

In recent years, there has been growing interest in carbohydrate restricted diets for the management of T2D. Remission from T2D with standard treatment is rare [7] and severe carbohydrate restriction is showing potential for not only delaying the progression of T2D but also reversing the disease and related conditions [8]. However, the totality of the evidence is ambiguous and opinion on carbohydrate restriction ranges from promoting it as first-line treatment for T2D [9] to suggesting that it may be actively harmful to patients [10]. Typically, carbohydrate restricted diets recommend increased consumption of animal fats, place no limits on saturated or total fat intake, and limit the consumption of wholegrains, complex carbohydrates, fruits, and starchy vegetables. These recommendations are at odds with many official dietary guidelines [11]. Additionally, due to the strong connection between T2D and cardiovascular disease, and the promotion of the diet-heart and lipid hypotheses which hold that dietary fat is an important cause of heart disease through its action in raising blood cholesterol concentrations, physicians are understandably cautious about prescribing high-fat diets for T2D patients [12,13]. The nutritional adequacy and sustainability of carbohydrate restricted diets, especially those very low in carbohydrate, are also areas of controversy [10,14,15].

Randomised controlled trials (RCTs) and prospective cohort studies are the two main lines of evidence used to evaluate the efficacy and safety of carbohydrate restriction for T2D patients [16-18]. These designs are considered the highest level of evidence from intervention and observational study designs, respectively. However, both of these study designs have major limitations when it

comes to investigating the role of nutrition in chronic lifestyle diseases. Results from controlled trials and observational studies also tend to be contradictory and opinion on carbohydrate restriction is polarised predominantly according to which of these two designs researchers consider to be less flawed. Those who feel that RCTs fail to address nutritional questions generally prefer prospective cohort data and are therefore antipathetic towards carbohydrate restriction [19-21]. By contrast, those that feel that prospective cohort data is of questionable value tend to favour results from controlled trials and have a more positive outlook towards carbohydrate restriction [9,22,23]. An additional complication is that low fat, high carbohydrate dietary guidelines and national policies have been promoted to the general public since the late 1970s and early 1980s [24]. These guidelines discouraged the consumption of foods high in fat and saturated fat which have been labelled as unhealthy. Despite a lack of strong evidence in formulating these guidelines and policies [24-26], they have had a large impact on how carbohydrate restricted diets are currently perceived by the public, clinicians, and researchers.

There is still a lack of conclusive scientific evidence on the best diets and foods to eat for health. Carbohydrate restricted diets are particularly controversial, even though the available evidence is at least as strong as that for other diets. The interpretation of observational and controlled trial studies, as well as the influence of official dietary policies have all had a role in creating this controversy. This review will discuss the strengths, limitations, and interpretation of nutritional research in order to better understand current perceptions of carbohydrate restricted diets. The literature on carbohydrate restricted diets, within the context of T2D, will be reviewed under three headings: 1) Observational Studies; 2) Controlled Trials; and 3) Dietary Policy. The definitions of carbohydrate restricted diets proposed by Feinman et al (2015)[9] will be used in this review to categorise low carbohydrate diets (Table 1). The words *very low*, *low*, *moderate*, and *high* will be italicized when these specific definitions are referenced.

Table 1. Definitions of different types of low carbohydrate diets		
Category	**Grams per day**	**Percentage of calories**
Very low	< 50	< 10
Low	50 – 130	< 26
Moderate		26 – 45
High		> 45

1.1) Observational Studies

1.1.1) Strengths and limitations

One of the challenges in studying chronic lifestyle diseases, including T2D, is that the disease process occurs over many decades. It also involves a multitude of lifestyle, environmental, and genetic factors, and the relevant aetiological exposures usually occur long before disease outcomes become apparent. For practical reasons, such as cost and the reliance on high levels of participant commitment and compliance, controlled trials are poorly suited to investigating this decades-long disease process [27]. By contrast, observational studies require minimal participant commitment and are relatively cheap. It is therefore possible to recruit very large numbers of participants and to follow them for many decades. The strongest observational study design is the prospective cohort study, in which participants are recruited and examined prior to the development of the outcomes of interest. This study design allows researchers to investigate the relationships between pre-disease dietary exposures and hard disease endpoints, such as T2D diagnosis, cardiovascular events, and all-cause mortality. Prospective cohort studies are currently the only practical way to study the relationship between long-term health outcomes and dietary exposures. For this reason, there is currently a heavy reliance on observational data to inform dietary policies [27-29].

The main limitation of observational studies is a lack of control for confounding factors. Confounders are factors, known or unknown, which are related to both the exposure and the outcome, thereby distorting the measured relationship. Due to the potential for confounding, prospective cohort

studies can reveal the relationship between exposures and outcomes but cannot determine if the relationship is causal or whether it is explained by a common cause [30,31]. Well-designed RCTs can negate this problem by distributing potential confounders evenly between groups. Observational studies have no such control and researchers attempt to reduce the impact of confounders by identifying and adjusting for their effects using statistical models [31]. Additionally, RCTs are considered superior to other study designs because they eliminate many sources of potential bias, either by distributing bias evenly between groups, or due to other design characteristics, such as blinding participants and researchers from knowing which treatment was received. Observational studies are more vulnerable to bias than RCTs and the prospective cohort is considered the strongest observational design because it reduces the likelihood of recall bias, selection bias, and reverse causation, all of which can be a problem in other observational designs [27]. Recall bias is most problematic in retrospective observational designs in which participants who already have experienced a health outcome are asked to recall their prior exposures [32]. In this instance, knowledge of the outcome can alter their perceptions of how participants developed the disease. Selection bias occurs in case-control designs, as investigators can influence the characteristics of the control group. Reverse causation occurs if an 'exposure' is actually caused by the 'outcome' [32]. For example, ex-smokers may appear more likely to die of lung cancer than current smokers, but this association is explained by the cancer diagnosis causing individuals to stop smoking.

Therefore, the strength of observational research is that it enables the study of long-term health outcomes in large numbers of people, which would otherwise not be possible. The weakness is that causation cannot be proven from associations, as potential confounding and bias limits the explanatory power of the data. However, even though association is not causation, there are certain circumstances where observational data may be sufficient for causal relationships to be inferred. An historical example of this is the causal relationship between cigarette smoking and lung cancer, which was established predominantly from observational data. Concluding causation from association requires careful consideration of numerous factors and involves scientific judgement [33], as was outlined in the 1964 Surgeon General's report on smoking [34] and elaborated on by Sir Austin Bradford Hill in 1965 [35]. Hill provided nine 'viewpoints' to consider when assessing the possibility of causation from association. These have become known as Hill's Principles and will be referred to as such throughout this review. The nine principles are:

- Strength
- Consistency
- Specificity
- Temporality
- Biological gradient
- Plausibility
- Experiment
- Analogy
- Coherence

Hill was cautious in his approach and emphasised that none of these principles could prove causation: "What I do not believe is that we can usefully lay down some hard-and-fast rules of evidence that must be obeyed before we accept cause and effect." Rather, they outline ways to think about associational data which may provide insight into the likelihood of causation. The observational literature dealing with nutrition is vast and carbohydrate restriction impacts many aspects of the diet. As such, these nine principles will be used as an outline for the following review of the observational data as it relates to carbohydrate restriction and T2D.

1.1.2) Prospective cohort results

The Seven Countries Study was the first large scale prospective observational study designed specifically to investigate the relationship between nutritional factors and cardiovascular disease [36]. In this ecological study, over 12 000 men were recruited and examined from 1958 to 1964 and followed for 25 years. The study was instrumental in providing supporting data for the *Diet-Heart Hypothesis;* the hypothesis that dietary saturated fat increases serum cholesterol concentrations, which in turn cause heart disease. The Seven Countries Study found that increased saturated fat, dietary cholesterol, and trans fatty acids were associated with increased serum cholesterol concentrations and increased risk of cardiovascular disease [36,37]. The study also associated monounsaturated fatty acids with reduced risk of cardiovascular disease [38] and established the concept of the 'heart-healthy Mediterranean diet'. This was defined as a diet low in cholesterol, saturated fats, red meat and animal fats such as butter and lard, and high in fruits, vegetables, and

legumes [39]. Fish and plant protein sources are favoured over red meats and animal fats, and monounsaturated fatty acids are the preferred source of fat [39]. With respect to T2D, the Seven Countries Study found that high total fat and saturated fat intakes were associated with increased risk, while fish, potatoes, vegetables, and legumes were associated with decreased risk of T2D [40].

Since the Seven Countries Study, there have been a number of considerably larger and more representative prospective cohort studies. Some examples are the original Nurses' Health Study which included over 85 000 women recruited in 1976 (diet data was collected in 1980) and followed-up over 26 years and the Health Professionals' Follow-up Study, which included 44 000 men recruited in 1986 and followed-up over 20 years [41]. The ongoing European Prospective Investigation into Cancer and Nutrition (EPIC) study includes more than 500 000 individuals, recruited between 1993 and 1999, from 10 countries and the Prospective Urban Rural Epidemiology (PURE) study includes 225 000 participants, recruited from 2003 to 2013, from 25 countries and has been running for 12 years [42,43]. Many publications are generated from these and other prospective cohort studies so that there is now a huge body of literature reporting on the associations between dietary exposures and health outcomes.

Several of these publications have reported health outcomes in relation to low carbohydrate diet scores. Diet scores consider the whole diet, as opposed to individual nutrients, and rank diets according to the relative mix of carbohydrate, fat, and protein. Low carbohydrate diet scores have been associated with increased risk of total mortality [44,45] and cardiovascular disease [46] in some studies but no association was found in others [47,48]. Low carbohydrate diet scores have also been associated with an increased risk of T2D in some [49,50] but not all [51] studies. In cases where low carbohydrate diet scores were further categorised as either animal-based or plant-based, animal-based diets were associated with increased risk whereas plant-based low carbohydrate diets were associated with decreased risk of poor health outcomes [49,52,53], including T2D [54]. Associations have also been reported with carbohydrate as a single macronutrient. Recent results from the PURE study found that lower carbohydrate intake was associated with reduced risk of total mortality and no association was found between carbohydrate intake and cardiovascular disease mortality. By contrast, both high (> 70 % of calories) and low (< 40 % of calories) intakes of carbohydrate were associated with an increased risk of mortality by Seidelmann et al. (2018)[53] based on over 15 000 participants from the Atherosclerosis Risk in Communities cohort.

Reduced carbohydrate diets are typically high in dietary fat and therefore, the associations of dietary fats with health outcomes are very relevant to the topic of carbohydrate restriction. The association between saturated fat and coronary heart disease has received particular attention since dietary

goals recommending reduced fat and saturated fat intake were adopted in 1977 in the United States. Some prospective observational studies have since found that high saturated fat intake is associated with increased risk of heart disease [36,55-57] but recent publications and meta-analyses suggest that there is no association [58-60]. Dietary fats have also been implicated in the development of T2D. Increased total fat intakes have been associated with increased risk of T2D in a few studies [61,62] but a more common finding was that replacing trans fatty acids or saturated fatty acids with polyunsaturated fatty acids was associated with reduced risk of T2D [61-64], although these relationships were not always present after adjusting for body mass index [62,63].

There are many other nutritional epidemiologic associations that are relevant to a discussion of the health risks and benefits on low carbohydrate diets. Some examples include: increased risk of cardiovascular disease mortality and total mortality associated with egg consumption [65]; increased risk of colorectal cancer, heart disease, and obesity associated with red and processed meat consumption [50,62,66-68]; reduced risk of cardiovascular disease, total mortality, cancer, and T2D with the consumption of fruits, vegetables, wholegrains, and dietary fibre [46,69-75]; and reduced risk of negative health outcomes with the consumption of nuts, nut butters, and dairy [76-78]. There are many more associations in the literature and this overwhelming volume of work includes many publications that cover all foods, nutrients, vitamins, and minerals.

For the reasons discussed above in *Section 1.1*, associations cannot be assumed to represent cause and effect relationships and interpretation is required. The remainder of this section will consider the interpretation of nutritional observational data using Hill's nine principles to guide the review.

1.1.3) Strength

In prospective cohort studies, the strength of an association is measured by relative risk - the ratio of the probability of an outcome occurring at an assigned reference level of exposure to the probability of it occurring at a higher/lower level of exposure [79]. Hazard ratios are similar to relative risk but consider the rate at which outcomes occur over time as opposed to the risk of an outcome at a specific point in time. Whether reported as relative risk or hazard ratios, the strength of an association can be evaluated similarly. There are no firm criteria as to what constitutes *weak*, *moderate*, or *strong* associations and a range of different values have been proposed [80-84]. However, there seems to be some consensus that a minimum of a 2-fold increase (i.e. relative risk value of 2.0 or 100 % increased risk) or decrease (relative risk value of 0.5 or 100 % decreased risk) in relative risk would constitute a *strong* association, and this is the criteria used by the Grading of

Recommendations Assessment, Development and Evaluation (GRADE) approach [81]. Historical examples of *strong* associations of lifestyle factors to disease endpoints include the 6 to 30-times increased risk of lung cancer in heavy cigarette smokers and the over 6-times increased risk of liver cirrhosis with heavy alcohol consumption [85]. By contrast, relative risks greater than 2.0 are very rare in nutritional prospective cohort studies. Indeed, the field is characterised by associations which rarely exceed 1.5, and the majority are *weak*, ranging from 0.8 to 1.2 [82,86-88].

All of the nutritional associations outlined above in section 1.2.2 are *weak* and the relative risks (with 95 % confidence intervals) are rarely outside the range of 0.8 – 1.2. For example, the strength of the association between lower carbohydrate intake (< 40 % of calories) and total mortality reported by Seidelmann et al (2018)[53] was 1.20 with a 95 % confidence interval (CI) of 1.09 – 1.32. This could also be expressed as a 20 % increased risk of death from any cause in participants who reported carbohydrate intakes less than 40 % of total calories. The association between increased carbohydrate intake and total mortality in the PURE study was 1.28; 95 % CI 1.12 – 1.46 and the association between increased saturated fat intake and total mortality was 0.86; 95 % CI 0.76 – 0.99. The relative risk of T2D with a high animal protein and fat diet was reported by de Koning et al. (2011) [50] as 1.37; 95 % CI 1.20 – 1.58, which was reduced to 1.11; 95% CI 0.95 – 1.30 after adjustment for red and processed meat. Many better-studied nutritional associations are even weaker, such as the reduced risk of cancer with increased fruit and vegetable intake, which was 0.97; 95 % CI 0.96 – 0.99 from the EPIC study [89]. Fruit consumption was associated with a 0.98; 95 % CI 96 – 0.99 decreased risk of T2D in a meta-analysis of three cohorts containing over 187 000 participants [90]. Increased vegetable consumption was associated with a 0.90; 95 % CI 0.80 – 1.01 reduced risk of T2D in a meta-analysis containing over 400 000 participants [91].

According to Hill, *strong* associations are one of the most important factors which can strengthen an argument in favour of a causal relationship. There have been some *strong* associations reported in nutritional observational studies. However, these associations are usually from smaller studies that used case-control or retrospective study designs. The vast majority of *strong* and *moderate* nutritional associations have either not been replicated or were found to be *weak* in prospective cohorts [87,92]. Further, *strong* and *moderate* nutritional associations can lead to absurd conclusions if interpreted literally as causation. Ioannidis (2018)[88] provides the example of the finding that eating 12 hazelnuts daily would extend life expectancy by 12 years from a baseline 80 years [88]. For this reason, the role of strength in evaluating nutritional associations may actually be the opposite of what Hill proposed. Rather than strengthening the argument in favour of causation, *strong* nutritional associations raise concerns over bias and/or error [87,93]. By contrast, *weak* and *very weak*

associations are more likely to represent a realistic causal relationship and have become a necessary requirement to take nutritional associations seriously [79]. However, *weak* associations also increase the possibility that even small amounts of bias and/or confounding could reasonably explain the measured effects [27,86,87,94,95]. For this reason, nutritional associations are particularly vulnerable to the effects of confounding, bias, and/or measurement error [82,96,97]. Importantly, the reason that *strong* associations are rare, and *weak* and *very weak* associations are the expected norm is an intrinsic property of the field. This will be discussed in more detail later in this section under *specificity*.

1.1.4) Consistency

An association is consistent if it is repeatedly observed in studies by different personnel, laboratories, and settings. Consistency strengthens the argument for a causal relationship as different laboratories and experimental designs are less likely to be exposed to the same biases [98,99]. Consistency is a challenge to nutritional observational research as there tends to be large heterogeneity in findings [82]. Early examples of heterogeneity are the 'paradoxes' identified in the Seven Countries Study, where some countries had similarly high saturated fat intakes but very different rates of heart disease [100,101]. The association between saturated fat intake and health outcomes is one of the most studied nutritional associations. A relatively strong association with negative health outcomes was initially identified from small observational studies; early prospective cohort studies confirmed the association but the strength of the association was weaker; and as more evidence has accumulated over time, the association between saturated fat intake and negative health outcomes has disappeared [58,60,102-104]. This is the predominant trend in nutritional observational research, especially if associations were *strong* or *moderate* when they were first reported. Health associations with many foods and nutrients have diminished, reversed, or disappeared with increased data and synthesis into meta-analyses [31,87,105-111].

Some of the factors which contribute to inconsistencies in observational research may relate to genuine methodological or regional differences [98]. For example, different studies often use different dietary instruments and other data collection tools. This can lead to heterogeneity and make it difficult to compare results between studies. Differences in regions and populations will also make it difficult to collect data in a homogenous way and it becomes challenging to differentiate between genuine differences between populations and bias related to regional differences. The same applies to gender differences. Some of the heterogeneity in findings is likely due to genuine physiological

differences between genders but there may also be differences in the way men and women are examined and/or answer questionnaires. However, as with the requirement for *weak* and *very weak* associations discussed above, the reason for the lack of consistency in nutritional epidemiology is largely an intrinsic property of the field. This is considered next when discussing Hill's third principle: *Specificity*.

1.1.5) Specificity

Specificity refers to the directness of the relationship between the exposure and the outcome. In general terms, a more specific relationship strengthens an argument in favour of causation as it limits the number of possible confounders and alternative explanations [79]. Hill provides the example of an increased risk of a very rare cancer in workers in the chemical processes used in nickel refineries. The uniqueness of the exposure and the rarity of the disease in the general population made this a very specific association which therefore is more likely to represent causation. The relationship between cigarette smoking and lung cancer is also specific, in that it is a *strong*, consistent association compared to the *weak* and less consistent associations of cigarette smoking with other cancers.

In nutritional research, the exposures (diet) and the outcomes (T2D, cardiovascular disease, and death) are both very broad and complex. In terms of the exposures, everybody has to eat and everybody therefore has some level of exposure to the majority of dietary constituents. Nutritional exposures are also highly interrelated and separating out the individual constituents of the diet, such as saturated fat, for example, is difficult, if not impossible [63,87,98,112]. For example, removing saturated fat from the diet requires that the lost calories must be replaced by calories from another foodstuff. If this is associated with an adverse or a beneficial outcome, it is not possible to conclude whether it was the removal of one foodstuff or the addition of another that produced the measured outcome. There are also many other factors which have important effects on how food and nutrients affect health. These include the combinations in which foods and nutrients are eaten and the various methods used for cooking and preparation of meals. Additionally, factors specific to individual participants affect how dietary compounds are absorbed and metabolised, and there is variation in the neurohormonal response of individuals to different foods. Many nutrients can also be synthesised within the body if dietary intake is lacking and threshold effects, where a wide range of intakes above a low minimum threshold is sufficient to prevent obvious health problems, are

common for most vitamins and minerals. All of this creates a 'biological action package' which is highly interrelated and complex [27,113].

The problem of untangling the interrelated web of foods and nutrients is further compounded by the difficulties involved in accurately measuring dietary intake in the first place [27,109,114-116]. The food frequency questionnaire (FFQ) is the most commonly used tool in nutritional epidemiologic research and it is notoriously poor at quantifying what subjects actually eat [113,117]. The FFQ asks participants to report on how often they have eaten portions of listed foods over a certain period, usually the past year. It relies on participants remembering and estimating the foods and quantities that they had eaten during this period. Apart from relying on recall, one of the problems is that quantities are often difficult to accurately report as portion sizes for some foods can be unintuitive (e.g. cups of broccoli versus heads of broccoli). There is also the assumption that responses to a single FFQ reflect eating behaviours over the past year and that they are representative of all future behaviours. Even if the diet was remembered and reported accurately in the FFQs, nutrient databases are often limited or unavailable and factors such as food quality and seasonality are not considered [27,98]. The food environment has experienced a rapid change over the past few decades and it is questionable whether FFQs completed in the prior decades have external validity to the current food climate. For example, data for the Seven Countries Study was collected from 1958 to 1964 and it is questionable whether these data are relevant to the choices facing consumers over 50 years later [118].

The outcomes of nutritional observational research are also non-specific. Lifestyle diseases such as cardiovascular disease, cancer, and T2D are complex conditions that are prevalent in society and which have multiple poorly-understood and interrelated aetiologies. Additionally, many of the hard disease endpoints studied in prospective cohort studies are interrelated and strongly associated with each other, such as T2D and cardiovascular disease. These endpoints are also associated with the cluster of health markers that comprise the metabolic syndrome and it is these health markers that are predominantly studied in short-term RCTs. The way in which diet exerts its effects on the body is extremely complex, as it is the cumulative effects of tens of thousands of individual physiological interactions with food over decades that determines future health or ill-health. The transition from a healthy metabolic response after a meal to an unhealthy response is a progressive process and isolating the points at which damage was done by food is not possible. These complex health conditions are also not isolated to the failure of a single organ or system within the body but the progressive deterioration of many related systems. T2D affects the whole body and the liver, pancreas, skeletal muscle, heart, mitochondria, adipose tissue, brain, and more, which are all altered

in various ways over time. Given the whole-body nature of these diseases, it is unlikely that the pathways and exposures which cause T2D and other diseases are homogenous for all individuals.

The high degree of non-specificity in the potential nutritional effects on health introduces some further methodological challenges. Firstly, there are a large number of potential confounding variables and lifestyle factors which could conceivably contribute to the development of T2D and/or which would be associated with the way people eat. Some confounders may be easy to identify and measure accurately, such as age and smoking. However, many potential confounders are as non-specific and poorly measured as diet, such as physical activity and exercise [27]. There are also many potential confounding factors which go unmeasured, such as sleep, stress, eating behaviours, pollution, pesticide residues in food and water, and the emotional and psychological relationships of participants with food [27]. Additionally, some potential confounders are so recent that little is known about their effects, such as the electromagnetic radiation generated by cell phones and WiFi networks. Taboos such as illegal drug use may also not be reported accurately and lastly, there is the distinct possibility of unknown confounders. As mentioned above, an advantage of *strong* associations is that confounders would need to have large effects in order to significantly alter the measured relationships. However, as nutritional associations are *weak* and *very weak*, these myriad potential confounders could be relevant, even if their effects are small.

A second methodological problem of non-specificity is that it increases the potential for bias in the analyses [40,87,119,120]. Researchers have a large number of exposures, outcomes, participant subgroups, confounders, models, and statistical adjustments from which to choose when conducting analyses and selecting which associations to report and emphasise [109,121-123]. The following excerpt from Miller et al (2010)[109] illustrates this property of nutritional observational studies due to non-specificity: 'In our early case control study of colorectal cancer we found an inverse association for cruciferous vegetables in females for colon cancer, but not for males, but we did not evaluate potential associations for all vegetables or for vegetables combined with fruits. In a case-control study of diet and lung cancer we found a protective effect of vegetables, but not of fruits. The effect of fruits and vegetables upon lung cancer was later evaluated in the EPIC study, where a protective effect of fruits was found, but not of vegetables. A similar association with fruits (and a weaker one with vegetables) was found in the pooling project. However, when these studies were considered by an IARC working group, it was concluded that residual confounding by smoking could not be excluded, in spite of the care taken in all these analyses to adequately adjust for smoking.'

Non-specificity pervades nutrition research and all but eliminates the possibility of establishing robust, consistent associations. The observational research on the health properties of eggs provides

a further illustration of the problem. Eggs contain energy, fat, a mixture of saturated, monounsaturated, and polyunsaturated fatty acids, protein, carbohydrate, cholesterol, choline, and many other vitamins and minerals. Eggs are also seldom eaten in isolation and many of the nutritional elements within eggs are present in these other foods or interact with those in eggs during the digestion, absorption, and metabolism of the egg. Within the literature, all of these individual nutritional constituents have variously been associated with an increased and/or decreased risk of various health outcomes. Unsurprisingly, the associations of egg consumption with health outcomes is also ambiguous. Most recently in a meta-analysis of 6 prospective cohort studies containing 29 615 participants, Zhong et al (2019)[65] reported that each additional half egg eaten per day increased incident cardiovascular disease risk (HR: 1.06; CI 1.03 – 1.10). However, this association is no longer significant after adjustment for dietary cholesterol (0.99; 95 % CI 0.93 – 1.05). By contrast, the results of the Nurses' Health and Health Professionals' Studies, which were conducted in the same country and consisted of approximately 120 000 participants, found no association of increased risk of cardiovascular disease with egg consumption [124]. However, that study found that the subgroup with T2D had increased risk of cardiovascular disease with high egg intake [124]. Similarly, a meta-analysis of nine publications found no increased risk of coronary heart disease with increased egg consumption but reported that the subgroup with T2D had increased risk of coronary heart disease but decreased risk of haemorrhagic stroke with higher egg consumption [125]. On the other hand, over 500 000 participants in a Chinese population had reduced risk of cardiovascular disease with increased egg consumption (HR 0.89; 95% CI 0.87 – 0.92) [126].

The above example illustrates the non-specificity in nutritional epidemiologic research which leads to ambiguity and inconsistencies. This point was highlighted by Ioannidis et al (2016)[87] who demonstrated that 50 % of all possible analyses relating nutritional variables with outcomes from the National Health and Nutrition Examination Survey (NHANES) data would produce statistically significant results. Non-specificity may therefore be the most important of Hill's principles in evaluating whether nutritional associations represent causation. Non-specificity makes it unlikely that meaningful associations can be elucidated from the data. Indeed, the underlying assumption that specific food choices can be linked to specific diseases may ultimately be flawed [88].

1.1.6) Temporality

In order for an association to be causal, the exposure must occur before the onset of the disease. Reverse causality is the situation where the 'outcome' actually explains the 'exposure' and it can be

problematic in retrospective and case-control designs. The prospective cohort design theoretically mitigates the problem of reverse causation by recruiting and examining participants prior to the development of any outcomes of interest [32]. Exposures are therefore guaranteed to have occurred before the outcomes. However, the requirement of temporality cannot be completely fulfilled in nutritional epidemiology. For one, many nutritional associations were initially established via observational research designs other than prospective cohorts [24,87,109,127]. The knowledge of these associations could have introduced various biases into the designs, data collection, and analyses of the prospective cohort studies that followed [87]. Even small amounts of bias could be sufficient to increase the likelihood of confirming the association in prospective cohort studies. Another possibility that cannot be completely ignored is that there may already be differences between participants 10 or 20 years before T2D diagnosis that would explain both dietary and disease outcomes, even though these differences are unknown and/or cannot be measured. Participants with the outcomes of interest are excluded from prospective cohort studies but given the lengthy disease processes, there are almost certainly participants at different stages of disease development that are included in the study. The prospective design may therefore not be sufficient to completely eliminate all possibility of reverse causation.

A more pervasive challenge to temporality is that advice consistent with the 1977 *Dietary Goals for the United States* has been promoted and taught to the general public. This creates an interesting problem, as the promotion of certain foods and behaviours as 'healthy' has very likely influenced beliefs and behaviours in the cohorts being studied [27]. Additionally, health-conscious people would have been more likely to adopt these behaviours, more likely to adopt other 'healthy' behaviours, and more likely to be healthier than individuals who completely ignored these guidelines [27]. It is therefore possible that results from post-1977 prospective cohort studies simply reflect the nutritional advice which was promoted as healthy in the first place. The promotion of advice could also have affected the accuracy of diet reporting, as health-conscious people may have underreported what they understood to be less desirable eating behaviours and overreported 'healthy' behaviours [93]. The promotion of dietary guidelines to the public in the absence of strong evidence would also have increased the likelihood of confirmation and publication bias by researchers, as results concordant with the guidelines would have been received more favourably and the reputations of many researchers would have been linked to the dietary guidelines being proved correct [128,129].

1.1.7) Biological gradient

By biological gradient, Hill was referring to a dose-response relationship between an exposure and an outcome. Distinct biological gradients are rarely observed in nutritional epidemiology as there is usually overlap in the 95 % confidence intervals of several different levels of intake. Relative risks are therefore most commonly reported for the differences in the extreme (low versus high) levels of intake. One interpretation of this principle is that in nutrition, a statistically significant linear or otherwise increasing trend would strengthen the argument in favour of causation [82]. However, it is common for a statistically significant trends to exist despite no apparent trend at the different levels of exposure, especially with very large sample sizes. For example, the quartiles for the association between whole grain intake and T2D reported by Kyro et al (2018)[75] were 1.00 (reference); 0.93 (95% CI 0.84 – 1.02); 0.97 (95% CI 0.88 – 1.06); and 0.78 (95% CI 0.70 – 0.86). The p value for a linear trend was < 0.0001 but the 3rd quartile had a higher relative risk than the second and only the fourth quartile had 95 % confidence intervals that were different to the reference value. A possible contributing factor for the lack of biological gradients in nutrition research is classification error. This occurs when measurement error or poor dietary reporting results in participants being placed in the wrong quartile of exposure [82]. Sometimes statistically significant U-shaped and J-shaped models provide better fits for certain associations than do linear models. This was the case for the association between carbohydrate intake and total mortality reported by Seidelmann et al (2018)[53]. However, the majority of the 95 % confidence interval of the U-shaped curves fitted in this study were not different to a hazard ratio of 1.0. Searching for dose-response patterns and fitting models to the data potentially increases the risk of bias as a variety of models can be fitted [87,94,108,130]. Whether *biological gradient* is an important factor for causation in nutrition is debatable but weak associations, inconsistencies, and the non-specificity of the field combine such that clear biological gradients are not found in nutritional epidemiology.

1.1.8) Plausibility

Plausibility refers to whether there are plausible biological mechanisms by which the exposure could cause the outcome. Hill was aware that knowledge of mechanisms is limited by the current level of scientific advancement and that a lack of a plausible mechanism does not rule out causation. On the other hand, general acceptance of a plausible mechanism may turn out to be entirely misleading. With the large amount of nutrition research available today, there are no shortages of proposed

mechanisms from animal, genetic, *in vitro*, and other scientific studies. Biological plausibility is probably the least useful of Hill's principles with respect to nutritional epidemiology, as there are proposed mechanisms for and against carbohydrate restriction. When evaluating nutritional observations in the context of potential mechanisms, *post hoc* justifications should not hold the same evidentiary status as *a priori* hypotheses [82]. This may be relevant in the evaluation of the *Diet-Heart Hypothesis*, which made the *a priori* prediction that saturated fat intake would be closely associated with the risk of heart disease.

1.1.9) Coherence

According to Hill, a cause and effect interpretation 'should not seriously conflict with the generally known facts of the natural history and biology of the disease.' He provides the example of the 'histopathological evidence from the bronchial epithelium of smokers and the isolation from cigarette smoke of factors carcinogenic for the skin of laboratory animals' as coherent with the association between smoking and lung cancer. Whereas consistency refers to the reproducibility of findings and the results of similar research done in different settings and laboratories, coherence considers whether other types of research contradict or support the associations. The enormous volume of published literature in genetics, cell cultures, animal studies, etc makes this a slightly ambiguous principle as there are likely supporting and contradicting studies for any hypothesis [87]. However, short-term dietary controlled trials are particularly relevant to the hypotheses generated by nutritional epidemiology.

Results from controlled trials tend to contradict many established nutritional observational findings [27,131]. The association between dietary fat and increased risk of T2D is a particularly pertinent example of this incoherence. There have been many RCTs examining various forms of low carbohydrate high fat diets and meta-analyses of these trials report that intervention diets are either similar or superior to lower-fat control diets at improving the symptoms and health markers of T2D [132-136]. Recent research is also finding potential for T2D remission with *very low* carbohydrate diets. Participants in an open-label controlled trial were instructed to follow a *very low* carbohydrate diet which targeted a daily carbohydrate intake below 30 g per day and to eat sufficient dietary fat to achieve satiety [137,138]. There was no restriction placed on saturated fat intake. After two years of following this intervention, more than half the participants had improved their T2D to the point that they met the T2D remission criteria of the study [139]. Controlled trials demonstrate that low carbohydrate high fat diets are, at worst, equivalent to control diets and are often superior at

improving health markers of T2D [132-136]. Further, RCTs demonstrate that these diets are safe over the short-term (up to 2 years) and there are no known adverse effects compared to control diets. Published clinical and case reports from practicing clinicians also tend to support carbohydrate restriction [140-147]. These reports do not prove that carbohydrate restriction is safe and effective but they are incoherent with the observational associations from nutritional epidemiological studies.

The association between dietary saturated fat, cholesterol, and total fat with negative health outcomes was at the core of the *Diet-Heart Hypothesis* and underlies almost four decades of dietary policies promoting low fat eating patterns to the public [103,104]. However, results from RCTs investigating the impact of low carbohydrate high fat diets on health markers of cardiovascular disease risk are also not coherent with this hypothesis. Whereas the *Diet-Heart Hypothesis* was based on the association of serum cholesterol with heart disease, the current understanding of the relationship between dietary fat and cardiovascular risk factors is much more complex [102,148,149]. The basic premise that dietary composition can alter serum cholesterol concentrations has been proven true using a variety of different experimental methods, including very-tightly controlled metabolic ward studies [150]. Dietary RCTs investigating low carbohydrate diets demonstrate that low carbohydrate diets compared to control diets have the following effects on blood lipid profiles: blood triglyceride concentrations are reduced; HDL-cholesterol is increased; LDL-cholesterol is variable; total cholesterol is variable; the total cholesterol to HDL ratio is decreased; and LDL particle size is increased [151-158]. Therefore, low carbohydrate high fat diets induce some objective improvements in lipid profiles, such as HDL and triglyceride concentrations, total cholesterol to HDL ratio, and LDL particle size. However, the potential for increased LDL- and total cholesterol concentrations is an area of concern for cardiovascular disease risk for some authors [10,14] but not others [159].

It becomes more complicated when other aspects of insulin resistance and metabolic syndrome are considered in the determination of cardiovascular disease risk, as meta-analyses of RCTs show that low carbohydrate diets are equivalent or superior to (higher carbohydrate) control diets for weight loss, systolic and diastolic blood pressure, fasting blood glucose concentrations, and HbA1c [132-134,151,160,161]. Therefore, controlled trials show that low carbohydrate high fat diets improve many of the health markers considered to predict future risk of cardiovascular disease, including aspects of the lipid profile, but do not reliably improve total cholesterol or LDL-cholesterol concentrations. The argument has been made that the net changes in cardiovascular disease risk are positive, although this remains a controversial opinion [152,162]. However, the above changes in cardiovascular disease risk factors highlights the lack of coherence between observational and controlled studies. A

coherent finding would have been that low carbohydrate high fat diets cause a clear and objective deterioration in all markers of cardiovascular disease risk compared to lower-fat control diets, not that a majority of health markers either stay the same or improve, with the exception of LDL and total cholesterol concentrations.

Incoherence is also apparent in RCTs that have examined meat consumption. A systematic review and meta-analysis of red meat consumption in 36 randomised controlled trials found that higher consumption of red meat had no effect on LDL cholesterol, HDL cholesterol, apolipoproteins A1 and B concentrations, or on blood pressure [163]. There was heterogeneity in the included studies and red meat improved health markers in some subgroup analyses but produce detrimental outcomes in others. Once again, these trials do not prove that red meat consumption is healthy or that it does not contribute to disease, but the lack of a deterioration of health markers, and in some cases the beneficial effects of red meat, is incoherent with the hypotheses that animal-based diets cause negative health outcomes.

Even when taken to the extreme of a 100 % meat diet, there is no evidence of harm from case-study interventions. Two Arctic explorers, Vilhjalmur Stefansson and Dr. Karsten Anderson, famously spent 1 year eating a 100 % meat diet in a controlled environment [164-167]. The diets eaten during this experiment consisted entirely of animal meat and fat. Fatty cuts and organ meats were commonly eaten and fat comprised 80 % of energy intake. Rather than the expected decline in health, researchers found no health deterioration, noting normal kidney and liver function, no signs of nutrient deficiency, decreased blood pressure in one man, and some weight loss. After 1 year the two subjects were "mentally alert, physically active, and showed no specific physical changes in any system of the body."[165] Researchers concluded: "It seems that man can live on a meat diet without physical deterioration occurring and that the use of meat in a general diet is not the cause of all the harmful effects which have been ascribed to it."[164]

There are several modern examples of case-studies using a similar diet - a 100 % meat diet with a composition of roughly 80 % fat and 20 % protein [168-176]. In these case-studies, participants had been following this diet for approximately 2 years and no negative health effects were detected except for increased LDL-cholesterol and total cholesterol concentrations in some cases. Additionally, many health markers improved and patients experienced improvements in a number of serious health conditions such as type 1 diabetes, Crohn's disease, epilepsy, cancer, and T2D. A 100 % meat diet containing 80 % of energy from fat is not commonly studied and to my knowledge, there are no published case-reports or intervention studies that have demonstrated any ill effects of such a diet. These examples of all-meat diets do not prove that such a diet is healthy or that it does not cause ill-

health over the long-term. However, they are at odds with nutritional epidemiological data which associates ill-health with animal-based diets. Coherent data would have established a clear deterioration in health markers of cardiovascular disease, T2D, and cancer after following such a diet.

Dietary controlled trials have many limitations, the largest of which is that intermediary health markers are studied rather than long-term (hard) disease endpoints like death or the development of specific chronic diseases. For this reason, the interpretation of results from RCTs is ambiguous. The *Controlled trial* section of this review will provide more details on RCT evidence for carbohydrate restriction in T2D. It is also noteworthy that genuine *low* and *very low* carbohydrate diets are very rare in the general population. The lowest categories of carbohydrate intake in the majority of nutritional epidemiology studies is around 30 - 40 % of intake and sometimes it is higher. At best this would fit the definition of *moderate* carbohydrate restriction, whereas many controlled trials investigate diets that would be classified as *low* or *very low* carbohydrate and which have been designed by researchers to meet certain nutritional standards. The lack of coherence between nutritional epidemiology and clinical trials is very important in understanding the controversy in nutrition as it relates to low carbohydrate diets.

1.1.10) Experiment

Experiment refers to the testing of nutritional associations with an experimental design from which causation can be concluded. It was mentioned above that short-term RCTs contradict many nutritional associations. However, a major limitation of these experimental trials was that they were not testing the same hypotheses as were generated by the prospective cohort studies. The short-term duration of these studies meant that health markers were used as proxies, rather than actual hard disease endpoints. This limits the conclusions of these RCTs to the short-term (~ 2 years) [133]. There have been several attempts at long-term RCTs to directly examine the same exposures and outcomes as the prospective cohort studies. These included the Women's Health Initiative Dietary Modification Trial with a 7.5 year follow up and the Look AHEAD (Action for Health in Diabetes) trial with a 9.6 year follow up [177,178]. However, both studies failed to show significant health improvements with dietary modification. The methodological challenges involved in conducting long-term lifestyle RCTs may have contributed to these outcomes. Direct RCT testing of the long-term relationships which are examined in nutritional epidemiology may therefore not be possible [179,180], which is why nutritional epidemiology is regarded by some as the only approach available to

study these long-term relationships [120,181]. The methodological limitations that potentially preclude definitive findings from long-term RCTs are discussed in the *Controlled trials* sections.

1.1.11) Analogy

Analogy is the consideration of evidence from within the literature which could provide guidance on how to interpret the observed associations [182]. Care should be taken with this principle as analogy may only be limited by the imaginations of the researchers. However, a very useful analogy in understanding the state of nutrition research may be that of smoking. It is absolutely clear that nutritional epidemiology differs from the epidemiology of cigarette smoking in a number of key ways. Most importantly, there is not a single nutritional association that is analogous to the association between cigarette smoking and lung diseases. This association is *strong*, consistent, specific, and coherent with other available evidence [35,183]. The conclusion of causation from association in the 1964 Surgeon General's report was uncontroversial and set the foundation for government intervention based on the scientific findings in a mature field. By contrast, nutritional observations are *weak* and *very weak*, inconsistent, non-specific, and incoherent with controlled trial data. There is no consensus today amongst scientists on how to interpret the data which is why nutrition is a field mired in controversy. There was even less known about nutrition back in 1977 when government policy was adopted to alter diets in accordance with a particular scientific theory. The impact of policies and guidelines on dietary research are discussed in section 1.4 in this review. All of these characteristics which made cigarette smoking and lung diseases a success story for epidemiology are absent from nutritional epidemiology. It is therefore unsurprising that scientists express very little agreement on how to interpret nutritional data.

1.1.12) Observational studies summary

This section provided a broad overview of the nutritional observational literature as it relates to carbohydrate restriction and T2D. Results from prospective cohort studies have associated low carbohydrate diet scores with increased risk of negative long-term health outcomes. Additionally, many foods associated with carbohydrate restricted diets have been associated with negative health outcomes, and foods eliminated with carbohydrate restricted diets are associated with positive health outcomes. Association does not prove causation and therefore, interpretation of the data is required. The nine principles provided by Hill were used to guide this review and are summarised in Table 2. The lack of *specificity* in nutritional epidemiologic research is probably the overriding

characteristic of the field and it explains to a large extent why *weak* associations and inconsistencies are the norm. It also creates a problem in that bias and confounding are almost impossible to eliminate. Diet is also very difficult to measure accurately and a single FFQ is often used to determine dietary exposures. As nutritional associations are *weak*, even small amounts of bias, confounding, or error could affect the measured associations in a meaningful way. Ultimately, whether Hill's principles are useful, the interpretation of nutritional associations, and the value of nutritional associations in informing clinical and population-level dietary decisions, comes down to subjective evaluation of data. As an analogy, nothing in nutritional epidemiology is anywhere near as conclusive as the effects of smoking on lung cancer. The ambiguity in nutritional research is reflected in the diversity of viewpoints and divisiveness in the literature. This is particularly apparent with respect to carbohydrate restriction as researchers often hold completely opposite views based on their evaluation of the same data. The incoherence of observational studies with findings from controlled trials is a large component of this continuing intellectual division in low carbohydrate nutrition.

Table 2. Summary of Hill's Principles as each relates to nutritional epidemiology	
Strength	Associations are *weak* and *very weak*. *Strong* and *medium* associations suggest potential bias and/or error.
Consistency	Nutritional associations are heterogeneous. Some inconsistencies may be due to methods and regional differences but heterogeneity is also intrinsic to the field.
Specificity	Dietary exposures and chronic lifestyle diseases are very non-specific. This principle is likely the reason that *strong*, consistent associations do not occur in nutrition.
Temporality	Dietary advice has been promoted to the public for approximately four decades. The provision of this advice has very likely biased results of prospective cohort studies since 1977.
Biological gradient	Rarely observed. Most often only the extreme categories of intakes have 95 % confidence intervals that are different from each other.
Plausibility	Potentially not that useful in nutrition. The volume of available literature is enormous and plausible mechanisms abound.
Coherence	Observation results and controlled trial results are often incoherent. This is particularly true for low carbohydrate high fat diets and T2D.
Experiment	Long-term RCTs have failed to provide conclusive evidence for or against nutritional observations. Conclusive long-term RCTs may not be feasible.
Analogy	Nutritional epidemiology is not analogous to cigarette smoking in which observational data was accepted to have proven that smoking causes lung disease. Rather, observational data is ambiguous and this accounts for the large number of diverse viewpoints in nutrition.

1.2) Controlled Trials

1.2.1) Strengths and limitations

RCTs and meta-analyses of RCTs are considered the highest level of evidence in biomedical research. The strength of the RCT design is that, if done properly, potential sources of bias and confounding are equally distributed between groups so that the effects of the intervention are isolated. This allows a causal link to be established between the intervention and the outcomes. RCTs are well suited to clinical drug research but have some major limitations for dietary and lifestyle interventions [184]. Perhaps the biggest challenge with dietary RCTs is the high level of motivation and commitment required from participants. Indeed, the participants become fully responsible for the successful implementation of an intervention which alters their behaviours and choices throughout the day [185]. Participants in a family environment face the additional challenge of eating with, and sometimes cooking for, individuals following completely different diets.

The substantial commitment required by participants has implications for compliance [184]. Participants who agree to the informed consent, while committed to several aspects of such an intervention, may have no ability or intention to follow every aspect of the intervention with equal vigour. This is particularly true in instances where the whole diet is overhauled and/or where many aspects of the intervention differ substantially from habitual diets. Often interventions also contain other aspects of behaviour change, such as recommendations to exercise, which further increases demands on participants [185]. Participants may also compensate for diet and/or behaviour changes in unanticipated ways and adapt their diets and other behaviours to accommodate their preferences within what they perceive to be the rules of the intervention. The high level of motivation required by participants typically results in declining levels of adherence to both intervention and control groups over time [186]. Researchers can attempt to maintain high levels of compliance via regular contact and support but this introduces potential biases when interpreting the results. In general, there is a trade-off between the explanatory power of a trial versus real-world applicability, or external validity. For example, a metabolic chamber study where all foods are provided gives the researchers total control and the effects of that specific dietary intervention on metabolism can be determined [187]. However, these findings have limited external validity as they lose relevance in a setting in which participants control their own food choices and quantities. With RCTs in which participants are given dietary advice to follow on their own, the effectiveness of the dietary advice can be investigated but it is difficult to isolate the effects of the diet *per se*. Similarly, high levels of researcher engagement may help participants adhere to the prescribed diets and allow for the

examination of the effects of the diet, but it also reduces the external validity of the study as high levels of engagement and support are not usually available in clinical settings.

An additional consequence of participants having to enact lifestyle and dietary interventions is that it is impossible to blind them from whether they were assigned to the intervention or control group [184,187]. A lack of blinding undermines much of the value of randomisation and new sources of bias and confounding are introduced into the experiment [179]. Knowledge of their group assignment could differentially affect participant motivation and behaviour. The majority of participants who volunteer for a dietary RCT would likely have identified the intervention as something from which they feel they would benefit. Participants assigned to the intervention group may struggle for motivation if expected benefits are not realised or if the intervention fails to live up to expectations in other ways. Conversely, participants assigned to the control group may adopt certain aspects of the intervention anyway, a phenomenon known as control group reactivity. This is particularly problematic as all participants would have been exposed to the details of the intervention through the informed consent process prior to randomisation and some aspects of the intervention were likely attractive to the participants.

Another problem with dietary RCTs is that the RCT design is best suited to focused interventions, ideally where only a single factor is different between groups [184]. However, dietary factors are all interrelated and there are also differences in how foods are digested, absorbed, and metabolised, depending on the mixture of foods and nutrients or the 'biological action package' of the diet. In this respect, the non-specificity of nutritional research, which was discussed in relation to observational studies in section 1.2.5, is also very relevant to controlled trials. Due to this property of dietary research, intervention and control diets mostly differ in multiple aspects [185]. For example, a low carbohydrate diet is lower in carbohydrate than a control diet, but it may also be lower in fibre, wholegrains and fruits, and higher in saturated fat, animal products, and dairy. It is very difficult, perhaps impossible, to have only one difference between an intervention and control diet. In recognition of this problem, there has been a shift away from single nutrient research towards food-based dietary patterns [188,189]. This does increase the impact of intervention but helpful, harmful, and neutral components of the changes cannot be determined.

Even if the sole difference between groups was a handful of nuts per day, the diet with the addition of nuts would contain more daily energy intake unless some other dietary constituent was reduced to compensate. Even if it were possible to change only a single factor, a new RCT would have to be planned, run, and analysed for each individual difference, or RCTs would have to contain many different groups to account for all the possible differences in diet. Single vitamin studies come

closest to achieving an ideal RCT study design in nutrition. However, even these studies cannot easily overcome the problem of non-specificity, due to the complexity of how vitamins are metabolised within the body, which often depends on the combinations of other foods and nutrients in the diet and the general nutrient status of the body [187,190,191]. Additionally, for ethical reasons, participants in the control group will have a baseline vitamin intake from food which is sufficient to prevent acute deficiencies [191]. Therefore, any conditions resulting from the differences between the intervention and control groups may take a long time to present and may only occur based on interactions with other nutrient deficiencies or specific food combinations [190,192].

The problem is compounded by difficulties in accurately measuring dietary intake in RCTs [114]. Researchers face many of the same challenges as in observational studies but one advantage is that there are fewer participants which makes it possible to spend the time and effort on diet logbooks and/or 24-hour food recalls [193]. However, these methods, like FFQs, have severe limitations. The biggest problem is that participants can change their dietary behaviour during the logbook and 24-hour recall periods, or even report dishonestly. Participants always know when they are recording logbooks and may therefore follow their intervention prescriptions more closely during these days. The same could happen if participants are aware of when 24-hour diet recalls are going to occur. Most commonly, 24-hour recalls and logbooks are kept in the days preceding check-ups and participants may be more compliant during this period to be seen by the researchers to be following the protocols. Diet logbooks are also very demanding of the participants' time and this can lead to fatigue and a decline in accuracy, especially once the novelty of being involved in research has worn off. Even if the logbooks and 24-hour recalls are an accurate reflection of dietary intake during the study period, nutrient databases have limitations. They are often not specific to the foods in the region being studied, do not account for differences in food quality or seasonality, and may not contain detailed information on all food options.

Results from RCTs are very specific to the exact populations and interventions being studied [187]. This means that RCTs often study diseases in populations that may not be that relevant or at the wrong time in the development of the disease. Studying high-risk populations is one way to allow for hard disease endpoints to be used as outcomes in short-term RCTs. However, these high-risk populations may differ substantially from the general population and the key exposures that led to the development of the disease may have already occurred. Smaller RCTs need to recruit as homogenous a group of participants as possible to increase the power of their studies. Participant selection and exclusion criteria can therefore be very specific, which can further reduce the external validity of a trial. Lastly, RCTs need to be very carefully designed and the protocols are not adaptable

once the experiment has begun [184]. It can take many years to design and approve a protocol and concepts in the study may be outdated by the time the study is under way. There is also the ethical problem of whether to continue with a trial if the intervention shows no benefit (i.e. it is unethical to waste participants' time and funders' money), as was the case with the Look AHEAD trial, or if the intervention does show a benefit (i.e. it is unethical to continue offering inferior advice to the controls), as was the case with the Prevención con Dieta Mediterránea (PREDIMED) trial [178,194].

All of the above factors make it incredibly difficult to design, plan, and implement a dietary RCT with good participant compliance and in which the final results are unambiguous [179]. It is easier for participants to comply with protocols over the short term and it is also much cheaper and less risky from the perspective of the researchers and funding bodies. The vast majority of dietary RCTs are therefore under 1 year in duration and some of the longer trials are 2 years [27]. These short-term trials use health markers as endpoints as the duration of the trials are too short for diseases to progress and hard disease endpoints to develop. There have also been a number of long-term RCTs which did use hard disease endpoints as outcomes. However, for all of the reasons described above, these trials have failed to add clarity to nutritional questions [27]. The Women's Health Initiative Dietary Modification Trial randomised 48 835 postmenopausal women into either a control group or an intervention group that received advice to reduce fat intake and increase vegetable, fruit, and grain intake. After 8.1 years, it was found that "a dietary intervention that reduced total fat intake and increased intakes of vegetables, fruits, and grains did not significantly reduce the risk of CHD, stroke, or CVD in postmenopausal women."[177] The Look AHEAD trial investigated the effects of an Intensive Lifestyle Intervention versus Diabetes Support and Education. The Intensive Lifestyle Intervention focused on calorie restriction and targeted less than 30 % of calories from dietary fat. The trial was stopped early after a median follow-up period of 9.6 years on the basis of futility as there was no significant difference in cardiovascular disease events between groups [178]. Long-term benefits via reductions in hard disease endpoints have never been 'proven' for any dietary patterns [195]. Despite this, these findings are generally not interpreted to mean that lifestyle modification has no impact on disease outcomes [196,197]. Rather, it has been interpreted as demonstrating the methodological challenges with long-term RCTs outlined above [179].

The PREDIMED trial provides another example of how a lack of blinding, the interrelatedness of food and nutrients, and the requirement for focused interventions limits the effectiveness of RCTs for dietary research. Participants were assigned to receive advice on how to follow either a Mediterranean diet with supplemented olive oil, a Mediterranean diet with supplemented mixed nuts, or a low-fat control diet [198]. The trial was stopped early, after 4.8 of 6 years, due to the two

Mediterranean diet groups having a statistically significant reduction in the primary endpoint (composite cardiovascular disease score)[199] and it being deemed unethical to continue with the control group. However, a number of factors make it very difficult to interpret the results [194]. Firstly, the Mediterranean and control diets were quite similar as the study was conducted in a Mediterranean country and the targets of the low-fat dietary advice were not achieved [200]. Therefore, according to the authors: "The main nutrient changes in the Mediterranean-diet groups reflected the fat content and composition of the supplemental foods."[198] Rather than comparing whole diet patterns, the trial may have been investigating the effects of daily supplementation with olive oil or nuts[194]. It is unclear from this study whether the control diet would have performed similarly to the two supplemented Mediterranean diet groups had olive oil or nuts been provided. It is also unclear how Mediterranean dietary advice without the provision of olive oil or nuts would have performed against the un-supplemented control group. Further, it is not possible to determine whether the reported improvements with the supplemented Mediterranean diets were due to the supplementary olive oil and nuts, increased total fat content, increased monounsaturated fatty acid content, or some combination of factors in the intervention diets. It is also unknown how the PREDIMED Mediterranean diets would have performed against different control diets, such as a *very low* or *low* carbohydrate diet. In order to attempt to answer these questions, a PREDIMED equivalent study with one intervention group for each possible dietary comparison, as well as multiple control groups to account for the olive oil and nut supplements, would be required. The above example illustrates many of the limitations of RCTs in dietary research.

1.2.2) RCTs investigating carbohydrate restriction in T2D

There are a relatively large number of RCTs that have investigated the effects of low carbohydrate dietary interventions for the management of T2D. These trials tend to contain around 30 to 300 participants and range from several weeks to over 2 years in duration. There have been seven systematic reviews and meta-analyses since 2017 which have synthesised results from RCTs which investigated the effects of carbohydrate restriction in T2D [132-136,201,202]. Reasons given for this spate of recent systematic reviews are: growing interest in carbohydrate restriction for the management of T2D; recent RCTs not included in earlier reviews; and the contradictory findings of earlier reviews [203-209]. These seven reviews include approximately 55 different RCTs between them and provide a comprehensive overview of the state of the RCT evidence for low carbohydrate diets in T2D. None of the RCTs contained in these reviews had hard disease endpoints as outcomes.

There was agreement in all seven reviews that numerous factors inherent to the underlying RCTs limited the certainty with which conclusions could be made. One of the biggest limitations in synthesising the results into meta-analyses was the wide variation in intervention and control diets between individual RCTs. The definition of what constituted a low carbohydrate diet also differed widely in individual studies. To give an idea of the heterogeneity in RCT study design, of the 55 different RCTs included in the 7 recent systematic reviews, only 1 study met the inclusion criteria and was included in all seven systematic reviews [210]. Additionally, 10 studies were included in meta-analyses as low carbohydrate diets in only a single review. A 'network meta-analysis' by Schwingshackl et al (2018)[211] highlights this heterogeneity in dietary interventions as they attempted to compare the efficacy of nine different named dietary interventions on glycaemic control in T2D. Studies amongst the 55 included in the low carbohydrate systematic reviews are variously categorised by Schwingshackl et al (2018) [211] as low carbohydrate, moderate carbohydrate, high protein, Mediterranean, and low glycaemic index/load. Similarly, a review by Ajala et al (2013)[203] performed a meta-analysis of RCTs on the Mediterranean diet. Two of the three Mediterranean diet RCTs included in this meta-analysis are commonly included in low carbohydrate diet meta-analyses [212,213]. Proponents of multiple different diets for T2D could therefore be using the same RCT evidence but using different names or selecting to emphasise different properties of the diet.

As discussed in section 1.3.1 above, participant adherence is one of the major problems with dietary RCTs. Most commonly, RCTs are incorporated into meta-analyses based on the diet that the researchers intended the participants to eat, regardless of whether their intervention was achieved by participants. The review by Huntriss et al (2018)[132] tried to address this problem by only including studies in which the actual carbohydrate intake in the carbohydrate restricted diets was significantly lower than actual carbohydrate intake in the control group. Several reviews commented on adherence, and there was agreement that adherence to *very low* carbohydrate diets was poorer than either *low* or *moderate* carbohydrate diets [132,133]. It is worth noting that adherence was assessed as the difference between intended and actual intakes, which would have favoured intervention or control diets which were similar to habitual intakes. It is also very difficult to distinguish between whether intrinsic properties of the diet or an ineffectual intervention was responsible for participants' poor adherence. An extreme example of this is Iqbal et al (2010)[214], which was a 24-month RCT in which 70 participants were assigned to follow a *very low* carbohydrate diet of 30 g per day. Only 28 participants completed the study and at no point during the study, did actual carbohydrate intake in the intervention group change from baseline. In fact, after 2 years, carbohydrate intake was reported as higher in the low carbohydrate group than in the low-fat group. As adherence was very poor, the authors concluded that this study 'demonstrates that low-

carbohydrate diets may be difficult to sustain.'[214] A 2-year study which failed to achieve any change in diet in either group, even after 6 months, forms part of the meta-analysis evidence that *very low* carbohydrate diets are difficult to sustain [134].

Protocol variation and poor adherence were noted in all seven systematic reviews and prevented any strong conclusions in the four studies that used GRADE [133,136,201,202]. Risk of bias was assessed in each review [215] and, with the exception of Snorgaard et al. (2017)[136] which assessed the overall risk of bias as low to moderate, the majority of RCTs were assessed as having either high or unclear risk of bias. Some of this risk is unavoidable in dietary and lifestyle RCTs, such as the lack of blinding of participants and study personnel from the interventions. Other factors which increased risk of bias included: poor descriptions of the randomisation sequence and allocation concealment (i.e. that researchers did not know what the next treatment allocation would be); no description of pre-intervention diets; and a lack of blinding of outcome assessors from the intervention. Publication bias was not found to be a problem [133,135]. It was also noted that the studies which had the biggest impact on outcomes were often those with the highest risk of bias [133].

Despite all these sources of heterogeneity, there was agreement in all seven reviews that the carbohydrate restricted intervention was superior to control diets for reducing HbA1c in studies up to 1 year in duration. Additionally, the intervention diets resulted in greater reductions in glucose-lowering medications than control diets, which may have masked some of the differences in glycaemic control between diets. Some reviews analysed subgroups based on duration and found that the superiority of the carbohydrate restricted diets for producing reductions in HbA1c was most pronounced in studies with durations of 2 to 6 months [133,136,202]. By contrast, there was no difference in HbA1c between intervention and control diets in the subgroup of studies which followed up after 1 year. Another subgroup analysis was based on the level of carbohydrate restriction intended by the researchers [134,201]. Here it was found that the largest differences in HbA1c between groups had occurred in the *low* carbohydrate diet category (50 - 130 g/d). These studies were also generally shorter than 1 year and were assessed as high risk of bias. By contrast, *very low* (< 50 g/d) carbohydrate diets were found to be less effective than *low* carbohydrate diets, which was attributed to poor adherence to the diet. Snorgaard et al. (2017)[136] was unique in that they found that the magnitude of carbohydrate restriction that was actually achieved corresponded to greater improvements in HbA1c. Their analysis was conducted in *low* to *moderate* carbohydrate diets over a 3- to 6-month period and excluded some of the *very low* carbohydrate RCTs where actual intakes were not changed from baseline. The other marker of glucose control that was assessed in some reviews was fasting plasma glucose concentrations [135,202]. There was no benefit for the carbohydrate

restricted diets compared to controls in fasting glucose concentrations, even though HbA1c may have improved.

Other health markers which were commonly assessed in these reviews were weight loss, blood pressure, and lipid profiles. Weight loss favoured the carbohydrate restricted diet over the short term (2 - 6 months) but this difference was not apparent at 1 year or longer. A common outcome of individual RCTs was that similar levels of weight loss occurred in both the carbohydrate-restricted and control groups. Often this was by design, as the carbohydrate restricted and control diets were meant to be matched and to produce an equivalent calorie deficit. Sometimes no attempt was made to match energy intakes. Regardless of the intentions of the researchers, a possible confounder of weight-loss results was that energy intakes cannot realistically be controlled in these types of lifestyle interventions.

There was also heterogeneity in starting body weight amongst RCTs, which may have impacted the meta-analyses of weight-loss results. Blood pressure was analysed in several reviews [132-134]. It was noted that improvements in individual RCTs were often similar between carbohydrate-restricted and control diets. Korsmo-Haugen et al (2019)[133] and McArdle et al. (2019)[134] found no benefits of the lower carbohydrate diets in reducing blood pressure but Huntriss et al. (2018)[132] reported lower systolic and diastolic blood pressure in the carbohydrate restricted diets after 1 year compared to control diets. Blood lipid differences between intervention and control diets were often small but there were consistent findings amongst all seven reviews. There were no differences between the carbohydrate restricted and control diets for changes in total or LDL cholesterol concentrations. By contrast, the carbohydrate restricted diets usually resulted in greater increases in HDL cholesterol and larger reductions in triglyceride concentrations than the control diets. A common recommendation made in the individual RCTs was to replace dietary carbohydrate with unsaturated fats and to limit saturated fat intake [132,133]. This was presented as a possible explanation for the finding that changes in total and LDL cholesterol did not differ between carbohydrate-restricted diets and control diets.

The overall picture created by these reviews is that carbohydrate restricted diets may be superior, but are at least as effective, as control diets for improving HbA1c, reducing the need for glucose-lowering medications, lowering blood pressure and blood triglyceride concentrations whilst raising blood HDL cholesterol concentrations in T2D patients. Carbohydrate restriction also had similar effects to control diets for changes in LDL, total cholesterol and fasting blood glucose concentrations. Those RCTs that did report on adverse events found no differences between intervention and control diets. Not one review found the control diets to be superior in any risk

factor, over any duration, or at any level of carbohydrate restriction. Due to the underlying problems with the literature, most reviews concluded that although there may be apparent benefits for carbohydrate restriction in T2D, the overall evidence is uncertain and of low quality. Concern was also expressed over the absence of studies of long-term health outcomes. Additionally, concerns were also raised over the potential for adverse effects and the nutritional adequacy of the low carbohydrate diets, although these concerns were not based on data from the RCTs which were reviewed.

1.2.3) Type 2 Diabetes Remission

Until recently, T2D has been categorized as a lifelong, progressive disease. The rates of remission from T2D based on conventional treatment is very low. A higher rate of T2D remission with bariatric surgery provided one of the first indications that T2D could be reversed. Since then, severe calorie restriction and severe carbohydrate restriction have gained attention as possible dietary strategies to reverse T2D. The potential of a calorie restricted diet containing 600 kcal per day to reverse T2D was confirmed by Lim et al. (2011). In that study fasting plasma glucose concentrations were reduced to non-T2D levels within 1 week in 11 T2D patients [216]. Additionally, these participants had large improvements in body weight, HbA1c, lipid profiles, inflammatory markers, insulin secretion, and insulin sensitivity after 8 weeks. The possibility of remission with calorie restriction was tested in a much larger and longer trial: the open-label, cluster-randomised trial (DiRECT) [217]. This calorie-restricted diet contained approximately 850 kcal per day. After 12 months, 68 of 149 participants (46 %) had achieved T2D remission, defined as HbA1c less than 6.5 % and not taking any diabetes medications [217]. The authors highlighted the role of weight loss in the management of T2D as 86 % of the 36 participants who lost more than 15 kg were in T2D remission. After 2 years, 36 % of participants met the above T2D remission criteria and 11 participants had lost more than 15 kg since baseline [218].

The carbohydrate restriction RCT evidence summarised above suggests some benefit of the diet for managing T2D but it does not demonstrate that remission is a likely outcome. However, potential for remission has been observed based on certain individual responses and medication reductions in controlled trials. As an example, the authors of a clinical trial in 16 obese T2D patients said: “An important issue is the fact that some patients do become completely free of disease as soon as they are presented with a low-carbohydrate option. It is unknown what factors make these persons succeed now despite complete failure in the past.”[219] The potential of severe carbohydrate restriction to induce remission has recently been supported by the results of an open-label

controlled trial which prescribed a *very low* carbohydrate diet for the management of T2D (Virta Health) [138]. The goal of the diet was to raise blood beta-hydroxybutyrate levels to above 0.5 mmol/l; a metabolic state known as nutritional ketosis. This occurs when there are very high rates of whole-body fat mobilisation and oxidation after several days of *very low* carbohydrate intakes. Beta-hydroxybutyrate concentrations at this level would indicate compliance to a diet that is sufficiently low in carbohydrate to induce nutritional ketosis. The exact carbohydrate content of the diet necessary for nutritional ketosis varies according to each individual but less than 50 g of carbohydrate per day is usually advised. Participants in this study were asked to routinely measure beta-hydroxybutyrate. The aim of this biofeedback was to increase the motivation and compliance of participants to the diet [138]. After 1 year, 58 % of participants in the intervention group met the remission definition of HbA1c less than 6.5 % with no T2D medication other than metformin [137]. An average weight loss of 12 % of body weight had been achieved along with large reductions in T2D medications. After 2 years, 54 % of participants met the above T2D reversal criteria and weight loss in the intervention group was 10 % of body weight [139].

Both the DiRECT (850 kcal diet study) and Virta Health studies have some similarities. Both trials identified that compliance is very important and both had a strong focus on sustainability of the intervention by providing regular support to participants. Both trials were open-label in that participants were aware of the intervention they were signing up for rather than randomized into groups. The control groups in both trials received conventional treatment, defined as following best practice care according to current T2D guidelines. The control groups therefore did not receive the same level of contact and support as the intervention groups. Both control groups experienced gradual progression of T2D, as would be considered normal with current best-practice guidelines. One of the differences between these studies was in the types of participants that were enrolled. Virta Health had no selection criteria related to how long participants had T2D, the types of medications they were on, or their starting body weight. By contrast, DiRECT excluded patients with T2D for more than 6 years, if body mass index (BMI) was less than 27, or if patients were taking exogenous insulin.

An important consideration is that both calorie restriction and severe carbohydrate restriction may be operating on similar mechanisms. The 850 kcal diet prescribed by DiRECT and the 600 kcal used by Lim et al. (2001)[216] would have contained 125 g and 70 g of carbohydrate, respectively. Both of these diets would therefore be classified as *low* carbohydrate diets. Additionally, a spontaneous reduction in calorie intake is well described with *low* and *very low* carbohydrate diets [159,220]. It is therefore likely that the patients in the Virta Health study reduced calorie intake and lost weight

based on a reduction in appetite. The mechanisms of health and T2D improvements with severe calorie and carbohydrate restriction may therefore be similar. Some of the likely mechanisms involved with T2D reversal are: reductions in hepatic fat and glycogen content; reduced muscle fat and glycogen content; reduced pancreatic fat; and increased fat oxidation [216,221-223]. All of the above have been shown to occur with calorie restriction and carbohydrate restriction. A focus on severe carbohydrate and calorie restriction for the management of T2D marks a return to treatment guidelines prior to the discovery and synthesis of insulin in the 1920s [224]. Both of these approaches were once standard practice but high carbohydrate diets have dominated the management and treatment of T2D over the past 60 years. There is currently a resurgence in interest in nutritional strategies such as severe calorie restriction and *very low* carbohydrate diets and this is an exciting area of future research due to the potential for T2D remission.

1.2.4) Summary of experimental trials

Results of RCTs investigating carbohydrate restriction for the management of T2D suggest that low carbohydrate diets are at least as effective as control diets for improving short-term markers of health. Carbohydrate restricted diets seem particularly effective at reducing diabetes medication requirements and may also benefit HbA1c, body weight, blood pressure, and lipid profiles over the short-term (~ 6 months) but not long-term (> 2 years). The reduced efficacy of dietary interventions over the long term may be due to reduced compliance and motivation of participants in both the intervention and control groups. Compliance is one of the greatest challenges with dietary RCTs and poor compliance in intervention and control groups contributes towards the ambiguity of RCT data. Recent open-label controlled trials with a strong focus on compliance have demonstrated impressive results in reversing T2D with calorie restriction and *very low* carbohydrate diets. However, the lack of randomisation of the DiRECT and Virta Health studies makes this evidence difficult to evaluate in relation to RCT evidence. The biggest limitation of low carbohydrate RCTs is a lack of long-term data with hard disease endpoints. Concerns over the safety of carbohydrate restriction over the long term have therefore not been addressed. However, historical attempts at long-term RCTs in other dietary patterns have been inconclusive and the efficacy of any particular dietary pattern on hard disease endpoints remains unproven. It is unlikely that this limitation in the available dietary RCT literature can be resolved in the foreseeable future.

1.3) Dietary policy

1.3.1) The Case for Action

There is currently no definitive scientific answer as to what constitutes a healthy diet, for healthy individuals or for the prevention and management of T2D [225]. As discussed earlier in this review, the available data has many limitations: it is unclear whether associations from prospective cohort studies should be interpreted as causation; results from short-term RCTs often contradict findings from prospective cohort studies; and long-term RCTs are not feasible and prior attempts at such trials have failed to add clarity [27,180,226]. Yet, it is clear to all concerned that nutrition has a large impact on health and is perhaps the single most important lifestyle factor in the development and treatment of T2D [227]. Hill recognised that the level of evidence required for scientific certainty may be different from that required to take action. He wrote: "All scientific work is incomplete - whether it be observational or experimental. All scientific work is liable to be upset or modified by advancing knowledge. That does not confer upon us a freedom to ignore the knowledge we already have, or to postpone the action that it appears to demand at a given time."[35]

As with the nine principles described earlier, Hill provided a framework for considering 'The Case for Action'. He suggested that the level of evidence required to take action based on an association depends largely on the consequences of being wrong. He wrote: "Thus on relatively slight evidence we might decide to restrict the use of a drug for early-morning sickness in pregnant women. If we are wrong in deducing causation from association no great harm will be done. The good lady and the pharmaceutical industry will doubtless survive. On fair evidence we might take action on what appears to be an occupational hazard, e.g. we might change from a probably carcinogenic oil to a non-carcinogenic oil in a limited environment and without too much injustice if we are wrong. But we should need very strong evidence before we made people burn a fuel in their homes that they do not like or stop smoking the cigarettes and eating the fats and sugar that they do like."[35]

The last of Hill's examples seems particularly pointed, as at the time momentum was building amongst preeminent epidemiologists to attribute dietary fat and saturated fatty acids as a cause of heart disease via effects on serum cholesterol - i.e. the *Diet-Heart Hypothesis* [25,228-232]. This momentum culminated in the release of *Dietary Goals for the United States* (*Dietary Goals*) in 1977, which set out goals to alter the eating behaviours of the American population [233]. The goals were to: increase carbohydrate consumption to ~48 % of calories; reduce dietary fat intake to ~30 % of calories; decrease saturated fat use to ~10 % of calories; target ~10 % each for mono- and polyunsaturated fats; reduce cholesterol consumption to ~300 mg per day; and limit salt intake to ~5

g/d [233]. The stated impact of these goals on food selection were to: increase consumption of fruits, vegetables and wholegrains; decrease consumption of refined and processed sugars; decrease consumption of animal fats and favour poultry and fish which are lower in saturated fat; choose low-fat or non-fat milk over whole milk; decrease consumption of butter, eggs and other cholesterol-containing foods; and decrease consumption of salt [233].

According to Hill, this type of population-wide intervention should require 'very strong evidence' as the consequences of being wrong could be severe. At the time, evidence was available from a number of published prospective cohort studies and dietary RCTs [24,26]. There were six primary prevention prospective cohort studies that consisted of 31 445 male participants [26]. Not one of these studies found a significant association between coronary heart disease deaths and dietary fat and only one found a significant association between coronary heart disease deaths and saturated fat intake. The *Diet-Heart Hypothesis*, that dietary saturated fat increases serum cholesterol concentrations which in turn causes heart disease, was only supported by one study. The six RCTs consisted of 2 467 male participants but not one study was a primary prevention trial nor tested the specific dietary recommendations of the 1977 *Dietary Goals* [24]. Even if the available evidence wholly supported the *Diet-Heart Hypothesis*, the fact that no women were included in prospective cohort studies or RCTs, and that no primary prevention RCTs were available, severely limits the external validity of the data. Additionally, the dietary committee did not consider all of the available evidence and made use of various other sources of data such as weaker observational studies, animal studies, and unpublished data from the Seven Countries Study [24,26]. Another major limitation of the time was that knowledge of the current diets of Americans, the diet which the *Dietary Goals* set out to change, was based entirely on US Department of Agriculture Food Disappearance Data [233,234]. It was therefore unknown what was actually being eaten and how much this varied by region and by individual.

The debate during the development of the 1977 dietary goals was similar to the current debate between some scientists and public health authorities today: debate over interpretation of data, methodological limitations, and causation [235]. Scientists of the time were aware of the lack of conclusive evidence, including those most in favour of the *Diet-Heart Hypothesis*. Throughout *Dietary Goals for the United States*, areas of uncertainty are mentioned [233]:

- “Because of these divergent viewpoints, it is clear that science has not progressed to the point where we can recommend to the general public that cholesterol intake be limited to a specific amount.”;
- “A similar divergence of scientific opinion on the question of whether dietary change can help the heart illustrates that science can not yet verify with any certainty that coronary heart disease will be prevented or delayed by the diet recommended in this report.”;
- “The problem with all of these [clinical] trials is that none of them showed a difference in heart attack or death rate in the treated group. Only when soft-end points were used in fact was there any subjective difference, and this was only in studies that were not blinded.”;
- “The question of whether dietary changes alone such as those suggested in these goals can reduce the leading causes of death in the United States remains controversial. Individuals, in exercising freedom of dietary choice, should recognize that these dietary recommendations do not *guarantee* improved protection from killer diseases. They do, however, increase the *probability* of improved protection.”;
- “There have been no studies that have found whole wheat flour to be superior nutritionally to white flour when consumed in a normal diet; and surprisingly few studies have even considered the question.”;
- “After hearing additional testimony from witnesses, discussing these goals with a number of experts and reading rather convincing correspondence from a variety of informed sources, I have become increasingly aware of the lack of consensus among nutrition scientists and other health professionals…”

Two broad approaches were apparent amongst scientists during the development of *Dietary Goals*: those in the majority who felt the *Diet-Heart Hypothesis* was probably correct and that being wrong would have no consequences; and those, akin to Hill, who were cautious in the absence of strong evidence and wary of the potential for harm and unintended consequences. Of the former:

- Professor D. Mark Hegsted from Harvard School of Public Health said at the press conference announcing the goals: “There will undoubtedly be many people who will say we have not proven our point; we have not demonstrated that the dietary modifications we recommend will yield the dividends expected … What are the risks associated with eating less meat, less fat, less saturated fat, less cholesterol, less sugar, less salt, and more fruits,

vegetables, unsaturated fat and cereal products — especially whole grain cereals. There are none that can be identified and important benefits can be expected."[236]

- Assistant secretary for health and former director of the National Heart and Lung Institute, Theodore Cooper, underscored that even if the dietary fat and cholesterol hypothesis was completely unsubstantiated by future research, there is nothing ". . . in the recommendations to reduce fat intake [that] would in any sense be harmful."[235]
- Dr Jeremiah Stamler, chairman of the Department of Preventative Medicine, Northwestern School of Medicine said: "The potential risks are essentially nil, the potential benefits vast, and that constitutes an optimal public health and medical care situation."[235]

On the other hand:

- Dr. E. H. Ahrens, Jr., Professor of Medicine at Rockefeller University said: "Senator McGovern, I recognize the disadvantage of being in the minority as I have been on this…. Yet it is an issue in which I have an enormous stake, and which I hope will someday be resolved. But I feel as a scientist that the most difficult thing in science is the getting of adequate proof.... The proof is not there yet…. My contention is, however, that this is a matter of such enormous social, economic and medical importance, that it must be evaluated with our eyes completely open…. They are betting, and they are hoping. I am betting and I am hoping, too, for I have changed my diet to some degree, no question about it. I have done so in the hope that I am stepping off in the right direction. But I have no conviction nor foreknowledge that what I am doing is prolonging my life or that of my family."[235]
- The American Medical Association, in a letter to the Nutrition Committee states: "The evidence for assuming that benefits to be derived from adoption of such universal dietary goals as forth in the report is not conclusive and … potential for harmful effects … would occur through adoption of the national goals."[233]

Thus, in 1977, on the basis of weak evidence, and with no trials demonstrating that lowering serum cholesterol concentrations via dietary changes could reduce disease, policies aimed at changing many dietary aspects of the general public were adopted [25,234].

1.3.2) Impact of Dietary Goals for carbohydrate restriction

The *Dietary Goals for the United States* have had a large impact on nutrition over the past 40 years [237]. The United States adopted National Dietary Guidelines in 1980 based on these goals, which have been updated every 5 years since 1980 [18]. The core of these guidelines has not differed from *Dietary Goals* until the 2015 edition, when recommendations to avoid foods containing dietary cholesterol were dropped [18,238]. Following the example of the US, national dietary guidelines were adopted by several other countries and health organisations, including the UK in 1983 [24]. However, some medical organisations remained sceptical of the benefits of the *Dietary Goals*, until evidence that cholesterol-lowering drugs could reduce coronary heart disease was attained and interpreted by many as proof of the *Diet-Heart Hypothesis* [232]. Guidelines based on *Dietary Goals* were then accepted and adopted by countries and health organisations around the world [239], including the World Health Organisation, the American Diabetes Association, the American Heart Association, and many more. South Africa followed suit with national dietary guidelines in 2003 and the Society for Endocrinology, Metabolism, and Diabetes of South Africa (SEMDSA) first published guidelines for T2D in 2012 [240]. The 1977 *Dietary Goals* had a major influence on the majority of national and organisational dietary guidelines. For example, the 2017 SEMDSA dietary recommendations for T2D include the specific recommendations to limit total fat consumption to less than 30 % and saturated fat to less than 10 % of calories [241]. Both of these values originate from the 1977 *Dietary Goals* and not from any specific trials or other body of evidence [233].

The appearance of national dietary goals and guidelines coincided with the start of the current obesity and T2D epidemic [242,243]. Whether the specific advice within the dietary guidelines are responsible for harm is controversial and arguments exist for and against [244-247]. Regardless, *Dietary Goals* has had unintended consequences for the field of nutrition research. Firstly, a precedent was set that weak observational data could impact dietary policy [29,31,242]. A rigorous and thoughtful process of considering the totality of the evidence, as was outlined by Hill, has largely been abandoned in nutritional science [88]. As long-term RCTs are not possible, the strength of prospective cohort data has been elevated to the highest level of evidence in nutrition when considering policy decisions [27,31]. This is apparent in the way many nutritional observational studies use language consistent with causation [88]. For example, Seidelmann et al (2018)[53] said: "These data also provide further evidence that animal-based low carbohydrate diets should be discouraged." This statement implies causation and encourages action based on weak associations. It is also very common for associational studies to appeal to policy makers within their manuscripts. The recent review of egg

consumption by Zhong et al (2019)[65] concluded their paper with: “These results should be considered in the development of dietary guidelines and updates.”

A second major consequence of national dietary goals and guidelines has been the political disruption of the scientific process in an unsettled field [248-251]. Political promotion of the *Diet-Heart Hypothesis* created the impression that a high level of evidence and certainty backed fat and saturated fat restriction [25,244,252]. The dietary advice recommended by guidelines attained the status of proven, without any of the uncertainties outlined in the original *Dietary Goals* having been resolved [244]. The political acceptance of *Dietary Goals* created a division in nutrition research: supportive research was accepted and seen as collaborating with a large body of evidence for a proven hypothesis; while contradictory research and/or hypotheses were held to the much higher standard of scientific proof [253]. De Souza et al (2019)[19] illustrates the dual standards to which evidence is held in a recent editorial entitled: “Low carb or high carb? Everything in moderation ... until further notice.” Talking about low carbohydrate high fat diets, the authors conclude the paper by saying: “Until these ratios have been studied in the general population for long periods of time or in long-term randomized trials, the best advice seems to be to select whole foods from a variety of sources and avoid dietary extremism. For now, for carbohydrates, everything in moderation carries the day.” Mazidi et al (2019)[20] were even more explicit when they concluded their review of prospective cohort studies by saying: “In conclusion, despite the usual limitations of observational data (and so causality impossible to determine), our study highlights an unfavourable association of low-carbohydrate diets (LCD) with overall and cause specific mortality, based on both individual data and pooling previous cohort studies. Given the fact that long-term LCDs may well be associated with greater long-term harm, it might be important to consider whether we should routinely recommend such diets in clinical practice for weight loss until further higher quality studies evaluate this issue in greater detail. Rather, other dietary patterns that have been associated with both short-term weight loss and long-term benefits (such as low fat diets, with plentiful fibre) may be preferable.” In other words, the current dietary paradigm, although adopted and accepted without study in the general population for long periods of time and yet to be proven effective in a single long-term RCT, should be considered superior to alternative hypotheses that have similar levels of supporting evidence. The level of evidence required to challenge the current paradigm far exceeds the level of evidence on which the paradigm is based [254].

The comprehensive systematic review and meta-analysis of low carbohydrate diets in T2D by Korsmo-Haugen et al (2018)[133] provides another example of the double standard. Their review discussed all the issues with the underlying evidence, the methodological difficulties with dietary

RCTs, and the limitations of the available data. Based on their meta-analysis of 19 RCTs, they said that although there may be some benefit with low carbohydrate diets, the data is ambiguous, there is an absence of long-term studies, and that low carbohydrate diets therefore cannot be recommended in general terms. They then concluded their review by saying: "On the basis of currently available systematic reviews and meta-analyses there is an appreciable body of evidence to suggest that a traditional Mediterranean-type diet is particularly appropriate for individuals with T2D." The cited references for this statement included two meta-analyses, both of which consisted of the same three RCTs. Two of these trials investigated "low carbohydrate Mediterranean diets" and are frequently incorporated into low carbohydrate meta-analyses (indeed, one was actually in the Korsmo-Haugen et al (2018)[133] meta-analysis). The duration of these three RCTs was 4 years, 1 year, and 6 months, and health markers were used as endpoints. The quality of the evidence and the problems with the underlying research in these Mediterranean diet RCTs is identical to the quality and limitations with the low carbohydrate literature. Further, more RCTs have studied low carbohydrate diets than Mediterranean diets for T2D and the only RCT that has directly tested a Mediterranean diet versus a *low* carbohydrate diet found no difference in weight loss between diets and some health markers changed more favourably on the *low* carbohydrate diet [255]. Despite this, they concluded that low carbohydrate diets cannot be recommended, but the Mediterranean has proven benefits. This is not to say that low carbohydrate diets should be recommended and adopted in guidelines. Rather, these examples serve to illustrate that the status of proven, which low fat and Mediterranean dietary advice has attained, is a consequence of policies and not of a significantly superior evidence base.

The major concerns that researchers have about low carbohydrate diets have been unchanged over the past 40 years [10,256,257]: low carbohydrate diets are unsustainable, there is no long-term proof that they are safe, they may increase the risk of heart disease due to increased saturated fat intake, weight loss is due to calorie restriction and not carbohydrate restriction, and they may be nutritionally deficient. Despite low carbohydrate diets being perhaps the most studied dietary pattern in short-term RCTs investigating T2D patients, none of these commonly expressed concerns are demonstrable and they remain theoretical [258]. Therefore, that low fat and Mediterranean diets are promoted and encouraged whilst low carbohydrate diets are cautioned against and discouraged has more to do with policy decisions than scientific evidence. Weinbert et al (2004) described the situation as follows: "Defence of the low-fat high-carbohydrate diet, because it conforms to current traditional dietary recommendations, by appealing to the authority of its prestigious medical and institutional sponsors or by ignoring an increasingly critical medical literature, is no longer tenable. The categoric rejection of experience and an increasingly favourable medical literature, though still

not conclusive, which suggests that the much maligned low carbohydrate high-protein diet may have a favorable impact on obesity, lipid patterns, type II diabetes, and the metabolic syndrome, is also no longer tenable."[239] The situation is unchanged 15 years later and the double standards by which different dietary patterns are evaluated remains apparent [18,242,259]. The adoption of dietary policies to endorse low fat diets is therefore one of the most important factors in how researchers and clinicians currently perceive carbohydrate restriction.

1.4) Summary and conclusions

The most important take-away message from Chapter 1 is that the nature of nutrition research precludes definitive answers to many food- and diet-related questions. Foods and nutrients are highly interrelated, have complex interactions during absorption and metabolism, and stimulate highly individualised hormonal and metabolic responses which combine to cause diseases after many decades. The two main study types which have been used to attempt to determine which foods and diets are healthy are the prospective cohort and the RCT. However, both designs face severe challenges in nutrition research to the extent that no clear answers exist and additional studies of the same quality as existing research are unlikely to add clarity. One of the notable sources of ambiguity in the RCT literature is the large heterogeneity in dietary interventions that are described as 'low carbohydrate diets' by the investigators. Diets used in intervention trials range from almost no carbohydrate to approximately 45 % of daily intake as carbohydrate. 'Low carbohydrate' interventions have also differed widely according to which sources of fat are promoted or discouraged, whether they are high or low in protein, and on other aspects of the interventions, such as calorie control strategies. The controversy surrounding carbohydrate restricted diets stems from multiple available interpretations of ambiguous research and the different values of the researchers who interpret this information. Additionally, dietary policies in favour of low fat and Mediterranean diets have created the impression that these eating patterns are better supported by evidence than carbohydrate restricted diets. Determining the health effects of various foods and diets is incredibly challenging. For this reason, research focused on understanding the underlying physiological and metabolic effects of carbohydrate restricted diets may be an alternative approach to alleviate or validate some of the concerns being expressed about LCHF diets. Limitations inherent to the study of nutrition need to be carefully considered in order to formulate future hypotheses which address clearly defined questions which are testable.

Chapter 2

Diet, diabetes status, and personal experiences of individuals with type 2 diabetes who self-selected and followed a low carbohydrate high fat diet *

* Please note that findings from this Chapter have been accepted for publication in Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy.

Introduction

Type 2 diabetes mellitus (T2D) is a complex disease that results from a myriad of genetic, environmental, and lifestyle factors that interact over many decades. It is strongly associated with obesity and cardiovascular disease and is often described as a chronic progressive disease, in which glycaemic control and health are expected to decline gradually even with the use of appropriate medications [2]. The primary goals of standard treatment are to reduce weight in overweight or obese patients and to reduce hyperglycaemia to within an individualised target range, usually HbA1c below 7 %, via lifestyle changes and glucose lowering medications [2,3]. Very recently, there has been growing interest to not only delay the progression of T2D but also to reverse if [7,8,15]. Remission from T2D with standard treatment is rare [7] but occurs with greater frequency after bariatric surgery [260] and severe calorie restriction (850 kcal per day or less) [217]. More recently, severe carbohydrate restriction has also gained attention as another possible intervention to put T2D into remission [8,139,261].

There has been much scientific interest in carbohydrate restricted diets to manage T2D, with at least 22 randomised control trials, 10 non-randomised control trials, and 10 systematic reviews (8 with meta-analyses) that have investigated the effects of carbohydrate restriction on glycaemic control in patients with T2D [259]. While there is considerable heterogeneity in findings, this evidence generally shows that the lower carbohydrate dietary interventions are at least as effective as control diets for improving HbA1c, T2D medication requirements, body weight, and dyslipidaemia [132-134,259]. However, the prescribed low carbohydrate diet interventions varied considerably amongst individual studies and ranged from less than 20 g of carbohydrate per day to less than 50 % of total energy intake [132]. Interventions also varied considerably in other ways, such as recommended sources of dietary fat and protein, *ad libitum* versus prescribed energy intake, timing of meals, level of participant support,

and duration. Further, the actual diets consumed by participants in these studies was often very different to the one prescribed by the intervention [134]. Indeed, a common criticism of carbohydrate restricted diets, particularly those which target less than 50 g of carbohydrate per day, is that they are difficult to sustain over the long term [134].

The heterogeneity in carbohydrate restricted interventions has made it difficult to assimilate findings into clear and consistent public messages. This has been a source of frustration for clinicians and has produced confusion for patients. Despite this, carbohydrate restricted diets have grown in popularity with numerous anecdotal reports emerging of individuals having 'reversed' their T2D. Individuals who have followed their own version of a carbohydrate restricted diet, rather than one which was prescribed, supported, and incentivised by an intervention trial, have rarely been studied.

It is therefore largely unknown what actually constitutes a sustainable carbohydrate restricted diet in a real-world setting. Additionally, the remission claims of these individuals have not been investigated. Studying this population would present clinicians and researchers with a better understanding of the foods, behaviours, and other diet-related factors that patients feel are important, as well as provide insight into why some patients may wish to follow these diets. It may also reveal aspects of their dietary interventions that are challenging or potentially dangerous. The aim of this study was therefore to characterise the diet, eating patterns, T2D status, and personal experiences of individuals with a confirmed history of T2D who claimed to have followed a carbohydrate restricted diet for at least the previous 6 months.

Methods

Ethical considerations

This study was approved by the University of Cape Town (UCT), Faculty of Health Sciences, Human Research Ethics Committee (REF: 608/2016, first approval 1-Sep-2016). All participants were informed of the nature of the study and written informed consent was obtained before participants were enrolled in the study.

Study design

This was a multi-method descriptive study which investigated the habitual diets, diabetes status, and personal experiences of individuals with T2D who claimed to have followed a carbohydrate restricted diet for at least the previous 6 months. Recruitment material broadly defined the diet as one which deliberately avoided carbohydrate rich foods but did not restrict dietary fat intake. Therefore, the term low carbohydrate high fat (LCHF) diet will be used throughout Chapter 2 to refer to the habitual diets of the participants. Diet, body weight, and diabetes status was assessed when participants were first enrolled in the study (*First-Assessment*). Copies of past laboratory blood test results from the time of diagnosis (*Diagnosis*) and prior to starting the LCHF diet (*Pre-LCHF*) were obtained from the participant or from their medical practitioners. Participants were also interviewed in depth about their experiences with the LCHF diet. Participants were contacted again approximately 15 months after *First-Assessment* and invited to participate in an additional assessment (*Follow-up*). Diet, body weight, diabetes status, and remission from T2D was assessed at *Follow-Up*. No attempts were made to alter participants' diets or behaviours at any point during this study. An overview of the timepoints and data collected in this study is shown in Table 1.

Table 1. Definition of the timepoints in this study and the measurements taken

Time point	Description	Measures
Diagnosis	Medical records with proof of T2D diagnosis.	• HbA1c from medical records
Pre-LCHF	Medical records prior to starting the LCHF diet.	• HbA1c from medical records
Start-LCHF	Self-reported date that participants started their LCHF diets.	• Self-reported body weight • Medications history
First-Assessment	Participants were enrolled in the study and assessed.	• Blood draw for HbA1c • Body weight • Medications history • Food frequency questionnaire • 1-day diet recall • 3-day diet logbook • In-depth interviews • Detailed questionnaires
Follow-Up	Participants were reassessed approximately 15 months later.	• Blood draw for HbA1c • Body weight • Medications history • Food frequency questionnaire

T2D, type 2 diabetes; LCHF, low carbohydrate high fat.

Participants

Adult participants were eligible for inclusion if they had been diagnosed with T2D by a medical practitioner, if they claimed to be currently following an LCHF diet, and they reported that at least 6 months had passed since the date that they started following an LCHF diet (*Start-LCHF;* **Table 1**). Participants were excluded from the study if they had changed their diet significantly within the past 6 months, their T2D diagnosis could not be confirmed either through past medical records or T2D medication prescriptions, or they were unable to understand and speak English. Participants were respondents to social media adverts, press releases, or posters placed at local clinics (**Appendix A**). Respondents received detailed information about the study and were interviewed over the phone to confirm that they had been previously diagnosed with T2D and that they claimed to have been following an LCHF diet for the previous 6 months. Once the informed consent process had been completed, researchers obtained a copy of T2D diagnosis medical records to confirm eligibility. There were 53 respondents and 28 participants who met the inclusion criteria, completed the

informed process, and were enrolled in the study. Of the other respondents, 8 were interested in participating in an LCHF intervention and were not currently following an LCHF diet, 7 had been following an LCHF diet for less than 6 months, 3 had type 1 diabetes, T2D diagnosis could not be confirmed in 1, and 5 were not responding and could not be contacted after they had received detailed information about the study.

Data collection

At *First-Assessment*, participants completed health and lifestyle questionnaires (**Appendix B**), a detailed medications history (**Appendix B**), a food frequency questionnaire (FFQ) (**Appendix C**), a 24-hour food recall, a 3-day diet record, and an in-depth one-on-one interview. All the questionnaires were completed online by the participant. The medications history questionnaire asked for details about medication names, start and stop dates, and doses. Participants were able to complete the questionnaire in their own time (they were able to save and return later) and they were encouraged to look up any details for which they may have records. The questionnaires had conditional formatting and participants were only presented with questions that were relevant to them based on prior answers. For example, if a participant had never taken insulin, they were not asked dose and date information for that medication. A researcher analysed the completed questionnaires and reviewed it in discussion with the participants to resolve any ambiguities or inconsistencies.

Eighteen participants lived in the Cape Town area and visited the laboratory for the interview and food recall. Height, body weight, body fat percentage, and blood pressure were measured during the visit and a blood sample was drawn for measuring HbA1c, fasting plasma glucose, fasting serum insulin, and fasting serum lipid concentrations (*Lancet Laboratories, South Africa*). Body fat percentage was calculated from skinfolds (*Cescorf Innovare 3 calipers, NutriActiva, Minnesota, USA*) taken at 4 sites (subscapular, bicep, tricep, suprailiac) according to the equations of Durnin and Womersley [262]. Blood pressure was measured in duplicate after the participant had been seated for 10 min using an automatic blood pressure monitor (*Omron 711, Automatic IS, Kyoto, Japan*). A third blood pressure reading was measured if diastolic or systolic blood pressure differed by more than 5 mmHg. Ten participants were unable to visit the laboratory. These participants completed the interview and food recall via Skype and had their blood tests performed through a medical practitioner or directly through a pathology laboratory. For practical reasons we were unable to collect fasting blood samples in all participants. At *Follow-up,* participants completed a questionnaire (**Appendix D**), repeated the FFQ, and had a blood sample drawn for the measurement of HbA1c.

Diet assessment

The types of foods eaten by participants during the 6 months prior to *First-Assessment* and *Follow-up* was estimated using a 110-item semi-quantitative FFQ. Participants were asked to report the frequency with which they ate portions of the listed foods, on average, over the past 6 months. This FFQ was adapted from the South African Medical Research Council (MRC) FFQ to include LCHF foods. The FFQ (with the serving sizes of each food item) is available as **Appendix C**. Habitual diet was quantified at *First-Assessment* using 4 sequential days of dietary intake data that included a 1-day food recall and 3 days of food records. The food recall was interview-based and used open-ended questions and visual aids to guide the participant on quantifying all foods and drinks consumed during the previous day. This also served as familiarisation for participants to keep a detailed record of all foods and drinks consumed over the next 3 days. Nutrient composition from the food recall and 3-day record was analysed using the MRC SAFOODS database. Some LCHF foods, such as full-fat and double-cream dairy, and bone broth, did not appear in this database. Nutrient information for these foods was obtained from either the FatSecret or NutritionDataSelf online databases (**Appendix E**). Total fat, carbohydrate, protein and alcohol composition data was available for all foods. As such, reported values for these 4 macronutrients indicate an estimate of actual intake. Fibre, sugar, saturated fat, monounsaturated fatty acids, polyunsaturated fatty acids, or cholesterol data was not available in some of the missing foods. As such, reported values of these nutrients indicate an estimate of minimum intake. Micronutrient data was not available for most of the missing foods. In addition, vitamin and mineral supplementation and added salt was not recorded. The reported micronutrient data therefore represents an underestimate of intake.

Remission assessment

The criteria used to assess remission are in line with those described by Hallberg et al (2019) [8]. There are two categories of T2D remission, both of which require that improvements in T2D status are sustained for at least 1 year. The 15-month *Follow-Up* period therefore allows for the assessment of T2D remission. Partial T2D remission was defined as HbA1c below 6.5 % without taking diabetes medications other than metformin at both *First Assessment* and *Follow Up*. Metformin is included in this definition as it is a low-risk medication (for hypoglycaemia) which does not need to be urgently stopped after improvements in glycaemic control. Metformin also has indications beyond T2D [8].

Complete T2D remission was defined as HbA1c of 5.7 % or less without taking any diabetes medications (including metformin) at both *First Assessment* and *Follow Up*. Participants with partial or complete remission would have sustained their improvements in T2D status for at least 15 months. Remissions were referred to as 'potential' if these criteria were only met at *Follow-Up* (i.e. the HbA1c and medication component of the remission criteria was met but the time component could not be confirmed).

Statistical analysis and quantitative data reporting

Normality was tested using the Shapiro-Wilk test. Data are reported as either mean ± SD or median with interquartile range (IQR) in parentheses, depending on the distribution. Data was not normally distributed and non-parametric tests were used for comparisons between time points. The Wilcoxon signed-ranked test was used for comparisons over 2 timepoints. The Friedman test was used for comparisons over 3 timepoints and if significant, post-hoc analysis was performed using the unadjusted Pairwise Wilcoxon Rank Sum tests. Statistical analyses were performed using the Python-based library SciPy and R-3.5.1 statistical software.

In-depth interviews

Individual in-depth interviews were conducted with all 28 participants. Interviews followed a semi-structured interview guide (**Appendix F**), were once-off, and were conducted at the division campus for 18 participants who lived in Cape Town and over Skype for 10 participants. The interviews and subsequent thematic analysis were conducted by researchers CW (34 y/o male) and TM (32 y/o female). Both CW and TM were registered health professionals (biokinetics and dietetics respectively) and were full-time researchers (PhD and MSc candidates respectively) at the time of the interviews. The researchers received extensive training in qualitative interview techniques and had conducted several pilot interviews prior to the start of data collection. Particular attention was placed on asking open-ended questions and probes, remaining neutral throughout the interviews, and avoiding leading questions. Each researcher conducted 14 interviews and all interviews were conducted in English. Rapport between the interviewer and participant had been established prior to the interviews through the informed consent process and by assisting participants with lifestyle and medications history questionnaires.

Many participants were aware of the involvement of researcher TN in the study, who is well known for publicly promoting the LCHF diet for the management of T2D. Participants had no contact with TN during the study. Participants were aware that CW and TM were involved in the research for the fulfilment of their degrees. Prior to the start of the interviews, participants were informed that: the interviews would be confidential; that they should speak freely and honestly about their opinions rather than what they thought the researchers wanted to hear; that the researchers were interested in negative as well as positive experiences; and that there were no right or wrong answers. Interviewers used an interview guide, developed during pilot interviews to ask about the participants' experiences and perceptions of their LCHF diet and T2D (**Appendix F**). The interview guide contained open-ended questions and probes on topics including: diabetes status prior to their LCHF diet; lifestyle prior to their LCHF diet; how they started an LCHF diet; their understanding of LCHF diets in terms of foods eaten and avoided; the effect of their LCHF diet on their health and diabetes; interactions with medical professionals; and challenges and negative experiences with following an LCHF diet. Both CW and TM felt that data saturation had been achieved as no new ideas were being introduced in the last few of each interviewer's respective 14 interviews.

Qualitative analysis

The thematic analysis method was used to identify, analyse, and report emergent themes from the interviews, as described by Braun and Clarke (2006) [263]. This method is commonly used to analyse qualitative data and produces a rich description of the data by identifying and examining patterns of meaning (i.e. themes). Interviews were audio recorded and transcribed intelligent verbatim (excluding fillers such as 'um'). CW and TM had familiarised themselves with the data set through conducting the interviews, checking the transcripts for accuracy against the original audio recordings, and reading through all the transcripts. Codes (labels used to identify concepts which were relevant to participants' experiences with the LCHF diet) were developed based on the interview questions and the researchers' knowledge of the transcripts (**Appendix G**). Sentences and passages in the transcripts were then organised and categorised based on these codes. Inter-coder reliability was established in 6 (20%) interviews, which were independently coded by CW and TM with an accuracy of 97 % and a mean kappa of 0.74 ± 0.24 [264]. This indicates that there was good agreement in how CW and TM were coding the transcripts. The remaining 22 interviews were then randomly assigned to be coded by either CW or TM. Once coding was complete, the researchers searched for and identified themes and subthemes across and within codes. This process was

dynamic and the themes and subthemes were altered several times to best fit the researchers' understanding of the data. The themes (supported by quotes) are reported in the results section. Coding and analysis of the codes was done using Nvivo 11 Pro. Finally, the interviews were reviewed to ensure the relevance and accuracy of the themes and subthemes across the entire dataset.

Qualitative data reporting

Reported quotations were selected to illustrate the emergent themes being discussed as they capture the essence of the theme [263]. Expanded lists of quotations are available as **Appendix H**. All quotations were checked against the original audio recordings to ensure that the meaning was correctly captured. When reporting the prevalence of themes, quantification was avoided so as to maintain an emphasis on the perspectives of the participants [265,266]. Quotations are shown in quotation marks and italics. Square brackets indicate text added by the authors to provide clarity or context and an ellipsis indicates the omission of irrelevant parts of the quote.

Results

Participants

A total of 28 eligible participants (14 women and 14 men) completed *First-Assessment*. Characteristics of the participants at *First-Assessment* are displayed in **Table 2**. Ethnicity was self-reported by 26 participants as 'white' and by 2 participants as 'coloured/mixed ancestry'. Monthly income was reported to be in the highest bracket (>R25000 (~ $1700) per month) by 18 participants; R10000 – R25000 (~$670 – $1700) by 6 participants; and 4 were in the lowest bracket (<R10000 (~$670) per month). The participants reported level of education as 10 tertiary postgraduates; 10 tertiary undergraduates; and 8 having completed high school. At *First-Assessment*, time since T2D diagnosis ranged from 6 months to 28 years and time following an LCHF diet ranged from 6 months to 6 years (**Figure 1**). Participants were contacted 15 months after *First-Assessment* and invited to complete the *Follow-Up*. Two participants could not be contacted and 2 no longer followed an LCHF diet. Reasons given by the 2 participants for stopping the diet were: 1) goal weight had been achieved; and 2) work and personal difficulties led to eating junk foods. Therefore, 24 participants were following an LCHF diet at *Follow-Up*.

Table 2. Participant characteristics at *First-Assessment*

Female / male (n)	14 / 14
Age (years)	57 ± 10
Body mass index (kg/m^2)	30 ± 6
Body fat (%) *	36 ± 9
Time since T2D diagnosis (years)	7.4 (3.5 – 13.2)
Time on LCHF diet (years)	1.2 (0.8 – 3.2)
Systolic BP *	136 ± 15
Diastolic BP *	83 ± 12

Data presented either as mean ± SD or Data expressed as median (IQR). n, number of participants. * indicates the subset of 18 participants that visited our laboratory. Two participants self-identified as coloured.

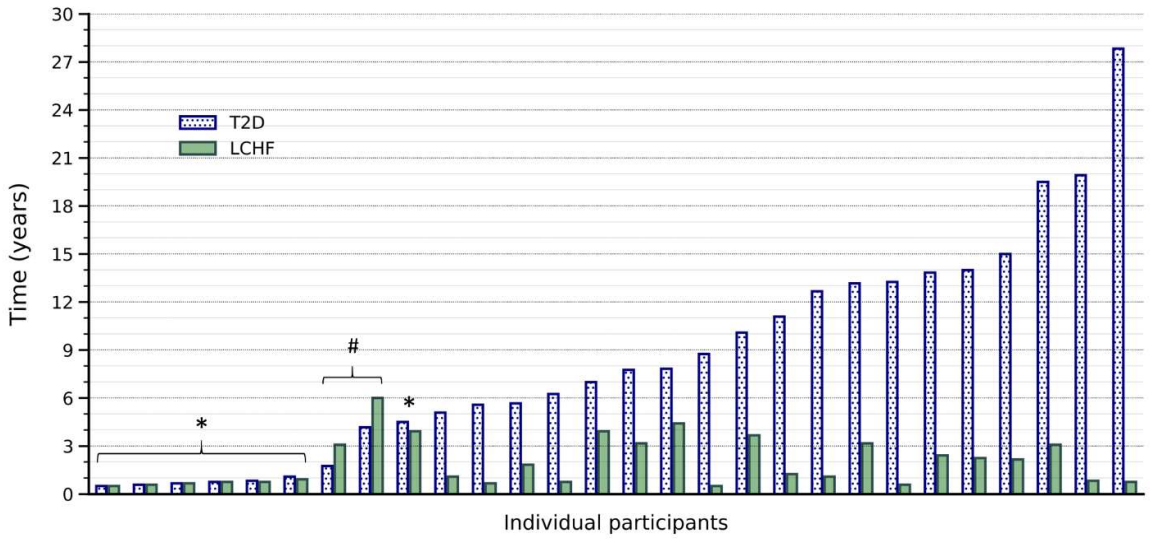

Figure 1. Time with T2D and time following the LCHF diet at First-Assessment

Time since T2D diagnosis and time on the LCHF diet is shown for each individual participant at *First-Assessment*. Participants are ordered from left to right based on time since T2D diagnosis. * indicates the 7 participants who started the LCHF diet shortly after diagnosis; # indicates the 2 participants who were following an LCHF diet prior to formal diagnosis. The remaining 19 participants had a period of conventional diabetes treatment prior to starting an LCHF diet.

Seven participants started an LCHF diet soon after their T2D diagnosis, with just 24 (3 – 32) days between *Diagnosis* and *Start-LCHF*. They had been following the diet for 9 (7 – 11) months at *First-Assessment* (**Figure 1**). Two participants had been following the diet for 24 and 16 months respectively prior to their T2D diagnosis (**Figure 1**). These participants had normal HbA1c and FPG concentrations at *Diagnosis* and their formal T2D diagnosis was based on 120-minute plasma glucose concentration during the oral glucose tolerance test. The remaining 19 participants had T2D for 9.8 (4.7 – 12.2) years before *Start-LCHF* and had followed the LCHF diet for 22 (10 – 38) months at *First-Assessment* (**Figure 1**). The follow-up assessment was completed 15 ± 2 months after *First-Assessment*. At *Follow-Up*, 24 participants had been following the LCHF diet for 35 (26 – 53) months.

General health and medical conditions

Participants reported current and previous medical conditions as part of the detailed questionnaires. **Table 3** shows a selection of the most frequently reported medical conditions. Many participants reported having had various medical conditions which resolved since following the LCHF diet. For example, 10 participants reported hypertension at *First-Assessment*, and 20 participants reported that they previously had hypertension at *Start-LCHF* but no longer consider themselves to have the condition (**Table 3**). The qualitative findings in the next chapter provide more details on this aspect of the participants' health and explore their perceptions and experiences of these and other medical conditions. In terms of major cardiovascular disease, 2 participants previously had strokes, 1 participant had severe angina, and there were no reported cases of myocardial infarction or heart failure. There were a few positive and negative changes in medical conditions reported at *Follow-Up* but the overall health status of the participants was largely unchanged from *First-Assessment* as shown in **Table 3**. No cardiovascular events occurred in the Follow-Up period.

Table 3. Commonly reported medical conditions

Condition	***Start-LCHF*** **(n)**	***First-Assessment*** **(n)**
Hypertension	20	10
Dyslipidaemia	18	11
Peripheral neuropathy	11	7
Retinopathy	11	2
Intermittent claudication	14	7

The table shows a selection of the most commonly reported medical conditions at *Start-LCHF* and *First-Assessment*. Data was collected from a detailed medical history. n, number of participants.

Diet

Table 4 shows reported habitual daily energy and macronutrient intake for the group, derived from 4 days of diet data at *First-Assessment*. Estimated total carbohydrate intake was 61 g/d, including at least 38 g/d that was digestible (glycaemic) and at least 14 g/d that was fibre (**Table 4**). Total carbohydrate intake was between 20 and 50 g/d for 10 participants, meeting the definition of a '*very low carbohydrate*' diet [13]. It was between 50 and 115 g/d (i.e. '*low* carbohydrate') for 17 participants and was 142 g/d (i.e. '*moderate* carbohydrate') for one participant [13]. Participants' reported diets had similar amounts of saturated fatty acids and monounsaturated fatty acids, which made up almost 90% of the 121 g/d of fat that was reported (**Table 4**). The majority of protein was derived from animal sources (**Table 4**). A minimum estimate of daily vitamin and mineral intake from food sources (excluding supplementation) is reported in **Table 5**. Intakes of folate, vitamin D, vitamin E, and calcium were below 50 % of the recommended daily allowances (RDAs). Thiamin, vitamin B_6, vitamin C, magnesium, manganese, and potassium intakes were between 50 % and 100% of RDAs. Intake of the other 11 vitamins and minerals quantified were above the RDAs.

The FFQ was used to analyse the types of foods that participants reported eating during the months preceding *First-Assessment* and *Follow-Up*. The 25 most frequently reported foods are shown in **Figure 2**. Dairy was one of the most reported food groups and cream, butter, cheese, milk, and yoghurt were commonly reported at *First-Assessment* and *Follow-Up*. Non-starchy vegetables, coconut oil, eggs, and tree nuts were amongst the most commonly reported foods. Unprocessed red meat with fat was the most frequently reported protein at both timepoints. Poultry with fat, seafood, bacon and biltong (cured, dried meat) were the other common protein sources. Participants reported predominantly eating fatty cuts of meat as well as full-fat dairy. Lean meats and low-fat / fat-free dairy were items on the FFQ which were rarely reported. Fruit (excluding olives, avocado and berries), grains, breakfast cereals, oats, breads, crackers, pasta, rice, beans and legumes, starchy vegetables, and vegetable and canola oil were amongst the least frequently reported foods. Although most participants reported that they did not drink alcohol, some reported regular daily intakes. No participants reported adding sugar to food or drinks but non-sugar sweeteners and sugar-free soft drinks were fairly commonly reported. The foods that participants reported commonly eating were similar at *First-Assessment* and *Follow-Up*. One difference was that coconut milk was commonly eaten at *First-Assessment* but was not in 25 most-eaten foods at *Follow-Up*.

Table 4. Dietary composition

Energy (kcal)	**1794 (1366 - 2474)**
Carbohydrate (% energy)	**12 (11 - 16)**
Carbohydrate (g)	**61 (45 - 75)**
Glycaemic (g)	38 (28 - 55)
Total sugar (g)	28 (17 - 34)
Added sugar (g)	0 (0 - 5)
Fibre (g)	14 (10 - 19)
Protein (% energy)	**20 (17 - 23)**
Protein (g)	**92 (67 - 124)**
Animal sources (g)	70 (49 - 101)
Plant sources (g)	8 (5 - 12)
Fat (% energy)	**66 (62 - 69)**
Fat (g)	**121 (99 - 176)**
Saturated fat (g)	49 (40 - 73)
MUFA (g)	45 (37 - 60)
PUFA (g)	13 (9 - 21)
Cholesterol (mg)	616 (408 - 799)
Alcohol (g)	**0 (0 - 11)**

Reported data is from the 1-day food recall and 3-day diet record. Data expressed as median (IQR). MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids. Macronutrient information was available for all foods but a further breakdown of nutrients was not available for some LCHF foods. Therefore, the nutrient breakdown for each macronutrient is incomplete and represents a minimum intake.

Table 5. Vitamin and mineral intake

	Intake	RDA	% RDA
Vitamins			
Vitamin A (mg/d)	1.6 (0.7 – 2.4)	0.8	200
Thiamin (B_1) (mg/d)	1.0 (0.6 – 1.3)	1.2	83
Riboflavin (B_2) (mg/d)	1.6 (1.2 – 2.1)	1.2	133
Niacin (B_3) (mg/d)	20.7 (16.4 – 26.4)	15.0	138
Pantothenic acid (B_5) (mg/d)	5.8 (5.1 – 8.3)	5.0*	116
Vitamin B_6 (mg/d)	1.3 (0.9 – 1.8)	1.6	81
Biotin (B_7) (µg/d)	35.5 (28.7 – 51.9)	30.0*	118
Folate (B_9) (µg/d)	98.2 (76.9 – 142.6)	400	25
Vitamin B_{12} (µg/d)	6.4 (4.1 – 10.1)	2.4	267
Vitamin C (mg/d)	75.2 (50.3 – 133.7)	82.5	91
Vitamin D (µg/d)	6.2 (4.0 – 9.2)	15.0	41
Vitamin E (mg/d)	6.8 (5.4 – 12.6)	15.0	45
Minerals			
Calcium (mg/d)	502 (375 – 679)	1100	46
Copper (µg/d)	1.5 (0.8 – 2.1)	0.9	167
Iron (mg/d)	10.1 (8.1 – 13.8)	8.0	126
Magnesium (mg/d)	230 (152 – 311)	370	62
Manganese (mg/d)	1.8 (1.2 – 2.5)	2.1*	86
Phosphorus (mg/d)	1073 (864 – 1569)	700	153
Potassium (g/d)	2.4 (1.8 – 3.2)	4.7*	51
Sodium (g/d)	1.8 (1.1 – 2.5)	1.3*	138
Zinc (mg/d)	11.0 (8.1 – 16.7)	9.5	116

Reported data is from the 1-day food recall and 3-day diet record. Data expressed as median (IQR). Micronutrient data was not available for many LCHF foods and vitamin and mineral supplements were not captured in the study. Data therefore represents a minimum estimate of nutrient intake from food sources. RDA, Recommended Daily Allowances according to Dietary Reference Intake recommendations from the Institute of Medicine. RDAs are set 2 standard deviations above the Estimated Average Requirement for that nutrient. The reported RDA is the average for males and females aged 51 - 70 years. * indicates that Adequate Intake, a value believed to be sufficient to cover the needs of all the individuals in that category, is reported.

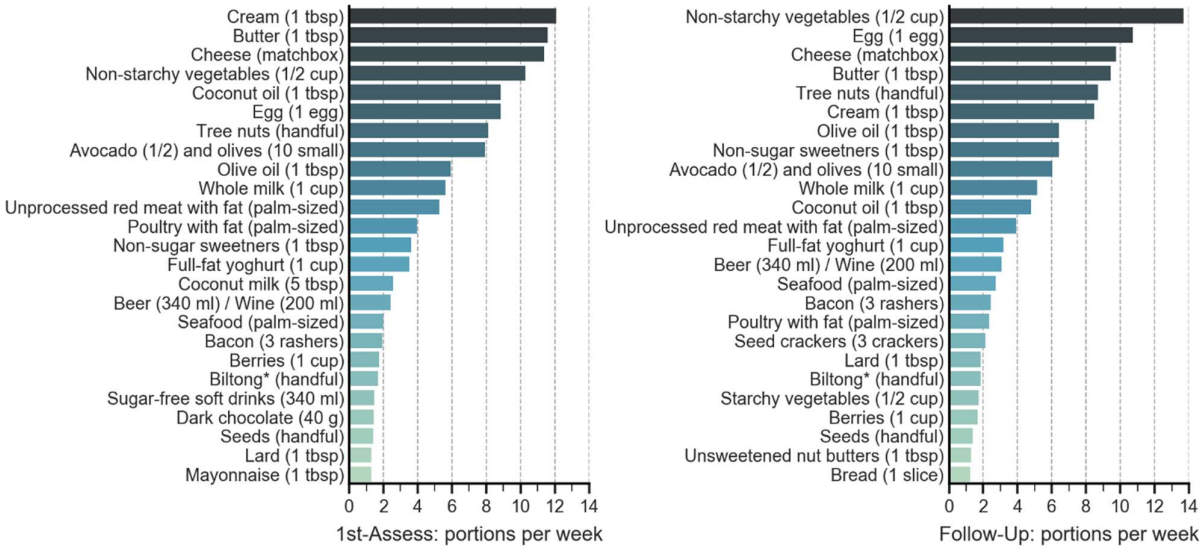

Figure 2. The 25 most frequently reported foods

Data shown is from the 110-item Food Frequency Questionnaire (FFQ) completed at *First-Assessment* (left) and *Follow-Up* (right). Figure shows the top 25 most commonly reported foods (portions per week). 1st-Ass, *First-Assessment*; Tbsp, tablespoon; * biltong is cured, dried meat, usually from beef or game.

Medications

Medications were reported at *Start-LCHF*, *First-Assessment*, and *Follow-Up*. Most participants reported large reductions in diabetes medications from *Start-LCHF* to *First-Assessment*. At *Start-LCHF,* 24 participants reported taking a median of 1850 (1000 – 2000) mg/d of metformin, compared with 16 participants taking 1000 (1000 – 2000) mg/d at *First-Assessment*. There were 11 participants taking 60 (39 - 94) U/d of exogenous insulin at *Start*-LCHF. At *First-Assessment*, 8 participants reported that they had discontinued insulin completely, 2 had reduced their dose from 88 to 26 U/d and from 40 to 32 U/d respectively, and one remained unchanged at 48 U/d. There were 2 participants using sulphonylureas at *Start-LCHF* and by *First-Assessment,* 1 had discontinued and the other had reduced their dose. The only other diabetes medication reported at *Start-LCHF* was a DPP-4 inhibitor used by one participant, which was discontinued by *First-Assessment*. Amongst all 28 participants, there was only 1 case of an increase in diabetes medications from *Start-LCHF* to *First-Assessment*, where 1 participant started a sulphonylurea. There were only minor changes in reported diabetes medications at *Follow-Up* compared to *First-Assessment*: 2 participants discontinued metformin; 1 decreased metformin dose; 1 increased metformin dose; 1 discontinued

a sulphonylurea; 1 started a sulphonylurea and DPP-4 inhibitor; and 1 started a GLP-1 receptor agonist and SGLT2 inhibitor.

In terms of non-diabetes medications, 7 of 14 participants reported stopping cholesterol-lowering medications from *Start-LCHF* to *First-Assessment*. This does not necessarily reflect improved lipid profiles in all these participants as cholesterol-lowering mediations were stopped for a variety of reasons which were explored in the qualitative study. Three participants reported stopping medications for blood pressure since *Start-LCHF* and there were no notable differences in the number of participants who reported medications for a variety of other conditions such as: inflammation; pain; allergies; gastrointestinal tract conditions; hormone imbalances; mental health conditions; and cancer. However, we only collected detailed dose information for diabetes medications and there may have been dose changes in these medications. Blood pressure medications were stopped by 3 participants and started by 1 at *Follow-Up* compared to *First-Assessment*. One participant had stopped cholesterol-lowering medications. Apart from the above, there were no major changes in medications at *Follow-Up* compared to *First-Assessment*.

Blood markers

Median HbA1c for all 28 participants at *First-Assessment* was 5.6 (5.3 – 6.1) %. There was no significant change in HbA1c in the 24 participants still following the diet at *Follow-Up* ($p = 0.48$). One participant had a notable deterioration in HbA1c, which increased to 14.1 % at *Follow-Up* (**Figure 3A & 3C**). The reason for this was unclear and he reported at *Follow-Up* that he was struggling with a variety of health and personal issues. There were 22 participants with HbA1c data at *Pre-LCHF*, *First-Assessment,* and *Follow-Up* (**Figure 3A**). The participants missing from this comparison were the 4 lost at *Follow-Up* and the 2 who started the diet prior to *Diagnosis* and therefore did not have a *Pre-LCHF* record. These 22 participants were further analysed in two subgroups: 6 participants who started the LCHF diet soon after diagnosis (**Figure 3B**); and 16 who had T2D for an extended period prior to *Start-LCHF* (**Figure 3C**). This was done as those participants who started the LCHF diet soon after their T2D diagnosis were unaware that they had diabetes at this time. In those that started the diet soon after *Diagnosis*, HbA1c was 9.5 (7.1 – 10.7) % at *Diagnosis*, 5.5 (5.4 – 5.7) % at *First-Assessment* and 5.4 (5.3 – 5.6) % at *Follow-Up* ($p < 0.001$, Friedman test; **Figure 3B**). By contrast, the 16 participants in **Figure 3C** were aware of their T2D status and *Pre-LCHF* values represent a managed state which included medications. In the participants who were managing their diabetes prior to *Start-LCHF*, HbA1c was 7.1 (6.5 – 8.3) % at *Pre-LCHF*, 6.1 (5.4 – 6.5) % at *First-Assessment*

and 6.1 (5.7 – 7.0) % at *Follow-Up* (p = 0.002, Friedman test; **Figure 3C**). The majority of medication changes described above were in this second subgroup and therefore the ~1 % reduction in HbA1c from *Pre-LCHF* to *First-Assessment* occurred in conjunction with these medication reductions (**Figure 3C**).

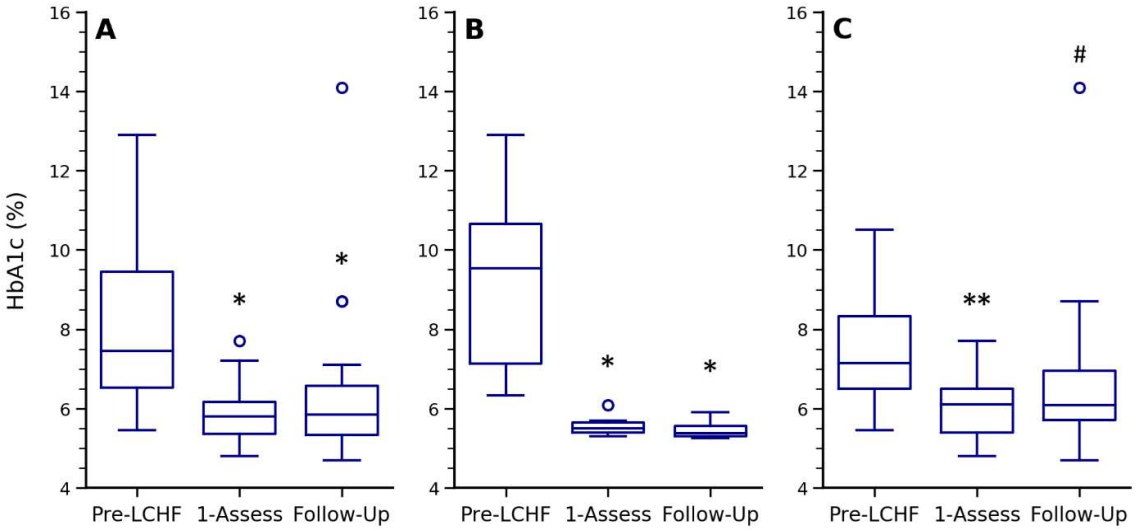

Figure 3. Change in HbA1c over time

HbA1c expressed as median, interquartile ranges, and outliers at *Pre-LCHF*, *First-Assessment* and *Follow-Up* for: **A**) all participants with valid HbA1c records at these three timepoints (n = 22, p < 0.01); **B**) the subset of participants who started LCHF shortly after diagnosis (*Pre-LCHF* is equivalent to *Diagnosis*) (n = 6, p < 0.01); and **C**) the subset of participants who were receiving conventional T2D management prior to starting an LCHF diet (n = 16, p = 0.03). P values were determined using the Friedman test. * and ** indicates a significant difference to *Pre-LCHF* (p < 0.05 and <0.01 respectively); # indicates that the p value for the difference between *Pre-LCHF* and *Follow-Up* changes from 0.08 to 0.02 if analysed without the 14.1 % outlier. Post-hoc p values were determined using the unadjusted pairwise Wilcoxon post-hoc test.

HbA1c was prioritised in this study as fasting serum lipid and plasma glucose concentrations were only present on some of the *Pre-LCHF* medical records. We were also unable to get fasting blood samples for all participants, particularly those not based in Cape Town. We therefore did not take any blood samples other than HbA1c at *Follow-Up*. The values that we did collect at *First-Assessment* are available in **Table 6.** Only 8 participants had lipid profiles on their *Pre-LCHF* medical records and at *First-Assessment*. At *First-Assessment versus Pre-LCHF* respectively, HDL-cholesterol was higher (1.4 (1.2 – 1.7) vs 1.2 (1.0 – 1.2) mmol/l; p = 0.02), triglycerides were lower (0.8 (0.7 – 1.0) vs 1.7 (1.2 – 3.0) mmol/l; p = 0.02), and there was no significant difference in total cholesterol (4.5 (3.8 – 5.1) vs 4.1 (3.9 – 4.9) mmol/l; p = 0.58) or LDL-cholesterol (2.5 (2.0 – 3.3) vs 2.2 (1.7 – 3.0) vs mmol/l; p = 0.33) in these 8 individuals. Every individual of these 8 participants had an increase in HDL cholesterol concentrations and all but one had a decrease in triglyceride concentrations since their LCHF diet. Seven participants had fasting plasma glucose concentrations at *Pre-LCHF* (7.7 (6.8 – 8.8)) and *First-Assessment* (5.1 (4.7 – 6.6)) and there was no significant difference at these timepoints (p = 0.18). Insulin concentrations were not measured on any of the *Pre-LCHF* or *Diagnosis* medical records.

Table 6. Blood glucose, insulin and lipid concentrations

Marker	***First-Assessment***
Fasting plasma glucose (mmol/l)	6.0 (5.1 – 7.4) n = 23
Fasting serum insulin (µU/ml)	13 (5 – 15) n = 21
HOMA-IR	3.4 (1.1 – 4.8) n = 21
Total cholesterol (mmol/l)	5.2 (4.0 – 5.5) n = 19
LDL-cholesterol (mmol/l)	3.2 (2.3 – 3.7) n = 19
HDL-cholesterol (mmol/l)	1.4 (1.1 – 1.) n = 20
Triglycerides (mmol/l)	1.0 (0.8 – 1.4) n = 20

Available results from *Pre-LCHF* medical records and at *First-Assessment*. LDL, low-density lipoprotein; HDL, high-density lipoprotein.

Weight loss

Self-reported weight loss was 16 (7 – 31) kg from *Start-LCHF* to *First-Assessment* ($p < 0.001$) and there was no change in body weight from *First-Assessment* to *Follow-Up* ($p = 0.64$) (Figure 4). All 28 participants reported weight loss, ranging from 2 to 63 kg, from *Start-LCHF* to *First-Assessment* and median weight loss as a percentage of *Start-LCHF* body weight was 17 (7 – 25) %. At *Follow-Up*, the 24 participants following an LCHF diet had sustained weight losses of 17 (6 – 26) % of *Start-LCHF* weight. At *Follow-Up*, there were 10 participants who had sustained weight losses of more than 25 kg, including 4 participants who had lost over 50 kg.

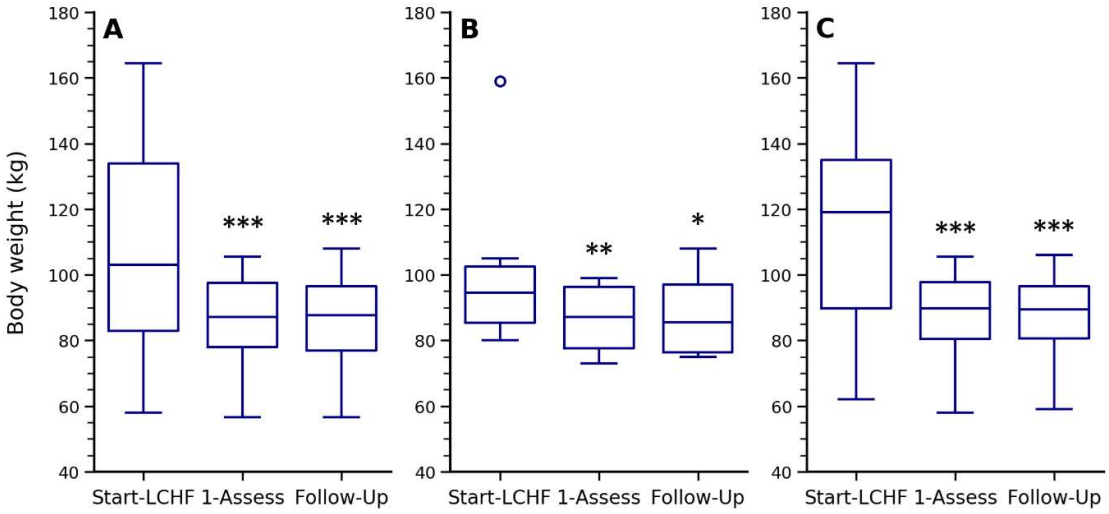

Figure 4. Body weight at Start-LCHF, First-Assessment and Follow-Up

Body weight expressed as median, interquartile ranges, and outliers at *Start-LCHF*, *First-Assessment* and *Follow-Up* for: **A**) all participants who completed *Follow-Up* ($n = 24$, $p < 0.001$); **B**) the subset of participants who started LCHF shortly after diagnosis ($n = 6$, $p = 0.03$); and **C**) the subset of participants who were receiving conventional T2D management prior to starting an LCHF diet ($n = 16$, $p < 0.001$). P values were determined using the Friedman test. *, **, and *** indicates a significant difference to *Start-LCHF* ($p < 0.05$, 0.01, and 0.001 respectively); Post-hoc p values were determined using the unadjusted pairwise Wilcoxon post-hoc test.

Diabetes remission

Of the 24 participants tested at *Follow-Up*: 7 were in complete T2D remission (HbA1c < 5.7 % and no T2D medications at *First Assessment* or *Follow-Up*); 3 were in potential complete remission (they met the definition of complete remission at *Follow-Up* but partial remission at *First-Assessment*); and 7 were in partial remission (HbA1c < 6.5 % and taking no T2D medications other than metformin at *First-Assessment* and *Follow-Up*). Seven participants were not in remission as they were taking glucose lowering medications in addition to metformin and/or had HbA1c greater than 6.5 % at *Follow-Up*. Of the 2 participants that could not be contacted for the *Follow-Up*, 1 met the definition of complete remission at *First-Assessment* and the other met the definition of partial remission. Of the 2 participants who stopped their LCHF diets, 1 was taking insulin at First-Assessment and *Follow-Up* (not in T2D remission) and the other participant was in partial T2D remission (HbA1c < 6.5 % and taking no T2D medications other than metformin at *First-Assessment* and *Follow-Up*).

Thematic analysis

The duration of interviews ranged from 21 to 64 min, with an average length of 38 min. Five themes, comprising 19 sub-themes, were identified during the thematic analysis and are reported in **Table 7**. Findings from each theme are presented below and additional quotes not including in the results are available in **Appendix G**.

Table 7. Themes and sub-themes from the in-depth interviews	
Themes	**Sub-themes**
Control of eating, hunger, addiction	Increased control of eating Reduced hunger Changed eating patterns Reduced cravings for sweetness Preference for whole foods Awareness of food and body
Control of diabetes, weight, health	Increased control of health Increased quality of life Reduced medications Positivity and energy
Sustainability	Lifestyle Ease
Social aspects	Difficulty eating out General negativity of public Lack of external control
Interactions with medical professionals	Participant independence Critical tone of medical professionals Apathy of doctors Scepticism and fear of medications

Eating, hunger, addiction

A major theme to emerge was that participants had increased feelings of control over their eating behaviour since following an LCHF diet, as well as a more positive relationship with food. Reductions in both hunger and addictive eating behaviours were reported as powerful facilitators of this control. Most participants expressed the sentiment that they "*do not get hungry*" on an LCHF diet and they often made comparisons with previous diets or weight loss attempts where they were "*always hungry*". A 48 y/o male said of his previous diet: "*I was low GI, low fat, hungry 24/7. Every time I ate, I was thinking about my next meal, I dreamt of food.*" Another participant compared his previous "*balanced diet*" to a LCHF diet, saying: "*I used to wear ear plugs to sleep…. I remember waking up chewing on my earplug. I was so hungry that in a dream I thought: 'this is food.' The biggest difference when introducing the fat was that the hunger was sated.*" Instead of hunger being ever-present and permeating their daily lives, participants found themselves satiated by meals and untroubled by hunger for much of the day. In addition to feeling hungry less often, participants experienced a milder form of hunger on an LCHF diet, rather than an urgent "*need*" to eat which they had felt previously. A 59 y/o female said of her hunger: "*When I got hungry* [prior to LCHF

eating], *it wasn't: 'I think I would like to eat something now', it was: 'if you don't eat something now, you'll die!'"* Similarly, a 59 y/o male said: "*The beauty of the whole LCHF scenario is you don't get the hunger cravings. So, it's not that you go somewhere and feel, 'well I have to eat'. You don't have to eat.*"

Participants spoke of how they had developed an awareness of the relationship between food choices, hunger, and their bodies, which led to organic changes in eating behaviours over time. Where previously hunger was feared, many participants now found it successfully guided them on when and how much to eat. Most participants expressed sentiments that they "*eat until I'm not hungry*" and "*eat what I want and when I am hungry*". Additionally, participants made changes to the timing and frequency of their meals. A 51 y/o female said of this process: "*I got to the stage where I don't actually feel like this egg in the morning…. It was probably after about a month* [on an LCHF diet] *that I stopped eating breakfast in the mornings before work*." Most participants said they now ate 1 or 2 meals per day. "*I stuck to* [previous patterns of eating] *and then realized, you shouldn't be eating if you are not hungry. So, I pushed my breakfast later and found I wasn't hungry*," said a 63 y/o female. Similarly, a 65 y/o female said: "*I realized, 'Hang on, I am getting it wrong. I am eating because it is meal time. I am not eating because I am hungry and I am really following the same bad patterns. I am just changing what is on the plate*.'" Another common eating change described by most participants was a large reduction, and in many cases a complete cessation, of snacking between meals. Some participants mentioned that they were able to fast for longer periods such as 48 hours or more. However, these longer fasts were often done with the intention of breaking weight loss plateaus rather than as a natural adaptation to changes in hunger. Some participants monitored blood glucose after meals and were aware of specific foods which spiked their blood glucose concentrations. A small minority of participants said they counted calories in order to control calorie intake.

In addition to altered feelings of hunger, participants described how the LCHF diet helped them control binging and addictive eating behaviours. A powerful "*craving*" for carbohydrates, and sweetness in particular, emerged as the main driver of these eating behaviours. Participants found their taste for sweetness had changed since following an LCHF diet, and that they now had either a reduced desire, or no desire for sweetness and sweet foods. Several participants described no longer feeling "*bothered*", "*triggered",* or "*turned-on*" in situations such as shopping aisles and parties containing cake, sweets and baked goods. One 65 y/o male said: "*I can happily sit through a party where people are eating everything under the sun and I am not tempted by it."* A 66 y/o female described this process as "*breaking the sugar barrier*" and a 67 y/o female said of her experiences with sugar: "*I miss the sweet stuff. But the less you have of it the less you want."* Several participants

identified themselves as carbohydrate or sugar addicts, such as one 65 y/o male who said: "*I have come to understand about LCHF, that I'm an addict. Carbs are something that I'm probably never going to be able to eat for the rest of the life.*" These participants described how their addictive response to certain foods meant that total elimination of these foods was their only option. *"If I have 1 biscuit, it becomes 3 biscuits, it becomes the rest of the packet,"* said a 58 y/o male. Similarly, a 48 y/o male said: "*Not having bread at all, I could cope with, but having one piece of bread was just infuriating and difficult for me.*" All participants spoke of their diets in terms of whole foods and were wary of any manufactured foods. Some participants were concerned about food products with the potential for hidden ingredients, such as a 64 y/o female who said: *"I don't eat sausages because I'm aware that there is cereal added to sausages."* Many participants said that they would avoid anything in *"packets*" and a 49 y/o female said: *"If it's got a label, I generally tend to avoid it."* Similarly, a 67 y/o female said: "*I don't eat anything that comes from a box or a tin, where I have to read the ingredients.*"

Despite their reduced desire for sweetness, participants remained "*wary"* and actively avoided sweet tasting foods. To this effect, many participants stopped eating sweet tasting foods which were low in carbohydrate – a category of foods predominantly made up of low-carbohydrate products and substitute foods. According to one 67 y/o male: "*I took one bite* [of a low carb product] *and it was just full of xylitol and very, very sweet. Immediately I wanted to eat the whole thing, and I would have had that, and another one. That was the immediate response.*" Many participants developed an awareness for how non-carbohydrate-containing foods as well as their behaviours affected their future control of eating. For one 41 y/o female, this resulted in a change in alcohol intake. She said: "*I don't drink alcohol anymore, because … you have a couple of glasses of wine and the next morning, it's like you're starving.*" There were a few participants who struggled to restrain their eating even with LCHF foods. One 59 y/o female said: "*If I binge on things, it would be something like pork scratchings, which are on the top shelf, so I would have to get a ladder out.*" Another example was a 56 y/o male who said: "*If I cook for 2 days, then I get the urge to eat because it was delicious and I couldn't leave it alone, I couldn't take my hands off it…. Although it was never with carbs, never with rice or bread or potatoes, always vegetables and meat and eggs and fish.*" A number of participants were aware of eating despite knowing they were not hungry (on their LCHF diets). Some of the reasons they ascribed to these situations were mindless snacking, daily habits such as eating when they got home, boredom, and stress.

Diabetes, weight, health

Participants felt increased control, confidence and a sense of accomplishment at having improved their diabetes status, weight, general health, as well as other specific health conditions. Every participant said their diabetes status had improved since following an LCHF diet and they described their diabetes with phrases such as: "*under control*"; "*gone*"; "*no trace of it*"; "*doesn't exist*"; "*has not progressed*"; and "*definitely improving*". According to one 56 y/o male: "*It cured my diabetes, that's for sure. I am diabetes free now. As long as I keep doing LCHF, I have no diabetes anymore.*" A concurrent decreased reliance on diabetes medications further empowered the participants and added to their feelings of control. A 63 y/o female said: *"[I am] very positive in the fact that I feel more under control. That I can do this by diet - I really do not want to go onto medications. I can control it by diet, which is very positive for me.*" A 46 y/o male expressed similar sentiments when he said: "*All I can say is that it's an amazing, positive feeling…. I eat right, I drink less medication, and I'm still weaning myself off it. So, yes, that's a huge positive.*" Participants who stopped injecting insulin, reduced their insulin dose, or felt they had avoided starting insulin, described these outcomes as extremely empowering and motivating. For some, it was the most important feature of the LCHF diet, such as one 67 y/o female who said: "*My goal was to get off the Insulin. That was my ultimate goal.*"

Most participants experienced weight loss and improvements in general health which they felt had impacted positively on their quality of life. For many participants, following an LCHF diet was the first time they had lost weight without hunger. It was also the first time most had been able to sustain weight loss, despite many previous dieting attempts. Some participants lost life-changing amounts of weight, such as one 48 y/o male who said: "*The function of the diet* [LCHF] *is the weight loss and the function of the weight loss is that I am not carrying seventy-three extra kilos. There are just so many positives in having lost this weight."* Together with weight loss, just about every participant described an aspect of their health, something that had bothered them for years, that had improved or disappeared. Often participants said they were surprised by these improvements as it was not something they had anticipated. Every individual had a different experience and the wide variety of improvements described by participants included, amongst other things: skin and skin conditions; headaches; general pain; joint pain; neuropathy pain; fertility; cramping; gout; sleep; and hypertension. The majority of participants emphasised the impact that weight loss and general health improvements had on their quality of life. According to a 63 y/o female: "*Knowing how good I feel health-wise and weight-wise, I would never want to go back. I wouldn't want the battles that I*

had with excess weight and with diabetes particularly, and all the other health problems, which have gone. It is like being a miracle."

A dominating theme to emerge was the positivity participants felt from having increased energy levels and improved mobility that they associated with weight loss and an LCHF diet. Increased energy levels were described both as a general excitement and enthusiasm for life as well as the specific ability to focus and concentrate better. A 52 y/o male said: "*I'm far more positive about the future. The pain in my knees and my back have disappeared. I'm more focused and I'm able to concentrate more on a particular task.*" This sentiment was expressed by most participants. One said: "*All of a sudden I had so much energy,*" and others described themselves as feeling: "*hyped about life*"; "[having] *more energy and a new sort of enthusiasm*"; "*positive about myself*"; and "*more alert, more awake, more alive.*" A 59 y/o male said: "*I can concentrate longer and work longer. I'm more alert…. My energy levels are up as well. I feel more energetic.*" For some participants, increased energy levels resulted in them being more active. According to a 41 y/o female: "*Just in terms of walking around the block with my child every day, that kind of thing, 3 or 4 years ago, you must be joking. I'd be like, do what!?*" Similarly, a 63 y/o female said: "*Before I was always sleepy and tired and just had no energy. Now I am up and clearing the house and spring cleaning and my husband is very amused - I am always active, like I used to be in my twenties.*" Not one participant said that their exercise capacity or activity levels had decreased since starting an LCHF diet and most said they had more desire and energy for physical activity than previously.

The only negative physical symptom to emerge as a theme was constipation, which was reported by a minority of participants. For most who experienced constipation, it resolved after 2 to 3 weeks on the diet. However, one said it took longer than a year to disappear and a couple of other participants said they were still battling with constipation. For example, a 67 y/o female said: "*The one thing that I do have a problem with is extreme constipation. It is really uncomfortable and I don't know what to do about it.*"

Sustainability

Almost all participants said that an LCHF diet was very sustainable for them, and many participants specifically said it was a *"lifestyle"* rather than a diet. "*I think people hear the word diet, suggesting it's something that you do short term.... So, it's a way of living rather than a diet. I think that distinction is important,*" said one 49 y/o male. Many participants highlighted that the ease of sustainability was a defining feature of the LCHF diet for them. The general theme was that

participants did not feel "*deprived*" and were not using will power to sustain a diet. "*I believe every diet works. I simply came back to saying this is sustainable, it is sustainable,*" said a 58 y/o male. One participant, a 46 y/o male, compared his LCHF diet to his previous dieting attempts: "*I've never been able to lose weight for more than a few months* [before LCHF] *and I've been at LCHF for almost a year now, so I would say I'm going to eat that way for the rest of my life.*" For most participants, not having to measure quantities or count calories, contributed towards the ease and sustainability of LCHF eating. "*I think it's exceptionally sustainable. I've actually focused on making it sustainable. That is why I don't want to measure, I want to learn to judge what I need to eat and how I need to eat,*" said a 65 y/o male participant. Only one participant felt that LCHF eating was not sustainable. She said: "*It takes out much of the joy for me at this stage. I remember being very sorry for myself when I started with LCHF. I couldn't freely partake in everything anymore.*"

Quite a few participants mentioned that the initial adaptation phase was difficult, where they needed to overcome cravings for sweet foods or manage boredom or comfort eating. However, they all said that LCHF eating was easy to sustain after that period. A 63 y/o female said: "*The first two weeks is not so nice, but thereafter, just amazing and I will never go back.*" Some participants reported that prolonged periods of travelling or living outside of their own homes made it more difficult to sustain LCHF eating. A 64 y/o female highlighted this issue when she said: "*As far as sustainability, in my own home where I'm cooking, no problem. It could become a problem over the next month because I'm going to be living in my daughter's home.*" One participant said that sustainability for her was dependent on weight loss. She said: "*The centimetres are going but the kilograms are not falling as fast. I must say, I am sitting at a stage where I think a lot of people are saying, 'Is this the right thing?'*" No other themes emerged as a challenge to sustainability, including the cost of foods.

Social aspects

The main negative aspect of an LCHF diet to emerge as a theme was that socialising was difficult. Many participants found they no longer enjoyed eating out, such as one 46 y/o male who said: "*Having supper in a restaurant is a problem in the majority of restaurants. It's just not enjoyable anymore*." One problem raised by participants was that restaurants generally did not have appropriate foods available. A bigger problem, especially when eating out with friends or at friends' houses, was that participants felt conflicted as they wanted to be polite but also to stick to their diets. Sometimes participants felt they had to eat foods they would otherwise have avoided. For example, one 52 y/o female said: "*When people are going out for dinner and they are not aware of*

your [eating habits]*, occasionally you just need to put* [LCHF eating] *aside and eat what they give you.*" Other participants held a firmer stance. A 62 y/o male said: "*The odds are, if we are going out to eat, then we've either eaten beforehand or we're not going to eat and basically have water, because it's pointless. You just can't get people to give you what you need.*" Some participants' social lives were impacted to the point where they said they no longer wanted to go out, even though socialising used to be a big part of their lives.

Social situations were also made difficult for some participants who said that colleagues and acquaintances held a negative attitude towards LCHF diets and were often judgmental. These participants were frustrated that they received "*flak*" and negative comments from people they felt didn't understand the improvements that LCHF eating had made to their lives. A 33 y/o female said: "*You get the frowns, the 'Why are you not eating fruit anymore? Why are you not eating sugar anymore?' So, that has been my main battle.*" A 49 y/o male said: "[People] *can't put two and two together to see it's benefited me. 'You can have a heart attack if you eat all that fat.' All of those stereotypical comments do come out on occasion*." Several participants said that in certain social settings, such as casual work parties, people became irritated with them and often tried pressuring them into eating sugary foods. As one 63 y/o female said: *"People don't understand that I am trying to* [heal] *without medication and I am trying to lower my sugars with diet. They will say things like, 'Oh just have a piece of cake, it won't kill you.'"*

Interactions with medical professionals

Participants generally displayed a wide knowledge of health and nutrition and said they found out about LCHF diets on their own through books and online platforms. "*I guess I was lucky to find and to have access to knowledge and have the right mentality to discover what I did on my own,*" said a 49 y/o male. Only a few participants spoke about dietitians, despite being prompted about any interactions they had with them. Two described helpful interactions regarding LCHF eating, while the majority simply said they decided not to follow the advice of dietitians: "*I smiled politely and went away and did my own thing.*" Others said they "*could not relate*", or "*did not believe*" in dietitians. Every participant had an active relationship with at least 1 medical doctor. Only a few participants were given direct advice to follow LCHF eating by their doctors. One such participant said: "*My doctor told me to go completely onto meat, poultry, oily fish, cheese, green healthy vegetables, leafy vegetables and come back and see him in 3 months.*" Most participants had "*informed*" their doctors that they had already been following an LCHF diet, while some participants had decided not to tell their doctors about it, despite having followed an LCHF diet for several years.

The majority of participants said their doctors were cautious or concerned about LCHF diets but were supportive of their efforts to improve their health. Concerned doctors would often hand responsibility back to the participant, using phrases like: "*it is in your hands*"; "*if you're comfortable with it*"; "*well, if it works for you.*" Many participants expressed frustration that even though their doctors were "*amazed*", "*thrilled*" or "*impressed*" with their results, they were still reluctant to discuss or acknowledge LCHF eating. A 59 y/o male said: "*Well I think he is pleased with* [my results]*, but he obviously won't discuss* [LCHF]*."* A 67 y/o female said: "*I would like to see the point where the doctors that are seeing me, the regular doctors that are seeing me, recognized the fact that I have lost weight, that I am on a strict diet, that I take responsibility for my health*".

There were a few participants who had hostile interactions with doctors who were strongly opposed to LCHF diets. One participant said he formalised the following relationship: "*I made a deal with my doctors. I said to them, I am in charge of my health, not you.*" A 58 y/o male said: "*Not one of the 7 medical persons that I interacted with supported me, not one of them*." This conflict was usually related to concerns about blood cholesterol concentrations and cardiovascular disease, which was most intense when participants stopped taking cholesterol lowering medications against their doctors' advice. Some participants doubted whether they should continue LCHF eating after their doctors "*berated*" them for their blood cholesterol levels. However, most participants remained confident in their decision to follow LCHF eating and to reduce or stop taking statins. A 72 y/o male said: "*I immediately wrote to* [my doctors]*, saying, 'I have stopped statins on my own accord and I acknowledge that you have told me to take them.' They are so shit scared of the Medical Health Council.*" A 49 y/o male who stopped statins on his own said: "*The doctor tried to put me on statins and I refused. Because I'd read quite a lot of research and literature on statins and how they were, at best, probably a marginal benefit for the majority of people who took them.*"

In addition to being sceptical about statins, a broader theme to emerge was that many participants started questioning whether their other medications were appropriate or necessary. Most participants said they had benefited more from their recent LCHF diet than they had from years of taking various medications, including relief from long-standing conditions they thought were unrelated to diet. One 62 y/o male said: "*When you get involved in reading up about what goes on, you start understanding that maybe there are things that you've been prescribed which are inappropriate.*" This left many participants "*wondering if they couldn't reduce the meds*."

Discussion

This study investigated the habitual diets and diabetes status of individuals with T2D who claimed to be following an LCHF diet. An aim of this study was to provide an indication of the types of foods, nutrients, and eating patterns that constituted an LCHF diet that was sustainable for individuals with T2D in a real-world setting. All but one participant followed either a *very low* (< 50 g/day) or *low* (< 130 g/day) carbohydrate diet [9]. This low level of carbohydrate intake is noteworthy, given that participants were not screened for eligibility according to macronutrient intake. Their diets were also not prescribed or incentivised by a clinical trial and very few participants reported that they had input from medical professionals in developing their diets. Participants were generally well informed on publicly available LCHF information, suggesting that their diets were largely influenced by these sources of public messaging. Many participants also reported that they had developed an awareness of how all foods affected their hunger and health, irrespective of carbohydrate content. Therefore, the food and nutrient data described in this study reflects diets that each individual had evolved for themselves, based on both their nutritional knowledge and the result of their personal experimentation.

Participants in this study were affluent and well educated. The majority of participants were respondents to the social media recruitment campaign. The affluent study demographic may therefore reflect the type of individuals that have good access to social media. Alternatively, the demographic could be representative of the type of individual most likely to pursue a T2D treatment strategy that runs counter to conventional advice. An important characteristic of these participants was that they felt able to source and evaluate medical opinions independently of their physicians and dietitians. These participants trusted in their ability to interpret this information accurately enough that they were not persuaded to stop their intervention in the face of cautionary advice or hostility from medical professionals. Their understanding of the physiology of carbohydrate restriction, while not necessarily correct, demonstrated that participants were well informed about why they were following an LCHF intervention. This may explain the desire of these patients to engage with their doctors on the underlying principles and physiology of their diets [267]. Less affluent and less academic individuals may be less likely to pursue such an intervention as they may be unwilling to trust their own opinions over that of their doctors. Very little is known about the wider LCHF population in South Africa and it would a target for future research would be to better characterise the followers of LCHF-type diets in less affluent population groups.

There were a number of diet-related features other than macronutrient composition that were common amongst participants. Firstly, reported diets included predominantly *unprocessed* and *minimally-processed* whole foods according to NOVA classification [268], or processed foods like cured meat, dairy, and coconut products. *Ultra-processed* foods, including those specifically marketed as low carbohydrate products, were not frequently reported. Secondly, many participants naturally stopped snacking between meals and reduced the number of meals they ate each day. Thirdly, a large proportion of reported foods were from animal sources such as meat, poultry, dairy, and eggs. Finally, it is likely that participants reduced their energy intake after adopting their LCHF diets, given the large reductions in reported body weight. It is therefore not clear to what extent these factors may have influenced the health of these participants independent of carbohydrate restriction [241,269,270]. In order to recognise the above nuances, the term 'LCHF lifestyle' may be a more appropriate description of the interventions of participants in the current study. These factors are also important to consider when designing and interpreting future controlled studies, especially since LCHF diets may produce spontaneous reduction in the frequency of eating, associated with reduced calorie intakes.

The inclusion criteria for the current study would have favoured participants that had positive health experiences with an LCHF diet and would have excluded those who stopped the diet within 6 months of starting it. Even so, it is remarkable that so many of these participants were either in complete or partial T2D remission, given the assumed progressive nature of the disease and the rarity of remissions with conventional treatment [7]. That LCHF diets have the potential to put T2D into remission is becoming increasingly recognised, especially in trials with a high level of patient monitoring and support [8,139]. The 7 participants from the current study who started their LCHF diet soon after diagnosis responded particularly well, which agrees with the notion that an LCHF diet may be especially effective for remission in newly diagnosed T2D patients or those taking fewer medication [8,9]. The selection of highly motivated participants may explain why our findings are considerably more favourable than conclusions from a number of recent meta-analyses of RCTs [132-134]. These meta-analyses generally show a small advantage of the lower carbohydrate diet for improving glycaemic control, which was most prominent early in the trial but was often less clear or absent at trial end. The true efficacy of carbohydrate restriction is difficult to determine from these meta-analyses as the RCTs often had reduced dietary compliance (in both groups) over time, and included in the analysis participants that did not adhere to the intervention [184]. Additionally, many of the RCTs included in the meta-analyses achieved or targeted only *'moderate*' carbohydrate

restriction [132]. An important avenue for future research should focus on maximising dietary compliance.

There are also aspects of our participants' interventions that are particularly controversial. A large proportion of reported foods were from animal sources, including red meat and cured meats such as bacon. Saturated fat-containing foods were commonly reported whereas wholegrains and fibre intake was relatively low. Although low carbohydrate eating patterns have recently been included in the ADA and EASD guidelines, the high fat component of the diet has not been endorsed [2,271]. All of these features of the participants' diets have also been associated with negative health outcomes in long-term prospective cohort studies [66,272-276]. Additionally, low carbohydrate diet patterns (generally 30 - 40 % carbohydrate), and particularly animal-based low carbohydrate diet patterns, have been associated with increased risk of T2D incidence and mortality [44,50,53,277]. The above associations from prospective cohort studies likely underpin the lack of encouragement and support that many of our participants experienced from their physicians for the LCHF diets. A result of this was that many participants started their diets and/or reduced their medications without appropriate medical supervision, which is potentially dangerous [278]. It also exposes an interesting dilemma: should a patient who has improved their T2D, lost considerable body weight, reduced their medications, and subjectively improved their quality of life with an animal-based LCHF diet, be advised to change aspects of their intervention based on population level nutritional associational studies? Many T2D nutritional recommendations state that patients should be supported in an individualised diet that suits their preferences and lifestyle [6,279]. Clinicians are therefore encouraged to engage openly with patients about the potential harms and benefits of LCHF diets and make decisions which take into consideration individual patients' values and preferences [280,281].

It is well established that LCHF diets can lead to a spontaneous reduction in calorie intake [220,282,283]. In fact, it is common for randomised control trials to restrict calorie intake in the control diet group to more closely match the (reduced) calorie intake of the LCHF group, despite the latter being allowed *ad libitum* consumption of high-fat foods [133,134,136]. Outcomes from such trials are often similar between groups, which would suggest that the calorie deficit is important for health benefits, rather than a specific dietary composition [279]. However, long-term calorie-restriction induces compensatory responses of increased hunger, obsession with food, fatigue and lethargy [284-286]. Our participants described experiencing the reversal of these exact symptoms, and it was the lack of these symptoms to which they attributed the sustainability of their diets. They also associated their weight loss, increased energy levels, increased activity, reduced medication use, and improved sense of well-

being with their LCHF lifestyle. The precise relationship and relative importance of each of these factors is complex, but they all appear to be in some way facilitated by the diet, and likely act to reinforce each other [287,288]. These factors might explain the variable health outcomes and adherence rates observed in previous intervention trials and highlight the need for further research into the importance of conscious versus unconscious regulation of calorie intake when evaluating the efficacy of dietary interventions.

Participants in the current study revealed a complex interaction between food choice and appetite regulation, that went beyond carbohydrate content. For the remission of T2D with diet, severe calorie restriction [217] and/or severe carbohydrate restriction [8,139] appear to be essential. However, to achieve control over appetite, the removal of all foods which induce hunger and/or interfere with normal physiological regulation in a particular individual would appear to be important [289,290]. The foods that our participants identified as having these effects were predominantly sweet tasting, packaged, ultra-processed, and/or rich in carbohydrate. Descriptions of cravings, binging, increased tolerance, withdrawal, and reward, were also ubiquitous during the interviews when referring to these foods, suggesting that certain aspects of food addiction were present in our participants [291,292]. It is however possible that different foods (such as those high in fat) may elicit this effect in other individuals that would not included in this study. The impact of these foods in our participants is consistent with the idea that the physiological and psychological effects of highly-processed and highly-palatable foods may underlie the excess energy intake associated with the recent rapid increases in rates of T2D and other chronic lifestyle diseases [270,293].

Cholesterol-lowering medications were clearly a source of conflict between participants in the current study and their medical practitioners. Many participants were reluctant to take these drugs and doctors reportedly expressed concerns over how their diets would affect their blood cholesterol levels and consequently their potential risks for future heart disease. As with the decision to follow their diets, the majority of participants in the current study were well informed on the topic of cholesterol-lowering medications and their opinions had been carefully considered. The results of this study take no stance as to whether participants' opinions were correct; it reveals only that they had informed opinions on which they based their actions. Even though the serum cholesterol data was only available for comparison in 8 participants, it is interesting to note that the changes in lipid profile in these participants was in line with expected changes after an LCHF diet. Serum HDL-cholesterol concentrations increased, triglyceride concentrations decreased, and changes in LDL-cholesterol and total cholesterol were variable [132,157]. Regardless, our findings highlight that the

impact of high fat diets on blood cholesterol concentrations is currently of concern to physicians. Given the controversy in the literature over the role of LDL- cholesterol in the development of heart disease, it is unlikely that these concerns will be resolved in the near future [12,13].

Limitations

This was a descriptive study which would have selected for individuals who responded well to, and were able to sustain an LCHF diet. It therefore does not provide evidence that an LCHF diet is appropriate for a wider T2D population. The diets described in this study were those that participants had sustained and refined based on personal experience. As such, they do not necessarily represent an ideal LCHF diet but rather provide an indication of the types of diets being followed in a real-world setting. Much of the data are self-reported. Micronutrient data was not available for some LCHF foods and we did not record vitamin and mineral supplementation. For practical reasons we were unable to collect complete data for blood markers other than HbA1c.

Summary and conclusions

This observational study documented 28 T2D patients who reported following a self-administered LCHF diet that was rich in full-fat dairy, fatty meats, coconut oil, non-starchy vegetables, nuts, eggs, olives, olive oil, and avocados. Whilst following their LCHF diets, HbA1c, body weight, and T2D medication requirements were dramatically reduced, with the majority of participants having achieved complete or partial T2D remission. Participants perceived reduced hunger and cravings as one of the most important aspects of their new diets. Of concern, many participants felt unsupported by their doctors which may have resulted in inadequate medical supervision. This study described the characteristics and nuances of an LCHF 'lifestyle' that was sustainable and effective for certain T2D patients in a real-world setting. An important take-away message from Chapter 2 was that many interrelated factors contributed to the health improvements experienced by these participants, including factors such as: reduced hunger, less frequent meals, weight loss, improved psychological state, and increased energy and activity levels. Future research investigating the effects of LCHF diets on gluconeogenesis requires careful consideration for which of these factors should be controlled for versus included as part of the intervention.

Chapter 3

The quantification of gluconeogenesis in the early post-absorptive period using stable isotope tracers

Introduction

Glucose is a 6-carbon molecule that is an important energy substrate in the body. Blood glucose concentrations are homeostatically regulated to stay within a narrow target range of 4 − 6 mmol/l. Concentrations below this range (hypoglycaemia) can be acutely dangerous while long-term exposure to high blood glucose concentrations (hyperglycaemia) is ultimately toxic. There is a constant flux of glucose through the circulation. On the one hand, glucose is continually leaving the circulation to meet the glucose requirements of body tissues. On the other hand, there is a steady supply of glucose into the circulation to prevent hypoglycaemia. The rate at which glucose enters the circulation is referred to as the rate of appearance (*Ra*) and the rate at which glucose leaves the circulation is referred to as the rate of disappearance (*Rd*). *Ra* is inclusive of glucose entering the circulation from any source. Glucose absorption from the gut, after the ingestion and digestion of dietary carbohydrate, is termed exogenous glucose. Sources of blood glucose originating from within the body are referred to as endogenous glucose production (EGP).

EGP is important as it maintains a steady supply of glucose into the circulation regardless of exogenous carbohydrate availability. There are two metabolic pathways which contribute towards EGP: 1) the release of glucose from liver glycogen; and 2) the production of glucose from precursor molecules. The liver stores glucose as glycogen during periods of exogenous glucose availability and has the capacity to store 80 − 100 g of glycogen. Glycogen is then broken down into glucose and released into the circulation when exogenous glucose is not available – a process called glycogenolysis. The production of glucose from precursor molecules is called gluconeogenesis and occurs predominantly in the liver, whilst the kidneys make a small contribution [294]. The bulk of gluconeogenesis involves the synthesis of glucose from the 3-carbon precursor molecules: lactate; pyruvate; glycerol; and certain amino acids (such as alanine). The role of gluconeogenesis and glycogenolysis in the regulation of blood glucose concentrations in healthy and T2D individuals will be reviewed in the sections that follow.

3.1) Glycaemic regulation in healthy individuals

The regulation of glucose homeostasis in healthy individuals in the absorptive and postabsorptive states will be reviewed in this section. The postabsorptive state begins once the absorption of nutrients from the gut after a meal is complete, and extends until more nutrients are consumed. Therefore, by definition, in the postabsorptive state, there is no exogenous glucose contribution to *Ra*, and EGP is therefore equivalent to *Ra.* The postabsorptive state is characterised by relative stability: EGP and *Rd* are generally well matched, metabolic changes are gradual, and blood glucose concentrations are stable, although they may decline over time. By contrast, the absorptive state after a carbohydrate-containing meal is characterised by a large disturbance in glucose homeostasis which triggers an acute whole-body metabolic response aimed at restoring blood glucose concentrations to normal levels. Exogenous glucose disturbs the existing balance between EGP and *Rd* and results in increased blood glucose concentrations. In response, there is a large secretion of insulin from the beta-cells of the pancreas into the circulation. In peripheral tissues, the insulin-dependent transport of glucose into cells is stimulated, glucose oxidation and glycogen storage are stimulated in skeletal muscle, and the formation of triglycerides is stimulated in adipose tissue [295]. These effects result in a large increase in glucose *Rd*.

Additionally, high blood glucose and insulin concentrations act on the liver to reduce *Ra* via a reduction in EGP. Gluconeogenesis and glycogenolysis in the fed state have been difficult to study but it is clear that glycogenolysis is fully suppressed at physiological levels of hyperinsulinemia which would occur after a carbohydrate-containing meal [296-298]. Instead of glycogen breakdown, liver glycogen synthesis is stimulated and glycogen content is therefore increased [298]. The majority of glucose which is stored as glycogen originates from the circulation, which includes the recently ingested carbohydrate. This is referred to as the direct pathway of glycogen synthesis (i.e. glucose enters the liver and is converted into glycogen) [299-301]. Importantly, a significant fraction of stored liver glycogen originates from gluconeogenesis, indicating that some portion of gluconeogenic output is directed towards glycogen synthesis during hyperinsulinemia [302]. This is referred as the indirect pathway of glycogen storage (i.e. glucose produced by the liver is stored as glycogen rather than released into the circulation) [299,301,303]. In contrast to glycogenolysis, there is uncertainty of the extent to which gluconeogenesis is suppressed in the fed state. There seems to be some contribution of gluconeogenesis to EGP at physiological levels of hyperinsulinemia and un-physiologically high levels of hyperinsulinemia are necessary to fully suppress gluconeogenesis [296,302,304-306]. With the exception of Petersen et al (1996)[305], the studies on which this conclusion is

drawn involved the direct infusion of glucose into the circulation during a hyperinsulinemic normoglycemic "clamp". This involves the infusion of insulin to maintain hyperinsulinemia and the infusion of glucose to maintain blood glucose concentrations. It provides a measure of insulin sensitivity but may not accurately represent the metabolic and hormonal effects of a meal. The effects of an ingested meal on EGP and gluconeogenesis are less well studied.

In healthy individuals, glucose homeostasis is restored within 1 to 2 hours of a carbohydrate-containing meal and blood glucose and insulin concentrations return to baseline values. Metabolism transitions into the post-absorptive period in which *Ra* consists of EGP and is well matched to *Rd*. The equilibrium between EGP and *Rd*, and the relative contributions of gluconeogenesis and glycogenolysis to EGP, changes constantly as fasting progresses. After an overnight fast of 12 − 17 hours, when there is no exogenous glucose contribution to *Ra*, gluconeogenesis usually constitutes 40 − 60 % of EGP, with glycogenolysis making up the difference [303,305,307]. As fasting progresses, rates of glycogenolysis decline due to the progressive fall in liver glycogen content. Liver glycogen content is eventually reduced to the point that glycogenolysis no longer makes a meaningful contribution to EGP. Therefore, after 40 − 62 hours of fasting, gluconeogenesis accounts for upwards of 95 % of EGP [303,307]. An important observation from these studies is that *absolute* rates of gluconeogenesis appear relatively constant during this whole process, regardless of whether gluconeogenesis contributes 40 % of EGP after an overnight fast or close to 100 % of EGP after several days of fasting [294]. Therefore, the gradual decline in glycogenolysis as fasting progresses causes an equivalent decline in EGP, until such time that EGP is essentially equivalent to the rate of glucose production from gluconeogenesis. At this point EGP, Ra and rates of gluconeogenesis are essentially all the same.

The majority of studies which have investigated hepatic gluconeogenesis have investigated participants who followed conventional high or moderate carbohydrate diets so that it was common for standardised high carbohydrate meals to be provided to participants in the days prior to the experiments. However, there have been a few studies which have investigated the effects of *very low* carbohydrate diets on endogenous glucose production and gluconeogenesis. Gluconeogenesis has been quantified after 4 carbohydrate-free meals [308,309], after 11 days on a diet containing 2 % carbohydrate [310,311], and after long-term habituation to approximately 50 g/d of carbohydrate [312]. All of these studies quantified gluconeogenesis after an overnight fast. Compared to higher carbohydrate diets, glycogenolysis was significantly lower on the low carbohydrate diet. After an overnight fast, gluconeogenesis contributed upwards of 75 % of EGP on the low carbohydrate diets, compared to approximately 50 % of EGP with control diets. However, absolute rates of

gluconeogenesis were not statistically different, or were only slightly elevated, providing further evidence that rates of gluconeogenesis are relatively constant under a wide range of physiological conditions [294].

The decline in EGP during fasting or with adherence to severe carbohydrate restriction occurs concurrently with a reduction in *Rd* [303,313]. Metabolically flexible tissues, such as skeletal muscle, can oxidise any combination of glucose or fat for energy. These tissues favour glucose oxidation during periods of high glucose availability but shift to favour fat oxidation when carbohydrate availability is low [298,314]. There are other tissues which have a less flexible demand for glucose, the most notable example being the central nervous system. The blood-brain barrier limits the availability of fatty acids to the central nervous system, which is therefore heavily dependent on glucose oxidation under normal physiological conditions which occur with conventional high and moderate carbohydrate diets [315]. However, after several days without dietary carbohydrate, this glucose oxidation requirement is greatly reduced due to the production of ketone bodies [316]. Ketone bodies are produced when there are very high rates of fat oxidation in the liver [317]. When the production of acetyl-CoA from the beta-oxidation of fatty acids exceeds the oxidation capacity of the liver, the excess is converted into ketone bodies which are then released into the circulation. Beta-hydroxybutyrate is the most abundant ketone body and it can be used as an energy substrate by the central nervous system. After an extended period without carbohydrate (due to fasting or dietary carbohydrate restriction), gluconeogenesis is effectively the only source of glucose for the body. Rather than gluconeogenesis increasing to match the demand of body tissues, the demand for glucose in the body is drastically reduced, as metabolically flexible tissues oxidise fat, while ketone bodies contribute towards the energy requirements of the central nervous system [316].

3.2) Glycaemic dysregulation in type 2 diabetes

T2D is associated with many metabolic abnormalities in body tissues which combine to cause the dysregulation of glucose homeostasis in the absorptive and the post-absorptive states. As with healthy individuals, exogenous glucose from dietary carbohydrate results in increased *Ra* and a rise in blood glucose concentrations. This stimulates the release of insulin from the pancreas into the circulation. Depending on the severity and stage of T2D, this release of insulin is either very large (early stage T2D) or insufficient (late stage T2D with pancreatic beta-cell failure) [318]. Regardless, peripheral tissue insulin resistance limits the extent to which *Rd* can be increased [319]. Reduced glucose utilisation in the periphery is also associated with impaired skeletal muscle glycogen synthesis and reduced metabolic flexibility [222,314], as well as the continued mobilisation of free fatty acids from adipose tissue [320].

Additionally, abnormalities in liver metabolism reduce the extent to which EGP is suppressed after a carbohydrate-containing meal [321]. Whereas glycogenolysis is suppressed in healthy individuals, it is incompletely suppressed in T2D [311,322]. This has been associated with a reduced liver glycogen storage capacity and impairments in glycogen synthesis pathways in T2D [323-326]. Glycogen storage pathways are unable to effectively contribute towards glucose utilisation, which results in glycogen cycling (the simultaneous synthesis and breakdown of glycogen) in the liver [327]. Additionally, glucose produced via gluconeogenesis cannot be stored as glycogen via the indirect pathway [325], which may account for increased gluconeogenesis during hyperinsulinemia in T2D compared to non-T2D controls [322].

Importantly, glucose absorption from the gut does not appear to be impaired in T2D [328]. Therefore, individuals with T2D enter the post-absorptive state at approximately the same time after a meal as healthy individuals. Whereas blood glucose homeostasis is quite rapidly restored – usually within the first 2 hours in healthy individuals – it can take as long as 8 hours to return to overnight-fasted levels of hyperglycaemia and hyperinsulinemia in patients with T2D [321,329]. The early postprandial period is therefore of interest in understanding the effects of a meal on glucose regulation. Elevated overnight-fasted blood glucose concentrations are a defining and diagnostic symptom of T2D. In the overnight-fasted state, blood glucose concentrations are high but stable, indicating that EGP and *Rd* are matched and there is some degree of regulation [329]. Rates of EGP and *Rd* are often greater in poorly controlled T2D than in healthy individuals [321,330]. Therefore, elevated rates of EGP are

essential for the maintenance of fasting hyperglycaemia. Gluconeogenesis has been implicated as the main driver of overnight-fasted hyperglycaemia, especially in poorly controlled T2D [326,330-332].

As discussed in Chapter 1, Section 1.2.3 of this thesis, there is growing interest in *very low* carbohydrate diets for the management of T2D. This dietary intervention has the potential for large improvements in glycaemic control and can induce T2D remission in some individuals, as described in Chapter 2. Excessive EGP due to gluconeogenesis is a key defect in the development and maintenance of hyperglycaemia. Therefore, studying the effects of *very low* carbohydrate diets on gluconeogenesis could contribute towards an improved understanding of the physiology of T2D remission. In both healthy and T2D individuals, gluconeogenesis has predominantly been studied using the hyperinsulinemic normoglycemic clamp technique to model the post-absorptive state or after an overnight fast. The earliest measurements of gluconeogenesis in the post-absorptive state are usually after 12 to 14 hours of fasting. The effects of the ingestion of normal meals (as opposed to the infusion of glucose) on EGP and gluconeogenesis in the early post-absorptive state are poorly studied. Given the importance of the early post-absorptive period in T2D, future research into the effects of carbohydrate restriction (and other dietary interventions) on glycaemic control would benefit from quantifying gluconeogenesis as close to a normal meal as possible.

3.3) Average deuterium enrichment method

The location of the liver and complexity of liver metabolism presents significant methodological challenges in quantifying rates of gluconeogenesis. A variety of methods have been used in the past but the accuracy, invasiveness, and affordability of these methods has limited their use [333]. The average deuterium enrichment method is a relatively recent technique which overcomes these problems. The average deuterium enrichment method quantifies total gluconeogenesis, regardless of gluconeogenic precursor substrate and so is inclusive of gluconeogenesis from the kidneys [333]. Additionally, only small plasma sample volumes are required for the analysis (30 µl or less) [333]. The method is described in detail in the Methods section of this Chapter. Briefly, the average deuterium enrichment method requires the enrichment of body water with deuterium oxide (^2H_2O) to calculate the fraction of EGP being produced via gluconeogenic pathways. Deuterium/hydrogen ions in body water are in free exchange with those of carbon 2 (C2) of all endogenously produced glucose (i.e. glucose produced via both gluconeogenesis and glycogenolysis) [334]. Therefore, once equilibrated in

body water and at C2 in circulating glucose, the enrichment of ^2H_2O in body water is equivalent to the enrichment of C2 in all endogenously produced glucose. During gluconeogenesis, deuterium/hydrogen ions in body water are in free exchange with those of every carbon of glucose. Thus, once equilibrated, the average enrichment of deuterium of all carbons of glucose except C2 is equivalent to the enrichment of glucose produced via gluconeogenesis. The fraction of circulating glucose attributable to gluconeogenesis can then be calculated as the ratio of the average enrichment of all carbons of glucose except C2 to the enrichment of body water [335].

The accuracy of the average deuterium enrichment method is dependent on: 1) the equilibration of ^2H_2O in body water; and 2) the subsequent equilibration of the circulating glucose pool with enriched body water. During the absorptive state, a portion of liver glycogen is formed via the indirect pathway (i.e. gluconeogenesis to glycogen). Therefore, to minimise the risk of storing labelled glucose, the administration of ^2H_2O after a meal cannot begin until after liver glycogen storage subsides. For this reason, a common protocol is to allow 2 hours after a meal before starting ^2H_2O administration [312,333]. An additional complication is that the rapid ingestion of ^2H_2O is associated with the possibility of symptoms such as dizziness and nausea. The ingestion of ^2H_2O in small doses over an extended period is often used to minimise the risk of these symptoms. A common protocol is the ingestion of ^2H_2O in five doses over a 2-hour period and the administration of ^2H_2O is therefore only completed 4 hours after the meal.

There is no generally accepted minimum time requirement for the equilibration of ^2H_2O in body water and of the glucose pool with body water. Researchers using ^2H_2O to quantify gluconeogenesis have generally adopted a conservative approach by allowing equilibration to occur during an overnight fast [334,335]. This is why the majority of studies, including those that used the hyperinsulinemic clamp technique, quantified gluconeogenesis 12 − 17 hours after the most recent ingestion of a meal. However, there is some indication that the equilibration of body water after ^2H_2O ingestion can occur relatively rapidly and that once achieved, approximately 1 hour may be sufficient time for the equilibration of glucose enrichment [336-338]. It is therefore theoretically possible that the average deuterium enrichment method could be used to quantify gluconeogenesis as soon as 3 hours after ^2H_2O administration (5 hours after a meal). This early post-absorptive period after a meal is of great interest, especially in T2D in which the disruptions to glucose regulation after a meal can last for up to 8 hours. Additionally, the effects of different meals and diets on gluconeogenesis would be more apparent 5 hours after a meal than after an overnight fast.

3.5) Aims and hypothesis

The aim of this study was therefore to pilot the use of the average deuterium method for the quantification of gluconeogenesis 5 hours after a carbohydrate-containing meal. Tracer administration began 2 hours after the meal and we hypothesise that 3 hours is a sufficient length of time for equilibration of the stable isotope tracers that are used for this technique. The main outcome of this study was to determine if tracer equilibration had been achieved 5 hours after the meal by calculating the CVs of tracer enrichment and comparing the 5-hour results to the 7-hour results.

Methods

Ethical considerations

This study was approved by the University of Cape Town (UCT), Faculty of Health Sciences, Human Research Ethics Committee (REF: 560/2017, first approval 15-Dec-2017). All participants were informed of the nature of the study and written informed consent was obtained before participants were enrolled in the study.

Overview of study design

Ten participants that were representative of a range of ages, body weights, and habitual diets completed the study. On the morning of the experimental trial, overnight-fasted participants ate a standardised meal in the laboratory and stable isotope tracers were administered 2 hours after the meal. Three blood samples separated by 10 minutes were drawn 5, 6, and 7 hours after the meal (3, 4, and 5 hours respectively after the start of tracer administration) for the measurement of stable isotope enrichment levels. CVs of tracer enrichment were calculated at each timepoint to assess tracer equilibration. Rates of EGP and gluconeogenesis were calculated at 5, 6, and 7 hours post meal.

Participants

Healthy male and female participants aged 18 to 55 were eligible for inclusion in the study. Low-risk participants were enrolled for initial piloting of this technique. Participants were excluded from participating if they: reported a history of any chronic diseases except for T2D; were pregnant or lactating; had a body weight greater than 110 kg (); had an HbA1c greater than 6.5 % (measured); reported any current serious medical conditions; or reported taking any medications for metabolic conditions. We deliberately recruited for variety in age, body weight, and body fat percentage so that the protocol could be tested in a variety of participants.

Pre-experimental trial laboratory visit

Participants visited the laboratory prior to the experimental trial for the measurement of height, weight, and body fat percentage (DXA, Hologic Discovery W, Bedford, MA). A blood sample was drawn for HbA1c (EDTA containing tube), which was analysed on the same day at a local commercial

laboratory (Lancet Laboratories, Cape Town, South Africa). Participants were given detailed instructions on how to keep a 3-day diet logbook during the visit.

Habitual diet

Habitual diet was estimated from a detailed diet logbook which was kept during the 3 days prior to the experimental trial. Participants were instructed on how to record everything they ate or drank during these 3 days using the online app FatSecret South Africa. FatSecret South Africa has a comprehensive database that contains macronutrient information on most South African foods, including foods commonly eaten by LCHF individuals. The database does not include micronutrient data for all foods and therefore the breakdown of the diet beyond the macronutrient level was not analysed. On the night before the experimental trial, participants had an evening meal of their own choice between 19h30 and 20h00. This meal was the last food recorded in the diet logbook. During the experimental trial, a researcher went through the diet logbook with the participant to clarify any entries that looked unusual or incomplete.

Experimental protocol

An overview of the experimental trial is presented in **Figure 1**. Participants arrived at the laboratory at 07h00 after an 11-hour overnight fast. At 07h30, a cannula was inserted into an antecubital vein for blood sampling. Participants rested for 15 min, after which baseline respiratory gases were sampled. A blood sample was then drawn for baseline plasma glucose, serum insulin, and blood ketone concentrations. At 08h00, participants were provided with a standardised meal, which they ate over a 10 min period. The standardised meal had an energy content of 10 kcal/kg_{FFM} and a macronutrient composition of 50 % carbohydrate, 30% fat, and 20 % protein. The meal consisted of scrambled eggs and sweet potato, both of which were cooked in a microwave oven in the laboratory. This meal composition was chosen for this experiment after extensive pilot testing as it contained whole-foods and was acceptable to most pilot participants, including those following low carbohydrate and vegetarian diets. Reported timepoints are relative to the completion of the meal. Respiratory gases and blood samples for plasma glucose and serum insulin concentrations were collected every 30 min for the first 3 hours of the trial and every 1 hour for the last 4 hours of the trial.

Two stable isotope tracers were used in this experiment: [6,6-$^2H_2$]-glucose (99 atom percent ^2H; Cambridge Isotope Laboratories, Cambridge, MA, USA); and ^2H_2O (99.8 atom percent ^2H; Cambridge Isotope Laboratories, Cambridge, MA, USA). A blood sample for the determination of baseline ^2H

enrichments was drawn at 120 min, prior to the administration of any tracers. A priming dose of 5.4 mg/kg_{FFM} [6,6-$^2H_2$]-glucose was then injected, followed by a continuous infusion of [6,6-$^2H_2$]-glucose at a rate of 0.09 mg/kg_{FFM}/min for the remaining 5 hours of the trial using an infusion syringe pump (HK-400 Syringe Pump, Shenzhen Hawk Optical Instrument Electronic Instrument Co., Ltd, Shenzhen, China). Participants also ingested ^2H_2O to enrich body water to ~0.3 % ^2H_2O. Two different protocols were used for ^2H_2O administration: participants either had the full amount of ^2H_2O in a single dose; or they had 4 equal doses over 90 min (at 120, 150, 180, and 210 min). The ^2H_2O enrichment protocol was adapted during the study to minimise symptoms. Participants were free to drink water which was enriched to match the target body water enrichment. After the start of the infusion, a second cannula for blood sampling was inserted into the antecubital vein of the opposite arm to the one being used for the infusion. At each timepoint where gluconeogenesis and endogenous glucose production was quantified, three blood samples were drawn 10 min apart so that CVs could be determined. These sample timepoints were 290, 300, and 310 min (5-hour post meal), 350, 360, and 370 min (6-hour post meal in 5 participants only), and 410, 420, and 430 min (7-hour post meal). Ten minutes prior to, and throughout the tracer sample blood draws, a heated microwaveable wheat bag (Happy Hugger, The Wheatbag Company, South Africa) was wrapped around the hand and a pre-heated heating pad (Elektra Comfort Heating Pad, S&P Africa, Germiston, South Africa) was wrapped around the forearm to ensure that sampled blood was arterialised.

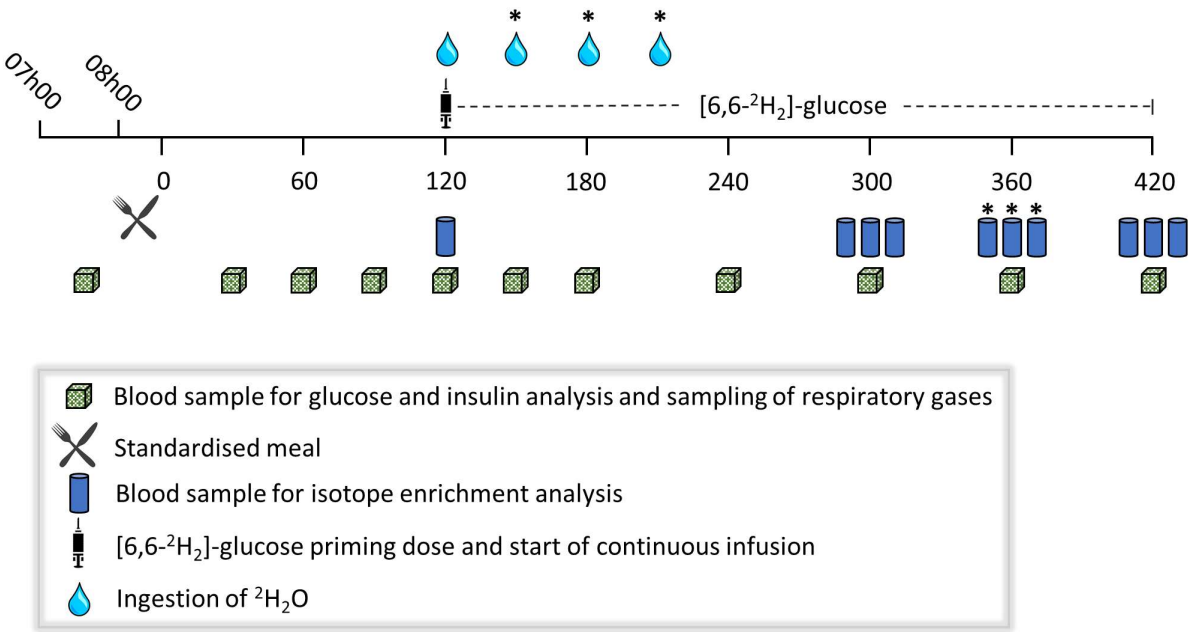

Figure 1. ***Overview of the experimental trial***

Clock times are shown above the line. The standardised meal was served at approximately 08h00 and was eaten over a 10 min period. Numbers below the line are minutes since the completion of the meal. A 5.4 mg/kg_{FFM} [6,6-$^2H_2$]-glucose prime was administered at 120 min, after the baseline blood sample for isotope enrichment analysis. This was followed by a continuous infusion of [6,6-$^2H_2$]-glucose for the remainder of the trial at a rate of 0.09 mg/kg_{FFM}/min. * indicates that the ingestion of ^2H_2O in multiple doses and the drawing of blood samples for isotope enrichment analysis at 360 min was only done in 5 participants. The other 5 participants drank the ^2H_2O in a single dose at 120 min and no blood samples for isotope enrichment analysis were drawn at 360 min.

Indirect calorimetry

Ventilation volume ($\dot{V}_E$), oxygen consumption ($\dot{V}O_2$), and carbon dioxide production ($\dot{V}CO_2$) were determined from the breath by breath analysis of respiratory gases (COSMED, CPET, Rome, Italy). Respiratory gases were sampled prior to the blood draws to minimally disrupt the participant during the measurement. Participants wore a mask for 5 min, during which time they were seated and instructed to remain still and not to talk. Reported data is an average of the measurements from 2:30 to 4:30 min of each 5 min sampling period.

Carbohydrate and fat oxidation rates and energy expenditure were calculated from $\dot{V}O_2$ and $\dot{V}CO_2$ using the stoichiometric equations and energy equivalents below [339]:

- Carbohydrate oxidation rate (g/min) = $(4.55 \times \dot{V}CO_2) - (3.21 \times \dot{V}O_2)$
- Fat oxidation rate (g/min) = $(1.67 \times \dot{V}O_2) - (1.67 \times \dot{V}CO_2)$
- 1 g carbohydrate = 4 kcal
- 1 g fat = 9 kcal

Sample collection

Blood samples for isotope analysis were collected in EDTA-containing tubes, and blood samples for insulin and glucose analysis were collected in tubes containing fluoride and oxalate and tubes with a clot activator and gel barrier respectively. Isotope analysis and glucose blood samples were centrifuged at 3000 g at 4°C for 10 min immediately after collection and were stored at −80 °C within 20 min of collection. Samples for insulin analysis were left at room temperature for 20 min before being processed and stored as described above. Whole blood was used immediately for β-hydroxybutyrate analysis.

Substrate analysis

Plasma and serum samples were analysed for glucose (c501 module, cobas 6000 analyser, Roche, Basel, Switzerland) and insulin (e601 module, cobas 6000 analyser, Roche, Basel, Switzerland) concentrations at a commercial laboratory (Lancet Laboratories, Cape Town, South Africa). Samples were analysed in the same batch in a randomised order. Blood β-hydroxybutyrate concentrations were determined immediately after sampling using a FreeStyle Optium Xceed β-ketonemeter and FreeStyle Optium H test strips (Abbott Laboratories, Abbott Park, IL, USA).

Endogenous glucose production

The M+2 mole percent enrichment of glucose was determined from plasma samples using gas chromatography–mass spectrometry (GC 6890, MS 5973N; Agilent Technologies, Wilmington, DE, USA) in the electron impact ionisation mode by selective ion monitoring of m/z 244/242. Deuterium enrichment of more than one carbon in a single glucose molecule during gluconeogenesis is negligible and does not interfere with the M+2 enrichment analysis provided body water ^{2}H enrichment is below 0.5% [335].

Rates of glucose appearance (R_a) and rates of glucose disappearance (R_d) into and from the circulation were determined using the non-steady-state equations of Steele, modified for use with stable isotopes [340]:

- $R_a = [F - V((C1 + C2) / 2)((IE2 - IE1) / (t2 - t1))] / [(IE1 + IE2) / 2]$
- $R_d = R_a - V[(C2 - C1) / (t2 - t1)]$

Where: R_a and R_d are in mg/kg_{FFM}/min; F is the infusion rate in mg/kg_{FFM}/min; V is the blood pool (0.145 l/kg) [341]; C is the plasma concentration of glucose (mg/dl); t is the sampling time (min); and IE is M+2 enrichment of plasma glucose expressed as decimal.

Fractional gluconeogenesis

The fraction of endogenous glucose production derived from gluconeogenesis (fGNG) was estimated from plasma samples using the average deuterium enrichment method [335]. The principle behind this technique is that body water forms the precursor pool for hydrogen/deuterium incorporation into glucose molecules during gluconeogenic pathways, and that this results in a nearly equal distribution of deuterium labelling on C-1, 3, 4, 5, 6, and 6 (as C-6 of glucose contains two hydrogen atoms) [335]. By contrast, deuterium labelling at C-2 does not represent gluconeogenic processes due to extensive glucose 6-phosphate to fructose 6-phosphate isomerisation, which rapidly achieves ^{2}H enrichment equivalent to that of body water in glucose molecules produced via both gluconeogenesis and glycogenolysis [334]. Assuming the complete equilibration of plasma $^{2}H_2O$ enrichment with deuterium enrichment of glucose at C-2, fGNG can be calculated as the ratio of the average deuterium enrichment of glucose at C-1, 3, 4, 5, 6 and 6 to the ^{2}H enrichment of body water.

To quantify these specific glucose isotope enrichments, the pentaacetate derivatives of plasma glucose were prepared. This was analysed using gas chromatography–mass spectrometry (GC 6890, MS 5973N; Agilent Technologies, Wilmington, DE, USA) in the positive chemical ionisation mode using methane as the reagent gas. Selective ion monitoring *m/z* 170/169 was performed to determine the M+1 enrichment of deuterium in the circulating glucose carbons (C-1, 3, 4, 5, 6, or 6), where M is the base mass 169, representing unlabelled glucose. To accurately measure the deuterium labelling of glucose from ingested ^2H_2O, the enrichment of M+1 resulting from natural abundance was subtracted. The deuterium enrichment in plasma water was determined by isotope ratio mass spectrometry (Delta$^+$XL IRMS; Thermo Finnigan, Bremen, Germany). The following calculations were used to estimate fGNG:

Average M+1 enrichment = $(M+1)(^2H)_{(m/z\ 170/169)}$ / 6
fGNG = Average M+1 enrichment / E^2H_2O

Where: $(M+1)(^2H)_{(m/z\ 170/169)}$ / 6 is the M+1 enrichment of deuterium of glucose measured at *m/z* 170/169; 6 is the number of ^2H labelling sites on the *m/z* 170/169 fragment of glucose; and E^2H_2O is the deuterium enrichment in plasma water.

Absolute rates of gluconeogenesis and glycogenolysis are estimated from fGNG as follows:

- Gluconeogenesis = Ra × fGNG
- Glycogenolysis = Ra - gluconeogenesis

Data reporting and statistical analysis

Results related to the validity of the tracer methodology are presented for each individual participant so that equilibration at each timepoint and within each individual can be assessed. CVs for each individual at each timepoint were calculated from the values at 3 blood draws by dividing the standard deviation by the mean. CVs of less of than 5 % are indicative of relative steady-state at that time point. For group statistics, data are reported as mean ± SD. Normality was tested using the Shapiro-Wilk test. The dependent t-test was used for comparisons between 2 timepoints. A one-way repeated measures ANOVA was used for comparisons of 3 or more timepoints. The Tukey test was used for post-hoc testing.

Results

Participant characteristics

Age, body weight, body fat percentage, BMI, and HbA1c of the 10 participants (6 female and 4 male) are shown in **Table 1**. Age ranged from 33 to 60 years, body weight from 58 to 111 kg, body fat percentage from 14 to 50 %, and BMI from 19 to 43 kg/m^2. All participants had HbA1c within the normal range, including two participants who had previously been diagnosed with T2D (3B-02 and 3B-04).

Table 1. Participant characteristics

Participant	**Gender**	**Age (years)**	**Body Weight (kg)**	**Body fat (%)**	**BMI (kg/m^2)**	**HbA1c (%)**
3B-01	Male	53	59	14	22	
3B-02*	Male	55	101	37	34	5.5
3B-03	Female	60	81	37	28	5.4
3B-04*	Male	59	76	17	24	5.3
3B-07	Female	34	70	27	25	5.0
3B-08	Female	45	102	47	40	5.5
3B-09	Female	43	59	23	22	5.2
3B-11	Male	44	78	28	27	5.6
3B-12	Female	55	111	50	43	5.2
3B-13	Female	33	58	26	19	4.8
Group		48 ± 10	80 ± 19	31 ± 12	28 ± 8	5.3 ± 0.3

Group results are mean ± SD; BMI, body mass index; * indicates the 2 participants with a previous type 2 diabetes diagnosis. Missing values: HbA1c of 3B-01.

Habitual diets of participants

Average daily calorie and macronutrient intakes during the 3 days prior to the experimental trial are shown in **Table 2**. Participants were not asked to change their diets so this logbook data is a reflection of their usual eating behaviours. Four participants reported on the screening questionnaire that their habitual diets were a mixed macronutrient diet and 6 reported that they followed an LCHF diet. However, based on the logbooks, 7 participants ate less than 16 % of calories as carbohydrate and 3 participants ate ~ 40 % of calories as carbohydrate.

Deuterium oxide protocol

The ingestion protocol for $^{2}H_2O$ was modified several times during testing because some participants experienced side effects (**Table 3)**. The first 2 participants received 4 g/kg $^{2}H_2O$ (target body water enrichment of 0.5 %), which was the enrichment percent used successfully in our previous study [312]. The dose was reduced to 4 g/kg_{FFM} $^{2}H_2O$ (target enrichment of 0.4 %) in 1 participant and 3 g/kg_{FFM} $^{2}H_2O$ was used for the remainder of the trial (target enrichment of 0.3 % body water) [342]. The first 5 participants received the $^{2}H_2O$ in a single dose, which was done in an attempt to rapidly reach target body water enrichment and increase the available time for the M+1 equilibration. The drinking of $^{2}H_2O$ in a single dose was tested in five pilot participants prior to the start of testing and no major symptoms were experienced. However, 2 of the first 5 participants experienced major symptoms, including severe nausea and vomiting (**Table 3**). For the second set of 5 participants, $^{2}H_2O$ was ingested in 4 equal doses over 90 min [334]. However, the first participant tested with this new protocol (3B-09) experienced severe symptoms including vomiting. A further adjustment was made for the remaining 4 participants in which participants were able to lie down on a bed in the laboratory if they felt dizzy and/or drowsy. Participants were asked to return to a seated position 20 min prior to the tracer sample blood draws. With this in place, one further participant (3B-13) experienced a severe headache but there were no further incidents of nausea or vomiting. In all participants who had symptoms, the onset of symptoms began ~ 60 min after the start of $^{2}H_2O$ ingestion (180 min in trial), peaked at 120 to 180 min after ingestion (240 to 300 min respectively in the trial), and generally subsided by 240 min (360 min in trial).

Table 2. Habitual diet and blood beta-hydroxybutyrate concentration							
Participant	**Self-reported eating pattern**	**Energy (kcal/d)**	**Protein (g/d)**	**Fat (g/d)**	**CHO (g/d)**	**CHO (%)**	**ßHOB (mmol/l) ***
3B-01	Mixed diet	2236	82	89	211	38	0.2 *
3B-02	LCHF	2105	122	132	64	12	0.3 *
3B-03	Mixed diet	1490	57	72	155	42	0.1 *
3B-04	LCHF	2550	218	152	71	11	
3B-07	LCHF	1612	109	101	35	9	0.6 *
3B-08	Mixed diet	2011	110	95	176	35	0.1 *
3B-09	LCHF	1952	74	141	69	14	0.8 *
3B-11	LCHF	1851	103	131	63	14	
3B-12	Mixed diet	1263	62	87	47	15	0.3 *
3B-13	LCHF	1609	95	94	40	10	
Whole Group		1868 ± 384	103 ± 46	109 ± 27	93 ± 63	20 ± 13	0.3 ± 0.3 *

Macronutrient data was estimated from 3 days of recorded diet using the FatSecret online application. CHO, carbohydrate; ßHOB, blood beta-hydroxybutyrate concentration. * indicates that beta-hydroxybutyrate was a fasted measurement on the day of the experimental trial. Missing values: ßHOB for 3B-04, 3B-11, and 3B-13.

Table 3. $^{2}H_2O$ enrichment of the participants

Participant	Target body water enrichment (%)	$^{2}H_2O$ ingestion protocol	Symptoms
3B-01	0.5	single dose	Dizziness, very mild nausea
3B-04	0.5	single dose	Minimal symptoms, some light dizziness
3B-08	0.4	single dose	Severe dizziness, strong nausea, vomiting, fatigue
3B-07	0.3	single dose	Minimal symptoms, felt lightheaded
3B-03	0.3	single dose	Severe nausea, vomiting, drowsiness
3B-09	0.3	5 doses over 120 min	Severe nausea, vomiting, mild dizziness, drowsiness, hunger
3B-11	0.3	4 doses over 90 min	Mild dizziness; drowsiness
3B-02	0.3	4 doses over 90 min	Mild dizziness
3B-12	0.3	4 doses over 90 min	No symptoms
3B-13	0.3	4 doses over 90 min	Severe dizziness and headache

Participants are listed in the order in which they were tested.

Stable isotope tracer enrichment

Deuterium enrichment was measured in body water, at M+1 in circulating glucose, and at M+2 in circulating glucose. Three blood samples were drawn 10 min apart at each tracer timepoint and the CV of deuterium enrichment was calculated from these 3 measurements. The deuterium enrichment percent of body water for each individual is shown in **Figure 2**. Participants were at target enrichment levels by 300 min and enrichment was stable at all timepoints. Deuterium enrichment of M+1 in circulating glucose for each individual is shown in **Figure 3**. The CV of the 3 measurements was less than 5 % at every timepoint (**Figure 3**). There was 1 % more variation of M+1 enrichment at 300 min (CV, 2.4 ± 1.4 %) compared to 420 min (CV, 1.4 ± 1.3 %; $p = 0.035$ determined by a dependant t-test). Deuterium enrichment at M+2 in circulating glucose for each individual is shown in **Figure 4**. The CVs for each measurement were below 5 % except for 300 min in participant 3B-08 (**Figure 4**). The variation in percent enrichment was similar at 300 min (CV, 2.6 ± 1.5 %) compared to 420 min (CV, 1.9 ± 0.9 %; $p = 0.12$ determined by a dependant t-test).

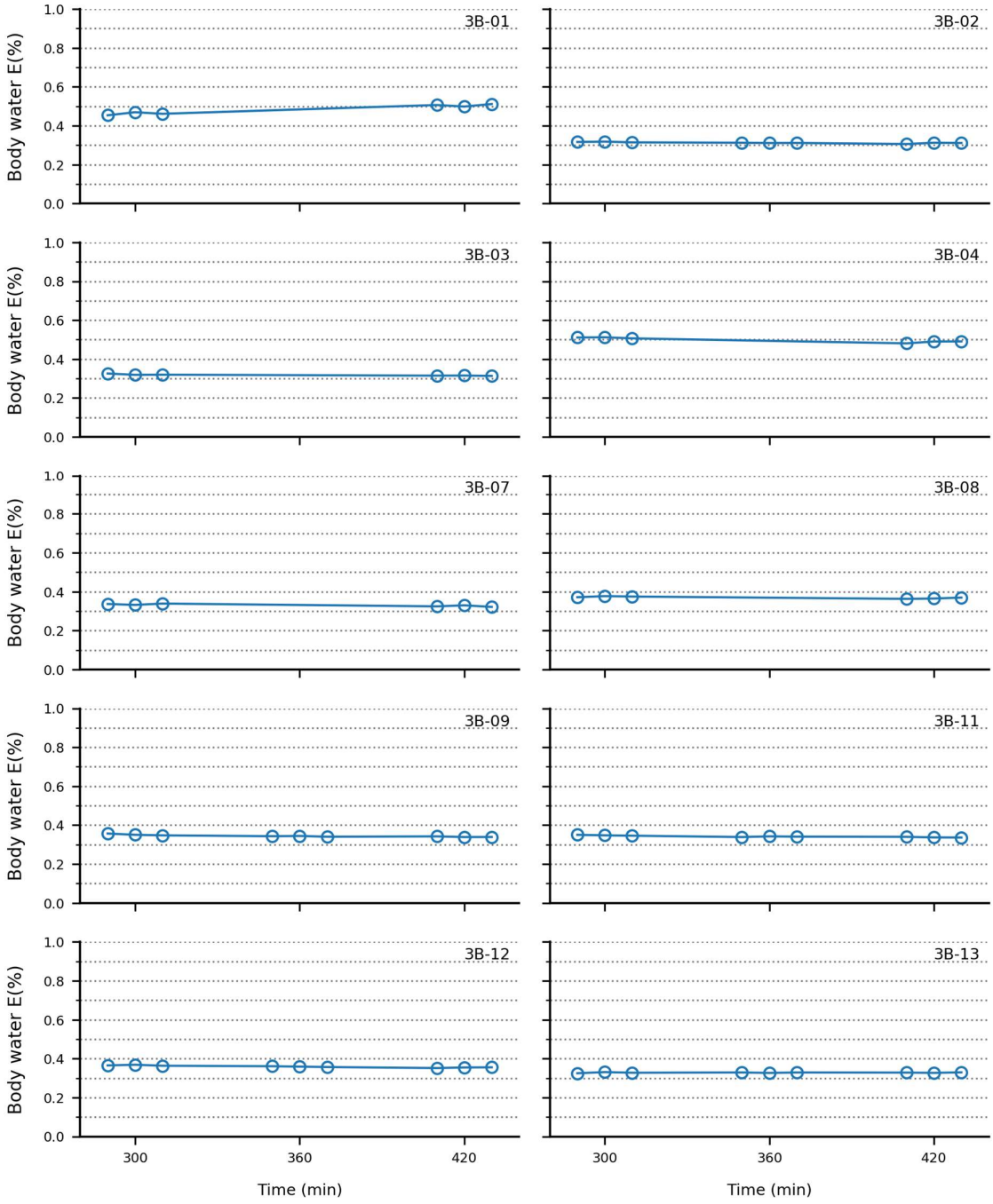

Figure 2. ***Deuterium enrichment of body water***

Deuterium enrichment percent of body water for each individual at 300, 360 and 420 min. Three samples were drawn 10 min apart at each timepoint. E(%), deuterium enrichment percent.

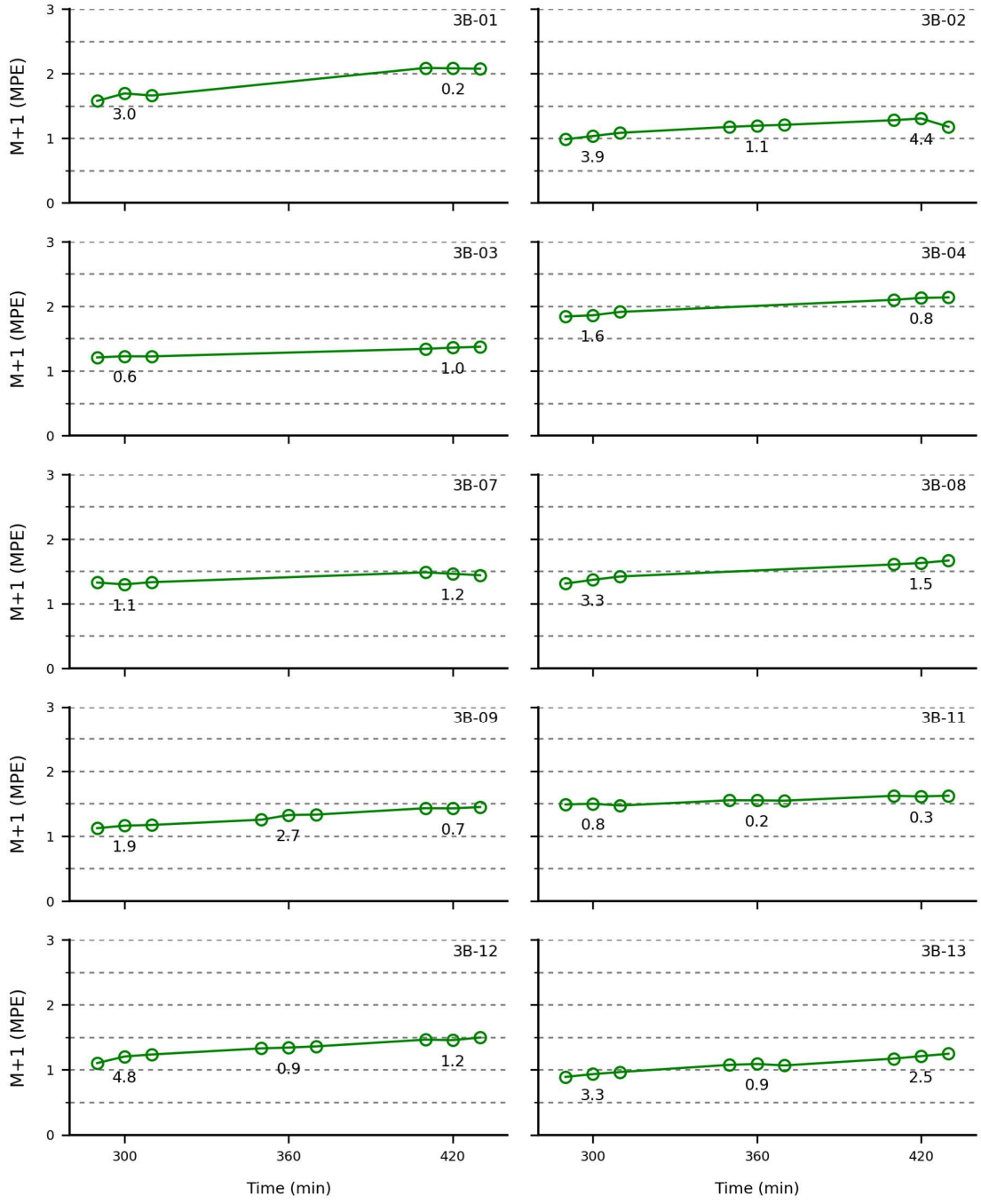

Figure 3. M+1 deuterium enrichment percent

Deuterium enrichment percent of M+1 of circulating plasma glucose. Three samples were drawn 10 min apart at each timepoint and the numbers below points on the graph are the coefficient of variation for each set of three samples. MPE, molar percent enrichment.

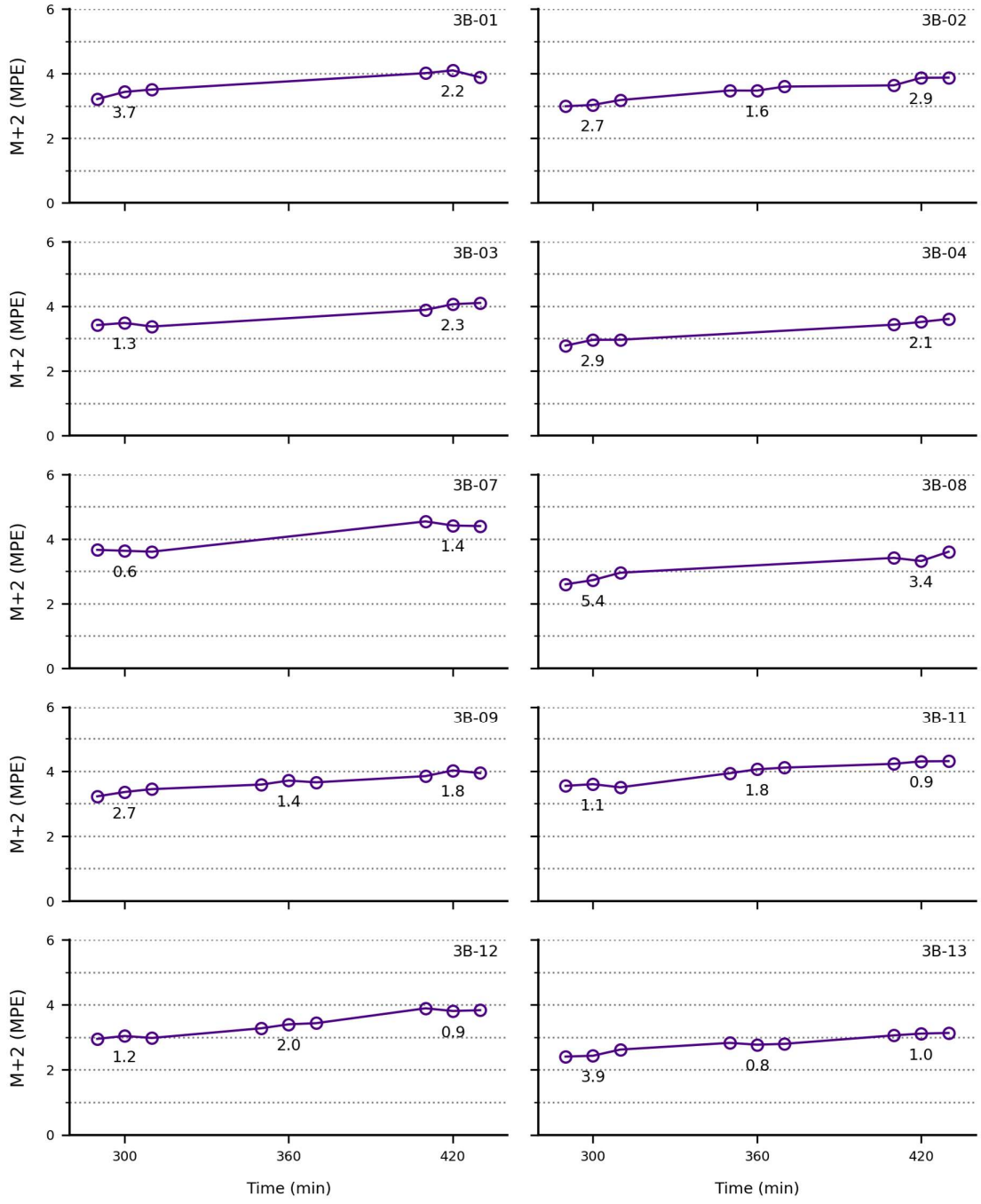

Figure 4. M+2 deuterium enrichment percent

Deuterium enrichment percent of M+2 of circulating plasma glucose. Three samples were drawn 10 min apart at each timepoint and the numbers below points on the graph are the coefficient of variation for each set of three samples. MPE, molar percent enrichment.

Fractional gluconeogenesis

fGNG at 300 min and 420 min for 10 participants was 0.59 ± 0.09 and 0.71 ± 0.05 respectively ($p < 0.01$; dependent t-test). fGNG at 300, 360, and 420 min for 5 participants was 0.56 ± 0.09, 0.64 ± 0.07, and 0.70 ± 0.07 respectively ($p < 0.01$; repeated measures ANOVA). This indicates that the contribution of gluconeogenesis to EGP was increasing over time.

Rates of EGP, gluconeogenesis, and glycogenolysis

Every individual had a similar pattern of EGP and gluconeogenesis (**Figure 5**). There was very little variation in rates of gluconeogenesis within each individual whereas rates of EGP and glycogenolysis were highest at 300 min and lowest at 420 min. Analysed as a group of 10 participants with values at 300 and 420 min (**Figure 6A**), mean rates of gluconeogenesis at 300 and 420 min were 1.59 ± 0.09 and 1.58 ± 0.07 mg/kg_{FFM}/min respectively ($p = 0.81$, dependent t-test). Mean rates of glycogenolysis at 300 and 420 min were 1.13 ± 0.32 and 0.66 ± 0.19 mg/kg_{FFM}/min respectively ($p < 0.001$; dependent t-test) and mean rates of EGP at 300 and 420 min were 2.71 ± 0.32 and 2.24 ± 0.24 mg/kg_{FFM}/min respectively ($p < 0.001$, dependent t-test).

In the subset of 5 participants with values at 300, 360 and 420 min (**Figure 6B**), there was no difference in gluconeogenesis between timepoints ($F(2, 8) = 0.11$, $p = 0.89$, repeated measures ANOVA). There was a significant reduction in glycogenolysis ($F(2, 8) = 56.2$, $p < 0.001$; repeated measures ANOVA) and EGP ($F(2,8) = 83.5$ $p <0.001$, repeated measures ANOVA) over time (Figure 7A). Glycogenolysis and EGP were significantly different between every timepoint in post-hoc testing (Figure 7A). The average rate of gluconeogenesis at all timepoints for all participants was 1.59 mg/kg_{FFM}/min. With an average FFM of 53.3 kg, this works out at 15 g of glucose over 3 hours. Assuming this rate of gluconeogenesis over a 24-hour period equates to the synthesis of 122 g of glucose.

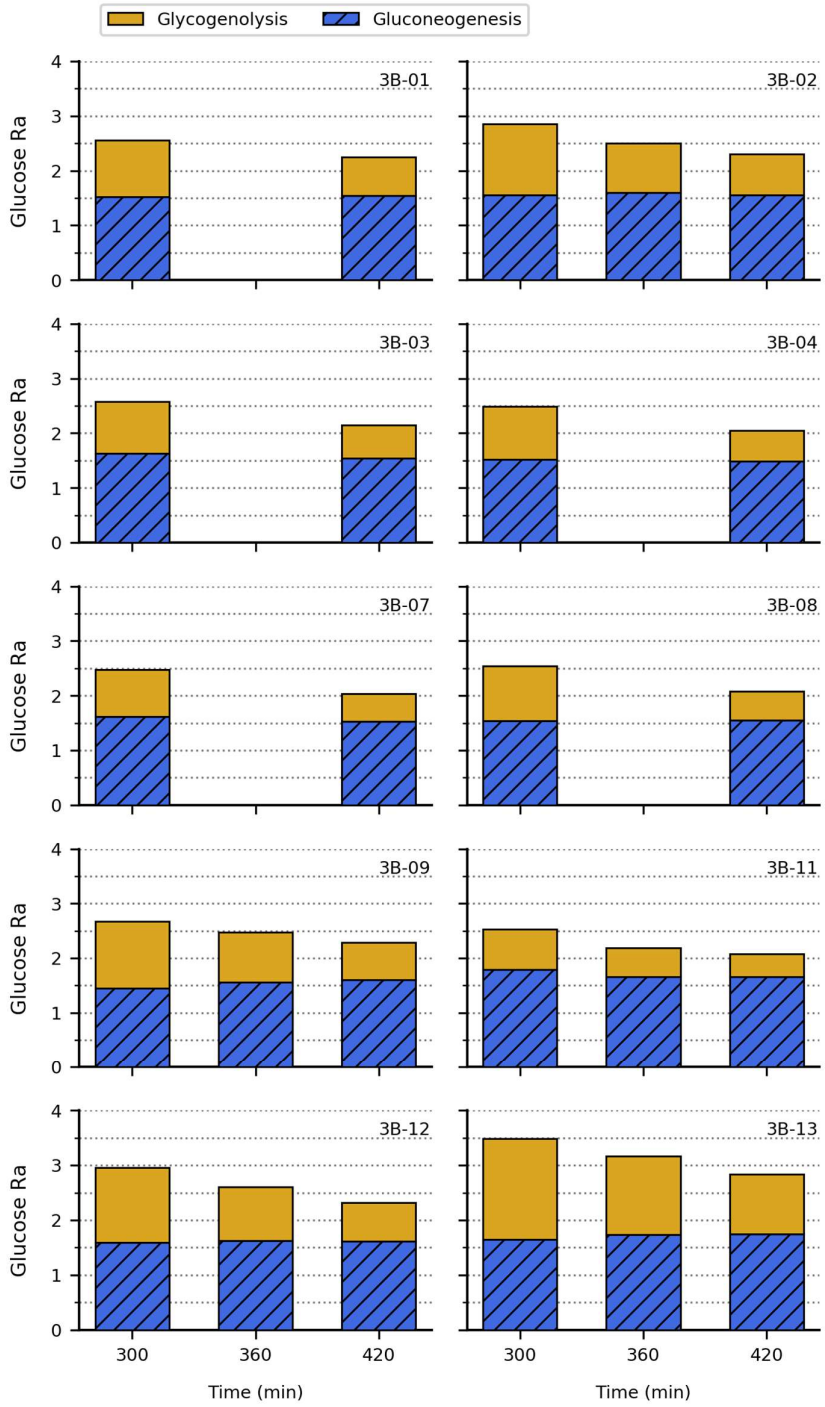

Figure 5. Individual rates of endogenous glucose production

The total rate of endogenous glucose production is shown as the sum of gluconeogenesis (blue section of the bar) and glycogenolysis (yellow section of the bar). Glucose Ra, rate of appearance of glucose into the circulation in mg/kg$_{FFM}$/min.

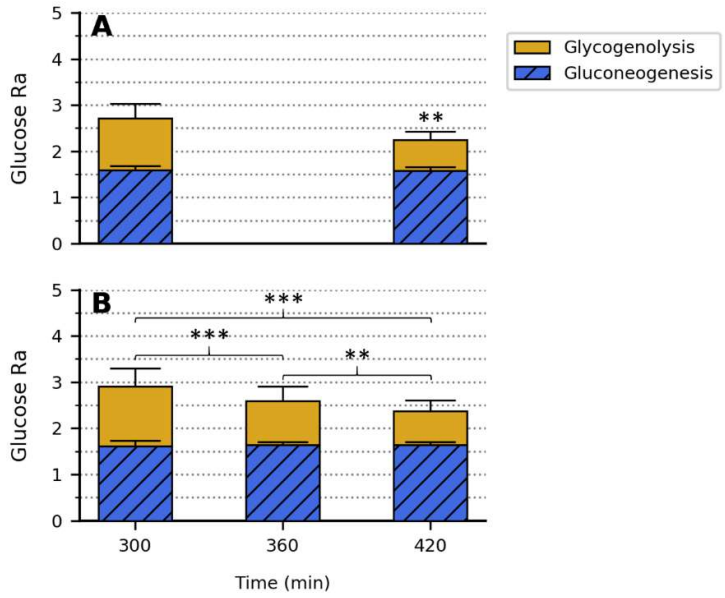

Figure 6. Group rates of endogenous glucose production

Rates of endogenous glucose production shown as the sum of gluconeogenesis and glycogenolysis in A) the entire group of 10 participants at 300 and 420 min, and B) the subset of 5 participants with measurements at 300, 360 and 420 min. ** and *** indicates a significant difference in EGP and glycogenolysis with $p < 0.01$, and 0.001 respectively. Glucose Ra, rate of appearance of glucose into the circulation in $mg/kg_{FFM}/min$.

Substrates and hormones

Plasma glucose concentrations were significantly elevated 30 min after the standardised meal but were not significantly different to baseline after 60 min (**Figure 7A**). Serum insulin concentrations were significantly higher than baseline at 30, 60, and 90 min post-meal (**Figure 7B**). Fat and carbohydrate oxidation rates did not differ from baseline at any point during the trial (**Figure 7C & 7D**). However, there were changes in fat ($F(10, 70) = 2.4$, $p = 0.016$, repeated measures ANOVA), and carbohydrate oxidation rates ($F(10, 70) = 4.08$, $p < 0.001$, repeated measures ANOVA) . Fat oxidation rates decreased until 120 min and then started to increase associated with a concomitant increase in carbohydrate oxidation rates until 120 min, before decreasing.

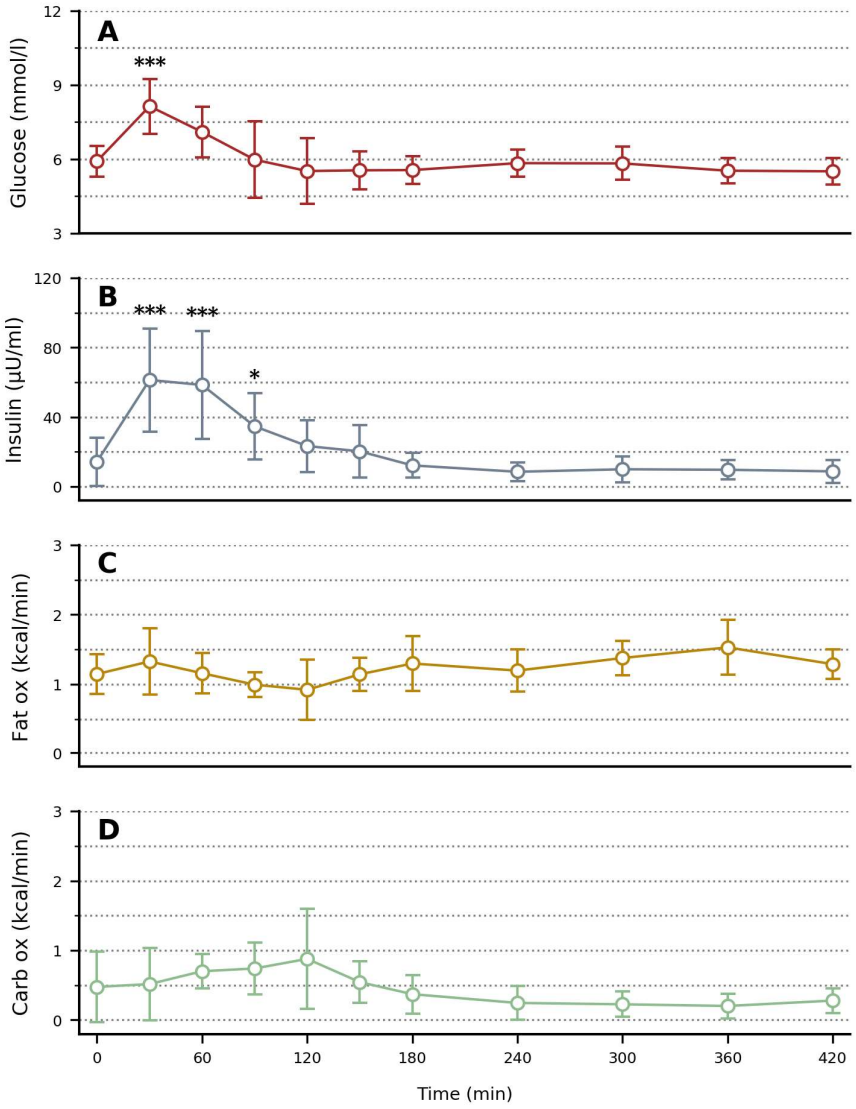

Figure 7. Plasma glucose and serum insulin concentrations and substrate oxidation rates

A) Plasma glucose concentrations; B) serum insulin concentrations; C) fat oxidation rates; and D) carbohydrate oxidation rates during the 420 min experimental trial. *, *** indicates a significant difference to baseline (0 min) with $p < 0.05$ and 0.001 respectively. P values were determined using a repeated measures ANOVA with Tukey post-hoc testing.

Discussion

The primary aim of this study was to determine whether the average deuterium enrichment method could be used to quantify gluconeogenesis 5 hours after a carbohydrate-containing meal. The main challenge in obtaining accurate measurements this soon after a meal is that the equilibration of M+1 in plasma glucose has to be achieved. Body water forms the precursor pool for hydrogen/deuterium incorporation into glucose molecules formed in the gluconeogenic pathways. Therefore, the equilibration of M+1 in the glucose pool requires the prior enrichment of body water with $^{2}H_2O$.

Ingestion of $^{2}H_2O$ began 2 hours after the completion of the meal to ensure that only unlabelled glucose was stored as glycogen in the absorptive state. The spike in plasma glucose and serum insulin concentrations after the meal had subsided by that time and glycogen storage after 2 hours was likely minimal [298,305]. The equilibration of $^{2}H_2O$ in body water had been achieved by the time we took samples for the measurement of gluconeogenesis (3 hours after the start of $^{2}H_2O$ ingestion) but we cannot be sure exactly when body water equilibration had occurred as we did not take samples prior to this point. However, the absorption and equilibration of $^{2}H_2O$ in body water has been shown to occur rapidly, within 15 to 30 min of ingestion [336-338]. Participants in the current study received either a single dose of $^{2}H_2O$ 2 hours after the meal or received 4 doses over a 90 min period. Although we did not measure it, it is likely that the equilibration of $^{2}H_2O$ in body water had occurred by at least 2 hours after the start of $^{2}H_2O$ ingestion. This would allow for at least 1 hour for M+1 equilibration to occur [336,337].

We measured M+1 enrichment at 3- and 5 hours after the start of tracer administration. The extra 2 hours of equilibration time for the 5-hour measurement is in line with the established methodology and known tracer equilibration times [335,337,338]. This later timepoint therefore serves as a reference for whether equilibration had been achieved at the earlier time point. Three blood samples were drawn 10 min apart at each timepoint so that CVs could be calculated to assess the equilibration of M+1 enrichment. It is important to note that the body is always in a state of metabolic adjustment and some variation is to be expected. CVs for M+1 enrichment were below 5 % for every individual at every timepoint. This indicates that tracer enrichment was relatively stable during the 20-min period when the 3 samples were drawn. The mean CVs of M+1 enrichment 3- and 5 hours after tracer administration was 1.4 and 2.4 % respectively. Although statistically different, this 1 % difference in variability may not be meaningful in terms of whether M+1 equilibration had been achieved. If the tracer was not close to or at equilibration, larger variability might be expected. A

comparison of CVs at these timepoints therefore indicates that equilibration of M+1 in plasma glucose was likely achieved at both time points.

A [6,6-$^{2}H_{2}$]-glucose infusion was used to determine the rates of EGP according to the non-steady-state equations of Steele [340]. In order for this measurement to be accurate, equilibration of M+2 in plasma glucose had to be achieved. The [6,6-$^{2}H_{2}$]-glucose infusion was started 2 hours after the meal and had therefore been running for 3 hours by the first blood sampling to measure EGP and for 5 hours by the later timepoint. A 5-hour [6,6-$^{2}H_{2}$]-glucose infusion is in line with a conservative approach that has been used in previous studies but shorter [6,6-$^{2}H_{2}$]-glucose infusion times of 90 min and 2 hours have also been used [312,342,343]. Comparison of the M+2 equilibration data after 3- and 5 hours of infusion suggests that equilibration had been achieved at both timepoints. CVs for M+2 enrichment were below 5 % and there was no statistical difference between CVs after 3- and 5 hours of infusion. This provides an indication that M+2 enrichment was equilibrated after 3 hours of infusion and rates of EGP can be calculated.

The combination of EGP (calculated from M+2 enrichment) and the fraction of EGP attributable to gluconeogenesis (calculated from M+1 enrichment) allows for an estimate of absolute rates of gluconeogenesis. We found that absolute rates of gluconeogenesis were constant at all timepoints. This is consistent with prior research which shows that gluconeogenesis is relatively constant under a variety of resting physiological conditions in healthy individuals, including during fasting and after changes in dietary macronutrients [303,310,329]. Therefore, constant rates of gluconeogenesis in the current study provide further evidence that our estimates of gluconeogenesis are accurate.

Glycogenolysis accounted for the reductions in EGP over a 2-hour period and was highest 5 hours after the meal, accounting for ~ 40 % of EGP, compared to ~ 30 % of EGP 7 hours after the meal. The extent of these changes over a 2-hour period highlights the importance of studying the early post-absorptive period. It would be interesting to follow the progression of glycogenolysis for a longer period to determine when, and at what rate, glycogenolysis stabilises. Glycogenolysis after an overnight fast is typically ~ 50 % of EGP in individuals on high- to moderate carbohydrate diets and ~ 25 % of EGP in individuals following *very low* carbohydrate diets [294,310]. Therefore, rates of glycogenolysis after 14 to 17 hours of fasting would be expected to be similar or even higher than values we measured 7 hours after a meal. There may be differences in glucose regulation during day fasting versus overnight fasting which account for this discrepancy. Alternatively, glycogenolysis may be stimulated in the mornings after an overnight fast during circadian cycles.

The results from the present study provide a good indication that gluconeogenesis was successfully quantified 5 hours after a carbohydrate-containing meal. However, we cannot be entirely certain as rates of EGP would be overestimated if M+2 was not equilibrated and fractional gluconeogenesis would be underestimated if M+1 was not equilibrated. The declining EGP and increasing fractional gluconeogenesis that we measured might represent tracer equilibration rather than physiological changes. An experiment to resolve this question would involve following exactly the same protocol and tracer enrichment procedures but without providing participants with a meal. Instead of fasting for 5 and 7 hours, participants would be fasting for 17 and 19 hours. EGP and gluconeogenesis would therefore be expect to be stable during this 2-hour period [337,338]. If the EGP and gluconeogenesis profile was similar to the current study, that would confirm that our results are an artefact of tracer equilibration and not representative of the underlying physiology. The experiment outlined above forms part of a post-doctoral fellowship grant application to continue with this work (see Chapter 4 for future perspectives).

Apart from quantifying gluconeogenesis in the early post-absorptive state, the protocol used in this study has a number of other advantages for researchers compared to the ingestion of $^{2}H_2O$ prior to an overnight fast. Firstly, the shorter timeframe gives researchers more potential for full control over the timing of the meal and $^{2}H_2O$ ingestion. The protocol requires only that the participant arrives at the laboratory after an overnight fast and is available for the full morning of the experiment. This is easier to accommodate than an overnight stay in the laboratory and more reliable than asking participants to ingest the meal and $^{2}H_2O$ in their own homes [300,312,335]. Secondly, the shorter protocol in the current study would make it feasible to measure gluconeogenesis in circumstances other than after an overnight fast. The current study estimated gluconeogenesis 5 hours after a mixed-macronutrient breakfast and the same protocol could be used to study the impact of a midday or evening meal on gluconeogenesis later during the same day.

The use of ^{13}C nuclear magnetic resonance (NMR) spectroscopy is an alternative methodology for the estimation of gluconeogenesis [333]. The natural abundance of ^{13}C in glycogen is used to quantify glycogen content in a section of the liver, which combined with an estimate of liver volume, provides an estimate of liver glycogen concentration. Measurements over a period of time can then be used to calculate the rate of liver glycogen synthesis or glycogenolysis. Rates of EGP are determined using tracers (similar to the current study) and gluconeogenesis is calculated as the difference between EGP and glycogenolysis. A major advantage of NMR is that it is a direct measurement of liver

glycogen content and therefore is not dependent on tracer equilibration periods. It can therefore be used in the absorptive state or in the early post-absorptive period which was investigated in the current study [298,305]. The two main limitations of NMR are the expense and the technical expertise required for the analysis [333]. This may limit the number of measurements which can be made. For example, Petersen et al (1996)[305] quantified glycogenolysis in the early post-absorptive period by measuring liver glycogen content 5 hours and 12 hours after a meal. Reported rates of glycogenolysis were therefore the average rate over this 7-hour period. The average deuterium method and NMR have different underlying assumptions, strengths, and weaknesses. For example, glucose and glycogen cycling in the liver may result in some degree of mislabelling of glycogenolysis and gluconeogenesis with the average deuterium method [333,337,338]. This is not a problem with NMR, which does make the assumption that the liver functions as a homogenous unit and that liver glycogen is evenly distributed [333]. These two methods should be seen as complementary rather than competing approaches at understanding the physiology of glucose regulation [333].

One of the challenges we faced with this protocol was that some participants experienced severe symptoms after the ^2H_2O ingestion. There was a large amount of variation in the symptoms and some participants had no symptoms or only mild dizziness whereas others had nausea and vomiting. Severe symptoms have been noted in other studies in some participants after ingesting ^2H_2O in a single dose [334,338]. We initially attempted the study using a single dose as this protocol did not produce severe symptoms during pre-trial testing in any of the pilot participants. Once some participants had experienced severe symptoms in the actual experiment, we adjusted the protocol by reducing the target ^2H_2O enrichment from 0.5 g/kg$_{FFM}$ to 0.3 g/kg$_{FFM}$. However, even with this modified protocol, some participants had severe symptoms. Participants in the current study were awake and seated during the ^2H_2O equilibration period. We were also measuring respiratory gases at frequent intervals which required wearing a gas mask. By contrast, participants would have been able to go to sleep shortly after ^2H_2O ingestion in those protocols where ^2H_2O was ingested the night before the measurements. An adaptation to our protocol was therefore to allow participants to lie down and keep their heads still after ^2H_2O ingestion. This appeared to help with symptoms and we are confident that we can minimise the risk of symptoms in future studies. However, this needs to be monitored carefully with future experiments and additional adjustments to the ^2H_2O ingestion protocol may be required. Interestingly, all of the participants who experienced severe symptoms were women and there may be some gender differences in the response to ^2H_2O. It is unclear whether symptoms from the ingestion of ^2H_2O have an effect on EGP or gluconeogenesis.

Limitations

We did not measure body water enrichment prior to 5 hours after the meal (for example at 2 hours after $^{2}H_2O$ ingestion) and therefore cannot confirm when $^{2}H_2O$ equilibration had been achieved. We cannot be sure that the gut was empty of all carbohydrates 5 hours after the meal and there may still have been some exogenous glucose contribution [334]. An additional experiment is required to confirm with certainty that changes in tracer equilibration from 5 to 7 hour represented physiological changes and not continuing equilibration of the tracer. The future perspectives of this research will be discussed in detail in Chapter 4.

Summary and conclusion

This study investigated the use of the average deuterium enrichment method for the quantification of gluconeogenesis 5 hours after a carbohydrate-containing meal in 10 individuals at low-risk for metabolic disease, who varied widely in body weight and body fat percentage. There are two main lines of evidence that our results represent an accurate estimate of gluconeogenesis: 1) CVs for tracer equilibration at M+1 and M+2 in circulating glucose were low and comparable at both the 5- and 7-hour timepoints; and 2) absolute rates of gluconeogenesis were constant at all timepoints. EGP and glycogenolysis were rapidly changing from 5 to 7 hours after the meal which highlights the importance of studying gluconeogenesis as close to meals as possible. We conclude that the low variation in tracer equilibration and constant rates of gluconeogenesis supports the hypothesis that the average deuterium enrichment method can be used to quantify gluconeogenesis 5 hours after the ingestion of a carbohydrate-containing meal. This protocol would allow for the study of gluconeogenesis and glycogenolysis in response to a meal in T2D patients. Findings from Chapter 2 will be used to design LCHF interventions to study with this protocol.

Chapter 4

Summary, conclusions, and future perspectives

The aim of this thesis was to lay a firm foundation for future research into the effects of carbohydrate restriction on EGP, gluconeogenesis, and glycogenolysis in individuals with T2D. A carbohydrate-containing meal has a large impact on blood glucose regulation in T2D but the impact of dietary composition on gluconeogenesis and glycogenolysis in the early post-absorptive period is not well understood. This thesis describes preliminary investigations that were conducted in preparation for this research. Carbohydrate restricted diets are controversial and the first chapter of this thesis was a narrative literature review aimed at understanding the nature of the controversy. Carbohydrate restricted diets have a long history in the literature and were once considered standard treatment for T2D. However, during the past 40 years, dietary policies and guidelines have promoted low fat diets as healthy for T2D and for the express prevention of heart disease especially in T2D patients who are at increased risk. By contrast, LCHF diets were discouraged and regarded as potentially harmful since according to the Diet-Heart and Lipid hypotheses, a higher fat diet must raise blood cholesterol concentrations increasing the risk for the development of heart disease. Prospective cohort observational studies and dietary RCTs are the two main study designs which have had the largest impact on the perception of the safety or otherwise of carbohydrate-restricted diets amongst researchers, medical professionals, and the public.

The strength of prospective cohort studies is that they can establish associations between dietary factors and long-term health outcomes. However, a major disadvantage is that associations cannot be assumed to represent causation. In Chapter 1, associations from dietary prospective cohort studies were reviewed. Concern over the long-term safety of LCHF diets is predominantly based on this body of evidence. The nine principles of Bradford Hill were used as a framework to consider whether these nutritional observational associations could be interpreted as firm and absolute evidence for causation. However, the review shows that the nutritional associations identified in these studies are weak, inconsistent, and non-specific. Non-specificity, in particular, is a major factor which may be preventing unambiguous findings in nutritional epidemiologic research. Results from observational studies also tend to be incoherent with those of short-term RCTs and the hypotheses generated by observational research cannot feasibly be tested with long-term experiments. Overall, it is not clear that the associations from observational research can be interpreted as definitive proof

of causation. A large component of the controversy and diversity of opinion on LCHF diets is due to a lack of consensus on how to interpret this body of research.

RCTs are considered the strongest study design, as many sources of bias and confounding can be eliminated by random allocation of participants into intervention and control groups. However, RCTs are poorly suited to the study of certain nutritional hypotheses. The design is best suited to focused interventions in which there are a few clearly defined differences between the intervention and control groups. In nutrition research, it is not possible to focus interventions, as nutrients and foods are highly interrelated. Randomisation and the blinding of researchers and participants from group assignments is a key component of the RCT design. It is also not possible to blind participants from their group assignments in dietary RCTs, as participants are responsible for preparing and eating their meals. Additionally, dietary RCTs place a high burden on participants and compliance to intervention and control diets is notoriously poor in these trials. For these reasons, it is not currently feasible to conduct long-term RCTs over the 10 to 30-year periods that would be required for hard disease endpoints to develop in a healthy population. A large number of short-term dietary RCTs have investigated the effects of LCHF diets on health markers in T2D. While results from these trials are generally favourable towards LCHF diets, the extrapolation of these results to the long term is a source of contention. Therefore, as with prospective cohort results, RCT results are ambiguous and require subjective interpretation.

The most important take-away message from Chapter 1 is that the nature of nutrition research precludes definitive answers to many food- and diet-focused questions. Foods and nutrients are highly interrelated, have complex interactions during absorption and metabolism, and stimulate highly individualised hormonal and metabolic responses which combine to cause diseases after many decades. Examples of questions which may not be possible to answer include: which diets and/or foods are healthiest over the long term; is dairy healthy or unhealthy for T2D; should eggs be recommended to T2D patients; and how much fat is the right amount in the diet? The two main study types which have been used to attempt to answer such questions, the prospective cohort and the RCT, have severe limitations to the extent that additional studies of the same quality as existing research are unlikely to add any further clarity. For this reason, research focused on understanding the underlying physiological and metabolic effects of carbohydrate restricted diets may be an alternative approach to alleviate or validate some of the concerns being expressed about LCHF diets. The intrinsic limitations in nutrition research need to be carefully considered in order to formulate future hypothesis which are likely to be testable.

One of the sources of ambiguity in the literature is the large heterogeneity in dietary interventions that are classified as 'low carbohydrate diets' by the investigators. The intervention diets used in these trials ranges from almost no carbohydrate to approximately 45 % of daily intake as carbohydrate. 'Low carbohydrate' interventions have also differed widely according to which sources of fat are promoted or discouraged, whether they are high or low in protein, and on other aspects of the interventions, such as calorie control strategies. Certain types of low carbohydrate diets, generally those considered *very low* in carbohydrate (i.e. less than 50 g per day), are showing potential to reverse T2D. This potential is predominantly based on results from non-randomised controlled trials, clinical reports, and anecdotal evidence. Our research group has received several such anecdotal reports of T2D patients who have reversed their conditions since following an LCHF diet. This is a small but growing population group which has not been studied and the properties of the diets that have been developed, adapted, and sustained in a real-world setting are unknown. Chapter 2 of this thesis was an investigation of the diets, T2D status, and experiences of T2D patients that had self-selected and followed an LCHF diet for at least 6 months. This was a multi-method study which had quantitative and qualitative components. The aim of the study was to investigate claims of large improvements in health and to provide insight into important aspects of the LCHF diets that are being implemented and sustained in the real-world, as opposed to prescribed during a clinical trial.

Twenty-eight confirmed T2D patients who claimed to be following an LCHF diet were recruited for the study. Participants had excellent glucose control, with a median HbA1c of 5.6 %, and had sustained weight losses of approximately 17 % of pre-LCHF-diet body weight. There was a large reduction in T2D medications since participants started their LCHF diets. Of 11 participants taking insulin, 8 had completely discontinued the medication since starting their diet. There were also large reductions in the doses and number of participants taking metformin. Of the 24 participants who completed a 15-month follow-up study, 17 were in complete or partial T2D remission. Quantitative findings from this study therefore confirmed that these T2D patients had achieved excellent glucose control and T2D outcomes whilst following their LCHF diets. The diets of these participants were quantified using three different methods: a food frequency questionnaire; a 1-day diet recall; and a 3-day diet logbook. Participants had a median carbohydrate intake of 61 g/day. Ten participants were following a *very low* carbohydrate diet (less than 50 g/day) and 17 were following a *low* carbohydrate diet (50 to 130 g/day). Approximately 45 % of dietary fat intake was saturated fat and 90 % of protein intake was from animal sources. In terms of foods, dairy was one of the most frequently reported food groups and cream, butter, cheese, milk, and yoghurt were commonly

reported. Non-starchy vegetables, coconut oil, eggs, and tree nuts were commonly reported and unprocessed red meat with fat was the most frequently reported protein. Poultry with fat, seafood, bacon and biltong (cured, dried meat) were other common protein sources. Participants reported predominantly eating fatty cuts of meat as well as full-fat dairy.

A major theme to emerge from the qualitative component of the study was that participants felt increased control of their eating since starting their LCHF interventions. They described feeling hungry less often, a less intense hunger when they did experience hunger, and reduced food cravings. A reduction in cravings was attributed to the removal of sweet-tasting foods and carbohydrates from the diet. Additionally, participants described an increased sense of control over their weight and their T2D since starting their LCHF diets. They also experienced increased energy levels and described feelings of increased positivity for the future. An area of concern from the qualitative study was that participants did not feel fully supported by their medical doctors. Some had negative interactions with medical professionals who were in opposition to their LCHF diets. The effects of the diet on serum cholesterol concentrations and the reluctance of some participants to take cholesterol-lowering medications was often the source of the more hostile interactions. The majority of participants said their doctors were unwilling to engage with them on the topic of their diets. Participants expressed frustration that even though doctors supported their efforts once improvements in health had occurred, they still would not discuss the role of the diet in these health improvements. Only a small minority of participants were prescribed an LCHF diet by their medical doctor or had any formal medical input from either doctors or dietitians in formulating their diets.

An important take-away message from Chapter 2 was that many interrelated factors contributed to the health improvements experienced by these participants. These included factors such as: reduced hunger, less frequent meals, weight loss, improved psychological state, and increased energy and activity levels. The LCHF diet was perceived by participants as an important catalyst for improved health but carbohydrate restriction *per se* may not have directly caused some or all of these positive health outcomes. To capture the importance of these factors, we referred to our participants' interventions as an LCHF 'lifestyle'. Future research with LCHF diets requires careful consideration of which of these interrelated aspects of LCHF lifestyles should be controlled for versus included as part of the intervention. For example, reduced hunger and cravings were important aspects of this diet for our participants. It likely led to spontaneous reductions in calorie intake, as well as changes in certain eating behaviours such as fewer meals, which in turn may have been important for weight loss. Therefore, reduced calorie intake, weight loss, and less frequent eating, all with less hunger, are

potentially an intrinsic property of the LCHF diet. A control group which is matched for calorie intake, weight loss, and/or eating timing would negate some of the potential benefits of the satiating effects of LCHF diets. Future studies examining the effects of diet on EGP, gluconeogenesis, and glycogenolysis need to be explicit about what aspects of the diet are being studied.

Chapter 1 and 2 of this thesis have important implications for practicing clinicians. The dietary advice that clinicians prescribe to patients is often based on national policies and/or guidelines from medical authorities. It is important for clinicians to be aware that this nutritional advice is not the result of conclusive scientific evidence but rather reflective of the prevailing nutritional hypotheses of the 1950s and 60s. An understanding of the extent to which nutrition research is ambiguous may help clinicians contextualise the controversies in nutrition. Further, an appreciation of these ambiguities would help clinicians be open to discussing alternative nutritional strategies with patients. Patients would also benefit from an honest discussion of the limitations of the science as well as the known harms and benefits of the various nutritional options. The participants in Chapter 2 of this thesis desired this level of engagement from their clinicians. An appreciation of the uncertainties and limitations in nutrition research would help clinicians build and maintain productive relationships with patients interested in nutrition.

Chapter 3 of this thesis was an experiment aimed at measuring gluconeogenesis in the early postprandial period. The dysregulation of gluconeogenesis is a key pathology involved in the development and maintenance of hyperglycaemia in T2D. Gluconeogenesis is relatively poorly studied due to available methodologies being invasive, inaccurate, and/or expensive. The average deuterium enrichment method is a relatively recent development which overcomes these challenges. This method relies on the enrichment of body water with deuterium oxide (^2H_2O) to estimate the fraction of endogenous glucose production attributable to gluconeogenesis (fGNG). A second tracer – [6,6-$^2H_2$]-glucose – is used to quantify rates of endogenous glucose production (EGP). Absolute rates of gluconeogenesis can then be calculated as the rates of EGP multiplied by fGNG. A limitation of this method is that the administration of ^2H_2O can only begin 2 hours after a carbohydrate-containing meal and the equilibration of both tracers has to be achieved before accurate measurements can be made. In prior research with this method, researchers have taken a conservative approach and allowed for an overnight fast to ensure sufficient time for tracer equilibration. Gluconeogenesis has therefore predominantly been measured after an overnight fast and there is limited research on gluconeogenesis closer to a meal. The time requirements for tracer equilibration to occur with the average deuterium enrichment method is unknown. We

hypothesised that this method could be used to measure gluconeogenesis in the early post-absorptive period. Therefore, the aim of Chapter 3 was to determine whether the average deuterium enrichment method could accurately quantify gluconeogenesis 5 hours after a mixed-macronutrient meal.

Ten participants (6 female and 4 male) were recruited for the study. Age ranged from 33 to 60 years, body weight from 58 to 111 kg, body fat percentage from 14 to 50 %, and BMI from 19 to 43 kg/m^2. Participants also varied in habitual diet and LCHF and mixed macronutrients diets were represented. Low-risk participants were chosen for the piloting of this protocol and no participants had a significant medical condition. Two participants had prior T2D diagnoses but had no signs or symptoms of T2D at the time of the experiment. Participants arrived at the laboratory after an overnight fast and ate a mixed-macronutrient meal which contain approximately 50 % of calories as carbohydrate. Two hours after the meal, ^2H_2O ingestion and a primed continuous infusion of [6,6-$^2H_2$]-glucose were started. Blood samples for the determination of tracer enrichment were taken 3- and 5 hours after the start of tracer administration (5- and 7 hours, respectively, after the meal). The 5-hour post-tracer-administration time point allowed an additional 2 hours for tracer equilibration and therefore served as a reference measurement to evaluate whether equilibration had occurred at the earlier time point. Three blood samples were drawn 10 min apart at each time point so that the coefficient of variation (CV) of tracer equilibration could be calculated.

We found that CVs were low (below 5 %) at each timepoint. Additionally, absolute rates of gluconeogenesis were the same at all time points. This is consistent with the available literature which suggests that rates of gluconeogenesis are constant under a variety of different physiological conditions. There was a relatively large drop in EGP from 5 to 7 hours after the meal. This was entirely due to a decline in glycogenolysis during this 2-hour period. We concluded that the low CVs and the constant rates of gluconeogenesis suggest that tracer equilibration may have been achieved within 3 hours of tracer administration.

A second important aspect of this experiment was to pilot the ^2H_2O ingestion protocol as severe symptoms of dizziness have been reported when ingesting ^2H_2O in a single dose. However, we did not observe these symptoms during pilot testing prior to the start of the experiment and initial participants in the experiment ingested ^2H_2O in a single dose. In the actual experiment, some participants experienced severe nausea and vomiting and we therefore altered the protocol to allow for the ingestion of ^2H_2O over a 90 min period. One participant still had severe symptoms despite

this modification and we later allowed participants to lie in a bed and rest during the equilibration period. We feel this has largely reduced the risk of severe symptoms, although symptoms were highly individualised and have to be monitored carefully during future experiments.

Based on the result from Chapter 3, there are a number of experiments which would be useful to confirm that our results are a true and accurate representation of the real rates of gluconeogenesis. Firstly, it would be useful to follow exactly the same protocol except without the pre-experimental meal such that measurements would be after approximately 17 and 19 hours fasted instead of 5 and 7 hours fasted. The large changes in EGP and glycogenolysis that we reported in Chapter 3 would not be expected after this length of fasting. Therefore, the measurement of stable rates of gluconeogenesis and glycogenolysis, using the same protocol but without the meal, would provide strong evidence that tracer equilibration in Chapter 3 had been achieved.

Additionally, it is possible that tracer equilibration is occurring rapidly and the quantification of gluconeogenesis may be possible even sooner than 5 hours after a meal. Assuming results from the fasting validation study outlined above are successful, it may be worth experimenting with the quantification of gluconeogenesis even sooner after a meal than 5 hours. Tracer equilibration could be measured 4 hours after a meal and it would also be useful to identify when the equilibration of body water is achieved. Lastly, although a variety of participants with different weights and body fat percentages were tested, hyperglycaemic T2D participants were not included in this study. A pilot study which confirms that tracer equilibration occurs at the same rate in poorly controlled T2D patients as these low-risk individuals is required. The experiments outlined above form the basis of post-doctoral fellowship grant applications to continue with this work.

Lesson from Chapter 1 and 2 are important in designing future experiments that build on the novel methodological foundation that has been laid in Chapter 3. There are many challenges and limitations that are specific to nutrition research that have to be considered in the design of these future experiments. It is also be important to be clear about the aims and objectives as well as what is beyond the scope of this research. In terms of investigations which involve low carbohydrate diets, there are many nuances that were identified in Chapter 2 which need to be considered and incorporated into the design and analysis of future experiments. One of the biggest problems in nutrition science seems to be the overinterpretation of data and an underappreciation of the complexities and interrelated nature of nutrition and chronic disease. These are large challenges but the foundation has been laid to take them on and produce meaningful research in the future.

An important outcome of this thesis was the implementation of the technical procedures and calculations required to measure gluconeogenesis. Although the measurement of gluconeogenesis is the main quantitative technique for future research, an invaluable aspect of this thesis has been an improved understanding of the complexities and controversies in nutrition research. Additionally, this thesis has highlighted the many nuances of LCHF diets and related lifestyle factors. A challenge for future research will be in formulating clear, well-defined hypotheses, which are likely testable within the parameters of the available study designs and methodologies. Ultimately, the focus of future research using the protocol outlined in this thesis is to better understand the physiology of disease reversal in T2D patients with the LCHF diet. A better understanding of the impact of diet on gluconeogenesis, glycogenolysis, and EGP in T2D patients could provide insight into the safety and appropriateness of LCHF diets for the management of T2D.

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MEDIA RELEASE
Sports Science Institute of South Africa
For immediate release
16 January 2017

PARTICIPANTS REQUIRED FOR UCT STUDY INVESTIGATING TYPE II DIABETICS (OR EX DIABETICS) FOLLOWING A LOW CARBOHYDRATE HIGH FAT DIET

Do you have or have you ever had **Type 2 Diabetes**? Do you follow **a low carbohydrate high fat (Banting) diet**? If so, we would like you to volunteer to participate in a short descriptive study.

The Division of Exercise Science and Sports Medicine at UCT based at the Sports Science Institute of SA (SSISA), is recruiting volunteers for a research study. The main aim of the study is to find out more about how you follow a low carbohydrate high fat diet and how your diabetes has responded and progressed. This will assist us in designing future studies.

If you volunteer to participate, you will be required to: complete an online health, diabetes, diet and exercise questionnaire; visit the Sports Science Institute on 1 occasion (90 min) for basic health screening; provide a blood sample for glucose testing; and keep a food diary for 3 days.

To be eligible, you must:

- have followed a low carbohydrate high fat diet for at least the **past 6 months** and made no major changes to the way you eat during this period
- be 18 years of age or older
- have been diagnosed with type 2 diabetes at some time in your life

What are the benefits and risks for partaking in the trial?

- By taking part, you will help us design future studies in this field that will potentially improve the health and well-being of patients with type 2 diabetes.
- You will receive a report of all results including dietary analysis, body measurements, blood pressure, and glucose, insulin and HbA1C blood concentrations.
- The risks and discomfort associated with taking part are small.

To apply or for more information, please contact Chris Webster before 24 February 2017.

Tel: 083 400 7206 **Email:** chris.webster@uct.ac.za

WE WOULD LIKE YOU TO VOLUNTEER TO PARTICIPATE IN OUR SHORT STUDY!

SPORTS SCIENCE INSTITUTE OF SOUTH AFRICA

DO YOU HAVE OR HAVE YOU EVER HAD TYPE 2 DIABETES?

University of Cape Town • iYunivesithi yaseKapa • Universiteit van Kaapstad

DO YOU FOLLOW A LOW CARB/BANTING DIET?

IF SO, WE WOULD LIKE YOU TO VOLUNTEER

TO PARTICIPATE IN OUR SHORT STUDY!

TO BE ELIGIBLE YOU MUST:

1. HAVE FOLLOWED A LOW CARBOHYDRATE HIGH FAT DIET FOR AT LEAST THE PAST 6 MONTHS,
2. BE 18 YEARS OF AGE OR OLDER AND
3. HAVE BEEN DIAGNOSED WITH TYPE 2 DIABETES

**YOU WILL NOT BE ABLE TO PARTICIPATE IF YOU HAVE SIGNIFICANTLY CHANGED YOUR DIET IN THE LAST 6 MONTHS*

IF YOU VOLUNTEER TO PARTICIPATE YOU WILL BE REQUIRED TO:

1. COMPLETE AN ONLINE HEALTH, DIABETES, DIET AND EXERCISE QUESTIONNAIRE.

2. VISIT THE SPORTS SCIENCE INSTITUTE IN CAPE TOWN ON 1 OCCASION (90 MIN) FOR BASIC HEALTH SCREENING.

3, PROVIDE A BLOOD SAMPLE FOR GLUCOSE TESTING.

TO APPLY NOW CONTACT:

EMAIL: LCHF.RECRUITMENT@GMAIL.COM
OR CALL
CHRIS WEBSTER | 083 400 7206 OR TAMZYN MURPHY CAMPBELL | 084 313 4103

FOR MORE INFO VISIT WWW.THENOAKESFOUNDATION.CO.ZA/NEWS.BLOG/RECRUITING

THE NOAKES FOUNDATION

This questionnaire will ask for details about your type 2 diabetes and will focus around three main periods:

1) The time around your initial type 2 diabetes diagnosis.

2) The time around when you started following a LCHF diet.

3) Your current diabetes status.

The questionnaire goes into detail around your use of chronic medications for the management of diabetes and other related conditions. In the event that you do not know exact dates, try to give as accurate an answer as possible.

The basic structure of the questionnaire is as follows:

Demographics and socioeconomic status Family history and your health history Details about the time when you were diagnosed with diabetes Details about the time when you started following a LCHF diet Details of your diabetic medication use Details of your non-diabetic medication use Current exercise Medical professionals and future study

It should take 30 min to 1 hour to complete. You are able to save the questionnaire and resume from where you left off by clicking the 'Resume Later' button.

Do not hesitate to contact a researcher if you experience problems with the questionnaire.

Section A: code

Participant code and eligibilty criteria.

A1. **Enter the code provided to you by the researchers.**

A2. **Have you previously been diagnosed with type 2 diabetes by a doctor?**

Yes ☐

No ☐

A3. **Have you been following a low carbohydrate high fat (LCHF) diet for at least the past 6 months?**

Yes ☐

No ☐

A4. **Are you 18 years of age or older?**

Yes ☐

No ☐

A5. **You are not eligible for this study due to your type 2 diabetes status.**

Please contact a researcher if you feel a mistake has been made.

End survey ☐

A6. **You are not eligible for this study due to your diet.**

Please contact a researcher if you feel a mistake has been made.

End survey ☐

A7. **You are not eligible for this study due to your age.**

Please contact a researcher if you feel a mistake has been made.

End survey ☐

Section B: personal

This section contains questions relating to your demographics and socioeconomic status status.

B1. **Date of birth:**

☐☐☐☐☐☐☐☐

B2. **What is your height?**

Answer in <u>centimetres</u> (cm)

☐☐☐☐☐☐☐☐☐☐

B3. **What is your current weight?**

Answer in <u>kilograms</u> (kg)

☐☐☐☐☐☐☐☐☐☐

B4. **Biological sex:**

Male ☐

Female ☐

Intersex ☐

B5. Ethnicity:

- Black ☐
- White ☐
- Mixed ☐
- Indian ☐
- Other ☐

Other

B6. Highest level of education:

- Primary school ☐
- High school ☐
- Tertiary undergraduate ☐
- Tertiary postgraduate ☐
- Other ☐

Other

B7. Usual monthly income:

- R 0 - R 9 999 ☐
- R 10 000 - R 24 999 ☐
- R 25 000 or more ☐

B8. How many people currently live in your household?

B9. How many rooms in total are there in your household?

Count an open plan area as the number of functional rooms.

Section C: family

This section cotains questions about your family history.

C1. Has your <u>mother or sister</u> had heart disease (e.g. heart attack / angina / heart failure)?

	Yes	No	Unsure
Before the age of 65?	☐	☐	☐

	Yes	No	Unsure
After the age of 65?	☐	☐	☐

C2. Has your <u>father or brother</u> had heart disease (e.g. heart attack / angina / heart failure)?

	Yes	No	Unsure
Before the age of 55?	☐	☐	☐
After the age of 55?	☐	☐	☐

C3. Have any of your close family members had any of the following conditions?

Close family members refer to siblings, parents and grandparents.

	No family history	Unsure	Father and/or mother	Brother(s) and/or sister(s)	Grandparent(s)	A combination of the previous options
Overweight or obesity	☐	☐	☐	☐	☐	☐
Pre-diabetes	☐	☐	☐	☐	☐	☐
Type 2 diabetes	☐	☐	☐	☐	☐	☐
High blood pressure	☐	☐	☐	☐	☐	☐
High cholesterol	☐	☐	☐	☐	☐	☐

Section D: health

This section contains questions on your health status, smoking history and alcohol intake.

D1. Do you have, or have you ever had, any of the following?

Every row requires an answer.

	Currently have	Previously had	No	Unsure
High blood pressure	☐	☐	☐	☐
Abdominal obesity	☐	☐	☐	☐
Dyslipidemia (high cholesterol and/or triglycerides)	☐	☐	☐	☐
Neuropathy (nerve damage leading to loss of sensation / numbness / tingling	☐	☐	☐	☐
Non-alcoholic fatty liver disease or other liver condition	☐	☐	☐	☐
Kidney conditions	☐	☐	☐	☐
Intermittent claudication (cramping pain in the legs, often caused by exercise)	☐	☐	☐	☐

	Currently have	Previously had	No	Unsure
Retinopathy (blurred or fluctuating vision)	☐	☐	☐	☐
Stroke or transient ischemic attack (TIA, temporary blockage in brain blood vessel)	☐	☐	☐	☐
Angina (chest pain)	☐	☐	☐	☐
Anemia (red blood cell deficiency)	☐	☐	☐	☐
Thyroid dysfunction	☐	☐	☐	☐
Vitamin D deficiency	☐	☐	☐	☐

D2. Do you have or have you ever had any of the following?

Every row requires an answer.

	Currently have	Previously had	No	Unsure
Lung disease	☐	☐	☐	☐
Pulmonary tuberculosis (TB)	☐	☐	☐	☐
Asthma	☐	☐	☐	☐
Epilepsy	☐	☐	☐	☐
Celiac disease	☐	☐	☐	☐
Crohn's disease	☐	☐	☐	☐
Depression / Anxiety	☐	☐	☐	☐
Cancer	☐	☐	☐	☐
Glandular fever	☐	☐	☐	☐
Gout	☐	☐	☐	☐
Drug (illigal or over the counter) / alcohol addiction	☐	☐	☐	☐

D3. Do you have any medical conditions that are not covered by the above questions?

Yes ☐

No ☐

D4. Please specify which medical condition(s).

1 ☐☐☐☐☐☐☐☐☐☐

2

3

4

5

D5. Do you currently smoke cigarettes?

Yes ☐

No ☐

D6. How long have you been smoking (years)?

D7. How many cigarettes do you smoke per day?

1 - 4 per day ☐

5 - 9 per day ☐

10 - 19 per day ☐

20 - 29 per day ☐

30 or more per day ☐

D8. Have you smoked in the past?

Yes ☐

No ☐

D9. When did you stop smoking?

D10. For how many years did you previously smoke (years)?

D11. How many cigarettes did you smoke per day?

1 - 4 per day ☐

5 - 9 per day ☐

10 - 19 per day ☐

20 - 29 per day ☐

30 or more per day ☐

D12. **Thinking back over the past 6 months, on average how many standard alcohol units per week did you usually drink?**

Note: 1 standard unit is equal to 200 ml of beer, 100 ml of wine or 25 ml of spirits. In other words: 2/3 of a can of beer; a small glass of wine; and a shot of spirits are 1 unit each.

- ☐ 0 units
- ☐ 1 - 2 units per week
- ☐ 3 - 6 units per week
- ☐ 7 - 10 units per week
- ☐ 11 - 14 units per week
- ☐ 15 or more units per week

Section E: women

E1. **Are you currently pregnant?**

- ☐ Yes
- ☐ No
- ☐ Unsure

E2. **Have you had a hysterectomy?**

- ☐ Yes
- ☐ No

E3. **Do you take hormones (HRT or birth control in the form of the pill, a patch, injection or hormone-releasing intrauterine device)?**

- ☐ Yes
- ☐ No
- ☐ Unsure

E4. **Do you have regular periods?**

- ☐ Yes
- ☐ No
- ☐ Unsure

E5. When was your last period?

- [ ] 3 months ago
- [ ] 6 months ago
- [ ] 1 year ago
- [ ] More than one year ago

Section F: diagnosis

This section contains questions which relate to your diabetes diagnosis.

F1. When were you diagnosed with diabetes?

F2. Did you follow a specific diet prior to your diabetes diagnosis?

- [ ] Yes
- [ ] No
- [ ] Unsure

F3. What diet did you follow prior to your diagnosis?

F4. What was your weight at the time of your diagnosis?

Answer in kilograms (kg)

F5. At the time of your diagnosis, how often did you exercise per week?

- [ ] 0 days per week
- [ ] 1 - 2 days per week
- [ ] 3 - 4 days per week
- [ ] 5 or more days per week

F6. What types of exercise were you doing around the time when you were diagnosed?

- [ ] Walking
- [ ] Jogging / running
- [ ] Cycling
- [ ] Swimming
- [ ] Strength training
- [ ] Yoga / pilates

Cardio activities at the gym ☐

Sports (i.e. soccer / hockey etc.) ☐

Other ☐

Other

F7. How long were your exercise sessions, on average, around the time when you were diagnosed?

Answer in minutes.

F8. Which best describes the intensity of your average exercise session around the time when you were diagnosed?

Light (very easy breathing / able to maintain a conversation) ☐

Moderate (heavier breathing but able to speak / building up a sweat) ☐

Hard (very heavy breathing and unable to speak) ☐

F9. Was your fasting blood glucose measured as part of your diagnosis?

Yes ☐

No ☐

Unsure ☐

F10. What was your fasting blood glucose concentration at the time of diagnosis?

Answer in mmol/l.

To convert mg/dl to mmol/l, divide your glucose value by 18.

F11. Was your HbA1c measured as part of your diagnosis?

Yes ☐

No ☐

Unsure ☐

F12. What was your HbA1c at the time of diagnosis?

Answer in % (DCCT).

You can covert IFCC (mmol/mol) to DCCT (%) at the following url: http://www.diabetes.co.uk/hba1c-units-converter.html

F13. Was an oral glucose tolerance test performed as part of your diagnosis?

- Yes ☐
- No ☐
- Unsure ☐

F14. Was insulin measured as part of your diagnosis?

- Yes ☐
- No ☐
- Unsure ☐

Section G: lchf

This section contains questions which relate to your diabetes around the time when you started eating a low carbohydrate, high fat diet.

G1. When did you start eating a LCHF diet?

G2. Did you follow a specific diet prior to starting your LCHF diet?

- Yes ☐
- No ☐
- Unsure ☐

G3. What diet did you follow prior to starting your LCHF diet?

G4. What best describes the progression of your diabetes from the time of diagnosis to the time when you started eating a LCHF diet?

- Drastic deterioration and worsening of symptoms ☐
- Some deterioration and worsening of symptoms ☐
- No change ☐
- Some improvement and reduction in symptoms ☐
- Drastic improvement and reduction in symptoms ☐

G5. Did you have your blood glucose or HbA1c checked prior to starting your LCHF diet?

- glucose ☐
- HbA1c ☐
- both ☐
- no ☐

G6. When was the closet time before starting your LCHF diet that you did have your blood glucose and/or HbA1c measured?

☐☐☐☐☐☐☐☐

G7. What was your blood glucose (mmol/l)?

To convert mg/dl to mmol/l, divide by 18.

☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

G8. What was your HbA1c at the the time when you started following a LCHF diet?

Answer in % (DCCT).

You can covert IFCC (mmol/mol) to DCCT (%) at the following url: http://www.diabetes.co.uk/hba1c-units-converter.html

☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

G9. What is the main reason that you started eating a LCHF diet?

☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

G10. What was your weight at the time when you started following a LCHF diet?

☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

G11. What best describes the progression of your diabetes since you started eating a LCHF diet?

- Drastic deterioration and worsening of symptoms ☐
- Some deterioration and worsening of symptoms ☐
- No change ☐
- Some improvement and reduction in symptoms ☐
- Drastic improvement and reduction in symptoms ☐

G12. Since you've been following a LCHF diet, have you experienced changes in any of the following?

Mark 'not applicable' if you are not affected.

	Not applicable	Drastic worsening	Some worsening	No change	Some improvement	Drastic improvement
Allergies	☐	☐	☐	☐	☐	☐
Asthma	☐	☐	☐	☐	☐	☐
Eyesight	☐	☐	☐	☐	☐	☐
Skin conditions	☐	☐	☐	☐	☐	☐
Gastrointestinal issues (e.g. irritable bowel syndrome / constipation)	☐	☐	☐	☐	☐	☐
Post nasal drip	☐	☐	☐	☐	☐	☐
Concentration	☐	☐	☐	☐	☐	☐
Sleep	☐	☐	☐	☐	☐	☐
Pre-menstrual syndrome (PMS)	☐	☐	☐	☐	☐	☐
Other	☐	☐	☐	☐	☐	☐

G13. What is 'other' referring to in the above question?

Section H: biguanides

The next series of questions will involve details of all the diabetic medications that you currently, or have previously, taken.

H1. Have you ever taken biguanides?

Examples of medications in this class include metformin / glucophage.

- ☐ Yes
- ☐ No
- ☐ Unsure

H2. What was the name of your biguanide medication?

H3. When did you first start taking this medication?

H4. **Were you taking this biguanide medication at the <u>time when you started following a lchf diet?</u>**

Yes ☐

No ☐

H5. **Did you ever stop taking this medication?**

Yes ☐

No ☐

H6. **When did you stop taking this medication?**

☐☐☐☐☐☐☐☐

H7. **Did you ever restart taking this medication?**

Yes ☐

No ☐

H8. **When did you restart taking this medication?**

☐☐☐☐☐☐☐☐

H9. **Did you re-stop taking this medication?**

Yes ☐

No ☐

H10. **When did you stop taking this medication?**

☐☐☐☐☐☐☐☐

H11. **Have you since restarted taking this medication again?**

Yes ☐

No ☐

H12. **When did you restart this medication?**

☐☐☐☐☐☐☐☐

H13. **Are you currently taking this medication?**

Yes ☐

No ☐

H14. **What is your current dose (mg daily)?**

☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

H15. **What was your maximum dose of this drug (mg daily)?**

☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

Section I: insulin

Diabetic medications continued...

I1. Have you ever taken insulin?

Yes ☐
No ☐
Unsure ☐

I2. When did you first start taking insulin?

☐☐☐☐☐☐☐☐

I3. Were you taking insulin at the <u>time when you started following a lchf diet?</u>

Yes ☐
No ☐

I4. Did you ever stop taking this medication?

Yes ☐
No ☐

I5. When did you stop taking this medication?

☐☐☐☐☐☐☐☐

I6. Did you ever restart taking this medication?

Yes ☐
No ☐

I7. When did you restart taking this medication?

☐☐☐☐☐☐☐☐

I8. Did you re-stop taking this medication?

Yes ☐
No ☐

I9. When did you stop taking this medication?

☐☐☐☐☐☐☐☐

I10. Have you since restarted taking this medication again?

Yes ☐
No ☐

I11. When did you restart taking this medication?

☐☐☐☐☐☐☐☐

I12. **Are you currently taking this medication?**

Yes ☐

No ☐

I13. **What is your current dose (units per day)?**

I14. **What was your maximum dose of this drug (units per day)?**

Section J: sulphonylureas

Diabetic medications continued...

J1.

Have you ever taken sulphonylureas?

Examples of medications in this class include chlorpropamide, glibenclamide, gliclazide, glimepiride andglipizide.

Yes ☐

No ☐

Unsure ☐

J2. **What was the name of your sulphonylureas medication?**

J3. **When did you first start taking this medication?**

J4. **Were you taking this sulphonylurea medication at the <u>time when you started following a lchf diet?</u>**

Yes ☐

No ☐

J5. **Did you ever stop taking this medication?**

Yes ☐

No ☐

J6. **When did you stop taking this medication?**

J7. **Did you ever restart taking this medication?**

Yes ☐

No ☐

J8. **When did you restart taking this medication?**

J9. **Did you re-stop taking this medication?**

Yes ☐

No ☐

J10. **When did you stop taking this medication?**

J11. **Have you since restarted taking this medication again?**

Yes ☐

No ☐

J12. **Are you currently taking this medication?**

Yes ☐

No ☐

J13. **What is your current dose (mg daily)?**

J14. **What was your maximum dose of this drug (mg daily)?**

Section K: othermed

All diabetic medications that you have ever taken must be entered into this questionnaire.

If you take, or took, any diabetic medications that you have not captured in the previous 3 questions, enter them here.

You can enter medications from any class of diabetic drugs here.

K1. **Apart from the medications mentioned in the previous questions:**

Have you ever taken any other medications for the <u>treatment of your diabetes</u>?

Yes ☐

No ☐

Unsure ☐

K2. **How many other medications have you taken for the treatment of your diabetes?**

1 ☐
2 ☐
3 ☐
4 ☐
5 ☐

K3. **What is the name of other medication 1**

K4. **When did you first start taking this medication?**

K5. **Were you taking this medication at the <u>time when you started following a lchf diet?</u>**

Yes ☐
No ☐

K6. **Did you ever stop taking this medication?**

Yes ☐
No ☐

K7. **When did you stop taking this medication?**

K8. **Did you ever restart taking this medication?**

Yes ☐
No ☐

K9. **When did you restart taking this medication?**

K10. **Did you re-stop taking this medication?**

Yes ☐
No ☐

K11. **When did you stop taking this medication?**

K12. **Have you since restarted taking this medication again?**

Yes ☐
No ☐

K13. **Are you currently taking this medication?**

Yes ☐

No ☐

Section L: othermed2

Diabetic medications continued...

L1. **What is the name of other medication 2**

L2. **When did you first start taking this medication?**

L3. **Were you taking this medication at the <u>time when you started following a lchf diet?</u>**

Yes ☐

No ☐

L4. **Did you ever stop taking this medication?**

Yes ☐

No ☐

L5. **When did you stop taking this medication?**

L6. **Did you ever restart taking this medication?**

Yes ☐

No ☐

L7. **When did you restart taking this medication?**

L8. **Did you re-stop taking this medication?**

Yes ☐

No ☐

L9. **When did you stop taking this medication?**

L10. **Have you since restarted taking this medication again?**

Yes ☐

No ☐

L11. **Are you currently taking this medication?**

Yes ☐

No ☐

Section M: othermed3

Diabetic medications continued...

M1. **What is the name of other medication 3**

M2. **When did you first start taking this medication?**

M3. **Were you taking this medication at the <u>time when you started following a lchf diet?</u>**

Yes ☐

No ☐

M4. **Did you ever stop taking this medication?**

Yes ☐

No ☐

M5. **When did you stop taking this medication?**

M6. **Did you ever restart taking this medication?**

Yes ☐

No ☐

M7. **When did you restart taking this medication?**

M8. **Did you re-stop taking this medication?**

Yes ☐

No ☐

M9. **When did you stop taking this medication?**

M10. **Have you since restarted taking this medication again?**

Yes ☐

No ☐

M11. Are you currently taking this medication?

Yes ☐

No ☐

Section N: othermed4

Diabetic medications continued...

N1. What is the name of other medication 4

N2. When did you first start taking this medication?

N3. Were you taking this medication at the <u>time when you started following a lchf diet?</u>

Yes ☐

No ☐

N4. Did you ever stop taking this medication?

Yes ☐

No ☐

N5. When did you stop taking this medication?

N6. Did you ever restart taking this medication?

Yes ☐

No ☐

N7. When did you restart taking this medication?

N8. Did you re-stop taking this medication?

Yes ☐

No ☐

N9. When did you stop taking this medication?

N10. Have you since restarted taking this medication again?

Yes ☐

No ☐

N11. Are you currently taking this medication?

Yes ☐

No ☐

Section O: othermed5

Diabetic medications continued...

O1. What is the name of other medication 5

O2. When did you first start taking this medication?

O3. Were you taking this medication at the <u>time when you started following a lchf diet?</u>

Yes ☐

No ☐

O4. Did you ever stop taking this medication?

Yes ☐

No ☐

O5. When did you stop taking this medication?

O6. Did you ever restart taking this medication?

Yes ☐

No ☐

O7. When did you restart taking this medication?

O8. Did you re-stop taking this medication?

Yes ☐

No ☐

O9. When did you stop taking this medication?

O10. Have you since restarted taking this medication again?

Yes ☐

No ☐

O11. Are you currently taking this medication?

Yes ☐

No ☐

Section P: chronicmeds

This section deals with chronic (long-term) medications taken for conditions other than diabetes.

P1. Have you ever taken any of the following chronic medications, or medications for any of the following conditions?

Chronic medication use means that the medication was taken over the long term.

In this case, any medication that you took continuously for a minimum of 3 months can be considered chronic.

Aspirin ☐

Blood pressure ☐

Heart disease / cardiac conditions ☐

Lipids (cholesterol / triglycerides) ☐

Allergies (excluding seasonal use) ☐

Asthma (excluding carrying a pump for occasional attacks) ☐

Post-nasal drip ☐

Lung or respiratory tract conditions ☐

Gastrointestinal conditions (stomach / abdominal pain) ☐

Skin conditions (such as psoriasis) ☐

Autoimmune diseases (such as rheumatoid arthritis / Hashimoto's thyroiditis) ☐

Muscle or joint pain (such as osteoarthritis) ☐

Depression / anxiety ☐

Sexual dysfunction or infertility ☐

Other ☐

None ☐

P2. When did you start taking aspirin?

☐☐☐☐☐☐☐☐

P3. **Were you taking aspirin <u>at the time of your diabetes diagnosis</u>?**

Yes ☐
No ☐
Unsure ☐

P4. **Were you taking aspirin at the time <u>when you started following a LCHF diet?</u>**

Yes ☐
No ☐
Unsure ☐

P5. **Are you <u>currently</u> taking aspirin?**

Yes ☐
No ☐
Unsure ☐

P6. **When did you stop taking aspirin?**

P7. **What is/was the name of your blood pressure medication?**

Note: if you have taken more than one blood pressure medication then just fill out the first one here.

P8. **When did you start taking this blood pressure medication?**

P9. **Were you taking this blood pressure medication <u>at the time of your diabetes diagnosis?</u>**

Yes ☐
No ☐
Unsure ☐

P10. **Were you taking this blood pressure medication at the time <u>when you started eating a LCHF diet?</u>**

Yes ☐
No ☐
Unsure ☐

P11. **Are you <u>currently</u> taking this blood pressure medication?**

Yes ☐

No ☐

P12. **When did you stop taking this blood pressure medication?**

☐☐☐☐☐☐☐☐

P13. **Have you ever taken any other chronic (long term) medications for the management of blood pressure?**

Yes ☐

No ☐

Unsure ☐

P14. **What is/was the name of your blood pressure medication?**

Note: only fill out the second one you have taken here.

☐☐

P15. **When did you start taking this blood pressure medication?**

☐☐☐☐☐☐☐☐

P16. **Were you taking this blood pressure medication at <u>the time of your diabetes diagnosis?</u>**

Yes ☐

No ☐

Unsure ☐

P17. **Were you taking this blood pressure medication at the time <u>when you started eating a LCHF diet?</u>**

Yes ☐

No ☐

Unsure ☐

P18. **Are you <u>currently</u> taking this blood pressure medication?**

Yes ☐

No ☐

P19. **When did you stop taking this blood pressure medication?**

☐☐☐☐☐☐☐☐

P20. **Have you ever taken any other chronic (long term) medications for the management of blood pressure?**

Yes ☐
No ☐
Unsure ☐

P21. **What is/was the name of your blood pressure medication?**

Note: If you have taken more than three blood pressure medications then please fill out the third one here. Any additional blood pressure medications can be filled out in the 'other' section.

P22. **When did you start taking this blood pressure medication?**

P23. **Were you taking this blood pressure medication <u>at the time of your diabetes diagnosis?</u>**

Yes ☐
No ☐
Unsure ☐

P24. **Were you taking this blood pressure medication at the time <u>when you started eating a LCHF diet?</u>**

Yes ☐
No ☐
Unsure ☐

P25. **Are you <u>currently</u> taking this blood pressure medication?**

Yes ☐
No ☐

P26. **When did you stop taking this blood pressure medication?**

P27. **What is/was the name of your heart/cardiac medication?**

Note: If you have taken more than one heart/cardiac medication then only fill out the first one here.

P28. **When did you start taking this heart/cardiac medication?**

P29. **Were you taking this heart/cardiac medication <u>at the time of your diabetes diagnosis?</u>**

Yes ☐

No ☐

Unsure ☐

P30. **Were you taking this heart/cardiac medication at the time <u>when you started eating a LCHF diet?</u>**

Yes ☐

No ☐

Unsure ☐

P31. **Are you <u>currently</u> taking this heart/cardiac medication?**

Yes ☐

No ☐

P32. **When did you stop taking this heart/cardiac medication?**

P33. **Have you ever taken any other chronic (long term) medications for the management of heart disease / cardiac conditions?**

Yes ☐

No ☐

Unsure ☐

P34. **What is/was the name of your heart/cardiac medication?**

Note: only fill out the second one you have taken here.

P35. **When did you start taking this heart/cardiac medication?**

P36. **Were you taking this heart/cardiac medication <u>at the time of your diabetes diagnosis?</u>**

Yes ☐

No ☐

Unsure ☐

P37. **Were you taking this heart/cardiac medication at the time <u>when you started eating a LCHF diet?</u>**

Yes ☐
No ☐
Unsure ☐

P38. **Are you <u>currently</u> taking this heart/cardiac medication?**

Yes ☐
No ☐

P39. **When did you stop taking this heart/cardiac medication?**

☐☐☐☐☐☐☐☐

P40. **Have you ever taken any other chronic (long term) medications for the management of heart disease / cardiac conditions?**

Yes ☐
No ☐
Unsure ☐

P41. **What is/was the name of your heart/cardiac medication?**

Note: If you have taken more than three blood pressure medications then please fill out the third one here. Any additional blood pressure medications can be filled out in the 'other' section.

☐☐

P42. **When did you start taking this heart/cardiac medication?**

☐☐☐☐☐☐☐☐

P43. **Were you taking this heart/cardiac medication <u>at the time of your diabetes diagnosis?</u>**

Yes ☐
No ☐
Unsure ☐

P44. **Were you taking this heart/cardiac medication at the time <u>when you started eating a LCHF diet?</u>**

Yes ☐
No ☐
Unsure ☐

P45. **Are you <u>currently</u> taking this heart/cardiac medication?**

Yes ☐

No ☐

P46. **When did you stop taking this heart/cardiac medication?**

☐☐☐☐☐☐☐☐

P47. **What is/was the name of your lipid/cholesterol medication?**

Note: if you have taken more than one lipid medication then just fill out the first one here.

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P48. **When did you start taking this lipid/cholesterol medication?**

☐☐☐☐☐☐☐☐

P49. **Were you taking this lipid/cholesterol medication <u>at the time of your diabetes diagnosis?</u>**

Yes ☐

No ☐

Unsure ☐

P50. **Were you taking this lipid/cholesterol medication at the time <u>when you started eating a LCHF diet?</u>**

Yes ☐

No ☐

Unsure ☐

P51. **Are you <u>currently</u> taking this lipid/cholesterol medication?**

Yes ☐

No ☐

P52. **When did you stop taking this lipid/cholesterol medication?**

☐☐☐☐☐☐☐☐

P53. **Have you ever taken any other chronic (long term) medications for the management lipids (cholesterol and/or triglycerides)?**

Yes ☐

No ☐

Unsure ☐

P54. **What is/was the name of your lipid/cholesterol medication?**

Note: If you have taken more than two lipid medications then please fill out the second one here. Any additional lipid medications can be filled out in the 'other' section.

P55. **When did you start taking this lipid/cholesterol medication?**

P56. **Were you taking this lipid/cholesterol medication <u>at the time of your diabetes diagnosis?</u>**

- Yes ☐
- No ☐
- Unsure ☐

P57. **Were you taking this lipid/cholesterol medication at the time <u>when you started eating a LCHF diet?</u>**

- Yes ☐
- No ☐
- Unsure ☐

P58. **Are you <u>currently</u> taking this lipid/cholesterol medication?**

- Yes ☐
- No ☐

P59. **When did you stop taking this lipid/cholesterol medication?**

P60. **What is/was the name of your allergies medication?**

If you have taken more than one chronic medication for the management of allergies, fill out one here and fill out the additional one(s) under 'other'.

P61. **When did you start taking this allergies medication?**

P62. **Were you taking this allergies medication <u>at the time of your diabetes diagnosis?</u>**

- Yes ☐
- No ☐
- Unsure ☐

P63. **Were you taking this allergies medication at the time <u>when you started eating a LCHF diet?</u>**

- Yes ☐
- No ☐
- Unsure ☐

P64. **Are you <u>currently</u> taking this allergies medication?**

- Yes ☐
- No ☐

P65. **When did you stop taking this allergies medication?**

P66. **What is/was the name of your asthma medication?**

If you have taken more than one chronic medication for the management of asthma, fill out one here and fill out the additional one(s) under 'other'.

P67. **When did you start taking this asthma medication?**

P68. **Were you taking this asthma medication at <u>the time of your diabetes diagnosis?</u>**

- Yes ☐
- No ☐
- Unsure ☐

P69. **Were you taking this heart/cardiac medication at the time <u>when you started eating a LCHF diet?</u>**

- Yes ☐
- No ☐
- Unsure ☐

P70. **Are you <u>currently</u> taking this asthma medication?**

- Yes ☐
- No ☐

P71. **When did you stop taking this asthma medication?**

P72. **What is/was the name of your post-nasal drip medication?**

If you have taken more than one chronic medication for the management of post-nasal drip, fill out one here and fill out the additional one(s) under 'other'.

P73. **When did you start taking this post-nasal drip medication?**

P74. **Were you taking this blood pressure medication <u>at the time of your diabetes diagnosis?</u>**

Yes ☐
No ☐
Unsure ☐

P75. **Were you taking this post-nasal drip medication at the time <u>when you started eating a LCHF diet?</u>**

Yes ☐
No ☐
Unsure ☐

P76. **Are you <u>currently</u> taking this post-nasal drip medication?**

Yes ☐
No ☐

P77. **When did you stop taking this post-nasal drip medication?**

P78. **What is/was the name of your lung/respiratory tract medication?**

If you have taken more than one chronic medication for the management of these conditions, fill out one here and fill out the additional one(s) under 'other'.

P79. **When did you start taking this lung/respiratory tract medication?**

P80. **Were you taking this lung/respiratory tract medication <u>at the time of your diabetes diagnosis</u>?**

Yes ☐
No ☐
Unsure ☐

P81. **Were you taking this lung/respiratory tract medication at the time <u>when you started eating a LCHF diet?</u>**

Yes ☐

No ☐

Unsure ☐

P82. **Are you <u>currently</u> taking this lung/respiratory tract medication?**

Yes ☐

No ☐

P83. **When did you stop taking this lung/respiratory tract medication?**

P84. **What is/was the name of your gastrointestinal medication?**

If you have taken more than one chronic medication for the management gastrointestinal conditions, fill out one here and fill out the additional one(s) under 'other'.

P85. **When did you start taking this gastrointestinal medication?**

P86. **Were you taking this gastrointestinal medication at <u>the time of your diabetes diagnosis?</u>**

Yes ☐

No ☐

Unsure ☐

P87. **Were you taking this gastrointestinal medication at the time <u>when you started eating a LCHF diet?</u>**

Yes ☐

No ☐

Unsure ☐

P88. **Are you <u>currently</u> taking this gastrointestinal medication?**

Yes ☐

No ☐

P89. **When did you stop taking this gastrointestinal medication?**

P90. **What is/was the name of your skin condition medication?**

If you have taken more than one chronic medication for the management of skin conditions, fill out one here and fill out the additional one(s) under 'other'.

P91. **When did you start taking this skin condition medication?**

P92. **Were you taking this skin condition medication at <u>the time of your diabetes diagnosis?</u>**

Yes ☐

No ☐

Unsure ☐

P93. **Were you taking this skin condition medication at the time <u>when you started eating a LCHF diet?</u>**

Yes ☐

No ☐

Unsure ☐

P94. **Are you <u>currently</u> taking this skin condition medication?**

Yes ☐

No ☐

P95. **When did you stop taking this skin condition medication?**

P96. **What is/was the name of your autoimmune disease medication?**

If you have taken more than one chronic medication for the management of autoimmune conditions, fill out one here and fill out the additional one(s) under 'other'.

P97. **When did you start taking this autoimmune condition medication?**

P98. **Were you taking this autoimmune condition medication <u>at the time of your diabetes diagnosis?</u>**

Yes ☐

No ☐

Unsure ☐

P99. **Were you taking this autoimmune condition medication at the time <u>when you started eating a LCHF diet?</u>**

Yes ☐
No ☐
Unsure ☐

P100. **Are you <u>currently</u> taking this autoimmune condition medication?**

Yes ☐
No ☐

P101. **When did you stop taking this autoimmune condition medication?**

P102. **What is/was the name of your joint and/or muscle pain medication?**

If you have taken more than one chronic medication for the management of joint and/or muscle pain, fill out one here and fill out the additional one(s) under 'other'.

P103. **When did you start taking this joint and/or muscle pain medication?**

P104. **Were you taking this joint and/or muscle pain medication <u>at the time of your diabetes diagnosis?</u>**

Yes ☐
No ☐
Unsure ☐

P105. **Were you taking this joint and/or muscle pain medication at the time <u>when you started eating a LCHF diet?</u>**

Yes ☐
No ☐
Unsure ☐

P106. **Are you <u>currently</u> taking this joint and/or muscle pain medication?**

Yes ☐
No ☐

P107. **When did you stop taking this joint and/or muscle pain medication?**

P108. What is/was the name of your depression/anxiety medication?

If you have taken more than one chronic medication for the management of depression/anxiety, fill out one here and fill out the additional one(s) under 'other'.

P109. When did you start taking this depression/anxiety medication?

P110. Were you taking this depression/anxiety medication <u>at the time of your diabetes diagnosis?</u>

Yes ☐
No ☐
Unsure ☐

P111. Were you taking this depression/anxiety medication at the time <u>when you started eating a LCHF diet?</u>

Yes ☐
No ☐
Unsure ☐

P112. Are you <u>currently</u> taking this depression/anxiety medication?

Yes ☐
No ☐

P113. When did you stop taking this depression/anxiety medication?

P114. What is/was the name of your sexual dysfunction or infertility medication?

If you have taken more than one chronic medication for the management of sexual dysfunction or infertility, fill out one here and fill out the additional one(s) under 'other'.

P115. When did you start taking this sexual dysfunction or infertility medication?

P116. **Were you taking this sexual dysfunction or infertility medication <u>at the time of your diabetes diagnosis?</u>**

Yes ☐
No ☐
Unsure ☐

P117. **Were you taking this sexual dysfunction / infertility medication at the time <u>when you started eating a LCHF diet?</u>**

Yes ☐
No ☐
Unsure ☐

P118. **Are you <u>currently</u> taking this sexual dysfunction / infertility medication?**

Yes ☐
No ☐

P119. **When did you stop taking this sexual dysfunction or infertility medication?**

☐☐☐☐☐☐☐☐

P120. **<u>Are there any other chronic medications that you take, or have taken, that you have not yet captured in this questionnaire?</u>**

Click 'yes' to report another medication.

Yes ☐
No ☐
Unsure ☐

P121. **What is/was the name of this medication?**

☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

P122. **What condition is/was treated with this medication?**

☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

P123. **When did you start taking this medication?**

☐☐☐☐☐☐☐☐

P124. Were you taking this medication <u>at the time of your diabetes diagnosis?</u>

Yes ☐
No ☐
Unsure ☐

P125. Were you taking this medication at the time <u>when you started eating a LCHF diet?</u>

Yes ☐
No ☐
Unsure ☐

P126. Are you <u>currently</u> taking this medication?

Yes ☐
No ☐

P127. When did you stop taking this medication?

☐☐☐☐☐☐☐☐

P128. Do you, or have you previously, taken any other chronic (long-term) medications that you have not yet entered into this questionnaire?

Yes ☐
No ☐
Unsure ☐

P129. What is/was the name of this medication?

☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

P130. What condition is/was treated with this medication?

☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

P131. When did you start taking this medication?

☐☐☐☐☐☐☐☐

P132. Were you taking this medication <u>at the time of your diabetes diagnosis?</u>

Yes ☐
No ☐
Unsure ☐

P133. Were you taking this medication at the time <u>when you started eating a LCHF diet?</u>

Yes ☐
No ☐
Unsure ☐

P134. Are you <u>currently</u> taking this medication?

Yes ☐
No ☐

P135. When did you stop taking this medication?

☐☐☐☐☐☐☐☐

P136. Do you, or have you previously, taken any other chronic (long-term) medications that you have not yet entered into this questionnaire?

Yes ☐
No ☐
Unsure ☐

P137. What is/was the name of this medication?

☐☐

P138. What condition is/was treated with this medication?

☐☐

P139. When did you start taking this medication?

☐☐☐☐☐☐☐☐

P140. Were you taking this medication <u>at the time of your diabetes diagnosis?</u>

Yes ☐
No ☐
Unsure ☐

P141. Were you taking this medication at the time <u>when you started eating a LCHF diet?</u>

Yes ☐
No ☐
Unsure ☐

P142. Are you <u>currently</u> taking this medication?

Yes ☐
No ☐

P143. When did you stop taking this medication?

P144. Do you, or have you previously, taken any other chronic (long-term) medications that you have not yet entered into this questionnaire?

Yes ☐
No ☐
Unsure ☐

P145. What is/was the name of this medication?

P146. What condition is/was treated with this medication?

P147. When did you start taking this medication?

P148. Were you taking this medication <u>at the time of your diabetes diagnosis?</u>

Yes ☐
No ☐
Unsure ☐

P149. Were you taking this medication at the time <u>when you started eating a LCHF diet?</u>

Yes ☐
No ☐
Unsure ☐

P150. Are you <u>currently</u> taking this medication?

Yes ☐
No ☐

P151. When did you stop taking this medication?

P152. Do you, or have you previously, taken any other chronic (long-term) medications that you have not yet entered into this questionnaire?

Yes ☐
No ☐
Unsure ☐

P153. What is/was the name of this medication?

P154. What condition is/was treated with this medication?

P155. When did you start taking this medication?

P156. Were you taking this medication <u>at the time of your diabetes diagnosis?</u>

Yes ☐
No ☐
Unsure ☐

P157. Were you taking this medication at the time <u>when you started eating a LCHF diet?</u>

Yes ☐
No ☐
Unsure ☐

P158. Are you <u>currently</u> taking this medication?

Yes ☐
No ☐

P159. When did you stop taking this medication?

Section Q: currentfitness

The following questions related to the amount of exercise that you do <u>currently.</u>

Q1. How physically active are you at work?

Low (sit for most of the day) ☐
Moderate (mostly sit but some light walking) ☐
High (walk / stand for most of the day) ☐
Very high (work involves a high level of physical labour) ☐

Q2. Do you routinely walk / run / cycle as a form of transport?

Yes ☐
No ☐

Q3. Do you currently exercise?

- [ ] Yes
- [ ] No

Q4. What is the main reason that you do not currently exercise?

Q5. How often do you <u>currently</u> exercise per week?

- [ ] 1 - 2 days per week
- [ ] 3 - 4 days per week
- [ ] 5 or more days per week

Q6. What type of exercise do you do currently?

- [ ] Walking
- [ ] Jogging / running
- [ ] Cycling
- [ ] Swimming
- [ ] Strength training
- [ ] Yoga / pilates
- [ ] Cardio activities at the gym
- [ ] Sports (i.e. soccer / hockey etc.)
- [ ] Other

Other

Q7. How long are your <u>current</u> exercise sessions on average?

Answer in minutes.

Q8. Which best describes the intensity of your <u>current</u> average exericise session?

- [ ] Light (very easy breathing / able to maintain a conversation)
- [ ] Moderate (heavier breathing but able to speak / building up a sweat)
- [ ] Hard (very heavy breathing and unable to speak)

Q9. How would you categorize your current fitness level?

- ☐ Very unfit
- ☐ Unfit
- ☐ Moderately fit
- ☐ Fit
- ☐ Very fit

Q10. Did the way that you used to exercise (type/time/intensity) <u>at the time just prior to starting a LCHF diet</u> differ from how you currently exercise?

- ☐ Yes
- ☐ No

Q11. How often did you exercise <u>at the time just prior to starting a LCHF diet?</u>

Answer in session per week.

- ☐ 0 days per week (do not exercise)
- ☐ 1 - 2 days per week
- ☐ 3 - 4 days per week
- ☐ 5 or more days per week

Q12. What type of exercise did you do <u>at the time just prior to starting your LCHF diet?</u>

- ☐ Walking
- ☐ Jogging / running
- ☐ Cycling
- ☐ Swimming
- ☐ Strength training
- ☐ Yoga / pilates
- ☐ Cardio activities at the gym
- ☐ Sports (i.e. soccer / hockey etc.)
- ☐ Other

Other

Q13. How long were your exercise sessions, on average, at the time just prior to starting your LCHF diet?

Answer in minutes.

Q14. Which best describes the intensity of your average exericise sessions at the time just prior to starting your LCHF diet?

- Light (very easy breathing / able to maintain a conversation) ☐
- Moderate (heavier breathing but able to speak / building up a sweat) ☐
- Hard (very heavy breathing and unable to speak) ☐

Section R: dietweighthistory

The following questions relate to your doctor and dietitian use.

R1. Are you currently seeing a doctor and/or dietitian to help manage your diabetes?

- No ☐
- Doctor ☐
- Dietitian ☐
- Both a doctor and a dietitian ☐

R2. Was a LCHF diet initially prescribed for you by a doctor and/or dietitian?

- No ☐
- Doctor ☐
- Dietitian ☐
- Both a doctor and dietitian ☐

R3. Is your decision to follow a LCHF diet supported by your doctors / dietian?

- No ☐
- Doctor ☐
- Dietitian ☐
- Both a doctor and a dietitian ☐

R4. Did a dietitian initially help you develop and follow your LCHF diet?

- Yes ☐
- No ☐

Section S: future

Involvement in future research.

S1. Would you consider being involved in more detailed future studies investigating LCHF diets in diabetics?

Yes ☐

No ☐

S2. Would you consider stopping all medication for a period of 1 month prior to being tested in a research trial?

This would only happen if your doctor and our medical team deemed it safe for you to do so and you would be carefully monitored by our medical staff during this time.

Yes ☐

No ☐

S3. Would you consider participating in an intervention where you are required to eat a 'best practice' mixed diet which contains carbohydrate for a period of 8 weeks?

Yes ☐

I would consider a 2 - 4 week intervention ☐

No ☐

You will be presented with a list of foods and asked to select how often you have eaten those foods on average during the past 6 months.

NOTE:

You are answering how often you have eaten the portion size (in brackets) of a food in past 6 months.

You are answering how often you have eaten that portion size on average. For example: if you eat a portion 7 times a week, that works out at 1 portion per day on average (even if you ate all 7 portions on the same day).

An answer is required for each item.

Be sure to include the foods that you have eaten during training and competition.

Section A: Food

A1. How often have you eaten the following foods on average in the past 6 months?

Remember to select the average of how often you have eaten the portion of the food in brackets over the past 6 months.

e.g. You eat 7 portion of chocolate but only on the weekend: select 1 per day.

	0	1 or less per month	2 - 3 per month	1 - 2 per week	3 - 4 per week	5 - 6 per week	1 per day	2 - 3 per day	more than 3 per day
Regular breakfast cereals / granola e.g. All Bran (1 cup / 250 ml)	☐	☐	☐	☐	☐	☐	☐	☐	☐

	0	1 or less per month	2 - 3 per month	1 - 2 per week	3 - 4 per week	5 - 6 per week	1 per day	2 - 3 per day	more than 3 per day
Low carb cereals / granola (1 cup / 250 ml)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Porridge / oats (1 cup / 250 ml)	☐	☐	☐	☐	☐	☐	☐	☐	☐
FutureLife (1 cup / 250 ml)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Added sugar to cereals / oats (1 teaspoon)	☐	☐	☐	☐	☐	☐	☐	☐	☐

A2. How often have you eaten the following foods on average in the past 6 months?

Remember to select the average of how often you have eaten the portion of the food in brackets over the past 6 months.

e.g. You eat 7 portion of chocolate but only on the weekend: select 1 per day.

	0	1 or less per month	2 - 3 per month	1 - 2 per week	3 - 4 per week	5 - 6 per week	1 per day	2 - 3 per day	more than 3 per day
White bread (1 slice / half roll)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Brown bread (1 slice / half roll)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Whole wheat bread (1 slice / half roll)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Rye bread (1 slice / half roll)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Low carb bread (1 slice / half roll)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Regular crackers / savoury biscuits (3 crackers)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Seed crackers (3 crackers)	☐	☐	☐	☐	☐	☐	☐	☐	☐

A3. How often have you eaten the following foods on average in the past 6 months?

Remember to select the average of how often you have eaten the portion of the food in brackets over the past 6 months.

e.g. You eat 7 portion of chocolate but only on the weekend: select 1 per day.

	0	1 or less per month	2 - 3 per month	1 - 2 per week	3 - 4 per week	5 - 6 per week	1 per day	2 - 3 per day	more than 3 per day
Pasta / noodles (1 cup cooked)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Rice (1 cup cooked)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Pap / mieliepap (1 cup cooked)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Heba pap (1 cup cooked)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Millet (1 cup cooked)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Cous cous (1 cup cooked)	☐	☐	☐	☐	☐	☐	☐	☐	☐

	0	1 or less per month	2 - 3 per month	1 - 2 per week	3 - 4 per week	5 - 6 per week	1 per day	2 - 3 per day	more than 3 per day
Quinoa (1 cup cooked)	☐	☐	☐	☐	☐	☐	☐	☐	☐

A4. How often have you eaten the following foods on average in the past 6 months?

Remember to select the average of how often you have eaten the portion of the food in brackets over the past 6 months.

e.g. You eat 7 portion of chocolate but only on the weekend: select 1 per day.

	0	1 or less per month	2 - 3 per month	1 - 2 per week	3 - 4 per week	5 - 6 per week	1 per day	2 - 3 per day	more than 3 per day
Potatoes (1 medium or 1/2 cup cooked)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Starchy veg - sweet potato / butternut / carrots / corn (1/2 cup cooked)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Non-starchy veg - broccoli / spinach / cabbage / salad greens etc. (1/2 cup cooked or 1 cup raw)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Olives (small hand full: 10 small / 5 large)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Avocado (1/2 fruit)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Banana / grapes (1 small banana / 12 grapes)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Berries (1 cup)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Fresh fruit excluding the above - e.g. apples etc (1 tennis ball sized / 2 golf ball sized fruit)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Dried fruit (3 pieces)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Jam (1 tablespoon)	☐	☐	☐	☐	☐	☐	☐	☐	☐

A5. How often have you eaten the following foods on average in the past 6 months?

Remember to select the average of how often you have eaten the portion of the food in brackets over the past 6 months.

e.g. You eat 7 portion of chocolate but only on the weekend: select 1 per day.

	0	1 or less per month	2 - 3 per month	1 - 2 per week	3 - 4 per week	5 - 6 per week	1 per day	2 - 3 per day	more than 3 per day
Fatty red meat - beef / lamb / pork etc (1 palm-sized portion)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Lean red meat - trimmed of fat (1 palm-sized portion)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Fatty poultry - with skin (1 palm-sized portion)	☐	☐	☐	☐	☐	☐	☐	☐	☐

	0	1 or less per month	2 - 3 per month	1 - 2 per week	3 - 4 per week	5 - 6 per week	1 per day	2 - 3 per day	more than 3 per day
Lean poultry - chicken breast / skin removed (1 palm-sized portion)	☐	☐	☐	☐	☐	☐	☐	☐	☐
How often was the poultry crumbed?	☐	☐	☐	☐	☐	☐	☐	☐	☐
Bacon / sausages (3 rashers / 1 sausage)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Organ meats (e.g. liver, kidneys) (1/2 cup)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Fish (1 palm-sized portion)	☐	☐	☐	☐	☐	☐	☐	☐	☐
How often was fish breaded / battered?	☐	☐	☐	☐	☐	☐	☐	☐	☐
Shellfish (1 palm-sized portion)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Eggs (1 egg)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Biltong (hand full)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Lunch meats - salami / sandwich ham / chicken roll (1 slice)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Protein shake/ powder (e.g. whey, casein, soy proteins) (1 serving)	☐	☐	☐	☐	☐	☐	☐	☐	☐

A6. How often have you eaten the following foods on average in the past 6 months?

Remember to select the average of how often you have eaten the portion of the food in brackets over the past 6 months.

e.g. You eat 7 portion of chocolate but only on the weekend: select 1 per day.

	0	1 or less per month	2 - 3 per month	1 - 2 per week	3 - 4 per week	5 - 6 per week	1 per day	2 - 3 per day	more than 3 per day
Full cream milk - including in tea / coffee (1 cup / 250 ml)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Low fat milk - including in tea / coffee (1 cup / 250 ml)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Fat free milk - including in tea / coffee (1 cup / 250 ml)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Cream / sour cream (1 tablespoon)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Full fat unsweetened yoghurt (1 cup / 250 ml)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Fat free / low fat plain yoghurt (1 cup / 250 ml)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Flavoured / sweetened low fat yoghurt (1 cup / 250 ml)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Hard cheese (1 matchbox sized portion)	☐	☐	☐	☐	☐	☐	☐	☐	☐

	0	1 or less per month	2 - 3 per month	1 - 2 per week	3 - 4 per week	5 - 6 per week	1 per day	2 - 3 per day	more than 3 per day
Soft cheese (2 tablespoons)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Cottage cheese (2 tablespoons)	☐	☐	☐	☐	☐	☐	☐	☐	☐

A7. How often have you eaten the following foods on average in the past 6 months?

Remember to select the average of how often you have eaten the portion of the food in brackets over the past 6 months.

e.g. You eat 7 portion of chocolate but only on the weekend: select 1 per day.

	0	1 or less per month	2 - 3 per month	1 - 2 per week	3 - 4 per week	5 - 6 per week	1 per day	2 - 3 per day	more than 3 per day
Butter (1 tablespoon)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Margarine (1 tablespoon)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Ghee (1 tablespoon)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Lard (1 tablespoon)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Medium Chain Triglyceride (MCT) oil (1 tablespoon)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Coconut oil (1 tablespoon)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Coconut milk / cream (1/4 tin or 5 tablespoons)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Coconut flakes / desiccated coconut (1 small hand full)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Olive oil (1 tablespoon)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Sunflower oil (1 tablespoon)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Canola oil (1 tablespoon)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Other oil (1 tablespoon)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Salad dressing (1 tablespoon)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Mayonnaise (1 tablespoon)	☐	☐	☐	☐	☐	☐	☐	☐	☐

A8. How often have you eaten the following foods on average in the past 6 months?

Remember to select the average of how often you have eaten the portion of the food in brackets over the past 6 months.

e.g. You eat 7 portion of chocolate but only on the weekend: select 1 per day.

	0	1 or less per month	2 - 3 per month	1 - 2 per week	3 - 4 per week	5 - 6 per week	1 per day	2 - 3 per day	more than 3 per day
Baked beans (1/2 cup)	☐	☐	☐	☐	☐	☐	☐	☐	☐

	0	1 or less per month	2 - 3 per month	1 - 2 per week	3 - 4 per week	5 - 6 per week	1 per day	2 - 3 per day	more than 3 per day
Soya beans (1/2 cup cooked)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Other beans - kidney etc (1/2 cup cooked)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Soya milk (1 cup / 250ml)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Soya meat substitutes (palm-sized)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Peas (1/2 cup cooked)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Lentils (1/2 cup cooked)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Peanuts (1 small handful / 30 g)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Cashews (1 small handful / 30 g)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Macadamia nuts / pecans / walnuts (1 small handful / 30 g)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Other nuts - e.g. almonds (1 small handful / 30 g)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Sweetened nut butter (1 tablespoon)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Unsweetened nut butter (1 tablespoon)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Seeds (small handful / 30 g)	☐	☐	☐	☐	☐	☐	☐	☐	☐

A9. How often have you eaten the following foods on average in the past 6 months?

Remember to select the <u>average of how often you have eaten the portion of the food</u> in brackets over the past 6 months.

e.g. You eat 7 portion of chocolate but only on the weekend: select 1 per day.

	0	1 or less per month	2 - 3 per month	1 - 2 per week	3 - 4 per week	5 - 6 per week	1 per day	2 - 3 per day	more than 3 per day
Fruit juice (1 glass / 300 ml)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Regular soft drink (1 can / 340 ml)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Diet soft drink (1 can / 340 ml)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Energy drink (1 can / 375 ml)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Sports energy drink (1 bottle / 500 ml)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Regular beer (1 can / 340 ml)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Lite beer (1 can / 340 ml)	☐	☐	☐	☐	☐	☐	☐	☐	☐

	0	1 or less per month	2 - 3 per month	1 - 2 per week	3 - 4 per week	5 - 6 per week	1 per day	2 - 3 per day	more than 3 per day
Wine (1 glass / 200 ml)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Alcoholic coolers - e.g. Savannah (bottle / 300 ml)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Hot chocolate / Milo (1 cup / 250 ml)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Added sugar to hot beverages (1 teaspoon)	☐	☐	☐	☐	☐	☐	☐	☐	☐

A10. How often have you eaten the following foods on average in the past 6 months?

Remember to select the average of how often you have eaten the portion of the food in brackets over the past 6 months.

e.g. You eat 7 portion of chocolate but only on the weekend: select 1 per day.

	0	1 or less per month	2 - 3 per month	1 - 2 per week	3 - 4 per week	5 - 6 per week	1 per day	2 - 3 per day	more than 3 per day
Regular pizza (1 medium 30 cm pizza)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Low carb pizza (1 medium 30 cm pizza)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Burger (1 medium)	☐	☐	☐	☐	☐	☐	☐	☐	☐
French fries (1 cup / 250 ml)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Pies (1 pie)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Sauces e.g. tomato, BBQ (1 tablespoon)	☐	☐	☐	☐	☐	☐	☐	☐	☐

A11. How often have you eaten the following foods on average in the past 6 months?

Remember to select the average of how often you have eaten the portion of the food in brackets over the past 6 months.

e.g. You eat 7 portion of chocolate but only on the weekend: select 1 per day.

	0	1 or less per month	2 - 3 per month	1 - 2 per week	3 - 4 per week	5 - 6 per week	1 per day	2 - 3 per day	more than 3 per day
Dessert - e.g. ice cream / pudding (1/2 cup)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Pastries / croissants etc. (1 item)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Slice of cake / muffin / brownie / waffle (1 item)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Sweet biscuits (1 biscuit)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Rusks (1 rusk)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Sweets (1 packet / 50g)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Crisps (1 small packet)	☐	☐	☐	☐	☐	☐	☐	☐	☐

	0	1 or less per month	2 - 3 per month	1 - 2 per week	3 - 4 per week	5 - 6 per week	1 per day	2 - 3 per day	more than 3 per day
Milk chocolate (1/2 slab / 1 individual-sized bar / 40 - 50 g)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Dark chocolate (1/2 slab / 40 - 50 g)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Honey / syrup (1 teaspoon)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Sweeteners (1 teaspoon)	☐	☐	☐	☐	☐	☐	☐	☐	☐

Thank you for completing the questionnaire. You may close this window.

Section A: diet

A1. **Are you currently following a low carbohydrate high fat (LCHF) diet?**

Yes ☐

No ☐

A2. **When did you stop following the LCHF diet?**

A3. **Outline the events / reasons that led to your stopping the LCHF diet.**

Please provide as much detail as you can.

A4. **What diet are you currently following?**

A5. **Did you temporariliy stop following a LCHF diet for a period longer than 2 weeks at any time during the past year since your participation in the study?**

Yes ☐

No ☐

A6. **When did this break from LCHF start?**

A7. **For how long did you stop following a LCHF diet.**

Answer in weeks.

A8. **Elaborate on the events / reasons which led to stopping LCHF for this period.**

Please provide a detailed response including what diet you ate during this period.

A9. **Has your LCHF diet changed or evolved in any way over the past year?**

Examples of changes:

Cutting out or introducing certain foods; Changes in timing of meals, number of meals, or length of fasts; Adjustments to carbohydrate, fat, or protein content.

Yes ☐

No ☐

A10. **Please provide the details of how your diet has changed or evolved over the past year.**

A11. **Which statement best describes how you feel about the sustainability of the LCHF diet?**

Very sustainable / a LCHF diet can be sustained forever ☐

Fairly easy to sustain ☐

Somewhat hard to sustain ☐

Very difficult to sustain / eating a LCHF diet is temporary ☐

A12. **Please comment on any difficulties or challenges you faced in terms of following a LCHF during the past year.**

Section B: health

B1. **What is your current weight?**

Answer in kg.

B2. **How much weight have you gained / lost in the past year?**

Weight gain: use a positive number (e.g. 5)

Weight loss: use a negative number (e.g. -5)

No change: put 0

B3. **Have you experienced any changes in your glucose control during the past year?**

- Yes ☐
- No ☐

B4. **What best describes the change in your glucose control during the past year?**

- Large improvement in glucose control ☐
- Some improvement in glucose control ☐
- No change in glucose control ☐
- Some deterioration of glucose control ☐
- Large deterioration in glucose control ☐

B5. **Have you experienced any changes in diabetes related symptoms during the past year?**

For example: eyesight; nerve pain in the feet; sleepiness during the day; etc.

Yes ☐

No ☐

B6. **Please provide details of what symptoms have changed and how these symptoms have changed.**

Provide as much detail as you can.

B7. **Please comment on the progression of your diabetes during the past year.**

Add as much detail as is necessary.

B8. **Have you developed any medical conditions or had any medical events in the past year?**

For example: developed high blood pressure; kidney problems; had a heart attack etc.

Yes ☐

No ☐

B9. **Please provide the details of the health condition or event that occurred during the past year.**

B10. **Have you had any negative health experiences, conditions or events during the past year which you, or your doctor, attribute to following a LCHF diet?**

- ☐ Yes
- ☐ No

B11. **Please elaborate in detail about the health experience, condition or event which you or your doctor attribute to following a LCHF diet.**

B12. **What best describes your interactions with medical professionals relating to your following a LCHF diet during the past year?**

- ☐ Overwhelming support and encouragement
- ☐ Reserved support and encouragement
- ☐ Neutral / non-committal
- ☐ Unsupportive and discouraging
- ☐ Openly hostile and discouraging

B13. **Please elaborate on your selection.**

B14. **Do you have comments related to health and LCHF that you would like to share?**

Please comment below.

Section C: exercise

C1. **How often do you currently exercise?**

- I do not exercise ☐
- 1 - 2 days per week ☐
- 3 - 4 days per week ☐
- 5 or more days per week ☐

C2. **Which best describes how your fitness has changed over the past year?**

- I am much fitter ☐
- I am slightly fitter ☐
- My fitness is unchanged ☐
- I am slightly more unfit ☐
- I am much more unfit ☐

C3. **Please provide details of how your fitness / exercise has changed during the past year.**

C4. **Please could you provide your opinion on the following statement:**

"Following a LCHF diet makes it harder to be active and maintain an exercise routine than following a diet higher in carbohydrate."

Section D: metformin

D1. **Do you currently take Metformin?**

Metformin includes: Glucophage; Diaphage; Mengen; Metforal; Mylan Metform

Yes ☐

No ☐

D2. **Did you stop taking metformin during the past year since your participation in the study?**

Yes ☐

No ☐

D3. **When did you stop taking this medication?**

☐☐☐☐☐☐☐☐

D4. **Did you start taking metformin during the past year since your participation in the study?**

Yes ☐

No ☐

D5. **What is your current dose?**

Answer in mg daily.

☐☐☐☐☐☐☐☐☐

D6. **Has there been any change in your dose of metformin over the past year since your participation in the study?**

Yes ☐

No ☐

D7. **What change has occurred in your use of this medication?**

Decreased the dose ☐

Increased the dose ☐

Section E: insulin

E1. **Do you currently take insulin?**

Yes ☐

No ☐

E2. **Did you stop taking insulin during the past year since your participation in the study?**

Yes ☐

No ☐

E3. **When did you stop taking this medication?**

☐☐☐☐☐☐☐☐

E4. **Did you start taking insulin during the past year since your participation in the study?**

Yes ☐

No ☐

E5. **What is your current dose?**

Answer in units of insulin daily.

☐☐☐☐☐☐☐☐☐☐

E6. **Has there been any change in your dose of insulin over the past year since your participation in the study?**

Yes ☐

No ☐

E7. **What change has occurred in your use of this medication?**

Decreased the dose ☐

Increased the dose ☐

Section F: meds

F1. Are you currently taking a statin?

Yes ☐

No ☐

F2. Are you currently taking medication for blood pressure / hypertension?

Yes ☐

No ☐

F3. Are you currently taking any chronic medications (other than metformin, insulin, statins, hypertensives)?

e.g. chronic allergies, gut health, anxiety, inflammation etc.

Yes ☐

No ☐

F4. List all the names of the other chronic medications that you are currently taking.

Chronic med 1

Chronic med 2

Chronic med 3

Chronic med 4

Chronic med 5

Chronic med 6

Chronic med 7

Chronic med 8

Section G: medications

G1. Are there any chronic medications that you have <u>STOPPED</u> taking during the past year since participating in the study?

Yes ☐

No ☐

G2. Which medications have you stopped taking during the past year?

Medication 1

Medication 2

Medication 3

Medication 4

G3. Are there any chronic medications that you have <u>STARTED</u> taking during the past year since participating in the study?

Yes ☐

No ☐

G4. Which medications have you started taking during the past year?

Medication 1

Medication 2

Medication 3

Medication 4

Appendix E

foodgrpID	FoodgrpNAME	SHORTNAME	Country_F
2	Vegetables	Samphire	UK
2	Vegetables	Capers	ZA
2	Vegetables	Seaweed, roasted	ZA
4	Legumes and legume products	Hummus	ZA
6	Milk and milk products	Yoghurt, double cream, plain	ZA
6	Milk and milk products	Yoghurt, double cream, Greek	ZA
6	Milk and milk products	Yoghurt, Greek	ZA
6	Milk and milk products	Yoghurt, full cream, plain	ZA
7	Eggs	Quiche,with Meat, Poultry or Fish	ZA
7	Eggs	Quiche, vegetable, crustless	ZA
8	Meat and meat products	Cheese grillers	ZA
8	Meat and meat products	Droewors, beef	ZA
8	Meat and meat products	Droewors, game	ZA
8	Meat and meat products	Pie, Pepper Steak, commercial, baked	ZA
8	Meat and meat products	Pate, duck liver	ZA
9	Fish and seafood	Sushi, california roll	ZA
10	Fats and oils	Mayonnaise, LCHF / Banting / creamy	ZA
10	Fats and oils	Ice Cream, LCHF / Banting	ZA
10	Fats and oils	Avocado oil	ZA
10	Fats and oils	Sesame oil	ZA
11	Sugar, syrups and sweets	Chocolate, dark, 90% cacao	ZA
11	Sugar, syrups and sweets	Chocolate, dark, 85% cacao	ZA
11	Sugar, syrups and sweets	Chocolate, dark, 70% cacao	ZA
12	Sauces, seasonings and flavourings	Cinnamon	ZA
12	Sauces, seasonings and flavourings	Banting tomato sauce	ZA
12	Sauces, seasonings and flavourings	Periperi sauce	ZA
12	Sauces, seasonings and flavourings	Pesto	ZA
16	Miscellaneous	Almond Milk	ZA
16	Miscellaneous	Psyllium husk	ZA
16	Miscellaneous	Konjac products	ZA
17	Sweeteners / sugar-substitutes	Erythritol	ZA
17	Sweeteners / sugar-substitutes	Equal, artifical sweetener	ZA
17	Sweeteners / sugar-substitutes	Xylitol	ZA
17	Sweeteners / sugar-substitutes	Sucralose, artifical sweetener	ZA
17	Sweeteners / sugar-substitutes	Natreen, artifical sweetener	ZA
18	Banting / LCHF products	Seed crackers	ZA
18	Banting / LCHF products	Banting / LCHF bread	ZA
18	Banting / LCHF products	Banting / LCHF granola	ZA
18	Banting / LCHF products	Heba Pap	ZA
18	Banting / LCHF products	Banting / LCHF pizza base	ZA
18	Banting / LCHF products	Snack Bars LCHF	ZA
18	Banting / LCHF products	Banting/LCHF wraps	ZA
19	Beverages	Kombucha	ZA

Semi-structured interview guide

1. How were you diagnosed with type 2 diabetes?

2. How has your diabetes progressed since diagnosis?

3. Describe your lifestyle leading up to your diagnosis.
    - Probe on diet;
    - Exercise;
    - Smoking, alcohol, stress, sleep.

4. Did you make any lifestyle changes when you were diagnosed with T2D?
    - Probe on diet;
    - Exercise;
    - Smoking, drinking, etc.

5. How would you describe your diabetes now?

6. Describe your current diet?
    - Probe on foods chosen;
    - Foods avoided;
    - Portion control (How do you decide how much to eat?);
    - Timing of meals and snacks (When do you eat during the day?).

7. When did you start following your current diet?

8. How did you find out about your current diet?

9. How did you learn about how to follow your current diet?

10. Has your diet changed from when you first started until now?

11. How sustainable is your diet?

12. How has your diet influenced your diabetes?
    - Probe on glucose control;
    - Medications.

13. Have you tried other diets to manage your diabetes?
    - Probe on impact on diabetes;
    - Which diets?

14. Has your diet affected any other aspects of your life?
    - Probe on other medical conditions;
    - Exercise;
    - Social settings;
    - Negatives (What negative effects has your diet had?);
    - Medical negatives (Any medical conditions related to your diet?).

15. How has your doctor responded to your diet?
    - Probe (When you first started the diet?);
    - How does your doctor feel currently?
    - How were medications managed?

16. Do you consult a dietitian?
    - How did your dietitian respond to your diet?

17. Anything else from your experience that you would like to share?

Supplementary Table. Codes and description used to code the data

Code	Description
Food control	Any mention of managing the amount of food being eaten or a loss of control. Examples include calorie counting, over-indulgence, binging, etc.
Frequency	Any mention related to timing of meals, number of meals, gaps between meals, fasting, etc.
Medical professionals	When participants mention perceptions of their treatment or of their medical professionals. Must contain a value judgement. Do not code descriptions of procedures: e.g. "my doctor took a fasting blood sample". But do code: "my doctor felt it was necessary to draw blood".
Social	Mentions of interacting in social settings such as eating out or eating with friends. Does not have to be related to eating, it could be any interaction in wider society. For example, work, public spaces etc.
Food choices	Descriptions of the types of foods or specific foods which participants specifically mention that they choose or avoid.
Progression of LCHF diet	Descriptions of ways in which participant's LCHF diet has changed or evolved over time.
Sustainability	Any mention of difficulties, challenges, or any aspects of sustainability and/or longevity of staying on the diet.
LCHF knowledge source	Any mention of sources from which participants gained information and learned about the diet or how to follow the diet. Examples: friends, family, medical professionals, books etc.
Cholesterol & statins	Any mention of blood cholesterol, dietary cholesterol, and/or statins.
Insulin	Any mention of exogenous insulin. Excludes when participants talk about blood insulin concentrations or insulin resistance etc.
Any medication (except statins and insulin)	Any mention of medications except those limited to the two codes above.
Other diets	Any mention of diets other than LCHF diets. Includes comparisons to LCHF. Includes mentioning a lack of a specific diet.
Blood markers and specific health conditions	Any mention of changes in specific diagnosed conditions or changes in blood markers since LCHF. Includes type 2 diabetes but also all other diagnosed conditions.
Soft conditions	Any mention of improvements in health which are subjective and not related to objective test results or specific diagnosed conditions since starting the LCHF diet. Includes mentions of mental health, e.g. coping better / less stress. Also includes perceptions of exercise capacity and changes in exercise since LCHF.
Emotions and/or reactions	Any emotionally charged / highly emotive language or reactive statements.
Negatives and challenges	Any mention of the aspects of following the LCHF diet which were challenging, negative, or said in the context of a negative.
Weight	Any mention of body weight

Selected quotes: **The impact of LCHF on Hunger**
"I have a very, very large appetite and I still eat a lot. But now I only eat at meals and I don't need to eat otherwise." C89 "When I got hungry [before LCHF], it wasn't: 'Oh, I think I would like to eat something now.' It was: 'If you don't eat something now, you'll die' kind of hunger. It was a really sharp pain in the stomach, like I just have to eat something. I haven't experienced any of those hunger pangs really ever since I started LCHF." C97 "[Around the time of diagnosis] I noticed that I was putting on weight around my tummy and the only symptom that I actually had was I was hungry all the time. And then when I started the low carb diet, I found that due to the high fat, it was easier." T139 "I fast because I don't get hungry anymore. So, I can actually go forty-eight hours without eating." C319 "I found that I was eating a nice substantial protein type breakfast later in the morning. I would start only getting peckish at about three in the afternoon and we have dinner early around five or six at the latest, so it didn't make sense to have another big meal in the middle of the day." C37 "I do get hungry. I know that Tim Noakes seems to eat like twice every 3 days. I get hungry so I eat." C53 "I just eat until I'm not hungry. I don't count calories, I don't weigh the food…. It satiates me." C83 "I got to the stage where I was thinking I don't actually really feel like this egg in the morning but then I was scared that if I didn't eat the egg I would feel hungry during the day…. It was probably after about a month or six weeks I stopped eating breakfast in the mornings before work." T101 "I just eat what I want and when I am hungry. In fact, sometimes I don't feel hungry at all." T103 "I stuck to [previous patterns of eating] and then realized, you shouldn't be eating if you are not hungry. So, I pushed my breakfast later and found I wasn't hungry" T127 "Since I have been Banting, I will not get hungry. I can easily eat twice a day." T131 "[After breakfast] I don't need to eat again until four or five in the afternoon. I just don't get hungry." T137 "Sometimes I am hungrier [than usual] at night. I do eat something, I'm not trying to go against my hunger." C29 "We eat when we are hungry. We don't eat regular meals and sit down at specific times" T149 "I don't restrict my calories but I do intermittent fasting." T157

Selected quotes: **Cravings and addiction**

"I've been brilliant since [LCHF]…. I don't have any cravings or any hunger." C83

"I was very wary about the sweet taste of things. If we had supper outside our house … when something tastes sweet I wouldn't eat it." C19

"I miss the sweet stuff. But the less you have of it the less you want. But I didn't know it then." C311

"I don't any longer get tempted by cake… That doesn't bug me anymore and I can happily sit through a party where people are eating everything under the sun and I am not tempted by it." T137

"I can walk through the cake department, the sweets department…the bread section, it doesn't turn me on anymore. If you knew me then and you know me know, you'd be amazed." - C319

"My hunger is pretty much controlled [now]. I went through a phase where I broke down and my sugar addiction kicked back in and I started raiding the biscuit cupboard at work. If I have 1 biscuit, it becomes 3 biscuits, it becomes the rest of the packet." - C13

"Sometimes I still crave, I feel I crave carbohydrates sometimes. So sometimes I feel like it's not as easy as other times." C343

"I have come to understand about banting, that I'm an addict. Carbs are something that I'm probably never going to be able to eat for the rest of the life, certainly not on a regular basis." C37

"When I go to the shop, the first thing I used to look for, was what are the specials on luxuries (chocolate cake, chips, chocolates, sweets, deserts)." C59

"I took one bite [of a low carb coconut product] and it was just full of Xylitol and very, very sweet. Immediately I wanted to eat the whole thing and I would have had that and another one, that was the immediate response." C73

"The beauty of the whole banting scenario in terms of lifestyle is you don't get the hunger cravings. So, it's not that you go somewhere and you feel you have to eat. You don't have to eat." C89

"Not having bread at all I could completely cope with but having one piece of bread was just infuriating for me and difficult for me, unsustainable for me." T109

"I thought this was wonderful, that I was able to have the erythritol, but then I realized actually I need to cut down on the sweetness, because my body is still craving sweetness. So, I have gone down on that as well, but there are occasions that I need to have something sweet." T139

"I stopped drinking cool drink. I drank a minimum of 2 litres of Coke every day of my life and I used to eat three chocolates and sometimes a 200 g slab of chocolate on my own, every day. I stopped that, not because the doctor told me to, I knew." T163

"It was a relief and I started getting addicted to LCHF." C31

Selected quotes: **Continuing struggles relating to eating / hunger**
"If I binge on things, it would be something like pork scratchings, which are on the top shelf, so I would have to get a ladder out." C97 "[My dietitian] said I can have the fat on food but nothing extra. I can't hunt for fat. So that's hard." C311 I struggle [with hunger], so it's quick to go for a sweet or a biscuit, so now it's a matter of drinking water or drinking herbal tea like chamomile. I struggle with hunger, but then it's all in the mind. I read something that says decide if you are hungry, or if you are just bored, so most of the time, you're bored, you just want to nibble, your mouth must just move." – C59 "If I'm not busy over a weekend, I'm snacking constantly. I've got to watch myself. I find myself wondering to the fridge every hour." - C19 "If I cook for 2 days, then I get the urge to eat because it was delicious and I couldn't leave it alone, I couldn't take my hands off it…. Although it was never with carbs, never with rice or bread or potatoes, always vegetables and meat and eggs and fish." C31 "I will often have a snack when I get home, like a habit, just head for the fridge." – C343 "I tend to do the fasting kind of thing, so I eat once a day probably. The rest of the time I'll drink coffee, I drink lots and lots of coffee." C79 "I restrict [cream] because I think it is so delicious and I have so much coffee usually, that's why I am jittering because I haven't had coffee [this morning]." T109 "I eat or suck a sugar free sweet or a sugar free bubble-gum, so that helps." C59 "Dinner is pretty early because I finish work at four. Then within the next hour I need to eat otherwise I go on a binge." T107 "I grew up at a time when food was a sign of love…. I have always been somebody who has taken comfort in food and I can remember whenever it got stressed… carbs are the cheapest things to buy, so I would eat." T137 "I have always been somebody who has taken comfort in food and I can remember whenever it got stressed, I would go and of course carbs are the cheapest things to buy, so I would eat." 137? "If I stick to a low carb thing properly I'm never ever hungry. I am more bored or need food because of boredom than I am actually feeling hungry." C343

Selected quotes: **Control of diabetes and other medical conditions**
"[I am] very positive in the fact that I feel more under control. That I can do this by diet. I really do not want to go onto medications and I can control it by diet, which is very positive for me." T139 *"*I have got my life back and for me that is really, that is really important. You know, I have got my life back." T137 "Knowing how I feel and how good I feel health wise and weight wise, I would never want to go back the other way, because I wouldn't want the battles that I had with the excess weight and with the diabetes particularly, and all the other health problems, which have gone and it is being like a miracle." T127 "I am aware of being able to cope with stress better having lost weight. Things that used to stress me don't so much, not that I am immune from stress but I am better able to cope with it…. I feel smarter. I also feel braver. Having succeeded to lose so much weight, it's given me a real confidence in myself." T109 "I think the most important [influence of LCHF] is psychologically…. I do think it is a panacea for more than people actually realize." T151 "I got the benefit and spin off of weight loss and all my blood numbers improving and normalizing. I'm saying but hang on, my life has normalized… my health has normalized and what a pleasure" C13 "It cured my diabetes that's for sure, I am diabetes free now. As long as I keep doing LCHF, I have no diabetes anymore." C31 "My goal was to get off the Insulin. That was my ultimate goal and I thought if I can lose weight, and also control and regulate my sugar that was my goal…. I find that I get excited, that I look good in what I wear and even if I stay the way I am, I am okay with that." T131 "The centimetres are going but the kilograms are not falling as fast, so I must say I am sitting at a stage where I think a lot of people are saying is [LCHF] the right thing? T107 "[Speaking about his previous high carbohydrate diet] I could never control my weight unless I ran 140 km a week or more." C73 C29 – "I feel a lot better because, and I'm 100% convinced because I'm a lot lighter. My knees are not sore, I'm up and about, so, yes."

Selected quotes: **Positivity and energy**
"I'm far more positive about the future. The pain in my knees and my back have disappeared. I'm more focused and I'm able to concentrate more on a particular task." C17 "All I can say is that it's an amazing, positive feeling…. I eat right, I drink less medication, and I'm still weaning myself off it. So, yes, that's a huge positive." C19 "I stopped injecting [insulin] and it made a tremendous difference. I had more energy, I could sleep much better and I didn't need the carbs anymore." C31 "It feels so much better when you are eating properly and taking care of yourself. It feels like it's supposed to be like that." C311 "It's taken me three years but my body has healed astronomically. It's been amazing…. The doctor took me off my blood pressure pills as well and it's been amazing" C319 "I'm just generally more alert, more awake, more alive, if you want to call it that." C79 "It's great, I love what I eat and I'm in a better place for it." T103 "So, that was my motivation - this fear of dying on the operating table. I mention that to say that my diabetes was really bad and to think that now I am on no medication at all, is extraordinary." T109 "I feel positive about myself." T139 "When people see me, they say that it is like I am glowing. I am, there is something different. And I feel healthier, I definitely do." T131 "I can concentrate longer and work longer. I'm more alert, because with teaching you have to be tuned in, and I've got more energy. My energy levels are up as well. I feel more energetic." C83 "I just have more energy, immediately I felt more energy and a new sort of enthusiasm, and then I was running, it really got me back into running." C73 "When that fat came off, suddenly I had the desire to get up and go. So, something came back, it was amazing." C13 "And that has been exciting as well and just generally within myself, feeling so much better, just hyped about life, whereas before, everything was a chore, you know? And being ill for so long with so many things and…" T127 "All of a sudden I had so much energy. Things that I can do, that [people] younger than me can't do, or [people] the same age as me, they can't even come close…. But I just got the energy levels." T163

Selected quotes: **Sustainability**

"I think people hear the word diet, suggesting it's something that you do short term.... So, it's a way of living rather than a diet. I think that distinction is important. It's great, I love what I eat and I'm in a better place for it." T103

"I don't see it as a diet, it is a lifestyle. I really don't see it as a diet and I feel like a missionary, I want to go out and tell people, 'Yay, this is what this diet has done to me.' And I get a lot of flak from people who say, 'Yes, but all the fat.' They don't understand." T149

"I believe every diet works. I simply came back to saying this is sustainable, it is sustainable. My CDE doctor asked me if I'm going to eat like this for the rest of my life and I said well why not." C13

"I think LCHF is easy…. I think you realize it is not difficult once you're in." T163

"Oh, LCHF is easy. It's a way of life. It's a lifestyle. I am always having this discussion with people, who haven't fully committed and are really scared of the fats. They just can't believe that I am not at all concerned. I am not going to go back." T151

"I just feel that rather than develop Type 2 complications one day, I would much prefer to sustain it the way I am doing it. For me, it is sustainable." T139

"The first two weeks is not so nice but thereafter, just amazing and I will never go back." T127

"There is no reason why I think I would change [my diet]." T109

"I've been doing LCHF since 2014, it is 2017 now and I don't intend changing it ever." C79

"If you don't make it a lifestyle change, then it's not going to work. So, for me, the low carb diet is a better option, because you can eat more, you stay full longer and it's healthier." C59

"I think it's exceptionally sustainable. I've actually focused on making it sustainable. That is why I don't want to measure, I want to learn to judge what I need to eat and how I need to eat." C37

"I think for me it's sustainable, I feel like it's become a lifestyle." C343

"I am going to stay on it for life. With the weight problems I've had all my life... Yes, I won't go back to eating potatoes and crisps and noodles and things like that. I am really not interested anymore in that kind of food. I am quite happy to eat what I am eating." C319

"It takes out much of the joy for me at this stage. Maybe it's just early days. Because I can remember being very sorry for myself when I started with the LCHF. I couldn't freely partake in everything anymore. Now it's even worse." C311

"I've never been able to lose weight for more than a few months and I've been at it for almost a year now, so I would say I'm pretty much going to eat that way for the rest of my life." C29

Selected quotes: **Socialising**
"It is a battle with regards to the general public, because they don't understand why I am doing low carb. And then you get the frowns, the 'Why are you not eating fruit anymore? Why are you not eating sugar anymore?' So, that has been my main battle." T157 "Having supper in a restaurant is a problem in the majority of restaurants. It's just not enjoyable anymore. That's the one thing that influenced my lifestyle, I would look at a menu and there would be nothing on it that I could have, that I would feel comfortable eating. It's different if you're not a diabetic and you crook on one meal, it's not the end of the world. I don't want to get suckered back into a lifestyle that I'm not comfortable with. So, there's no way that I'm going to have something or a hamburger bun - I would rather avoid it completely." C19 "The only problem is if you are on the road traveling as we do. If you feel hungry you can't just walk into a takeaway and find something LCHF friendly because it is not yet everywhere." C319 "When people are going out for dinner and they are not aware of your [eating habits]. Occasionally, you just need to put [LCHF] aside and just eat what they give you." C343 "The only time it becomes a little awkward, is if you get invited around to somebody's house for a dinner party. Now you're caught between the world where they're dishing up food that you don't want to eat, or you actually might want to eat but you shouldn't eat. You don't want to offend them and you don't want to be picky." C37 "The odds are if we are going out to eat then we've either eaten beforehand or we're not going to eat and basically have water, because it's a pointless. You just can't get people to give you what you need." C89 "I'm very careful when I go out. I'll either not have anything to eat because I don't trust what other people put in their food or I'll be very careful and have a chicken and salad and then people get ratty with you. Like come on." T101 "[People] cant' put two and two together to see it's benefited me. 'You can have a heart attack if you eat all that fat.' All of those stereo typical comments do come out on occasion." T103 "I think it is difficult for me at times in a social atmosphere. Other people don't understand that I am trying to do it without medication and I am trying to lower my sugars with diet. They will say things like, 'Oh just have a piece of cake, it won't kill you." And inside I am saying, well actually it may just." T139 "And I get a lot of flak from people, who say, 'Yes, but all the fat', and they don't understand." T149 "The negatives come mainly out of your social life. Because you are the person who is different, you are the person who is not doing it. You are the person who is not going out." T163

Selected quotes: **Interactions with medical professionals with respect to LCHF**
"He's always very impressed with my results when I have them out. But he's never really encouraged the low carb high fat diet." C343 "Well I think he is pleased with it, but he obviously won't discuss about it." C83 "I would like to see the point where the doctors that are seeing me, the regular doctors that are seeing me, recognized the fact that I have lost weight, that I am on a strict diet, that I, I take responsibility for my health." T149 "[My doctor] seemed to be a bit more positive about the whole thing. And I could discuss one or two [LCHF] things with him, without him saying: 'No, no it is rubbish.'" T127 "[My dietitian] wouldn't allow me to do LCHF. She said no, you have to take the 6 meals per day diet. […] Three months later, I made another appointment and when she saw me entering the office, she said like oh my God who, is this? […] She knows everything about LCHF now and she's very, very supportive." C31 "Not one of the 7 medical persons that I interacted with supported me, not one of them. The occupational health doctor and nurse, specialist physician, the CDE, the doctor, the medical advisor, the two dieticians that have been there, the Lilly person, nobody supported it." C13 "[My cardiologist] said, 'Those people who are [on LCHF] are going to come to me with heart disease. You wait. I'm just waiting for it. I'm going to be busier than ever.'" C53 "[My doctor] started off by saying, "This is where it is going to go wrong.' After about a year and a half, she suddenly lost a lot of weight and I said to her: 'Have you got a secret, you want to share?' And she laughed and said: 'Go away.'" T137 "[My doctor] is fully behind the concept of the low carb diet, which is probably why I am quite happy where I am" C79 "I bought the book and then I went away from that dietician and started my own journey with Banting. Trial and error." C311 "I purposely chose doctors, who are hostile. But I made a deal with them. I said to them, I am in charge of my health, not you." T103 "[My doctor] told me to go completely onto meat, poultry, oily fish, cheese, green healthy vegetables leafy vegetables and come back and see him in 3 months." C29

Selected quotes: **Medications**
"My goal was to get off the Insulin. That was my ultimate goal and I thought if I can lose weight and also control and regulate my sugar, that was my goal." T131 "[My wife's] been trying to persuade me to do LCHF for about 2 years and I resisted completely… But with the pressure of going to short-term insulin, I did a re-think" C37 "Just from my own personal psyche, I was terrified and I still am of having to deteriorate to a point where I have to start taking insulin injections and I wanted to avoid that." C89 "My doctor told me that it was okay for me to drive again, which was a huge difference and I stopped every medication again, the Metformin, the Statin and insulin." C31 "When you get involved in reading up a lot of what goes on, you start understanding that maybe there are things there that maybe you've been prescribed which maybe are inappropriate." C89 "This guy I go to is of the mindset that don't fix something that is not broken. And if you change that one thing, you can just cause a whole series of problems elsewhere. And I sometimes wonder if I couldn't reduce the medications slightly." T163 "My doctor feels you know, just stick with the medication." T109 "The belief was that as long as you are on these little tablets, life is cool." T107 "I have also cut down on animal fat. I think it was two months ago, when they told me my Cholesterol was so high." T149 "The one visit he said, 'So you're refusing to take Statins?' I said, 'Yes', so he said, 'Sign this form that I'm refusing to take treatment for my hypo whatever…' And I said, 'Sure, bring me a pen.'" - C13 "I stayed on the statins. I am actually not keen to stay on them I must say. The jury is out on the statins big time." C37 "I didn't want to subject my body to those poisons. So, it was my own choice [to go off statins] and I was foolish, I should have stayed on my medication. Well, that's what I've been told." C319 "My lipids have been good on this diet. On a high fat diet, you expect them to shoot up, don't you? But they don't." C83 "I immediately wrote to them, both of them, saying, 'I have stopped [statins] on my own accord and I acknowledge that you have told me to take them.' They are so shit scared of the Medical Health Council." T151

Participant age and gender by code		
Code	**Gender**	**Age**
C13	male	58
C17	male	52
C19	male	46
C29	male	46
C31	male	56
C311	female	67
C319	female	66
C343	female	52
C37	male	65
C53	female	64
C59	female	55
C73	male	67
C79	female	41
C83	male	59
C89	male	62
C97	female	59
T101	female	51
T103	male	49
T107	female	49
T109	male	48
T127	female	63
T131	female	67
T137	male	65
T139	female	63
T149	female	67
T151	male	72
T157	female	33
T163	male	67