



How low do you need to go?

Exploring the variability in the effects of keto-induction and individual outcomes from low-carbohydrate diets

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Abstract

Low-carbohydrate diets (LCDs) and very low-carbohydrate ketogenic diets (VLCKDs) are increasingly used for the management of a range of health conditions and in the general population for weight-loss and maintenance. However, there is little evidence for the superiority of greater carbohydrate restriction compared to more moderate restriction for most outcome measures, but potential benefits for some, like glucose, triglycerides (TG), and high-density lipoprotein cholesterol (HDL-c). There are also specific benefits from VLCKDs and the resultant ketonaemia, including reduced inflammation, inhibited tumour growth, amelioration of neurodegeneration, and increased metabolic flexibility in athletes.

Despite the popularity and benefits of VLCKDs, there is little consensus on what constitutes nutritional ketosis (NK) for clinical purposes, and there is an almost complete lack of research on the time taken to achieve NK of ≥ 0.5 mmol/L beta-hydroxybutyrate (BOHB) and the symptoms of carbohydrate withdrawal commonly described in mainstream media as ‘keto-flu’ that occurs during keto-induction. Dietary supplements and methods to improve ketonaemia, time-to-NK, and symptoms of carbohydrate withdrawal and mood during keto-induction are similarly not well understood.

This thesis begins with a narrative review of supplementary methods purported to increase ketonaemia and improve time-to-NK and symptoms of carbohydrate withdrawal. It provided, for the first time, a synthesis of research related to the time it takes for people to achieve NK and highlighted that there were no studies that had specifically evaluated adverse effects specifically during keto-induction. This review showed that there is a clear ketogenic effect of supplemental medium-chain triglycerides (MCTs) and a possible greater effect resulting from shorter-chain fatty acids such as butyric acid. However, the ketogenic effect of other supplements was unclear.

To understand the effect of increased ketonaemia on time-to-NK, symptoms of carbohydrate withdrawal, and mood, a randomised controlled trial comparing the use of MCTs in a ‘classic’ ketogenic diet providing a 4:1 lipid to non-lipid ratio of total energy vs a control oil rich in long-chain triglycerides (LCT) was then conducted. MCT resulted in higher BOHB at all time-points, and faster time-to-NK, but these results failed to reach significance. The magnitude of

symptoms of keto-induction were greater in the LCT control group, except for abdominal pain, which occurred with greater frequency and severity in the MCT-supplemented diet. There was a *possibly beneficial* effect on symptoms by MCT but the effect on mood was *unclear*. Based on these results, there was a clear effect of MCTs on ketonaemia compared with LCT and a likely reduction in symptoms of carbohydrate withdrawal.

Little research has been conducted on the qualitative experience of diet, and to deepen the understanding of the effects of carbohydrate restriction on individuals' mood and experiences, daily diary entries and focus group findings from the previous study were qualitatively analysed to illustrate the 'lived experience' of a following a VLCKD. Despite some challenges, especially gastrointestinal effects, the overall perception of the diet was positive, and benefits for wellbeing, mood, sleep, and sugar cravings were reported, with negative experiences decreasing as participants adapted to the VLCKD. These findings suggested that the overall experience of a VLCKD is positive but varies markedly between individuals.

The preceding study outcomes suggested that increased ketonaemia might positively affect symptoms of carbohydrate withdrawal during keto-induction, and mood, but it is unclear whether diets differing in carbohydrate content and resulting in differing levels of ketonaemia would elicit similar effects. The final study of this collective body of work was a randomised clinical trial comparing a VLCKD, LCD, and moderate-low-carbohydrate diet (MCD) consisting of 5%, 15%, and 25% of total energy (TE) from carbohydrate respectively, over 12 weeks. The first three weeks of this study was used to compare ketonaemia, symptoms of carbohydrate withdrawal, and mood between the dietary intervention groups. In 75 of 77 initial participants included for analysis, mean serum levels of BOHB were increased by 0.27 ± 0.32 , 0.41 ± 0.38 , and 0.62 ± 0.49 mmol/L for the MCD, LCD, and VLCKD respectively ($p = 0.013$). The achievement of NK was consistent for both VLCKD and LCD groups and sporadic for the MCD group. The overall mean change in symptoms scores was trivial (0.81 ± 2.84 , $p < 0.001$) and while symptoms were increased most in the VLCKD group (1.49 ± 2.47), compared to LCD (0.65 ± 2.70), and MCD (0.18 ± 3.3) the differences were small and did not reach the threshold for significance ($p = 0.264$). Only halitosis ($p = 0.039$) and muscle weakness ($p = 0.005$) differed significantly between the groups with the largest effects seen in the VLCKD group.

Mood improved significantly from baseline overall, but there was no significant difference between groups ($p = 0.181$). Interestingly, although participants were instructed to maintain habitual energy intake, energy restriction did occur, and it was more strongly associated with the magnitude of carbohydrate withdrawal effects than any other factor. Therefore, these findings suggest that reduced carbohydrate diets should be prescribed according to the projected benefits to the individual, rather than the desire to mitigate symptoms of carbohydrate withdrawal and that energy sufficiency could be a more important consideration for the avoidance of adverse effects of carbohydrate-restricted diets.

There were only small differences observed between symptoms of carbohydrate withdrawal and mood between the diets ranging from 5-25% TE from carbohydrate and outcome measures were also analysed to determine which diet was most effective overall. In completers of the 12-week study, significant reductions in TG, weight, and body mass index occurred, along with increases in HDL-c, low-density lipoprotein cholesterol (LDL-c) and total cholesterol concentrations. It was more difficult for those in the VLCKD group to achieve the carbohydrate allocation of 5% of TE (mean: 7.9%, SD = 4.9%), whereas the MCD (22.5%, SD = 4.5%) and LCD (14.1%, SD = 3.2%) groups adhered to the allocation. Despite this, the positive effect on markers of health trended towards greater improvement from greater carbohydrate restriction. The largest improvements in HDL-c and TG, and anthropometric changes occurred in the VLCKD group. However, between-group changes were not significant.

Over the 12-week period, adherence to the prescribed carbohydrate intake was less than allocation for both the MCD and LCD groups and was higher than the allocation for the VLCKD group. A linear trend was observed for reduction in carbohydrate intake as a proportion of TE for MCD relative to week (Beta = -0.137, $p = 0.24$). Conversely, increased intake by week was observed for LCD (Beta = 0.096, $p = 0.24$), and VLCKD (Beta = 0.174, $p = 0.15$) but these trends were not significant within groups, or between group allocations ($p = 0.108$).

There has been the suggestion that outcomes from lower- or higher-carbohydrate diets might be predicted by baseline cardiometabolic indicators such as insulin homeostasis but there is little consensus on the use of blood or anthropometric measures for dietary prescription.

Adverse effects, mood, and outcome measures differed by only a small amount between the LCDs but there was considerable variation between individuals. Baseline cardiometabolic measures were compared to changes in these measures, relative to carbohydrate allocation. Participants with 'poorer' baseline measures benefitted most from greater carbohydrate restriction, with 7 of 11 measures improved most by a VLCKD, relative to baseline measurements. However, only HDL-c reached between-group significance, with every 1 mmol/L lower HDL-c at baseline associated with a 0.5 and 0.2 mmol/L improvement in HDL-c for the MCD and LCD groups respectively, compared to a 0.4 mmol/L decrement for VLCKD ($p = 0.0006$). This study was the first to evaluate the use of baseline measures as predictors of outcomes resulting from differing carbohydrate-restricted diet interventions. Although the effects were equivocal, the findings do suggest that those with poorer baseline measures of cardiometabolic health might benefit most from greater carbohydrate restriction.

There are several novel findings from the studies presented in this thesis.

1. Medium chain triglycerides resulted in fewer overall symptoms of carbohydrate withdrawal when compared to a substitution oil rich in long-chain fatty acids.
2. The degree of dietary restriction of carbohydrate only had a trivial effect on increasing symptoms of carbohydrate withdrawal.
3. Very-low-carbohydrate diets were typically tolerated well and resulted in a range of self-reported health benefits, but results varied considerably between individuals.
4. There was a trend towards small improvements in overall benefits to cardiometabolic measures of health from a greater restriction of carbohydrate.
5. The benefits from greater restrictions of carbohydrate appeared to be greatest for those with poorer baseline measures of cardiometabolic health.

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Commonly used abbreviations

LCHF: low-carbohydrate, high-fat

VLCKD: very low-carbohydrate ketogenic diet

LCD: low-carbohydrate diet

BMI: body mass index

LDL-c: low-density lipoprotein cholesterol

HDL-c: high-density lipoprotein cholesterol

Total-c: total cholesterol

TG: triglyceride

LCHP: low-carbohydrate, high-protein

NK: nutritional ketosis

BOHB: beta-hydroxybutyrate

MCT: medium chain triglyceride

IR: insulin resistance

IS: insulin sensitive

TE: total energy

CRP: C-reactive protein

GGT: gamma-glutamyltransferase

ALT: alanine aminotransferase

AST: aspartate aminotransferase

ALP: alkaline phosphatase

Attestation of Authorship

I hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person (except where explicitly defined in the acknowledgements), nor material which to a substantial extent has been submitted for the award of any other degree or diploma of a university or other institution of higher learning.

Chapters two through eight represent papers that have been published or are in the submission process to peer-reviewed journals. My contribution and the contributions of various co-authors to these papers are outlined at the beginning of each chapter. All co-authors have approved the inclusion of the joint work in this doctoral thesis.

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Table 1. Co-authored Works

	Publication reference	Author contribution
Chapter 2.	Harvey CJdC, Schofield GM, Williden M. The use of nutritional supplements to induce ketosis and reduce symptoms associated with keto-induction: a narrative review. <i>PeerJ</i> . 2018;6:e4488.	CH: 85% GS: 10% MW: 5%
Chapter 3.	Harvey CJdC, Schofield GM, Williden M, McQuillan JA. The Effect of Medium Chain Triglycerides on Time to Nutritional Ketosis and Symptoms of Keto-Induction in Healthy Adults: A Randomised Controlled Clinical Trial. <i>JNutrMetab</i> . 2018;2018:9.	CH: 87.5% GS: 5% MW: 5% JM: 2.5%
Chapter 4.	Harvey CJdC, Schofield GM, Williden M. The lived experience of healthy adults following a ketogenic diet: A qualitative study. <i>The JHolistPerf</i> . 2018 May 4;7782018(1):3638.	CH: 90% GS: 5% MW: 5%
Chapter 5.	Harvey CJdC, Schofield GM, Zinn C, Thornley S. The effect of differing levels of carbohydrate restriction on the achievement of nutritional ketosis, mood, and symptoms of carbohydrate withdrawal in healthy adults: A randomised clinical trial. <i>Nutrition</i> [Submitted for review]	CH: 85% GS: 5% CZ: 5% ST: 5%
Chapter 6.	Harvey CJ, Schofield GM, Zinn C, Thornley SJ, Crofts C, Merien FL. Low-carbohydrate diets differing in carbohydrate restriction improve cardiometabolic and anthropometric markers in healthy adults: A randomised clinical trial. <i>PeerJ</i> . 2019 Feb 5;7:e6273.	CH: 82.5% GS: 5% CZ: 5% ST: 5% CC: 1.25% FM: 1.25%
Chapter 7.	Harvey CJdC, Schofield GM, Zinn C, Thornley S. Can baseline cardiometabolic markers predict efficacy of carbohydrate restriction in healthy adults? A pilot study. <i>JHolistPerform</i> [Submitted for review]	CH: 85% GS: 5% CZ: 5% ST: 5%
Chapter 8.	Harvey CJdC, Schofield GM, Zinn C, Thornley S. Can a 'carbohydrate tolerance questionnaire' predict outcomes from diets differing in carbohydrate content? A pilot study. <i>JHolistPerform</i> [Submitted for review]	CH: 85% GS: 5% CZ: 5% ST: 5%

We, the undersigned, hereby agree to the percentages of participation in the chapters outlined above:

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Professor Grant Schofield

Dr Caryn Zinn

Dr Simon Thornley

Primary

Secondary

Tertiary

Collaborators

Dr Micalla Williden

Dr Joseph A McQuillan

Professor Fabrice Merien

Dr Catherine Crofts

Acknowledgements

I never expected to be a doctor.

Thus, it's important to acknowledge my 'journey' in nutrition as critical to the achievement of this honour. My journey into the world of nutrition really began with an inauspicious call to the Dean's office in 1998. Approximately one year after being asked to leave high school, my attendance at nutrition class at the technical institute was no longer required. I was told that I had done enough to pass but that I was asking inconvenient questions about the role of carbohydrates, fat, and protein in the diet, and this was a distraction to the tutors and students. To my tutors and Dean, I thank you, as this set me free to explore the area of low-carbohydrate nutrition unfettered by orthodox and dare I say, outdated, nutrition guidelines.

Upon graduation (with a Diploma) I entered the field of nutrition, tangentially at first as a personal trainer cum nutrition coach and then, after further study, as a naturopath specialising in holistic nutrition and registered clinical nutritionist. Throughout this time, from the late 1990s onwards, I had applied various iterations of low-carbohydrate and ketogenic diets with enormous clinical success for both performance clients and those with severe metabolic disorder and other health conditions. Despite this success, the criticism from medical and nutrition practitioners was loud and clear; "you should not be doing this!" To say this had no effect on me would be a lie. It affected me deeply to know that on the one hand, I was helping people to recover their health and on the other that because of the methods I was using, was labelled a 'quack', 'dangerous' and much worse. I had a simple choice to continue or to 'fall into line'. I believed, and still do, that it would have been unethical for me to withdraw from these clients the very interventions that were helping them to normalise weight, improve blood measures of cardiometabolic health, and reduce their symptomology.

Over subsequent years I had practical experience with these diets as a world champion athlete and in the pursuit of my own improved health; and recognised even more than ever before the vital role that nutrition plays in health and resilience, both as I battled heavy weights in my chosen sport, and learned to live with the devastating effects of Crohn's Disease on my body and Bipolar disorder on my mind and emotions.

I had a brief flirtation with the idea of further study a few years into my clinical practice but was told, in no uncertain terms by advisors at the universities, that any thoughts of me pursuing later research in lower-carbohydrate and higher-fat or higher-protein nutrition would simply not be entertained! And so instead I continued to learn and evolve outside of academia. It wasn't until I was asked to provide guest lectures at institutes like Langara College, British Columbia, and provide workshops for athletes at the University of British Columbia in Vancouver, Canada, that I realised a shift might be occurring in the nutrition world.

On my return to New Zealand I was contacted by an old friend, then a doctoral candidate, now Dr—Joe McQuillan, who asked if I would like to meet and talk about concepts of low-carbohydrate nutrition with Professor Grant Schofield, Dr Caryn Zinn, Dr Mikki Williden and others at the Human Potential Centre, AUT Millennium. And thus, began this journey through the post-graduate certificate and diploma pathway, into the master's degree programme and doctoral candidacy, all without terminating at any point; the risk being that, if at any stage I stopped, I would not have gained any qualification, but if I pushed through to the end would complete the process as a Doctor of Philosophy in nutrition.

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My Dad always wanted to be a scientist but being from a humble rural family and without the opportunity to attend University, he worked his entire life to provide the means for my sister and me to receive the education that he didn't have the privilege to have. My sister Charlene was the first person in our family to ever achieve graduate (and postgraduate) qualifications, graduating from the University of Auckland with a master's degree in commerce in international business. I was so proud of her and I never expected to follow in her footsteps.

My Mum taught me that knowledge is power (growing up, we would never throw away a book...ever!) She was a voracious reader and prodigious intellect who, like my Dad, didn't have the opportunity to pursue the further education for which she would have been so well suited. But she instilled in me a love of learning, a love of reading, an immovable sense of natural justice, and a fierce and streak of independence that at times may have seemed a barrier but has ended up being the most important gift. I miss you every day Mum.

Ethics Approval

Ethics approval for chapters three and four was provided by the AUT University ethics committee, approval number 15/317. The trial was registered by the Australia New Zealand Clinical Trial Registry. ACTRN12616001099415.

The study providing data for chapters five through eight was registered by the Australia New Zealand Clinical Trial Registry. (ACTRN12617000421336p). Ethics approval for this study was granted by the Southern Committee of the Health and Disability Ethics Committee of New Zealand. 17/STH/60

Chapter 1. Introduction

Low-carbohydrate diets (LCDs) are increasingly being researched and used for the management of a range of health conditions, including neurological disorders, obesity, diabetes, metabolic syndrome, and various cancers. (1-11) They are also used widely in the general population for weight-loss and maintenance, (12) with improved satiety and control of hunger frequently reported by those who adhere to these diets. (13-15)

Despite the potential offered by LCDs, there is debate around several questions pertinent to clinical practice, including the superiority (or lack thereof) of greater or lesser restriction of carbohydrate, definitions of low-carbohydrate diets, ketogenic diet, and nutritional ketosis; along with an almost complete lack of research on time-to-nutritional ketosis and the true effects and implications of symptoms of carbohydrate withdrawal ('keto-flu').

Are low-carbohydrate diets superior to low-fat diets for anthropometric and cardiometabolic outcomes?

In studies up to 12-months, LCDs result in greater weight- and fat-loss than low-fat diets (LFDs), and favourably improve blood pressure, high-density lipoprotein cholesterol (HDL-c), triglycerides (TG), glycated haemoglobin (HbA1c) and fasting glucose, insulin, and c-reactive protein. (16) while LFDs result in improved low-density lipoprotein cholesterol (LDL-c) and total cholesterol. (17) Over longer timeframes, when energy intake is restricted, there is little difference in outcomes for weight-loss, total cholesterol, and LDL-c concentrations between diets that are higher or lower in carbohydrate. (18-22) However, after 12-months, there are persistent benefits for fasted glucose concentrations, (21) and greater improvements in HDL-c, TG, and HbA1c with greater degrees of carbohydrate restriction. (22-25) Bueno and colleagues in their systematic review of 13 randomised, controlled studies with > 12-month follow-ups, noted a greater overall weight loss from LCD vs LFD, along with greater improvements in cardiometabolic markers of health. (12) Similarly, Sackner-Bernstein et al. found that LCDs were associated with modest, yet significant improvements in weight and cardiovascular disease risk factors when compared to LFDs in studies between 8-weeks and 24-months. (26) In a review of 14 body composition change trials up to 2014, Hashimoto et al,

found that LCDs were associated with a significant reduction in body fat and sub-group analysis suggested that the results were limited to *very* low-carb diets. (27)

Taken together, these findings suggest that while effect sizes of outcome measures between diet types is small, LCDs are likely to be more effective for weight and fat-loss than low-fat interventions < 6 months and there might be persistent cardiovascular benefits past this time and that these benefits may be related to the magnitude of carbohydrate restriction.

What are low-carbohydrate and ketogenic diets?

Controversy also exists about the very nature of LCDs and very-low-carbohydrate ketogenic diets (VLCKDs). (28) Definitions for LCDs range from 20-200 g of carbohydrate per day, (29, 30) or up to 40-45% of daily energy from carbohydrate. (31, 32) Definitions for VLCKDs are similarly vague. The achievement of 'ketosis' is accepted to be the qualifying criteria for a ketogenic diet. The accepted definition for nutritional ketosis (NK) in the clinical nutrition field has become the achievement of ≥ 0.5 mmol/L beta-hydroxybutyrate (BOHB). However, the only evidence available for this criterion is that the majority of people following a very-low-carbohydrate diet typically achieve this level of blood ketones, (33) and this threshold has now been used by several studies as an indicator of entry into NK. (34, 35) Ketonaemia consistent with NK typically results from diets containing a 3:1 to 4:1 ratio of lipids to non-lipid macronutrients, or at least 75% of calories coming from lipids, very low carbohydrates (often less than 50 g) and low-to-moderate amounts of protein, (36, 37) but diets containing 60%-75% of calories from lipids and that include a high proportion of medium chain triglycerides (MCTs) are also functionally ketogenic. (38, 39)

There are potential benefits resulting from greater vs lesser carbohydrate restriction, as well as emerging benefits of ketogenic diets—including for the adjunct treatment of cancer, (40-42) neurodegenerative disorders, (43-48) metabolic syndrome and diabetes, (49-57), and for sports performance, especially for endurance and for the maintenance of weight in weight-dependent athletes, (58-64). However, few studies directly compare VLCKDs with other, less carbohydrate-restricted diets. In one of the only studies to date, Johnston and colleagues compared the effects of a non-ketogenic low-carbohydrate diet (fat 30% of total energy (TE); carbohydrate 40% of TE) to a ketogenic, low-carbohydrate diet (fat 60% TE; carbohydrate

5%TE) in twenty adults over six weeks, finding that the diets were equally effective in reducing body weight and insulin resistance. (65)

Barriers to adherence in low-carbohydrate and ketogenic diets

Adherence has also been touted as a barrier to the use of VLCKDs. While LCDs are, overall more easily complied with than LFDs, (23) VLCKDs with < 50 g carbohydrate per day can be difficult to sustain for longer periods. (22) More moderate carbohydrate restriction, still likely to result in ketosis; i.e. < 150 or < 100 g per day, might be preferable for some individuals. (66, 67)

The process of adapting to a ketogenic diet, known as keto-adaptation, or 'keto-induction' is also assumed to be a barrier to the uptake of a VLCKD. Adaptation from a standard diet to a VLCKD can cause various unpleasant symptoms. (68) These are referred to in common parlance as 'keto-flu' but not well elucidated in the scientific literature. For example, a Google search at the time of writing returned over 22,000 results for the term "keto-flu" but the same term searched in MEDLINE Complete, CINAHL Complete, Alt HealthWatch, Food Science Source, SPORT Discuss with Full Text, Psychology and the EBSCO Behavioral Sciences Collection produced no results. Symptoms of 'keto-flu' are characterised by constipation, headache, halitosis, muscle cramps, diarrhoea, and general weakness and rash. (68, 69) These effects are thought to occur because of increased natriuresis, kaluresis and diuresis in response to lowered insulin levels, (70-73) greatest between days 1-4 of a fast or ketogenic diet, (70) transient reductions in glucose provision to the brain, observed to occur on days 1-3, (74) and constipation resulting from reduced food volume or reduced fibre intake. However constipation may feature as a symptom due to the groups that have been studied, which have included children with disabilities who commonly experience constipation due to immobility. (75)

Due to the dearth of research specifically on keto-flu and symptoms of carbohydrate withdrawal and the time-period of keto-induction, it is unclear whether this set of symptoms is, in fact, a result of adaptation to VLCKDs or whether it is a more generalised response to either carbohydrate withdrawal or dietary change with calorie-restriction. (76) It is assumed though that adverse effects provide a barrier to the use of and compliance with a VLCKD. However, this early-induction time has not been specifically described in the literature. It is

likely there is considerable variation between individuals in symptoms arising from the keto-induction period, overall tolerability of the diet, and the results achieved from a VLCKD. Furthermore, there may be people for whom greater or lesser restriction of carbohydrate results in some combination of improved tolerability and better outcomes. For example, it may not be necessary to restrict carbohydrate to the degree necessary to achieve ketosis, if the symptoms of keto-induction are disproportionate to the outcomes from the diet. Conversely, if effects are not present or are minimal and results are improved, there would be a sound rationale to apply a more restrictive low-carbohydrate approach. At this time, this has not been investigated and this thesis will seek to address this important clinical topic.

Purported methods to indicate carbohydrate tolerance

It is clear from a practical perspective that diets containing differing amounts of the macronutrients (protein, carbohydrates, and fats) affect individuals differently, and whilst there are best-practice guidelines for macronutrient ranges for various desired clinical and performance outcomes, there is a large degree of individuality between nutritional prescriptions for individuals. However, there is no accepted or validated way to determine the macronutrient requirement of an individual except by trial and error, or by responding to signs and symptoms of metabolic disorder. This provides for a post-hoc provision of health and performance interventions and an opportunity exists to elucidate methods or protocols to aid the practitioner in the prescription of individualised, 'carbohydrate appropriate' diets based on tolerance to keto-induction and outcomes from low- and very-low-carbohydrate diets.

Relative insulin homeostasis offers promise as an indicator of physiological preference for diet type. It has been demonstrated that people with hyperinsulinaemia and insulin resistance (IR) respond more favourably to a low-carbohydrate diet. Pittas and colleagues demonstrated that those with above-median insulin response (30 min after glucose load) lost more weight when consuming a low glycaemic load diet compared with a high glycaemic load diet ($p < 0.05$). (77) The reverse was observed in the lower-insulin group, who lost more weight following a high-glycaemic load diet, but the difference was not statistically significant ($p = 0.25$). Similar results were demonstrated in a study comparing obese nondiabetic insulin-sensitive (fasting insulin < 10 microU/mL; $n = 12$) with obese nondiabetic insulin-resistant (fasting insulin > 15

microU/mL; $n = 9$) women, randomised to receive either a low-fat diet (LFD) (60% CHO, 20% fat) or LCD (40% CHO, 40% fat) hypocaloric diet. Insulin-sensitive women lost $13.5 \pm 1.2\%$ ($p < 0.001$) of their initial weight on a high-carb diet, whereas those on the LCD diet lost $6.8 \pm 1.2\%$ ($p < 0.001$; $p < 0.002$ between groups). In contrast, insulin-resistant women on the LCD diet lost $13.4 \pm 1.3\%$ ($p < 0.001$) of their initial BW as compared with $8.5 \pm 1.4\%$ ($p < 0.001$) for those on the LFD ($p < 0.040$ between two groups).⁽⁷⁸⁾ Likewise, in a 6-month, randomised controlled trial of 73 young adults with obesity, serum insulin concentration at 30 minutes after a 75 g dose of oral glucose was determined at baseline as a measure of insulin secretion. A lower-carbohydrate diet (40% carbohydrate and 35% fat) was compared to a lower-fat (55% carbohydrate and 20% fat) diet. While there was little difference between the two groups overall, those in the lower carbohydrate group that displayed values above the median insulin concentration at 30 min post glucose load (i.e. the 'more' insulin resistant participants) resulted in a greater reduction in weight (-5.8 vs -1.2 kg; $p = 0.004$) and body fat percentage (-2.6% vs -0.9% ; $p = 0.030$) than those in the low-fat group at 18 months. Cardiometabolic markers were not significantly different in relation to this modifier. ⁽⁷⁹⁾ A later pilot trial to investigate these effects in an ad-libitum diet over six-months found increased weight loss resulting from LCDs in insulin-resistant participants over insulin sensitive and improved weight loss resulting from low-fat diets for insulin sensitive participants, with these results failing to reach significance. Also noted were (non-significant) improvements in HDL-c, TG, fasting glucose and insulin, and blood pressure for LCD versus a higher-carbohydrate diet in those more insulin resistant. In those more insulin sensitive, LCD improved HDL-c and TG more than that of the low-fat diet, whereas the low-fat diet resulted in improved fasted insulin and glucose. ⁽⁸⁰⁾

The recent DIETFITS study ($n = 609$), a well-funded randomised trial, compared a healthy lower-fat vs lower-carbohydrate diet, and concluded *there was no significant difference in weight change between a healthy low-fat diet vs a healthy low-carbohydrate diet, and neither genotype pattern nor baseline insulin secretion was associated with the dietary effects on weight loss.* ⁽⁸¹⁾ However, it used an interesting methodology beginning with a baseline diet containing either 20 g of fat per day or 20 g of carbohydrate in the LCD and LFD groups respectively. Participants were then instructed to titrate their daily intake of either fat or carbohydrate up by 5 g or 15 g per day, each week until they found the *lowest level of intake they believed could be maintained.* This

led to a relatively modest carbohydrate restriction overall in the low-carbohydrate group; consuming $26.5\% \pm 0.7$ total energy from carbohydrate compared to the low-fat diet group which consumed $50.6\% \pm 0.7$ and the protein intake was modest at < 1 g protein per kg of bodyweight per day. It was also a study in relatively healthy overweight volunteers, and excluded people who might benefit most from a lower-carbohydrate regimen as indicated by previous research, i.e. those with “*hypertension or metabolic disease; diabetes; cancer; heart, renal, or liver disease*”. The mean 12-month weight change in this study was -5.3 kg (95% CI, -5.9 to -4.7) and -6.0 kg (95% CI, -6.6 to -5.4) for LFD and LCD respectively. While the authors stated that this difference was not significant, it is interesting that they did not include a p -value for this. The mean weight-loss in the lower-carbohydrate group was greater than the 95% CI threshold of the lower-fat group. A retrospective calculation to determine the p -value from the mean between-group difference with 95% CI gives a p -value of 0.13. While this does not meet the threshold for statistical significance, it does provide that the odds against chance are that there will be greater weight and fat-loss on a lower-carbohydrate diet. This implication is further strengthened by significantly greater improvements in BMI (0.33, 95% CI 0.01 to 0.64, $p = 0.04$), along with a greater reduction in waist circumference (0.67 95% CI -0.60 to 1.94), body fat % (0.18 95% CI -0.40 to 0.75), and blood pressure (0.54, 95% CI -1.07 to 2.16), and improved respiratory quotient (0.020, 95% CI 0.006 to 0.033). While these results are, on the whole relatively equivocal, it is interesting to note that 9 of 13 reported anthropometric variables were improved more by LCD relative to LFD. Consistent with the existing research, there were significant between-group differences for LDL-c favouring the low-fat group (-2.12 , 95% CI -4.70 to 0.47) vs LC (3.62, 95% CI 1.04 to 6.19). However, both TG and HDL-c were improved significantly more by the LCD intervention relative to LFD. There was a nearly 3-fold greater improvement in TG in LCD vs LFD and a 7-fold improvement in HDL-c for the LCD group vs LFD. The significant improvements in HDL-c and TG are likely to be more clinically meaningful than relatively minor changes in total cholesterol or LDL. (82-84) Overall, the multiplicity of benefits seen in DIETFITS shows a strong trend towards LCDs being *possibly* more effective overall for weight and fat-loss and the improvement of the most important predictors of cardiovascular and all-cause mortality, despite the ‘LCD’ being only modestly carbohydrate-restricted.

An important modifier of outcome that has been suggested is the 'carbohydrate-insulin model of obesity'. (85-88) This theory suggests that the increased ratio of insulin to glucagon concentrations after consumption of a meal with a high glycaemic load predisposes fuel towards storage in adipose tissue and results in reduced energy expenditure and increased hunger. (89, 90) Ebbeling et al., have recently demonstrated that a low carbohydrate diet containing 20% TE from carbohydrate resulted in increased energy expenditure (EE) when compared to a moderate- and high-carbohydrate diets containing 40% and 60% TE from carbohydrate respectively. There was a 52 Kcal per day (95% CI: 23 to 82) for every 10% decrease in TE from carbohydrate. In addition, this effect was modified by pre-study insulin status. Those in the highest third of insulin secretion at baseline (i.e. the most insulin resistant) had greater increases in EE with greater restriction of carbohydrate.

While there are conflicting results, there appears to be a likely effect of baseline insulin status, and perhaps other cardiometabolic markers, on outcomes resulting from diets differing in the magnitude of carbohydrate restriction.

Overarching research questions and thesis structure

There are, as indicated above, many unanswered questions and areas for further research and exploration in the area of LCDs and VLCKDs. The research which comprises this thesis will seek to address some of these questions and in doing so, add to the available literature and promote further hypotheses for exploration. Much of the research thus far indicates that those with obesity and metabolic syndromes will benefit most from LCDs and, in particular, VLCKDs, and there is already a relatively large body of research that has been conducted in these populations. There is little research on LCDs and VLCKDs in healthy people without metabolic syndromes or other health conditions. The outcomes of LCD research in healthy people are likely to be less clear due to expected smaller changes in blood and anthropometric changes from baseline, compared to those with more adverse baseline measures. However, it is important to study the effects of carbohydrate restriction in healthy people in order to identify changes in markers of future health risk and thereby help elucidate what, if any, role there is for LCDs as a preventative strategy for public health and the reduction of mortality and morbidity.

There are significant gaps in the literature for the achievement of, and negative effects arising from keto-induction, and for whom varying low-carbohydrate diets are more, or less effective. In this thesis, we sought to clarify several major themes arising from these gaps in the knowledge.

1. What effect do supplements, and diets differing in carbohydrate allocation, and resultant ketonaemia have on the achievement of nutritional ketosis?

Narrative review randomised clinical trial (RCT) 1, RCT 2.

2. What effect do ketonaemia and ketogenic vs non-ketogenic diets have on symptoms of carbohydrate withdrawal and mood?

RCT 1, RCT 2.

3. What are the *lived experiences* of people undertaking a ketogenic diet and how might that modify our understanding and application of ketogenic diets?

RCT 1

4. Can baseline cardiometabolic measures or other factors predict the efficacy of differing low-carbohydrate diets between individuals?

RCT 2

The thesis consists of a published narrative review and two RCTs. Due to the nature of the thesis, using published papers and those in the publication process, there is some repetition of methodology from chapter-to-chapter. An outline of the thesis and how the studies translate to the overarching research questions is provided in Figure 1.

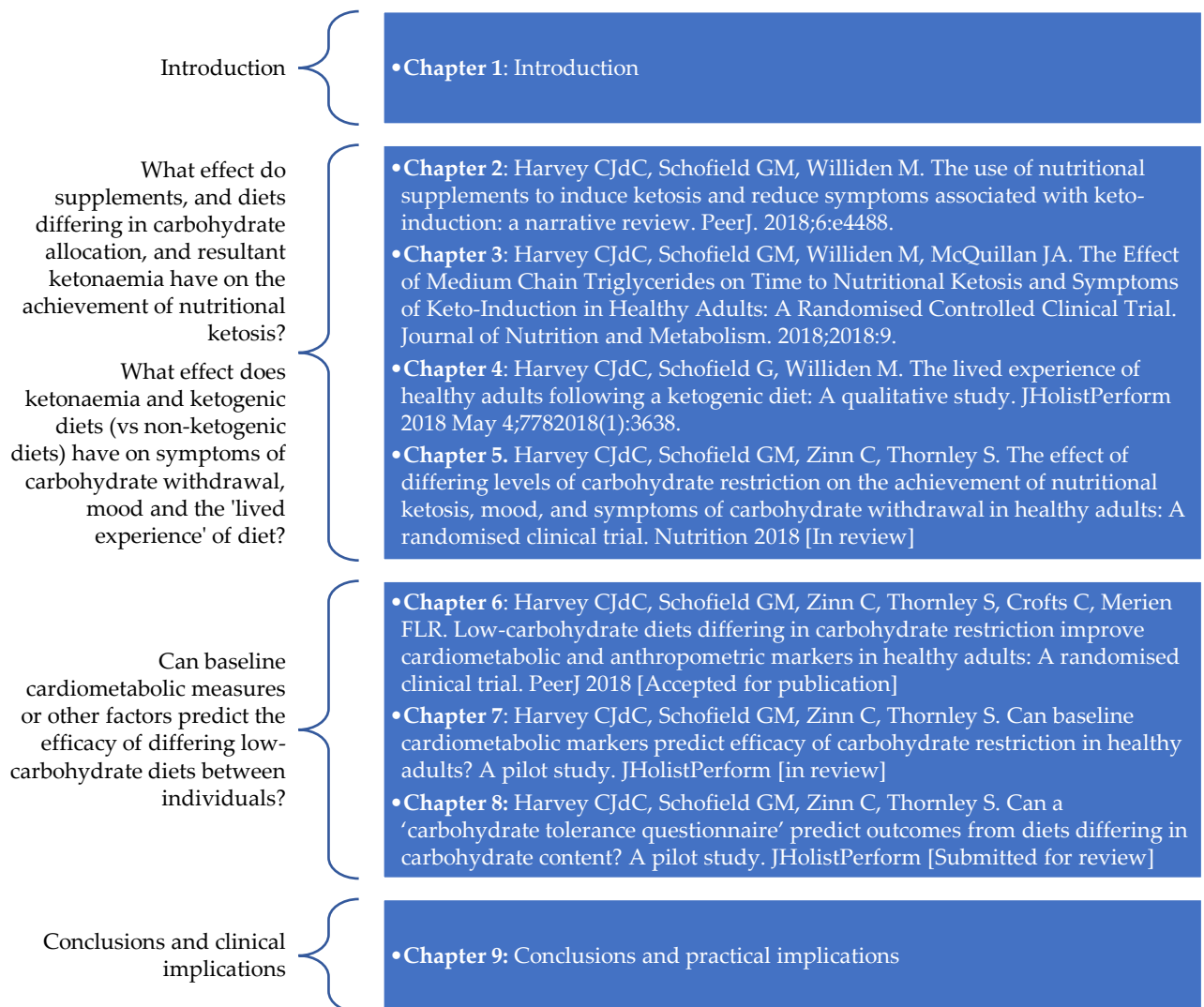


Figure 1. Chapter descriptions and thesis structure as it relates to overarching research questions

Chapter 2. The Use of Nutritional Supplements to Induce Ketosis and Reduce Symptoms Associated with Keto-Induction

This chapter comprises the following paper, published in *PeerJ*:

Harvey CJdC, Schofield GM, Williden M. The use of nutritional supplements to induce ketosis and reduce symptoms associated with keto-induction: a narrative review. *PeerJ*. 2018;6:e4488.

Author contributions:

CJdC H: 85%, GMS: 10%, MW: 5%

Preface

Despite the incredible popularity of both ketogenic diets and purportedly ketogenic supplements, there was an almost complete dearth of research describing the time it takes to induce ketosis, increase ketonaemia, and reduce the symptoms that have been associated with keto-induction. By synthesising the research on the effects of supplements to increase ketonaemia and induce ketosis, this review provides an important addition to the literature for illustrating more broadly, time-to-ketosis, and the symptoms of keto-flu and whether there are promising means by which these symptoms might be mitigated. This was the first review of ketogenic supplements and their effect on time-to-ketosis and symptoms of keto-induction and as such was well received in the academic community, becoming one of the top five ranked articles at *Peer J* in Public Health and Nutrition for 2018.

Introduction

Very-low-carbohydrate ketogenic diets (VLCKDs) are becoming increasingly popular for mainstream and athletic use for a range of outcomes including weight-loss and maintenance, (12) improved satiety and a reduction in hunger. (13-15) The diet also offers specific benefits for health conditions ranging from neurological disorders, obesity, and diabetes and other conditions on the spectrum of metabolic syndrome and offers the potential for the adjunct

treatment of various cancers. (1-11) Ketogenic diets elicit a state of ketosis known as 'nutritional ketosis' (NK), a state of hyperketonaemia distinct from pathological ketosis such as diabetic ketoacidosis. (91) Ketosis refers to the production of ketone bodies, derived from fats (and some amino acids) for use as an alternative fuel in times of fasting or drastic carbohydrate restriction. A restriction of carbohydrate, either by fasting or by restricting dietary carbohydrate, results in reduced insulin levels, thereby reducing lipogenesis (the creation of fats) and fat accumulation. When glycogen reserves become insufficient to supply the glucose necessary for normal β -oxidation of fatty acids, via the provision of oxaloacetate in the Krebs cycle, acetyl-CoA is then used instead in the biosynthesis of ketone bodies via acetoacetyl-CoA and β -hydroxy- β -methylglutaryl-CoA (92) to ensure provision of fuel to the Central Nervous System (CNS), which usually relies on glucose. The process of ketogenesis further allows coenzymes to be freed to ensure continued fatty-acid β -oxidation. (92) To elicit this carbohydrate restriction, while also providing sufficient alternate fuel to ensure sustainability of the diet, i.e. in comparison to fasting to achieve ketosis, VLCKDs have been used to encourage ketosis. Early research on VLCKDs focussed on children with epilepsy and for this purpose, the diet typically consists of a 3:1 to 4:1 ratio of lipid to non-lipid. This treatment for epilepsy was pioneered at Johns Hopkins University Hospital, (36, 37) and is referred to as a 'classic' or 'standard' ketogenic diet.

Ketogenic diets are now commonly applied, for a range of desired outcomes, and with differing definitions of what constitutes a ketogenic diet. Both low-energy diets and VLCKDs with fewer than 50 g of carbohydrate per day typically result in BOHB levels of ≥ 0.5 mmol/L. (33) This threshold has been used as a cut-off point for entry into ketosis by Guerci and colleagues, (34) and is commonly applied as a marker for entry into NK in the nutrition field, as compared to the typically higher levels expected in the medical field to elicit beneficial effects for seizure control in epileptic children. (93)

Time to ketosis

There is a paucity of research that identifies specific time points to the now-common definition of NK, as defined by BOHB levels of ≥ 0.5 mmol/L. (33, 34) In a study comparing fasted ketogenic protocols to a more gradual initiation of a ketogenic diet, Bergqvist and colleagues observed that participants fasting, achieved mean levels of ≥ 0.5 mmol/L BOHB, on the day

following initiation of the diet, whereas those on a 1:1 ketogenic diet (by weight) achieved the same level two days after initiation of the diet. (94) Other studies have measured either tangentially or directly, the achievement of 'ketosis' but have not specifically identified the time at which a level of ≥ 0.5 mmol/L was achieved. Berry-Kravis and colleagues observed a mean time-to-ketosis (urinary ketones > 80 mg/dl) of 42 hours. (95) Wirrell and colleagues have demonstrated a mean time-to-ketosis of 33 and 58 hours for any trace of urinary ketones or 'good ketosis' (> 0.8 mmol/L) respectively. (96) Wusthoff et al. recorded two cases of adults with prolonged nonconvulsive status epilepticus in which 'stable ketosis' was achieved after 8 and 10 days respectively, 3.6 and > 1.6 mmol/L, (97) but the definition for ketosis, in this study, was not mentioned and we cannot extrapolate the time-to-NK as defined in clinical nutrition. Strzelczyk et al. suggested ketosis as simply the presence of urinary ketones, some 3.5 days after initiation of a ketogenic diet, but at that time participants had achieved serum BOHB of 3.6 mmol/L. (98) Hoorn and colleagues observed no difference between fasted and non-fasted ketogenic protocols for time to ketosis, without specifically describing their definitions for ketosis or the time to ketosis itself. (99, 100) So, while the achievement of ketosis has been described in the medical literature, there are inconsistencies in the measurement of, and definition for ketosis in these papers.

Adverse effects of keto-induction — the 'keto-flu'

Adaptation to a VLCKD, or 'keto-induction', and the achievement of NK, when transitioning from a standard, higher carbohydrate diet, can cause various unpleasant effects. (68) Symptoms of keto-induction are predominantly constipation, headache, halitosis, muscle cramps, diarrhoea, and general weakness and rash. (69, 75) These occur because of increased urinary sodium, potassium and water loss in response to lowered insulin levels, (70-73) greatest between days 1-4 of a fast or ketogenic diet, (70) and transient reductions in glucose provision to the brain, observed to occur on days 1-3, with blood glucose normalising after day four. (74) Constipation may result from reduced food volume or reduced fibre intake, although this finding could be due to the groups that have been studied, which have included children with disabilities, who commonly experience constipation due to immobility. (75)

These symptoms are often referred to in the mainstream and grey literature as 'keto-flu' but are not well illustrated in the scientific literature. For example, a Google search returns over

22,000 results for the term “keto-flu,” but the same term searched in MEDLINE Complete, CINAHL Complete, Alt Health Watch, Food Science Source, SPORT Discus with Full Text, Psychology, and the EBSCO Behavioural Sciences Collection returns no results. Several studies have described adverse effects during ketogenic diets but to our knowledge, no studies have specifically described symptoms of keto-induction in the short time between commencing a ketogenic diet and the achievement of NK.

Adverse effects resulting from a VLCKD are likely to reduce compliance and tolerability, (101) and thus affect the efficacy of these diets as clinical interventions.

There have been several methods suggested to reduce symptoms of keto-induction and to reduce the time taken to achieve NK, including the ketogenic amino acid leucine, short chain fatty acids, medium chain fatty acids, and exogenous ketones.

The aim of this paper, therefore, is to elucidate the evidence for and against commonly applied nutritional supplements, purported to be ketogenic, to inform clinical practice in the growing field of ketogenic diets for common-use. This paper reviews the available scientific literature relevant to improvements in time to ketosis and symptoms of keto-induction, resulting from these nutritional supplements.

Methods

PubMed, Science Direct, CINAHL, MEDLINE, Alt Health Watch, Food Science Source, and EBSCO Psychology and Behavioural Sciences Collection electronic databases were searched online. Various purported ketogenic supplements, arising from a qualitative appraisal of forums, social media, message boards, and Google searches for ketogenic supplements, were searched along with the terms “ketogenic diet”, “ketogenic”, “ketosis” and ketonaemia (/ketonemia). Additionally, author names and reference lists were used for further search of the selected papers for related references. There is a paucity of studies on time to NK and mitigation of symptoms of keto-induction and as data related to the effects of various supplements on time to induction of ketosis and on symptoms of keto-induction are limited, and there is a lack of homogeneity between study objectives, outcomes, and measures, a narrative review style was chosen.

Results

Leucine

Leucine and lysine are solely ketogenic branched-chain amino acids (BCAAs). Thus, they do not contribute to gluconeogenesis. Higher leucine (and isoleucine) concentrations result from a ketogenic diet and are related to reduced glutamate-to-GABA ratio and this might explain some of the anti-seizure activity of a ketogenic diet in epilepsy. (102) There appears to be a high affinity of kidney cells for ketogenesis from leucine. (103)

Progression of fasting increases the conversion of leucine to ketone bodies and peripheral tissue is catabolised to provide leucine for ketogenesis. (104) Leucine can also be degraded in rat astroglial cells to the ketone bodies, including BOHB, and when released by these cells, used by neighbouring neurones as a fuel substrate. (105) Leucine also results in hepatic ketogenesis. (106) Studies in mice have shown that while ingested L-leucine can reduce seizure activity similarly to a KD, it does not independently increase blood levels of BOHB. (107) Evangeliou and colleagues have demonstrated that the addition of 20 g per day of BCAAs, including 9 g of leucine, in 17 children with intractable epilepsy, altering the ratio of lipid to protein from 4:1 to around 2.5, had no effect on ketosis, along with greater reductions in seizure activity. The authors postulated that this could be due to the ketogenic effect of leucine, but may also result from greater availability of BCAAs. (108)

Short chain fatty acids

Short-chain fatty acids (SCFAs) have carbon chains between two and five in length. These fatty acids include acetic acid (C:2), propionic acid (C:3), butyric acid (C:4), and valeric acid (C:5). Short chain fatty acids, especially butyric acid, are used extensively as a fuel substrate by intestinal epithelial cells. (109) It is generally accepted that chain length affects the relative deposition of fatty acids into either lymph or the portal vein. (110) Those short-chain fatty acids that escape metabolism by epithelial cells are, therefore, primarily absorbed via the hepatic portal vein and do not require 'bundling' with micelles and chylomicrons for absorption. (111) The highest quantities of short-chain fatty acids have been observed in portal blood, followed by hepatic, and far less in peripheral blood. (112) Thus, they bypass the usual route of absorption (for the more common long-chain fatty acids) into the lymphatics and

deposition into the bloodstream via the subclavian vein, and instead, are transported via the hepatic portal vein to the liver where they can be converted into the ketone bodies. (113-115)

Acetic acid

Acetic acid is a two-carbon SCFA. It comprises approximately 4-20% of vinegar. Vinegar has been demonstrated to improve postprandial insulin sensitivity in healthy and diabetic people and improve glycaemic responses to meals. (116-118) Urinary excretion of acetone (a ketone body) is increased in phloridzinised dogs and fasting rats after feeding with acetic acid. (119) Acetone is the spontaneous breakdown product of the ketone bodies acetoacetate and BOHB. Thus, it is likely that acetic acid is ketogenic, and has additional benefits for overall metabolic health, however, no research has been performed on acetic acid and its specific effects on the induction of ketosis or mitigation of keto-induction symptoms in humans. Interestingly, vinegar is commonly prescribed as a 'free food' in ketogenic diet trials, (120-122) and may provide an under-recognised stimulus for ketogenesis.

Butyric acid

Butyric acid (BTA) is a four-carbon, short-chain fatty acid found in the milk of ruminants and present in small amounts in many dairy foods. Most BTA in humans is produced by microbial intestinal fermentation of dietary fibre and resistant starch. Most of the BTA produced by this fermentation of starches is absorbed and used directly by colonocytes, with most of the remainder absorbed into the hepatic portal vein and transported to the liver where it can be converted to ketone bodies. (114, 115) A small amount is absorbed directly from the large colon and enters systemic circulation, to be used directly by peripheral tissue. (114) Butyrate exerts effects directly on the colonic mucosa, including inhibition of inflammation and carcinogenesis, decreasing oxidative stress, and promotion of satiety. (123, 124) Thus, it serves an important role in preserving the health of the colon, microbiota, and may have other beneficial roles for general and systemic health. Animal studies on the ketogenic potential of butyrate are mixed. For example, silage butyrate content has been shown to provide no significant effect on subclinical ketosis in dairy cows, (125) however, sub-clinical ketosis is higher in those receiving silage higher in butyrate content. (126)

In a recent study in humans, the effect of L-leucine, octanoyl-monoacylglycerol (O-MAG), a monoglyceride consisting of an 8-carbon fatty acid, L-carnitine, and butyric acid on acetoacetate and BOHB were studied. Both 2 g and 4 g of butyric acid were demonstrated to be more ketogenic than either 5 g of leucine, or 5 or 10g of O-MAG. (127)

Medium Chain Triglycerides

In medium chain triglycerides (MCTs) two-to-three of the fatty acid chains attached to the glycerol backbone are medium in length. These medium-chain fatty acids (MCFAs) are comprised of a 6–12 carbon chain. The MCTs are: caproic (C:6), caprylic (C:8), capric (C:10) and lauric acid (C:12). (128) Similar to the short-chain fatty acids and unlike long-chain triglycerides (LCTs), MCTs do not require the actions of bile, nor micellar-chylomicron mediated absorption into the lymphatics and instead are diffused directly into the hepatic portal vein and preferentially converted into bio-available ketone bodies in the liver. Huttenlocher and colleagues first demonstrated that diets containing fewer calories from lipids than a ‘classic’ ketogenic diet—around 60%-75% of calories—can induce NK if they include a high proportion of medium chain triglycerides (MCTs). (38) A VLCKD with 60% of energy derived from MCTs, a three-fold greater intake of carbohydrate (18% vs. 6%) and a ~50% (7% vs. 10%) increase in protein compared to a standard ketogenic diet induces NK with no appreciable difference in BOHB levels. (39)

Dietary MCTs are also known to promote both ketonaemia and ketogenesis in animals (129, 130) and humans with and without health conditions. (131, 132) MCTs promote ketonaemia and ketogenesis (useful to reduce the risk of night-time hypoglycaemic coma) in those with carnitine palmitoyltransferase deficiency, a rare genetic condition which inhibits the ability to produce ketone bodies from long-chain fatty acids. (133, 134) MCTs also increase BOHB when calorically dose-matched to either LCTs or carbohydrate in single feeding and non-ketogenic diet studies. (135-138) When fed intravenously, MCTs increase ketogenesis when compared to both structurally similar fats, (139) and LCTs. (140, 141) However, ketogenesis is reduced by the simultaneous application of glucose. (142) It has been demonstrated by Sandstrom and colleagues that in a hypercaloric diet, there are increased BOHB levels observed with the application of MCTs that aren’t seen in a hypocaloric state. (143)

MCTs increase BOHB in a linear and dose-dependent fashion. For example, when eleven pre-term infants were fed formulas with either 25% or 50% of fat calories coming from MCTs for at least 96 hours (30 Kcal/ml, around 50% calories from fat in total, 10% protein, 40% carbohydrate) the 50% MCT formula resulted in a mean plasma level of BOHB of 0.14 ± 0.03 mmol/L, a nearly three-fold increase over the lower MCT formula (0.06 ± 0.01). (144)

While there is a paucity of research on the effect of MCTs on the time taken to achieve NK, MCTs are demonstrably ketogenic and thus, allow induction of NK with a lower proportion of lipids in the diet, than that used in 'classic' 3-or-4:1 lipid to non-lipid (or 'ketogenic ratio') protocols. When 'classic' ketogenic diets with a greater than 3:1 ratio of lipid to non-lipid are compared to MCT ketogenic diets with 60% of calories from MCT, NK can be achieved with a lower lipid intake. Huttenlocher first observed higher BOHB levels in children with epilepsy aged 2-9 years, at up to one month on an MCT ketogenic diet, and marginally lower after this time, when compared to a classic ketogenic diet, although these differences were not significant. (39) In a study of 55 children with severe epilepsy, Schwartz and colleagues found modified ketogenic diets, MCT ketogenic diets, and classic ketogenic diets to all be 'ketogenic' (inducing NK) with peak ketone body concentrations of approximately 1 mmol/L, 1.5 mmol/L and 4 mmol/L respectively, after three weeks on the differing ketogenic protocols. (145) Nine children were subsequently trialled on a second diet and profiled three weeks later. Cumulative results over 24 hours of metabolic testing demonstrate that expression of ketone bodies rises (in order) from a normal diet (little change) to a modified MCT diet, an MCT ketogenic diet, and the greatest rise in ketone bodies over 24 hours resulting from a classic (4:1) ketogenic diet. In a 12-month study, a classic ketogenic diet resulted in higher levels of BOHB (and acetoacetate) over all time periods (three, six, and 12 months) but this was only statistically significant at three and six months ($p < 0.001$). (146)

After ingestion of MCT at a dosage of 30g MCT/m² body surface area by nine children (in a study of seizure control), BOHB levels rose progressively after administration from a mean of 0.2 ± 0.1 mmol/L after an overnight fast to 1.05 ± 0.3 mmol/L at 180 minutes. Participants reached NK on average at 30-60 min with most participants in NK by the 90th minute, but there was significant variation in BOHB between individuals. (147) With a lower dosage of

7.5 g of MCT taken three times per day after an acclimation period of 5 g MCT taken three times per day for one week, plasma BOHB was higher, yet not inducing NK. (148)

Exogenous ketones

Exogenous ketone supplements provide BOHB directly to the body without requiring ketogenesis and without concurrent elevations in free fatty acids. (149) They are considered to be a safe and effective way to increase ketone body concentrations. (150) Ketone supplements demonstrate promise as potential adjunct treatments for brain injury, (151) cancer, (152, 153) Angelman syndrome, (154) for reducing inflammation by suppressing activation of the NLRP3 inflammasome, (155) and Alzheimer's disease. (156) Ketone supplements might also improve fuelling during exercise, reduce lactate production, and improve performance due to glucose sparing, (157) and have positive effects on anxiety, (156) and mental performance and memory. (156)

Exogenous ketone supplements are available as either salts or esters of BOHB. Supplements containing ketone salts (KS) are some combination of sodium-, magnesium-, calcium or potassium-BOHB, and are available commercially from several companies under patent. (158) Ketone esters (KEs) at the time of writing, are only available for research, primarily as 1,3-butanediol monoester of BOHB (150) and thus, the animal and human research has mostly focused on the use of ketone esters. Both ketone esters and salts elevate BOHB to levels consistent with NK, (159) with ketone esters having greater effects on ketonaemia with ketone salts providing significantly higher reporting of gastrointestinal symptoms. (160) Ketone salts might provide a greater potential for long-term side effects if the inorganic ion load delivered is excessive for the individual. (160) Conversely, R-1,3-butanediol from ketone monoesters is readily metabolised in the liver to AcAc. (161) Clarke et al. detected no R-1,3-butanediol in the plasma of participants taking a ketone monoester supplement, except at the highest dosage of 714 mg/kg body weight, at which dose plasma R-1,3-butanediol was detectable at a level of ≤ 1.0 mmol/L and was undetectable 4 hours later. (161)

At a dosage of 395 mg/kg bodyweight, KE increased BOHB in healthy volunteers from 0.2 mmol/L (± 0.02) at baseline to 3.3 mmol/L (± 0.2) one hour later, (162) and from 0.16 mmol/L (± 0.02) at baseline to 3.16 mmol/L (± 0.14). (163) The same dose has been used to determine the effect on ketonaemia of KE taken with or without a meal. BOHB concentration (one-hour

post-KE) was lower in those having taken a meal, but both groups achieved levels of ketonaemia consistent with NK; 2.1 mmol/L (± 0.2) and 3.1 mmol/L (± 0.1) respectively. (164) In a study using higher dosages (0.573 g/kg BW) in healthy male athletes performing an hour of bicycle exercise at 75% of maximal exercise intensity BOHB levels rose from 0.1 to 3.4 mmol/L ($p < 0.01$) following ketone drinks. (165)

While it is clear that exogenous ketones increase serum BOHB, they are not ketogenic, and may, in fact, inhibit endogenous ketone production. (166) In other words, they promote ketonaemia but do not encourage the creation of ketone bodies in the liver. So, it is more accurate to say that exogenous ketones mimic the effects, many of which are positive, of NK, rather than inducing it.

Conclusions

It's unclear at this time whether an elevation in ketones over and above NK would mitigate the effects of keto-induction. It has, for example, been observed that mood is improved within the first two weeks of a diet irrespective of macronutrient composition, (167) and only one study, to our knowledge, has demonstrated a correlation between ketone levels and memory performance. (168)

Except for MCTs, there is limited research on the ketogenic potential of nutritional supplements, especially in human subjects. While the ketogenic amino acid leucine may not independently encourage ketogenesis to levels consistent with NK, more research is required, and the effect on time to NK and symptoms of keto-induction, particularly in a classic KD, are at this stage unknown.

Similarly, there is a paucity of research on the short-chain fatty acids and their effects on ketogenesis. Their mode of absorption and metabolism, like that of MCTs, but perhaps even more rapid, hints at a potential role for encouraging ketogenesis, and thus, the potential for improving time to NK and reducing symptoms of keto-induction.

There is a considerable amount of research demonstrating that MCTs promote both primary ketonaemia resulting from the conversion of medium chain fatty acids liberated from MCTs into bio-available ketone bodies, and longer-term ketogenesis by facilitating keto-adaptation. Expression of the ketone body BOHB is increased in a linear, dose-dependent manner in

response to oral loads of MCT but it is unclear whether MCTs independently improve time to NK. Modified MCT ketogenic diets do not significantly hasten the induction of NK over a classic ketogenic diet with a minimum of three parts lipid to one-part non-lipid, but they do allow NK to occur in diets containing greater amounts of non-lipid macronutrients.

There has, however, been little research performed on the application of MCTs to classic ketogenic diets and whether, if applied, they would; a) improve time to NK, b) result in significantly higher levels of BOHB, and c) significantly reduce symptoms of keto-induction. It is also unknown if, in the context of a ketogenic diet, MCTs provide additional benefits, for example for physical and mental performance and mood.

Exogenous ketones are unlikely to be ketogenic per se, and may inhibit ketogenesis, however, the rapid and substantial elevation of BOHB offers potential to mitigate effects of keto-induction, and thus, could play a role in improving adherence to a ketogenic diet. Newport et al. have reported improvements in mood and cognitive performance resulting from ketone ester treatment over 20-months in an Alzheimer's Disease case. In this case, cognitive performance tracked plasma BOHB concentrations. In a direct, dose-matched comparison, Kesl and colleagues evaluated the effects of ketone esters, salts, MCTs, and MCT + KS on blood BOHB in Sprague-Dawley rats at a dose of 5 g/kg. At 0, 30, and 60 min and 4, 8, and 12 hrs post administration (by intragastric gavage) KS+MCT and MCT supplementation rapidly elevated and sustained significant BOHB elevation compared to control for the duration of the 4-week study. Ketone salts did not significantly elevate BOHB at any time-point tested compared to controls. Ketone ester supplements significantly elevated BOHB levels for the duration of the 4-week study. This further demonstrates, albeit, in non-human subjects, the superiority of KE to KS for elevating BOHB and the utility of MCT for the same purpose but is likely to be limited in its applicability to health and performance as we have seen demonstrable increases in BOHB, consistent with NK levels with supplementation of KS in humans. (159, 160) Research performed on exogenous ketone supplements is, at this time, highly preliminary, and has been predominantly performed using animal subjects. Further clinical research is required to translate the potential benefits seen in these studies, to human models of disease and disorder.

This review was limited by a dearth of studies demonstrating the effect of supplementation on the time taken to achieve ketosis as defined by the *lingua franca* of NK of ≥ 0.5 mmol/L BOHB and on symptoms of keto-induction during this time.

While studies have described symptoms arising from a ketogenic diet, few studies have specifically evaluated symptoms and adverse effects of a ketogenic diet during the induction phase, and the studies that have been performed typically have not been designed to evaluate these as primary outcomes, and thus, our conclusions are extrapolated from a variety of sources. There is also little consensus on whether greater levels of BOHB (over and above NK threshold) are, in fact, associated with fewer symptoms of 'keto-flu', nor for that matter with improved outcomes but as previously noted, Newport and colleagues have observed a linear correlation between mood and cognition, and BOHB levels. (169) Adverse effects associated with the induction of NK might cause increased drop-out rates and preclude some of the positive effects for those that would otherwise benefit from a VLCKD. For example, Yancy and colleagues noted an 8% overall dropout rate due to difficulties adhering to an LCHF diet, with a further 5% withdrawing from their study due to adverse effects. (69) High attrition rates due to tolerability and gastrointestinal side effects have also been noted in childhood epilepsy research utilising VLCKDs. (3, 100)

Preliminary research suggests that increased BOHB levels and a faster time-to-NK might improve the acceptability of the KD and improve compliance rates, but more research is required to understand the role that supplementation could play in encouraging ketogenesis, improving time to NK, reducing symptoms associated with keto-induction, and the effect this might have on improving adherence to, and outcomes from a VLCKD.

Chapter 3. The Effect of Medium Chain Triglycerides on Time to Nutritional Ketosis and Symptoms of Keto-Induction in Healthy Adults

This chapter comprises the following paper, published in *The Journal of Nutrition and Metabolism*:

Harvey CJdC, Schofield GM, Williden M, McQuillan JA. The Effect of Medium Chain Triglycerides on Time to Nutritional Ketosis and Symptoms of Keto-Induction in Healthy Adults: A Randomised Controlled Clinical Trial. *JNutMet*. 2018;2018:9.

Author contributions:

CJdCH: 87.5%, GMS: 5%, MW: 5%, JAM: 2.5%

Preface

The previous review showed that medium chain triglyceride (MCT) supplementation is demonstrably ketonaemic and ketogenic. This suggests a possible role for this supplement to alleviate the symptoms associated with keto-induction and hasten entry into ketosis, but there is a lack of literature that indicates time-to-ketosis overall and explores symptoms of carbohydrate withdrawal and mood in very-low-carbohydrate ketogenic diets (VLCKDs). This paper describes the use of supplemental MCT in a 'classic' ketogenic diet consisting of a 4:1 lipid to non-lipid ratio, compared to a control oil rich in long-chain triglycerides to indicate whether MCT and the resultant, likely increase in ketonaemia, reduces time to nutritional ketosis (NK) and reduces symptoms of keto-induction and carbohydrate restriction, and improves mood.

Introduction

Ketogenic diets offer specific benefits for health conditions ranging from neurological disorders, cancer and obesity, diabetes and other metabolic conditions. (1-11) A restriction of carbohydrate, either by fasting or by restricting dietary carbohydrate results in reduced insulin levels, thereby reducing lipogenesis, fat accumulation, and glycogen reserves. Long chain fatty acids derived from common dietary lipids are almost always bound to albumin and are unable to cross the blood-brain barrier for use as fuel. Thus, when glycogen reserves become insufficient to supply the glucose requirement of the Central Nervous System (CNS), and for fat oxidation, an alternative fuel is required. During carbohydrate restriction acetoacetate accumulates and is converted into acetone and beta-hydroxybutyrate (BOHB), leading to the presence of these ketones in the blood and urine (ketonaemia and ketonuria respectively) and in the breath. Ketone bodies are utilised by tissue as a source of energy, with acetoacetate the primary ketone body and BOHB, not technically a ketone body (as the ketone moiety has been reduced to a hydroxyl group), functions as the primary fuel during ketosis resulting in two molecules of acetyl-CoA which enter the Krebs cycle. In ketosis, blood glucose (BG) levels stay within normal physiological limits due to the creation of glucose from glucogenic amino acids and via the liberation of glycerol during fatty acid oxidation.

Nutritional ketosis (NK), results from VLCKDs, as compared to starvation ketosis seen in fasting, and pathological ketosis such as the diabetic ketoacidosis resulting from uncontrolled Type 1 diabetes. (91) Both starvation or fasting ketosis, and nutritional ketosis result from evolutionary adaptations that allowed humans to survive in the absence of carbohydrate foods, and thus, glucose provision. (170) Nutritional ketosis allows for the maintenance of ketosis without starvation and so, NK allows for the maintenance of ketosis for longer than would be achievable with fasted ketosis. VLCKDs typically result in BOHB levels of ≥ 0.5 mmol/L (33) and this level has been used as a cut-off point for entry into NK. (34) Adaptation to NK when transitioning from a standard, higher carbohydrate diet, to a VLCKD, can cause various short-term adverse effects. (68) These effects of 'keto-induction' are constipation, headache, halitosis, muscle cramps, diarrhoea, and general weakness and rash. (69) These occur due to increased natriuresis, kaluresis and diuresis in response to lowered insulin levels (70-73) greatest between days 1 and 4 of a fast or ketogenic diet. (70) Transient reductions in glucose provision to the brain have been observed between days 1 and 3 with BG normalising

after day four, (74) while constipation and other gastrointestinal effects result from reduced food volume, increased fat intake or reduced fibre intake. (75, 100) Difficulties with adherence to ketogenic diets have been noted, (3, 69) but few studies specifically describe early-adverse symptoms associated with keto-induction.

Ketogenic diets typically contain a 3:1 to 4:1 ratio of lipids to non-lipid macronutrients, or at least 75% of calories coming from lipids, very low carbohydrates (often less than 50 g) and low-to-moderate amounts of protein. The 4:1 lipid to non-lipid ratio ketogenic diet pioneered at Johns Hopkins University Hospital, (36, 37) is now commonly used to induce ketosis and is referred to as a 'classic' or 'standard' ketogenic diet. Huttenlocher and colleagues first demonstrated that diets containing 60%-75% of calories from lipids induce NK if they include a high proportion of medium chain triglycerides (MCTs). (38) A VLCKD with 60% of calories derived from MCTs, a three-fold greater intake of carbohydrate (18% vs 6%) and a ~50% (7% vs 10%) increase in protein, induced NK with little clinical difference in BOHB levels when compared to a standard ketogenic diet. (39) Unlike long-chain triglycerides (LCTs), MCTs do not require the actions of bile nor micellar-chylomicron mediated absorption into the lymphatics and instead are diffused into the hepatic portal vein and preferentially converted into bio-available ketone bodies in the liver. Moreover, dietary MCTs promote ketonaemia and ketogenesis in both animals, (129, 171) and humans. (131)

Based on existing evidence, MCT supplementation is demonstrably ketogenic, (129, 131, 171) increases BOHB in a linear and dose-dependent manner, (135, 136, 138, 144, 172) and allows the achievement of ketosis with lower amounts of lipids (and concomitantly higher levels of protein and carbohydrate). (39, 145, 146) However, there is a paucity of research considering the role that MCTs may play in inducing ketosis more rapidly in a ketogenic diet with a 4:1 lipid to non-lipid ratio, or in improving symptoms of keto-induction and mood. NK is defined by the magnitude of ketonaemia (specifically ≥ 0.5 mmol/L of BOHB), and symptoms of keto-induction occur during the transition from a standard diet (with limited ketone expression in the blood) and the achievement of NK. Therefore, it is likely that the use of MCTs, resulting in ketonaemia and ketogenesis, could reduce time to NK and symptoms of keto-induction. These symptoms are likely to relate at least in part to the transition from a glucose dominant fuel system, to one in which BOHB becomes a primary fuel source. Further, by reducing the

time to NK, compliance with a VLCKD could be improved. Likewise, mitigation of symptoms of keto-induction is likely to result in improved adherence to the diet.

The aim of the present study, therefore, was to investigate, in a randomised, double-blind, placebo-controlled trial, whether MCTs reduce time to nutritional ketosis and symptoms of keto-induction and mood in a classic ketogenic diet. The primary outcome measured was the time taken to achieve NK. Secondary outcomes were symptoms and mood.

Materials and methods

Twenty-eight participants (2 males, 26 females: age \pm SD: 35 ± 4 y) (Table 2) were recruited between the 18th and 19th of October 2015 and gave written, informed consent to participate in this randomised, double-blinded, placebo-controlled study. Participants were required to be non-obese (< 30 BMI), not diagnosed as diabetic, not currently nor previously following a ketogenic diet and not a client of any of the researchers in clinical practice. The study took place between the 2nd and 21st of November 2015. Collection of data and analysis was performed at AUT Human Potential Centre, Auckland, New Zealand. (Figure 2.) The trial was registered by the Australia New Zealand Clinical Trial Registry. ACTRN12616001099415.

Table 2. Demographic characteristics of participants

	MCT	LCT
Gender (M/F)	1/11	1/10
Age (years); mean (range)	40 (33 to 47)	40 (32 to 48)
Ethnicity (<i>n</i>)	European (5)	European (11)
	NZ Maori (2)	
	Pacific (3)	
	Chinese (1)	
	Other Asian (1)	

MCT: medium chain triglyceride, LCT: long chain triglyceride, M: male, F: female.

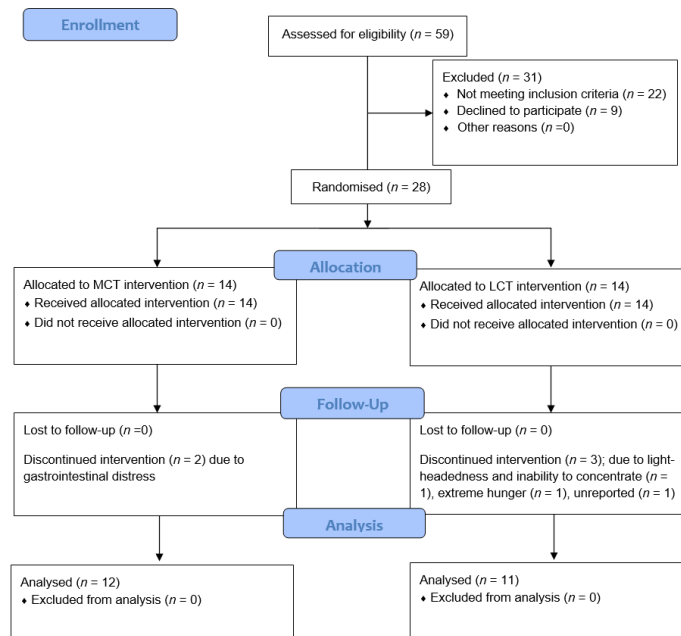


Figure 2. CONSORT flow diagram showing recruitment and retention of study participants

Participants were prescribed a ketogenic diet with a 4:1 lipid to non-lipid ratio. Males were allocated a diet containing 2200 Kcal per day and females 1800 Kcal per day each equating to 80% calories from fat (including supplemental oils), 13 to 17% from protein and 3 to 6% from carbohydrate. Minor differences in carbohydrate and protein were due to the use of protein intake of 1.4 g/kg of bodyweight per day (population means for male and female respectively), consistent with the International Society of Sports Nutrition (ISSN) guidelines for optimal protein intake for performance. (173) Participants were randomised to receive either an MCT supplement containing 65% caprylic acid (C:8) and 35% capric acid (C:10) triglycerides (Amtrade NZ limited) or a long chain triglyceride (sunflower) oil (Home Brand), 30 ml, three times per day, for 20 days. While MCTs are essentially non-toxic, (174) Ivy and colleagues observed that 100% of participants in their study experienced gastric distress (cramping and diarrhoea) with dosages of 50 and 60 g MCT, with only small GI effects noted at 30 g. (175) We used this dosage (30 g) as it was most likely to elicit an effect without unduly exposing participants to adverse effects arising from MCT ingestion. For our analysis, we considered the day in which each participant achieved ≥ 0.5 mmol/L BOHB as the time at which they had achieved NK. We proposed this threshold for NK (≥ 0.5 mmol/L) based on the level of BOHB

observed in those following a VLCKD (33) and as used as a cut-off point for ketosis by Guerci and colleagues. (34)

Participants were provided with a 'Freestyle Neo' blood-prick ketometer/glucometer (Abbott Industries) and were required to use the device to measure and record fasted BOHB and BG daily upon waking. Participants were also instructed to complete a questionnaire including a keto-induction symptoms questionnaire (Symptom-Q) and Profile of Mood States-Short Form (POMS-SF). The Profile of Mood States is a questionnaire commonly used to determine the overall mood state of study participants. (176) Saya Shacham developed a shortened, 37 question version of this form with correlation coefficients between the short and original scales all above 0.95 indicating the suitability of this shortened form for estimating mood. The symptoms questionnaire was developed by one of the authors (C.H) based on symptoms commonly observed in previous studies of ketogenic diets. The questionnaire asked *"In the past 24 hours to what extent have you experienced the following symptoms?"* answered on a Likert scale of 1) Not at all; 2) Mild; 3) Moderate; 4) Severe; and 5) Intolerable; for the following symptoms/effects: headache, constipation, diarrhoea, stomach or intestinal pain, intestinal bloating, halitosis (bad breath), muscle cramps, muscle weakness, skin rash, and difficulty concentrating.

An AUT University staff member who was not involved in data collection randomised participants using a simple randomisation technique of coin-flipping to generate a treatment sequence into two groups "A" and "B" and labelled the supplemental oils "A" or "B" for distribution and blinding and retained the blinding key. The primary researcher was unblinded to the supplement-oil key only after data analysis had been completed.

Participants were instructed to contact the primary and tertiary researchers for any assistance during the study duration. CH is a registered clinical nutritionist with the New Zealand Clinical Nutrition Association, and MW is a registered nutritionist with the Nutrition Society of New Zealand. The research was conducted in accordance with AUT ethical guidelines. Ethics approval was provided by the AUT University ethics committee, approval number 15/317.

Statistical analyses

Magnitudes based inferences (MBI) were used to compare the observed measures (BOHB, BG, Symptoms-Q, and POMS-SF). Pairwise comparisons were made between each of the 19 time-points for control (LCT) and experimental (MCT) trials for all using a customised analysis spreadsheet. (177) Pairwise comparisons were also made between observed measures relative to respective baseline values for each group. Data for BOHB and BG comparisons were log-transformed for analysis to reduce bias arising from non-uniformity of error and subsequently back-transformed to obtain changes in means and variations as factors. Raw data were used for comparisons between groups for Symptoms-Q and POMS-SF results. The sum of symptoms scores was used (Symptoms-Q) and the total mood disturbance score (TMDS) of the POMS-SF in which positive mood items are subtracted from negative mood items to give the TMDS score. (178) To make inferences about true (population) values of the effect on BOHB, Symptoms-Q, and POMS of MCT relative to LCT, the uncertainty in the effect was expressed as 90% confidence limits and as likelihoods that the true value of the effect represents substantial change (harm or benefit). An effect was deemed to be *unclear* if its confidence interval overlapped the thresholds for substantiveness; that is if the effect could be substantially positive and negative. (179) The smallest worthwhile change for between-group means for all blood and perceptual measures were calculated as 0.2 of the between-subject SD. (179) Inferences were based on threshold chances of harm of a difference between groups of 0.5%, and benefit of 25%. To determine the likelihood of clinical effects, the default values and qualitative terms were set at: < 0.5%, *most unlikely*; 0.5 to 5%, *very unlikely*; 5% to 25%, *unlikely*; 25 to 75%, *possibly*; 75 to 95%, *likely*; 95 to 99.5%, *very likely*; > 99.5%, *most likely*. Effect sizes (ES) were calculated using Cohens *d*, with an ES of < 0.2 considered *trivial*, > 0.2 *small*, > 0.6 *moderate*, > 1.2 *large* and > 2.0 *very large*. (179)

Time to NK comparison between LCT and MCT groups was made using a Kaplan-Meier survival analysis for time-to-event. (180) The 'event' analysed was the first recorded instance of NK (≥ 0.5 mmol/LBOHB) for each participant, and a log-rank test determined the significance of the survival analysis data. (180)

Finally, correlations were considered between BOHB and glucose, BOHB and symptoms (Symptom-Q), and BOHB and mood (POMS). Correlations were considered to be *trivial* $r <$

0.1, *small* > 0.1, *moderate* > 0.3, *large* > 0.5, *very large* > 0.7, *nearly perfect* > 0.9, or *perfect* = 1. (181)
Statistical significance of survival analyses, correlations, and additional analyses was determined by a *p*-value of ≤ 0.05 .

Results

A total of five participants withdrew during the data collection period—two from the MCT group (illness and gastrointestinal discomfort) and three from the LCT group (one due to extreme hunger, one unreported and a third due to light-headedness and inability to concentrate). The remaining participants' ($n = 23$) self-reported adherence to supplementation was 97% (combined) for both treatment groups. Thirty-two (7%) of BOHB measures failed to be recorded due to technical or operator error with the measurement device. Mean imputation analysis was used to adjust for the missing measures. (182)

Effects on beta-hydroxybutyrate

Supplementing MCT resulted in consistently higher blood levels of BOHB in our cohort of healthy adults relative to LCT treatment, with higher BOHB at all time-points in the MCT group (Figure 3). While clinically *trivial* effects were observed for days one to six, between-group effects for days seven to 19 were clear for MCT relative to LCT (Table 3). The magnitude of these effects was 0.2 ± 0.7 mmol/L (days 1 to 6), and 0.8 ± 0.7 mmol/L (days 7 to 19).

There was also a *very likely negative* effect of BOHB on glucose in both groups. I.e. higher BOHB levels were associated with lower glucose levels. This was further indicated by a *very large*, significant, inverse relationship of glucose to BOHB for both MCT ($r = -0.70$, $p = 0.0005$) and LCT groups ($r = -0.78$, $p = 0.00003$).

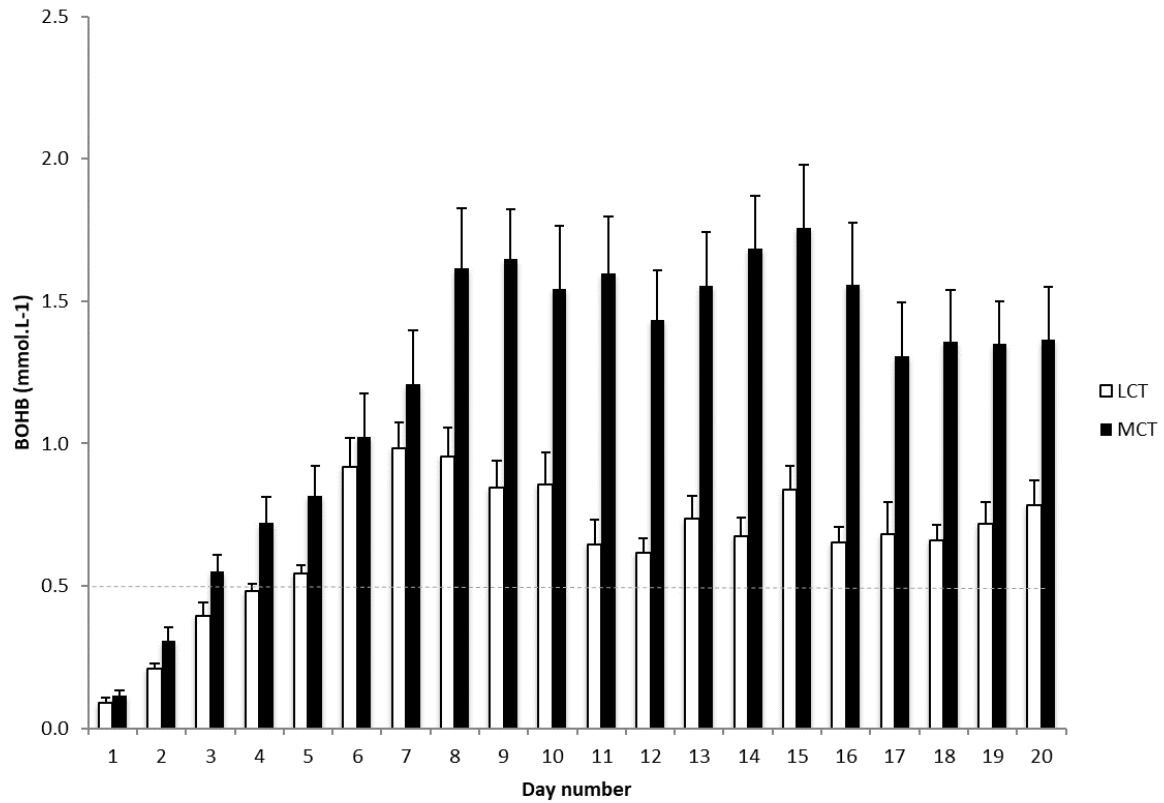


Figure 3. Mean BOHB for MCT vs LCT supplementation.

Error bars represent SE from the mean. BOHB: beta-hydroxybutyrate, MCT: medium chain triglyceride, LCT: long chain triglyceride

Time to nutritional ketosis

Overall, time to ketosis was more rapid with MCT supplementation. The achievement of NK within the first three days was higher with MCT vs LCT (17% vs 0% on day one and 33% vs 18% on day two) (Figure 3), and the mean time-to-NK was one day shorter with MCT supplementation but any observed differences between LCT and MCT for time to NK failed to reach significance ($p = 0.30$).

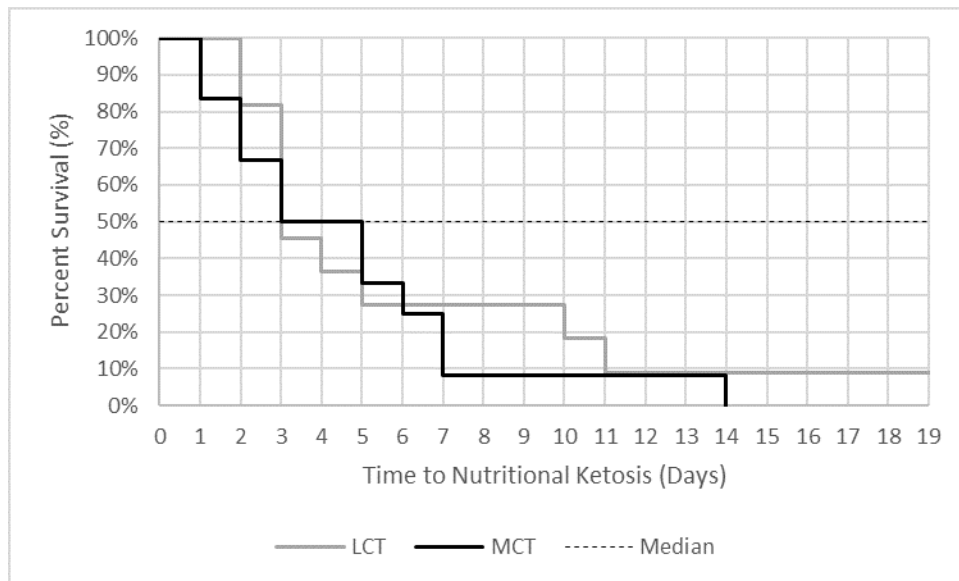


Figure 4. Kaplan-Meier Survival Graph and relative percentages of participants achieving nutritional ketosis (NK)

Day	LCT (n=11)					MCT (n=12)				
	Not in NK	In NK	In NK/d	% in NK		Not in NK	In NK	In NK/d	% in NK	
1	11	0	0	0		10	2	2	17	
2	9	2	2	18		8	4	2	33	
3	5	6	4	55		6	6	2	50	
4	3	8	2	73		6	6	0	50	
5	2	9	1	82		4	8	2	67	
6	2	9	0	82		3	9	1	75	
7	2	9	0	82		1	11	2	92	
11	1	10	1	91		1	11	0	92	
14	1	10	0	91		0	12	1	100	

LCT = long chain triglyceride, MCT = medium chain triglyceride, NK = nutritional ketosis

Symptoms of keto-induction

Supplementation with MCT vs control resulted in lower symptoms associated with keto-induction, with the mean sum of symptoms scores lower in the MCT group across all time points except for days 17, 19, and 20. (Figure 5.)

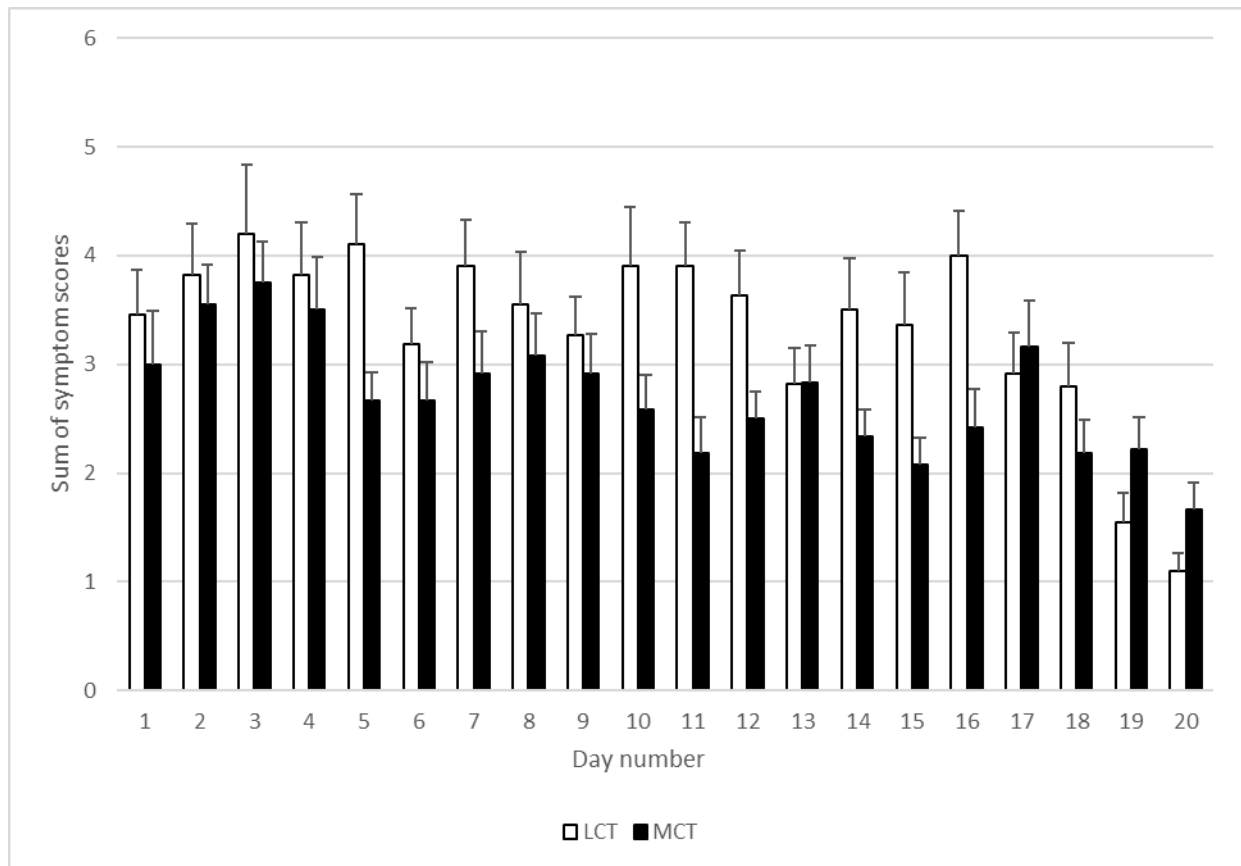


Figure 5. Sum of symptoms of keto-induction scores
Bars represent SE from the mean

Effects of MCT on the change-in-symptoms from baseline were *possibly beneficial* for days 4, 6, 9 to 11 and 13 to 15 but on all other days, effects were *unclear* relative to LCT. Improvements in symptoms scores from the preceding day indicated a *possibly beneficial* effect of MCT on 11 of 19 days. There was an *unclear* effect of BOHB as a predictor of symptoms for MCT relative to LCT (Table 3). However, there was a large inverse correlation ($r=-0.60$) observed between BOHB and Symptoms-Q in the MCT group ($p = 0.005$) only. We noted a small inverse correlation between BOHB and symptoms in LCT ($r = -0.23$), a result that failed to reach significance ($p = 0.30$).

Mood

Mood scores were improved at all time points from baseline in both groups with generally better mood reported by the LCT group compared to the MCT. As BOHB levels increased, reported mood improved in both groups. There was a significant, *large* inverse correlation between mean BOHB and mean POMS TMDS in both the MCT ($r = -0.70$, $p = 0.0006$) and LCT

supplemented groups ($r = -0.67, p = 0.001$) (Figure 5.). However, the effect of MCT relative to LCT on improvement in mood across all time points was *unclear* (Table 3).

When considering changes relative to the preceding day, there were approximately equal days of improvement in MCT vs LCT supplementation (nine and ten days respectively). A *possible beneficial* effect was observed across eight days for MCT supplementation (Table 2). MCT supplementation provides *very likely beneficial* effects on TMDS when BOHB is a predictor. A *large* correlation between glucose and TMDS in both MCT ($r = 0.50, p = 0.02$) and LCT ($r = 0.59, p = 0.01$) was also observed. There was a *possibly beneficial* effect from MCT supplementation when glucose was used as a predictor of mood. We observed an association between mood scores and symptoms scores across both groups (MCT $r = 0.61, p = 0.004$; LCT $r = 0.63, p = 0.003$).

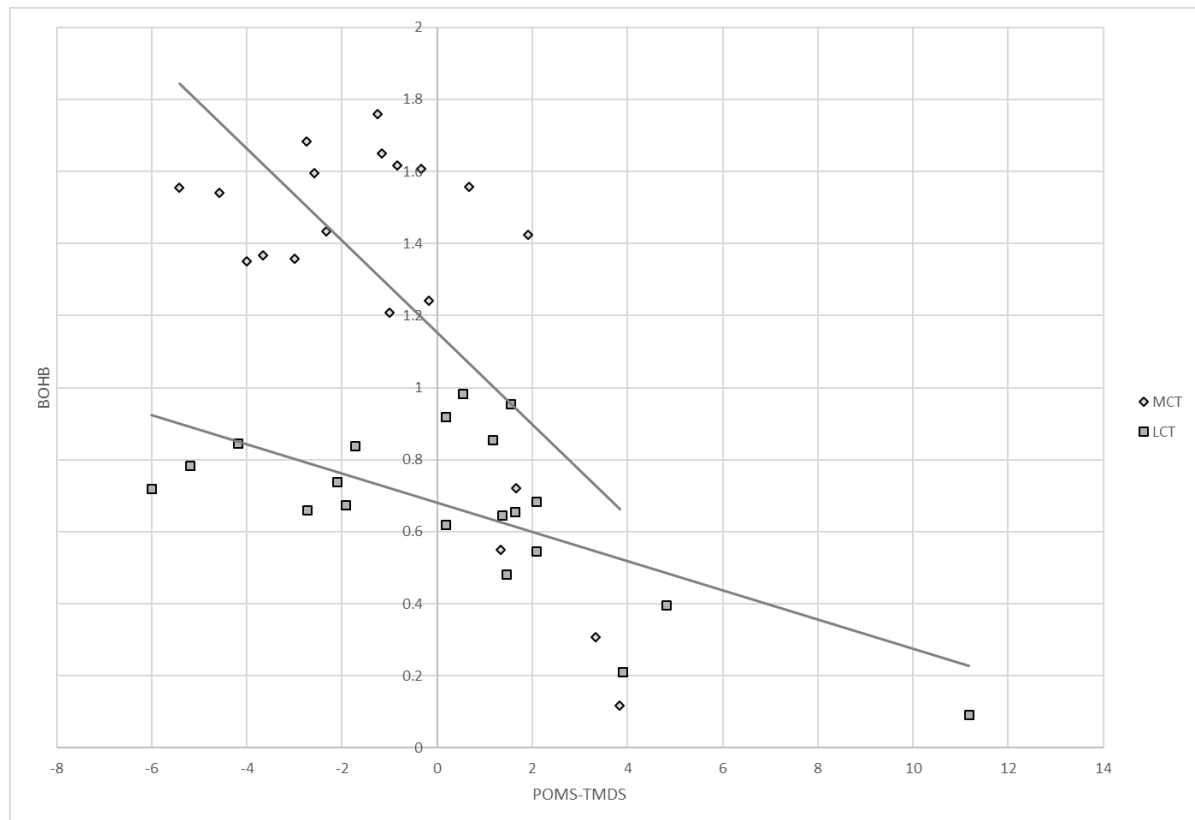


Figure 6. Mean BOHB compared to mean POMS-TMDS
BOHB: beta-hydroxybutyrate, POMS-TMDS: Profile of Mood States, total mood disturbance score

Table 3. Standardised effects for mean differences (LCT – MCT) in mood state, BOHB and blood glucose for days 1 to 19
LCT: long chain triglyceride, MCT: medium chain triglyceride, BOHB: beta-hydroxybutyrate

	Day →	Baseline (1)	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Δ Symptoms from baseline	Std. diff in means		-0.08	0.19	-0.27	-0.02	-0.17	0.00	0.03	-0.28	-0.24	-0.22	0.15	-0.12	-0.26	-0.36	0.23	0.09	0.79	0.67	0.86
CL 90%	±		0.86	0.99	0.66	0.70	0.67	0.83	0.77	0.74	0.72	0.66	0.70	0.76	0.75	0.74	0.89	0.83	0.65	0.70	0.66
Clinical inference			=			+	-	+	=		+			=	+			=			
Δ Symptoms By day	Std. diff means		0.06	-0.16	-0.03	-0.24	0.34	-0.24	0.38	-0.10	-0.26	-0.37	0.36	0.31	-0.18	-0.19	-0.15	0.60	-0.26	0.30	0.06
CL 90%	±		0.85	0.67	0.49	0.67	0.54	0.49	0.50	0.56	0.53	0.73	0.62	0.64	0.50	0.43	0.64	0.60	0.68	0.48	0.41
Clinical inference			=	+		=	+	=	+		=	=	+		=	+	=		+	=	
Δ POMS from baseline	Std. diff. in means		0.46	0.26	0.65	0.35	0.62	0.40	0.34	0.71	0.11	0.23	0.33	0.28	0.45	0.54	0.44	0.34	0.48	0.64	0.61
CL 90%	±		0.71	0.77	0.80	0.77	0.61	0.80	0.90	0.84	0.84	0.83	0.84	0.79	0.86	0.80	0.73	0.60	0.86	0.80	0.21
Clinical Inference			=																		
Δ POMS by day			0.46	-0.20	0.25	-0.17	0.27	-0.22	-0.06	0.37	-0.60	0.12	0.10	-0.06	0.17	0.09	-0.10	-0.10	0.15	0.16	-0.03
CL 90%	±		0.71	0.35	0.37	0.41	0.59	0.62	0.38	0.57	0.30	0.47	0.35	0.43	0.33	0.27	0.58	0.35	0.63	0.27	0.23
Clinical Inference			=	+	=	+	=	+		=	++	=	=	+	=	---	+	=		--	
BOHB	Diff. in means as a factor	1.16	1.24	1.16	1.06	1.00	1.04	1.48	1.90	1.87	2.45	1.91	1.85	2.31	1.84	1.76	2.06	1.67	1.93	1.71	1.16
CL 90%	⊙⊙⊙	1.75	1.71	1.61	1.68	1.74	1.79	1.89	1.79	1.90	1.91	1.71	1.76	1.69	1.82	1.86	2.01	1.98	1.90	1.97	1.75

Non-clinical inference		=								++		+++	++		+++	++					=
Glucose	Diff. in means as a factor	0.94	0.99	0.99	0.98	1.00	0.98	0.96	0.95	1.03	0.93	0.88	1.04	0.85	1.00	0.98	0.94	0.94	0.93	1.01	0.94
CL 90%	●●●	1.11	1.14	1.09	1.10	1.08	1.10	1.11	1.10	1.11	1.09	1.11	1.09	1.26	1.10	1.11	1.07	1.07	1.07	1.08	1.11
Non-clinical inference		+	=							++	--		+++	=					-	++	+

Standardised effects for mean changes (LCT – MCT) in Symptom-Q and POMS-TMDS for days 1 to 19, relative to baseline measures and for day-to-day changes. Clinical inferences based on threshold chances of harm and benefit of 25% and 0.5%. Non-clinical inferences based on threshold chances of 5% for substantial magnitudes. Positive or clinically beneficial: Very likely +++, likely ++, possibly +; Unclear = ; Negative or not clinically beneficial: Possibly -, likely --, very likely ---

Discussion

This study was the first to assess the impact of MCT on time to NK and symptoms of keto-induction and mood and, we believe, the first to specifically describe symptoms of keto-induction (keto-flu), within the first few days of a ketogenic diet.

Not surprisingly, there was a clear and significant effect of MCT supplementation on BOHB levels, relative to LCT control. MCTs are demonstrably ketogenic, (129, 131, 171) and this effect was expected. All MCT participants reached NK (≥ 0.5 mmol/L) with one participant in the LCT group failing to reach NK. A significant effect on time-to-NK wasn't demonstrated, despite the MCT group (based on mean BOHB levels) achieving ketosis two days earlier on day-2 vs day-4, for MCT and LCT respectively. We would consider an effect of MCT on time-to-NK to be likely due to the demonstrable effect on ketogenesis and ketonaemia resulting from MCT ingestion. (129, 131, 171) It must also be noted that this is likely to be a 'true' effect that we observed, as the participants' BOHB levels were tested when fasted in the morning, and so, increased BOHB cannot be solely explained by transient ketonaemia resulting from MCT ingestion. This effect is further indicated by the increased magnitude of the difference in BOHB between MCT and LCT groups over the study course. Studies with larger numbers of participants will be required to test this hypothesis adequately.

Symptoms initially worsened in response to both diet interventions, but these were ameliorated by day four five MCT and day six for LCT. At this time-point, mean BOHB levels were 0.8 and 0.9 mmol/L respectively. This result might suggest that the definition for NK of ≤ 0.5 mmol/L as suggested by Volek and Phinney (183) and previously used as a threshold for ketosis, (34) is not sufficient as a functional measure for ketosis. We also considered that a higher entry-point to NK could be 0.7 mmol/L based on the average of the BOHB readings on the day at which symptom scores had returned to baseline and the preceding day, as the questionnaire evaluated symptoms for the previous 24 hours. With this hypothetical higher threshold for NK, achievement of NK was greater in the MCT group for the first three days, but this result was also not significant, nor was the achievement of NK appreciably different after this time. Further exploration to define NK more appropriately to functional outcomes, and to determine evidence-based thresholds is warranted.

Symptoms were reduced in the MCT group relative to LCT. A *possibly beneficial* clinical effect was exhibited on symptoms by MCT application across almost all time-points and mean symptoms scores returned to baseline a day earlier with MCT supplementation. While the effect of MCT when BOHB levels were used as a predictor of symptoms was *unclear*, the large inverse correlation between BOHB and symptoms in the MCT group, not observed for LCT, suggests that the increase in BOHB resulting from MCT supplementation results in improved symptoms of keto-induction. This result further suggests that there might be a threshold level of BOHB required to mitigate some of the symptoms associated with keto-induction and that the higher BOHB exhibited in the MCT group may have caused this reduction in symptoms. This is a result that warrants further exploration. Relative to the MCT group, the LCT group reported factor increases of 0.5 ($p = 0.01$), 2.0 ($p = 0.01$), 1.7 ($p = 0.0004$) and 2.3 ($p = 0.0004$) for concentration difficulties, muscle cramps, intestinal bloating, and constipation, respectively. Relative to LCT, there was a 1.7 factor increase in the incidence of abdominal pain with MCT ($p = 0.003$). There are known gastrointestinal effects from ingestion of MCTs, (175) and in the amounts provided to participants, it is possible that symptoms noted with a higher incidence in the MCT group, especially diarrhoea and stomach pain, resulted from the use of MCTs. Abdominal pain, halitosis, and diarrhoea were, in fact, the most commonly observed effects in the MCT group with only abdominal pain as noted, reaching significance, and halitosis ('ketone breath') observed similarly in both groups. Reduced dosages of MCTs warrant further study to determine dose-dependent effects on symptoms and mood relative to control.

Mood scores correlated with symptoms scores, but the effects of MCT on mood-state were *unclear*. Interestingly, mood scores were typically more positive in MCT, whereas improvement from baseline was greater with LCT compared to MCT. Day-by-day improvements in mood were similar between MCT and LCT with some benefit from MCT supplementation observed on several days (Table 3). It is unlikely that MCT would worsen mood independently, except if resultant adverse effects (such as the stomach pain noted above) was sufficient to depress mood. When BOHB was considered as a predictor variable for mood, there was a likely beneficial effect from MCT supplementation.

There were several limitations to this study. Differences in compliance may have resulted from the free-living nature of this study. We did not adjust for exercise and activity, although

participants were advised to not change their current exercise habits. Standardised diets (both for male and female) were provided per age- and gender-adjusted average requirements. Thus, those participants that were more active may have exhibited differing results for BOHB, symptoms, and mood. Additionally, some participants experienced occasional difficulty taking readings with the ketometer / glucometer, resulting in several missed readings, which could have influenced results.

Because of the preliminary and exploratory nature of this study, we conducted many comparisons and hypothesis tests. We recognise the potential for multiplicity. A total of 114 pair-wise comparisons were made with 28 results, or 25%, reaching clinical significance. Since it is expected by chance that 5% of tests are expected to be false positives (or 6 results), our trial shows a clear excess of positive results over the number expected if chance were the only explanation for them. While it is not possible to distinguish which differences are true positives or negatives, an excess of observed over expected suggests some of the significant differences are true. Similarly, in correlations, 3 of 5 results were significant. We also note that magnitude-based inferences are not based on *p*-values, and thus, patterns of statistical significance with particular measures makes type-1 error less likely.

Furthermore, the numbers allocated to this study were limited by budgetary constraints due to its exploratory nature and this is likely to have reduced the statistical power of our results. We conducted a retrospective power calculation. The primary outcome of interest was the incidence of nutritional ketosis. The true proportion who achieved NK at seven days was 90% in the MCT group, and 70% in the LCT group, therefore, this study would have had 25% power to detect a significant difference in these proportions (alpha level = 5%), assuming the data conforms to a chi-square distribution. It is not surprising that we did not see a statistically significant difference between the two groups in the survival analysis. The chance of a false-negative result under these circumstances would be 75%. We consider that despite the relatively small numbers in the cohort, the findings are of interest considerable interest to both the scientific and lay community as there has been little direct research on keto-induction and 'keto-flu' specifically. We also recognise the limitations of our convenience sample arising from the snowball method of recruitment, via our networks on a first-come basis, as this led to an almost entirely female cohort.

Conclusion

MCT supplementation improves BOHB levels relative to an LCT control and has a possible, clinical application to reduce symptoms of keto-induction. It is unclear at this time whether MCTs significantly improve time-to-NK and mood but the large, inverse correlation between BOHB and mood disturbance scores, and the observed correlation between symptoms and mood suggest that in the context of a VLCKD, MCT supplementation may also improve mood. Due to the exploratory nature of this study, large variations between individual responses made many results unclear, especially concerning time-to-NK.

More research with larger sample sizes is needed in this area to elucidate the role of MCTs in a classic ketogenic diet more completely, and to understand the variability between individuals in their responses to ketogenic diets.

Chapter 4. The Lived Experience of Healthy Adults Following a Ketogenic Diet

This chapter comprises the following paper, published in the *Journal of Holistic Performance*:

Harvey CJdC, Schofield G, Williden M. The lived experience of healthy adults following a ketogenic diet: A qualitative study. *JHolistPerform* 2018 May 4;7782018(1):3638.

Author contributions:

CJdCH: 90%, GMS: 5%, MW: 5%

Preface

The previous chapter provided preliminary evidence that ketonaemia resulting from medium chain triglyceride (MCT) supplementation improved symptoms and mood during the keto-induction phase of a very-low-carbohydrate diet (VLCKD) as indicated by quantitative survey results. However, there is little qualitative research on the self-reported effects of a VLCKD on individuals. The qualitative impressions related to diet can help to inform clinical practice by providing additional information on the comfort, tolerability, and overall effects of diet and dietary change on individuals. This chapter describes the ‘lived experience’ of the participants in the previous study. We sought to understand more about the subjective experiences of participants undertaking a VLCKD to inform subsequent studies, which make up the remainder of this thesis.

Introduction

Low-carbohydrate, high-fat (LCHF) and VLCKDs offer specific benefits for health conditions ranging from neurological disorders, cancer, obesity, diabetes and other conditions on the spectrum of metabolic syndrome. (1-11) Adaptation to a ketogenic diet (keto-adaptation) facilitates the improved use of lipids for fuel and offers the potential for cognitive and physical performance enhancement. Thus, they are becoming increasingly popular for mainstream and

athletic use for a range of outcomes including weight-loss and maintenance, (12) improved satiety and a reduction in hunger. (13-15) VLCKDs result in 'nutritional ketosis' (NK) that is distinct from pathological ketosis, such as diabetic ketoacidosis, (91, 184) the condition characterised by a triad of hyperglycaemia (blood glucose > 11 mmol/L), increased total body ketone concentration of > 3 mmol/L, (185) and resultant metabolic acidosis. (184) VLCKDs typically result in beta-hydroxybutyrate (BOHB) levels between 0.5 and 5 mmol/L (33) and without hyperglycaemia, and this level has been used previously as indicative of entry into NK. (34)

Adaptation from a standard, higher-carbohydrate diet, to a VLCKD, and the induction of ketosis (keto-induction) can cause various unpleasant symptoms, for several days. (68) These symptoms can be referred to, in common parlance, as 'keto-flu' but are not well elucidated in the scientific literature. For example, a Google search returns over 22,000 results for the term "keto-flu" but the same term searched in MEDLINE Complete, CINAHL Complete, Alt Health Watch, Food Science Source, SPORT Discuss with Full Text, Psychology, and the EBSCO Behavioural Sciences Collection returns no results. Symptoms of keto-induction are predominantly constipation, headache, halitosis, muscle cramps, diarrhoea, and general weakness and rash. (69) These symptoms result from increased natriuresis, kaliuresis, and diuresis in response to lowered insulin levels, transient reductions in glucose provision to the brain, and constipation resulting from reduced food volume or reduced fibre intake. (70-73) These factors and resultant adverse effects are typically limited to the first 1-4 days of a ketogenic diet. (70, 74)

Yancy and colleagues noted an 8% drop out rate due to difficulties adhering to an LCHF diet, with a further 5% withdrawing from their study due to adverse effects. (69) High attrition rates due to tolerability and gastrointestinal side effects have also been noted in childhood epilepsy research. (3) However, few studies have specifically looked at the human experience of a ketogenic diet and symptoms of keto-induction, keto-adaptation and personal reflections on the challenges and opportunities of the diet. Studies have noted common adverse physical effects, such as dehydration and gastrointestinal (GI) disturbances. (75, 100) However, these studies did not investigate the qualitative, 'lived experience' of participants undertaking a VLCKD. The experience of any dietary intervention is likely to affect compliance and

adherence, and in the absence of significant differences between outcomes from different diets, an improved human experience whilst following a diet, could provide for relative superiority due to ease, comfort, and enjoyment.

The overarching aim of this study was, therefore, to evaluate both broad themes of experience within a ketogenic diet and to provide a 'snapshot' of individual experiences of a ketogenic diet in order to understand better the challenges and opportunities presented by the diet, and to help inform further research that may be of use to those following ketogenic diets.

Methods

Study design

This research reports on qualitative data collected within a randomised controlled trial comparing the use of medium chain triglycerides (MCT) to long-chain triglycerides in a classic (4:1 lipid to non-lipid ratio) ketogenic diet. Daily diary entries were recorded in an online questionnaire using Google Forms online software, and a post-study focus group was facilitated by one of the research team and recorded and transcribed by the primary researcher. The research was conducted in accordance with AUT ethical guidelines. Ethics approval was provided by the AUT University ethics committee, approval number 15/317. All participants were briefed twice on the intervention and study and gave written informed consent.

Participants and setting

Twenty-eight participants (2 males, 26 females: age \pm SD: 35 ± 4 y) (Table 4) were recruited between the 18th and 19th of October 2015 by a 'snowball method' (186) from the researchers' social media networks, with sharing encouraged to facilitate a 'viral' spread of recruitment and a broad demographic range of participants. All participants gave written, informed consent to participate in a randomised, double-blinded, placebo-controlled study. Participants were required to be non-obese (<30 BMI), not diagnosed as diabetic, not currently nor previously following a ketogenic diet and not a client of any of the researchers in clinical practice.

Table 4. Demographic characteristics of participants

	MCT	LCT
Gender (M/F)	1/11	1/10
Age (years); mean (range)	40 (33 to 47)	40 (32 to 48)
Ethnicity (n)	European (5) NZ Maori (2) Pacific Island (3) Chinese (1) Other Asian (1)	European (11)

MCT: medium chain triglyceride, LCT: long chain triglyceride, M: male, F: female.

The study took place between the 2nd and 21st of November 2015. Collection of data and analysis was performed at AUT Human Potential Centre, Auckland, New Zealand. (Figure 1.) The trial was registered by the Australia New Zealand Clinical Trial Registry. ACTRN12616001099415.

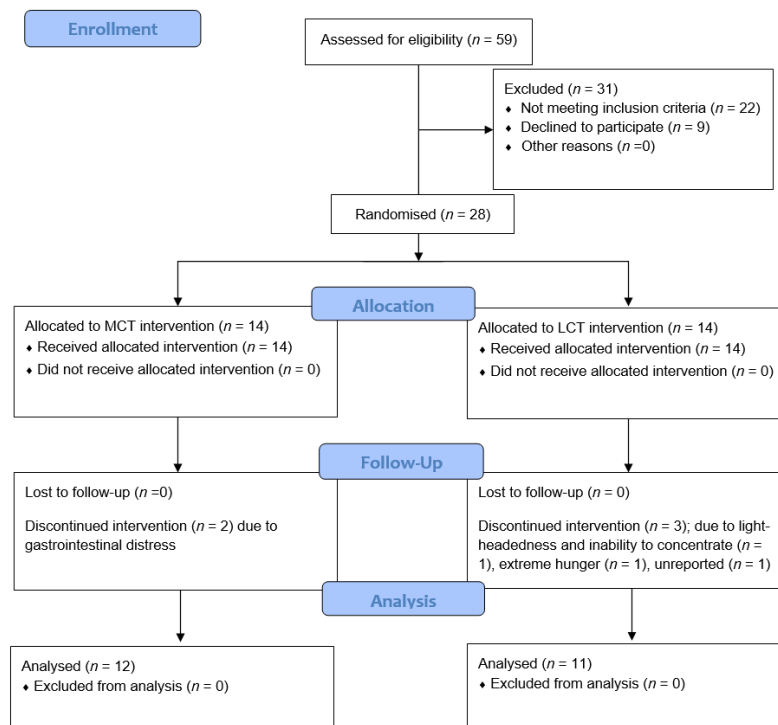


Figure 7. CONSORT flow diagram

Participants were prescribed a ketogenic diet plan with a 4:1 lipid to non-lipid ratio. Males were allocated a diet containing 2200 Kcal per day and females 1800 Kcal per day each equating to 80% calories from fat (including supplemental oils), 13 to 17% from protein and 3 to 6% from carbohydrate. Minor differences in carbohydrate and protein were due to the use of protein intake of 1.4 g/kg of body mass per day (population means for male and female

respectively), consistent with International Society of Sports Nutrition (ISSN) guidelines for optimal protein intake for performance. (173) Participants were randomised to receive either an MCT supplement containing 65% caprylic acid (C:8) and 35% capric acid (Amtrade NZ limited) or a long chain triglyceride (sunflower) oil (Home Brand), 30 ml, three times per day, for 20 days. Additionally, participants were provided with two introductory workshops to elucidate the dietary plan and its use. This paper reports the overall, qualitative impressions of a ketogenic diet across the intervention. Diary entries and focus group transcription were coded inductively and grouped into common themes. Twenty-three participants completed the study. Two withdrew from the MCT group (illness and gastrointestinal discomfort) and three (one due to extreme hunger, one unreported and a third due to light-headedness and inability to concentrate) from the LCT group. These results were omitted from this analysis. Participants were instructed to contact the primary and tertiary researchers for any assistance during the study duration. CH is a registered clinical nutritionist with the New Zealand Clinical Nutrition Association, and MW is a registered nutritionist with the Nutrition Society of New Zealand.

Qualitative measures

Daily diary entries were entered in an online questionnaire in addition to the measures taken for the aforementioned RCT, namely: blood glucose, beta-hydroxybutyrate, Profile of Mood States (POMS), and a ketogenic diet symptom questionnaire developed by the primary researcher (C.H.) based on commonly reported symptoms resulting from keto-induction. These quantitative measures will be reported in a separate paper.

The questionnaire asked the participants to respond to the open-ended, free-form question: "Please tell us about the experience of this diet. Describe in your own words anything that you may have felt or experienced (in the last 24 hours) because of this process". Additionally, an informal, semi-structured, post-study focus group was conducted. Questions posed in the focus group were also open-ended to facilitate ongoing discussion, e.g. "How did you feel while following the diet?", "How did the diet differ from your normal diet?", "Why did you enjoy, or not enjoy, the diet you were on?", "What effects, if any, did you notice while on the diet?"

Results

Diary entries and focus-group transcriptions were coded using an inductive approach by the lead researcher, with 'triangulation' (checking and confirmation) provided by the secondary and tertiary researchers to ensure robustness and validity of the themes and codes identified. In total 458 diary entries were submitted by the participants along with the post-study focus group. 830 references were extracted from the diaries and transcription. Physical effects accounted for over 28% of references. Other results were categorised as; mood, energy, and cognition (23%), satiety and hunger (16%), cravings and temptation (11%), and sleep (8%). Additionally, it was noted when participants directly stated that they were 'feeling good' and references related to behavioural change were noted to clarify the themes.

Overall, 49% of references were classified as 'positive' with 8% neutral, and 43% negative. Positive impressions were higher after participants had achieved NK and negative impressions higher during keto-induction. (Figure 8.) Adverse effects overall, both with respect to physical symptoms and feelings of mood and well-being, tended to improve over the course of the study, and conversely, positive impressions improved. However, it was noted that there was a large variation in responses and several respondents reported adverse effects throughout the course of the study.

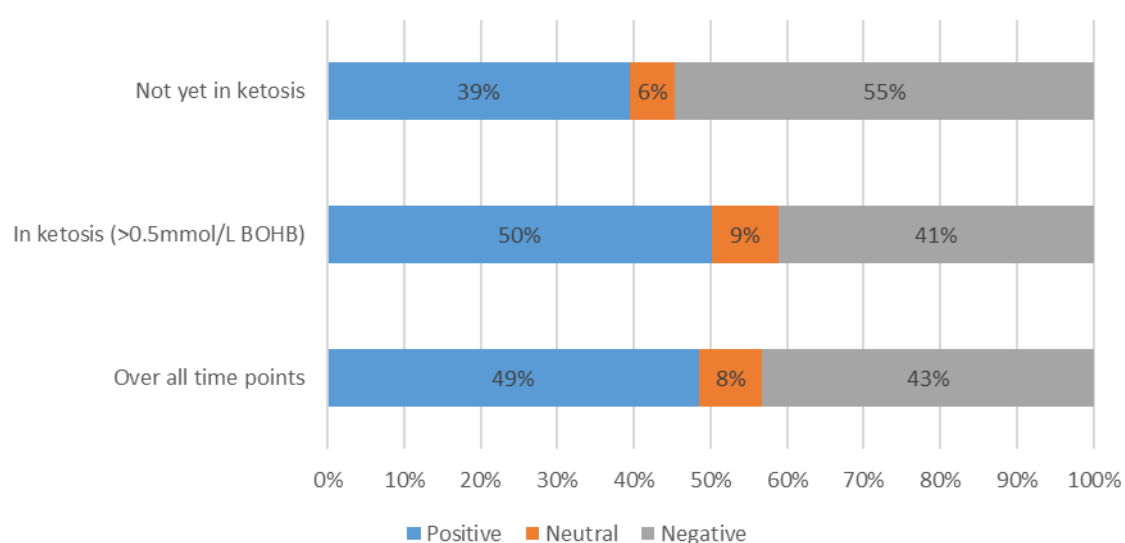


Figure 8. Percentage of experiences classified as positive or negative during keto-induction, nutritional ketosis, and over the study

Physical effects

Physical effects accounted for the largest category of reported responses. Gastrointestinal (GI) disturbances accounted for most adverse effects noted while on the diet. These included diarrhoea (the most commonly reported adverse effect), stomach cramps and constipation. Interestingly these GI effects were noted almost exclusively in the intervention group (those taking MCT oil). These symptoms, especially cramping and diarrhoea, are known effects associated with MCT supplementation. Ivy and colleagues have previously observed that 100% of participants in their study experienced gastric distress (cramping and diarrhoea) with dosages of 50 and 60 g MCT, with small effects at 30 g. (175) Less frequently reported were headaches, muscular cramps and physical weakness and one participant experienced a nosebleed. One participant considered pulling out of the study (unbeknownst to the researchers and revealed in the post-study focus group) due to extreme symptoms; *"I found it was very hard. Everything went wrong; headaches, shakes, dizzy, up all night just trying to get fluids in. I honestly yes, almost quit because I felt it was dangerous."* She sought medical advice and was provided with an electrolyte solution that caused the symptoms to abate. Interestingly, this participant stated that the 'hardship' of having these extreme symptoms and overcoming them, provided a reason to continue with the diet during, and after the study; *"Probably because it ended [the physical symptoms], and after the ending, I had a recipe for success."*

Some physical weakness was noted in association with exercise, but not concomitantly with lowered energy or mental cognition or mood. Instead, mood, energy, and cognition were improved (when compared to the perception of mood before the intervention), even though muscular weakness was present during exercise. It was noted that physical effects, in some cases, affected wellbeing; *"Feeling low in energy, headachy, stomach pain after meals is not encouraging me to be overactive and isn't making [me] feel very happy."* Interestingly, one participant's asthma symptoms disappeared completely on the diet; *"Asthma completely disappeared too."*

Mood, energy, and cognition

Mood, energy, and cognition were typically improved over the course of the diet. However, there were adverse effects on these at the beginning of the study. Mood and energy

suppression are likely to be related to the lowered provision of fuel (especially glucose) during this period; along with other factors such as electrolyte disturbance. (70-73) *"I found that just at the start was the hardest part" "but then once I was [in ketosis] I was pretty fine."*

Poor energy (fatigue and tiredness) were commonly reported in the early stages of the diet, when participants, for example, *"felt lightheaded" "quite low in energy", "difficulty focusing and concentrating"*. And for some, the reduction in energy was profound; *"Tired and wondering how on earth I'm going to do this for 20 days"*. However, for this respondent, and others, reported energy and mood did improve over the course of the study period. Conversely, some though suffered poor energy up until the end of the 20-day study; *"bit lightheaded and tired, so everything seems to take a little more effort. Quite looking forward to finishing diet"*, and others made no mention of poor energy at all, instead, only stable or improved energy.

Changes in energy patterns were noted, with either reduced or improved energy in the morning; or alternatively, a 'crash' in the evening. *"For most of the day, I felt great, at the end of the day I really seemed to crash."* Over the course of the study, energy mostly stabilised or improved for those reporting poor energy early in the study, along with improved or enhanced mental focus and clarity, e.g. *"really noticing a steady energy level especially at night when I am not exhausted going to bed. I just know I need to sleep but not shattered and 'over it' which is how I used to feel"* and *"Feeling very even in my moods (as in no real highs or lows and more 'mindful')"*.

Irritability was noted by several participants, predominantly in the early days of the study, and characterised by comments such as *"having a short fuse"* and *"tired with a short fuse"*. This irritability was also noted as a feeling of being 'wired,' i.e. *"[I] felt bloated, wired and tired!"* and was not necessarily related to increased energy, but instead, with fatigue. It is possible that this irritability and 'wired' feeling was related to increased sympathetic nervous system activity associated with carbohydrate withdrawal and the early keto-induction phase. This enhanced sympathetic nervous system activation, and increased epinephrine, norepinephrine, and cortisol has been observed in the early stages of carbohydrate-restricted diets and provides for the increased provision of glucose to preserve work capacity. (187-189)

Those who participated in exercise noted reduced energy in response to exercise, despite daily energy levels being stable or improved (in contrast to habitual peaks and troughs); *"Got an*

hour's bike ride in and felt rooted from the start. Energy levels still stable but just low". For two participants this was also exacerbated by mistakenly not taking the prescribed amount of supplemental oil. Omitting or taking the incorrect dosage of the supplemental oils, albeit accidentally, is likely to have affected energy levels significantly, as the major provider of calories on a ketogenic diet are lipids, and the ketone bodies into which they are converted, are the primary fuel substrate in this type of diet.

Despite lower-than-normal perceived energy during exercise earlier in the study, energy seemed to improve for participants over the duration of the study. For example, a participant noted on Day 3; *"Hard CrossFit session but no energy,"* yet by Day 18, despite being tired he did a *"Hard CrossFit session"* that he had *"heaps of energy for!"* Others shifted to a higher state of energy very quickly; *"Feeling really good, energy levels are great!"* (Day 4).

A potential confounding influence on energy levels was that calories were uniform based on a population- and age-appropriate eucaloric allocation of 2200 Kcal for males and 1800 Kcal for females. Thus, exercise would have provided a calorie deficit to hard-training participants. Training and exercise were not controlled in this free-living study.

It was also common for energy, mood and clarity to be improved even in the presence of negative physical effects such as diarrhoea, nausea and GI distress, and in the absence of quality sleep. *"I must have got about 4 good night sleeps over the whole thing but the really weird thing was that during the day like as soon as I ate breakfast my energy levels came up and it was like I had a super power...."*

Overall there seemed to be a general improvement in energy over the course of the study and a tendency towards improvements in 'stability' of energy following the VLCKD. While during-exercise energy did also improve over the course of the study, our impression was that this was reduced overall in relation to the diet.

Satiety and hunger

Satiety was drastically improved for most participants by the ketogenic diet. It has been well demonstrated that ketogenic diets improve satiety, reduce hunger, and reduce the desire to eat. (33, 190) Several participants were surprised at the volume of food, and especially vegetables, in the plan; *"Food portions [are] actually more than I would normally eat so, pretty*

bloated after lunch” and “veggie portions are just too big”. The vegetable portions recommended in the plan were approximately three cups with at least two meals per day to ensure that participants (who were not eating fruit due to the carbohydrate-restricted nature of the VLCKD) were consuming more than the recommended 5+ per day vegetable and fruit recommendation, in accordance with dietary guidelines for health. The difficulty of consuming this amount speaks to a common difficulty in dietary planning in general—compliance with vegetable and other ‘nutrient-dense’ (fruit, berry) food intake recommendations. For example, the New Zealand Adult Nutrition Survey of 2009 showed that many New Zealanders fail to eat the recommended servings of vegetables and fruits, and fail to consume recommended amounts of several vitamins and minerals from diet alone. (191)

This finding provides a hypothesis for vegetables as a ‘crowding’ food type, providing improved satiety and reducing the desire, within a meal, for other foods (such as optional ‘fuel’ foods—particularly sugars and starches). Interestingly, the absence of carbohydrate foods was perceived by some as a challenge not matched by how they *felt*. For instance, *“I was concerned at the amount of food and being hungry, but I was pleasantly surprised that I felt full after each meal for many hours.”*

Different portion sizes, in comparison to a habitual diet, appeared to be quite challenging. This was particularly evident in the morning when satiety was markedly increased; *“Finding it hard to eat so much at breakfast,” “Feeling much less hungry, couldn’t finish breakfast!”* Sometimes this was accompanied by increased afternoon hunger; *“extremely hungry after 3 pm”* despite improved morning satiety. Feelings of fullness were sometimes associated with a lack of interest in food, characterised by comments such as *“disinterested in food”* and *“no appetite.”*

The change in diet itself also caused some ‘confusion’ for participants in their perception of hunger. Several participants were used to eating more and snacking more frequently. Thus, they wondered whether they did, in fact, need so much food or whether they were simply habituated to it. *“It’s difficult to tell if it’s ‘real’ hunger (I don’t think so) or just ‘habit hunger.”* Because of this, some participants began to alter the diet – for example by consuming a smaller breakfast due to an inability to eat all the food prescribed.

A change was observed in response to snacking behaviours; *“Normally I will be hungry for each meal especially when waking up in the morning or going longer than usual without a meal, and now I am finding this is not so much the case.”* For instance, a participant noted on the first day of the study that it was a “shock” not snacking, but the following day noted, *“[I] am finding the not-snacking’ easier than anticipated”* and by the end of the study *“Not so hungry before meals, not starving or constantly thinking about my next meal.”* Others noted similar surprise at the elimination of the need for snacking, e.g. *“I was surprised at how well I coped given the lack of snacks I usually have”* and the ability to go for much longer periods than used to without needing to eat meals or snack; *“I’m used to eating every 3 hours” I went from 12.30 pm to 6.30 pm without eating—unheard of!!”* Occasionally, hunger led to cravings and non-compliance with the diet, i.e. *“Felt hungry at times and couldn’t resist some bread and a few hot chips.”*

The supplemental oils prescribed for the study appeared to improve satiety and provide a sense of ‘fullness.’ So, in addition to accountability provided by being involved in a dietary study, the satiating effect of the oils was a factor contributing to compliance. *“I think for me it wasn’t that I was following a strict regime, it was just that the oil made me feel so full”*. The oils, however, were associated with physical symptoms of nausea and GI effects (as noted earlier).

Overall responses and effects of the diet were very positive; *“I like that it’s a good amount of calories and I’m not hungry”, “I have felt full most of the time and have enjoyed eating more fat and protein”, “I definitely don’t feel hungry during the day on this diet”*.

In general, satiety was improved, with comments of ‘fullness’ common; *“I feel full most of the time”* and *“I have to force myself to eat as not feeling hunger”*. Improved satiety positively affected cravings *“I always feel full and no cravings”, “I didn’t crave the pizza or feel like I was missing out”, “I feel so surprised at how full I’ve been feeling on this program.”*

Cravings and temptation

It has previously been demonstrated that low-carbohydrate diets reduce cravings and desire for sugar and starches. (192) We noted a general improvement in sugar cravings in the study. For example, a comment from Day 1; *“craving sugar as I have a major sweet tooth”* but by Day 7 the same participant noted; *“Feeling great, no sugar cravings.”* Likewise, other comments indicated the reduced craving for sugar *“Sugar/junk food cravings are seriously diminished. First*

time ever!" and "Not craving much at all" Not craving carbs, sugar", "not fantasising about custard squares!" Satiety, possibly enhanced by the supplemental oils, reduced sugar cravings; "Have noticed that sugar cravings come back with a vengeance if not having enough fat with each meal." Participants who reported a tendency to have sugar cravings (or the "sugar demon" as one participant put it) tended to agree that if you have a little, you cannot help but have more; *"when you have carbs they make you crave more."* *"I feel so bad [when eating sugar], and the only thing that would make me feel better is to have more."* Ingesting carbohydrate (especially sugar) improves mood in people who experience sugar cravings. (193) In these diets, carbohydrates are quite literally 'off the table' and cannot continue to drive the positive feedback loop of craving more sugar. Evidence links dopamine release in the mid-brain to the pathophysiology of psychosis, addiction, and reward. Repeated ingestion of refined carbohydrate in a 'normal' western-style diet, stimulates the same dopaminergic pathway. (194)

Other comments indicated a behavioural tendency towards a mixture of moderation and abstinence, e.g. *"I would like to have one day where I can eat anything I like, but I am stricter on the other days like you know having that day to look forward to where I can eat anything."* Others lamented the ability not to be able to moderate food intake; *"I just wish I had the 'one bite ability,' you know, just to taste a cookie rather than a whole pack..."*

Although we noted a strong, general improvement in reported sugar cravings, over the course of the study, we noted an increase in the *desire* for, but importantly, not *craving* for carbohydrates (starches). This appeared to be related to a desire to return to more 'normal' dietary patterns.

Typically, during the study, cravings were resisted, when previously they may have been succumbed to; *"Really want carbs ... bread and butter never looked so good! Have refrained", "desperately wanted to just eat rubbish. Pure psychological warfare. Funny. Mostly resisted"*

Non-compliance with the diet was also associated with negative effects. *"Eating off plan yesterday left me with a huge food hangover"*, and there was a reluctance to 'give in' to cravings, especially where there were high perceived rewards from the diet (especially weight loss). *"Craving sugar today, but not wanting to go back to normal eating as I don't want to put the weight I've lost back on"*.

Cravings and temptation that did arise during the study did not appear to result from reduced satiety or increased hunger. *"[I] was tempted with my son's porridge today but amazed that I'm stronger than my sugar cravings and they're going too."*

Additionally, some participants became bored with the compendium of foods available; *"Getting a bit bored with the food"* and *"Sick to death of meat and eggs."*

Despite social situations providing a challenge, the diet, due to its restricted nature, enabled easier choices, especially at restaurants. *"I felt like restaurants were fine as you can order fish or meat and then get two sides of vegetables"*. This suggests that nutrition education ('what' to eat) and enabling strong, self-determined decision, is critical to the nutrition counselling process, rather than a simple dietary prescription.

Sleep

Overall, references related to sleep indicated improved sleep quality while following the VLCKD. improvement; *"Sleeping really well and feeling great."* *"Great, solid eight hours sleep."* *"I'm also falling asleep better, rather than tossing and turning how I used to."* Improvements in sleep quality have been noted in children with epilepsy following a ketogenic diet, (195) and ketogenic diets improve the GABA-glutamate ratio, providing a plausible explanation for greater relaxation and therefore, improved sleep. (102)

Difficulty getting to sleep was occasionally noted by some, however. This was often in association with a 'racing heart' indicative of a high stress-response such as *"I would wake up at about 2 am, so getting to sleep my muscles and everything felt quite restless, like jittery, and then I would get to sleep"* or *"Had difficulty getting to sleep and felt like I had a racing heart"*. As mentioned earlier, there are sympathetic nervous system effects during the early adaptation to a ketogenic diet, that might increase one's 'stress response'. One participant had extreme difficulties sleeping, noting early waking, trouble getting to sleep and sugar cravings; *"finding that I am not sleeping well at all - waking often and too early...so I am getting tired only because I feel like I am not getting quality sleep"*, *"really tired today - not sleeping well at all...huge sugar cravings - suspect due to tiredness...had to eat nuts to manage my way through"*. Interestingly, despite a real challenge with sleep, the participant felt good, and had improved mental clarity and focus; *"really struggling with sleep...waking incredibly early and having trouble getting off to sleep at night."*

Otherwise feel good!", "tired yesterday from terrible sleep - still great mental clarity though," "Not sleeping well at all. Totally exhausted today. Even had a day sleep which is not like me at all. Still mental clarity though".

Post-study reflections and intended changes.

Participants in the post-study focus group all stated that they desired to continue on a reduced carbohydrate diet. Several reasons for this being stated, most commonly, increased energy or cognition, reduced cravings and improved satiety, and weight loss. This was typified by statements such as; *"I really enjoyed it. I lost weight; my skin cleared" ...emotionally it was probably the most even-keeled I have felt in a really long time"* and *"I really liked the fact that I was not hungry, and I also wasn't emotional eating either, and I liked the kind of rigidity of the eating plan."* Moreover, this appeared to cause a 'shift' in habits and a desire to educate oneself about lower-carbohydrate food options; *"Because I felt so good, I felt like I need to carry this on, so I branched out and read a lot."*

Our impression from the post-study focus group was that most participants were likely to continue to follow some variation of carbohydrate-restricted nutrition plan. For example, *"I have kind of stuck with it somewhat" I'm certainly doing a modified version"*, but without the heavy use of supplemental oil, choosing instead whole-food dietary fats and perhaps adding in some whole food, nutrient-dense carbohydrate types (such as kumara (sweet potato) and yams); *"I found it hard that I couldn't have baked kumara so I was craving it when I would usually never crave that and I was kind of like 'give me the carbs!!' I feel long term a modified version of that plan would be really good."*

Even when returning to a diet closer to habitual, higher carbohydrate eating (albeit still lower in carbohydrate than previously consumed) there was a tendency to be mindful of carbohydrate portion size; *"I'm still eating bread and rice but maybe not as much"*. This greater attention to carbohydrate quantities in food could provide positive, long-term health benefits as reduced carbohydrate diets are likely to be more effective for weight loss for the obese and metabolically disordered (insulin resistant) than comparable low-fat diets, (77-80) and are more easily adhered to for those people who are insulin resistant., while offering no detriment to adherence for those insulin sensitive. (67)

Anecdotally, our clinical observations have indicated that cost can be seen as a prohibitive factor to the use of ketogenic and lower-carbohydrate diets. Although this wasn't a stated outcome of our study, the relative costs of the diet vs habitual eating were mentioned in the post-study focus group. Those that didn't habitually eat meat and other high-protein, higher-fat, lower-carbohydrate foods could find the diet more cost-prohibitive, e.g. *"[Cost was] Maybe a bit more because I do not usually cook or buy meat much"* and *"I was eating more meat so maybe a little bit more costly"*. Whereas those that habitually ate more meat and processed and refined foods found it cheaper than their usual diet; *"I thought it was cheap, especially as the protein/meat portions on a ketogenic diet are restricted."* *"And [[I'm] not eating as much meat as usual"*. A lower overall cost was also evident for those with a greater tendency to snack, compared to non-snackers; *"I feel like because I am [usually] eating so many snacks usually I found it actually quite cheap"*.

The consensus was that the diet intervention was a worthwhile experience for several reasons. Accountability was a major factor and is perhaps a confounding aspect of any dietary study; *"if I wasn't in the study I would not have persevered"*. The accountability provided by the daily ketone readings had the effect of almost 'gamifying' the experience and people, therefore, *wanted to comply*; *"You know with daily readings there was that sort of accountability with it"* but the ongoing cost (post-study) of the ketone testing strips provided a barrier to the continued use of this tool for accountability; *"the sticks ran out just as I finished and so I went to the chemist to buy more and they are \$3.15 per stick and for me that was just way too much."* Being involved in the study and having 'choice' taken away was a powerful contributor to compliance; *"What I have found is that the choice is actually taken away from you. Instead of thinking I could have one biscuit at home it is actually no gingernuts and you kind of think 'oh easy'. So, it isn't really a self-control issue, because when it comes to sugar, I do not have any"*.

Self-care was also mentioned as a motivating factor; *"another thing that was so great about doing this was that I was doing something for me, instead of doing something for everyone else in the household, I actually had the focus on me and what I was putting into my body and what I was doing for me and other people were sort of supporting me and they were supporting me with it and I really enjoyed that."* There was also a community aspect to this, between the participants, but also within the extended communities of the participants such as, *"People were always asking me*

what my numbers were, my brother or my mum or at work and you know everyone was quite interested you know how is it going kind of thing".

Discussion

There were significant challenges faced by the participants in this study, particularly with respect to physical effects. Many of these effects were GI and were mostly limited to the intervention group (taking MCT oil) for which GI effects are a known side effect. However, other side effects and physical symptoms also provided a significant challenge. Despite this, the most serious side-effects were experienced by people who, subsequently became the biggest advocates of continuing the diet, and this occurred once the keto-induction period had been transcended, or methods had been found to mitigate the negative symptoms (such as additional electrolytes).

The overall perception of the diet was positive, and the 'lived experience' of the diet likewise provided benefits for the overall feeling of wellbeing and mood, along with improved sleep and reduced frequency of sugar and carbohydrate cravings. Most participants in the focus group stated that they were continuing with some form of modified lower-carbohydrate diet due to the benefits, and this suggests that the 'lived experience' of a ketogenic diet overall is positive and that aspects of lower carbohydrate eating are both sustainable and may be preferred when compared to habitual, higher-carbohydrate eating patterns.

Negative experiences associated with the diet also appeared to diminish as participants adapted to a VLCKD, and so it is unlikely that negative effects are caused by a ketogenic diet per se, but more likely by the adaptation to the diet and its differential fuel use, transient, increased sympathetic nervous system activity, and most prominently by electrolyte imbalance, i.e. the increased natriuresis, kaliuresis, and diuresis in response to lowered insulin levels, (70-73) and transient reductions in glucose provision to the brain. These effects are typically minor and limited to the first 1-4 days of a ketogenic diet. (70, 74) More importantly for some participants, the diet itself provided a near epiphany, in that it offered a dietary option that provided a greater degree of freedom from hunger and cravings, and offered satiety, and enhanced mood and wellbeing.

The results though were highly variable between individuals. Submissions also varied considerably in quantity and depth, and we cannot exclude the possibility that those more enamoured with the dietary approach were also more effusive in their daily diary entries. Likewise, the voluntary post-study focus group was attended by eight people out of the original 28 participants, and this is unlikely to provide a comprehensive view of the effects on the lived psychological, psychosocial and psycho-emotional effects of a VLCKD. Despite this, the available qualitative data indicates that the experience of a ketogenic diet is generally a positive one.

There were several limitations to this study. Differences in compliance may have resulted from the free-living nature of this study. We did not adjust for exercise and activity, although participants were advised not to change their current exercise habits. Standardised diets (both for male and female) were provided per age- and gender-adjusted average requirements. Thus, those participants that were more active may have experienced the diet differently due to a relative calorie restriction. We also recognise the limitations of our convenience sample arising from the snowball method of recruitment, via our networks on a first-come basis, as this led to an almost entirely female cohort. There may be differences in the lived experience of a ketogenic diet between genders, but to our knowledge, this has not been elucidated in the literature, and although we don't have any reason to believe that the results would not apply similarly to men, our results need to be interpreted conservatively. We also recognise the limitations of qualitative research methods. While we sought to reduce bias by using an inductive, rather than a deductive approach to thematic identification, and through 'triangulation', checking and confirmation of codes by co-researchers, the research, and analysis, by its very nature cannot be wholly objective, nor should it be. The authors would like to humbly submit this work to the wider field of clinical nutrition for what it is, a qualitative appraisal by practitioner-researchers, of the experience of a ketogenic diet.

The lived experience of a ketogenic diet is likely to provide appreciable benefits for some people, but further research to better understand the individual tolerance and response to differing diets is warranted. In addition to this, further research to find methods to indicate clinically appropriate diets for individuals based upon physical, psychological or personality

characteristics, either known or yet to be elucidated would provide a valuable tool for practitioners.

Chapter 5. The effect of differing levels of carbohydrate restriction on the achievement of nutritional ketosis, mood, and symptoms of carbohydrate withdrawal in healthy adults

This chapter comprises the following paper, submitted to *Nutrition*:

Harvey CJdC, Schofield GM, Zinn C, Thornley S. The effect of differing levels of carbohydrate restriction on the achievement of nutritional ketosis, mood, and symptoms of carbohydrate withdrawal in healthy adults: A randomised clinical trial. *Nutrition* [Submitted for review]

Author contributions:

CJdCH: 85%, GMS: 5%, CZ: 5%, ST: 5%

Preface

The previous chapters provided preliminary investigations of ketonaemia and the effects of carbohydrate restriction on symptoms of carbohydrate withdrawal and mood. From those chapters, the hypothesis that the level of ketonaemia might positively affect both symptoms of carbohydrate withdrawal and mood is suggested. This chapter further investigates the differences in symptoms of carbohydrate withdrawal and mood between diets differing in the magnitude of carbohydrate restriction in order to demonstrate differences between different dietary interventions, and between individuals, in the achievement of ketosis, and whether greater or lesser restriction of carbohydrate, along with resultant ketonaemia, can mitigate symptoms of carbohydrate withdrawal and mood disturbance.

Introduction

Very-low-carbohydrate ketogenic diets (VLCKDs) benefit health conditions ranging from neurological disorders, cancer and obesity, diabetes and other metabolic conditions, (1-11) and are widely used in the general population for weight-loss and maintenance, (12) and

increased satiety. (13-15) Restriction of carbohydrate results in reduced insulin levels and depletes glycogen reserves, which reduces lipogenesis and fat accumulation. Nutritional ketosis (NK) is a natural state stemming from evolutionary adaptations that have allowed humans to survive in the absence of appreciable dietary carbohydrate, (170) and is distinct from the pathological state of ketoacidosis resulting from alcoholism and uncontrolled Type 1 diabetes. (91) These adaptations occurred because the Central Nervous System (CNS), which is typically reliant on glucose as a fuel substrate, requires an alternative fuel during periods of carbohydrate restriction. When glycogen levels become depleted, acetoacetate accumulates and converts to acetone and the preferred fuel ketone body—beta-hydroxybutyrate (BOHB), leading to the presence of ketones in the blood, urine (ketonaemia and ketonuria, respectively) and breath. Ketone bodies are utilised by most tissue as a source of energy.

Very-low-energy diets and VLCKDs with fewer than 50 g of total carbohydrate per day typically result in blood readings of ≥ 0.5 mmol/L BOHB. (33) This threshold has been used as a cut-off point for entry into ketosis by Guerci and colleagues, (34) and is now applied as a marker for entry into NK in the nutrition field, (35, 196) as compared to the typically higher levels warranted in the medical field to elicit acute seizure control in children with epilepsy. (93)

Many studies have measured the time taken to achieve 'ketosis', ranging from 1-8 days, (94-98) but there are inconsistencies in the definitions used for ketosis, and thus, there is a paucity of research that identifies specific time points to NK of ≥ 0.5 mmol/L BOHB. (33-35, 196) In a previous study comparing a classic ketogenic diet containing 80% total energy (TE) from lipids, to a medium chain triglyceride (MCT) supplemented diet also containing 80% TE from lipids, we observed the achievement of mean BOHB ≥ 0.5 mmol/L on day-3. (35) However, the specific achievement of NK, in diets differing in carbohydrate allocation, without the addition of MCTs to modify ketonaemia, has not been evaluated. In one of the few studies comparing a non-ketogenic, low-carbohydrate diet with a VLCKD, Johnston and colleagues recorded mean levels of BOHB at week-2 (of a 6-week intervention) of $0.7 \text{ mmol/L} \pm 0.2$, and $0.2 \text{ mmol/L} \pm 0.0$, for the VLCKD and NKLC groups respectively. (65)

Adaptation to a VLCKD, and the achievement of NK, when transitioning from a standard, higher carbohydrate diet, can cause various undesired effects. (68) Symptoms of carbohydrate

withdrawal or 'keto-induction' are; constipation, headache, halitosis, muscle cramps, bloating, diarrhoea, general weakness, and rash. (69, 75) These occur because of increased urinary sodium, potassium and water loss during the first 1-4 days of a fast or ketogenic diet in response to lowered insulin levels, (70-73) and transient reduction in glucose provision to the brain, with blood glucose normalising after day four of the initiation of a VLCKD. (74) These symptoms are often referred to in popular media as 'keto-flu' but are not well documented in the scientific literature. For example, a Google search returns over 22,000 results for the term "keto-flu," but the same term in the scholarly literature returns few results, and to our knowledge, only by the lead authors of the present paper. (35, 196) Many studies have described adverse effects during ketogenic diets, (68-75) but to our knowledge, few studies have specifically described symptoms of keto-induction in the short time between commencing a ketogenic diet and the achievement of NK. (35, 197) Adverse effects resulting from a VLCKD are likely to reduce the tolerability, and thus, compliance with these diets as clinical interventions. (101)

The aim of the present study, therefore, was to investigate, in a randomised clinical trial, the effect of low-carbohydrate diets differing in the magnitude of carbohydrate restriction on symptoms of carbohydrate withdrawal and mood and whether less restrictive low-carbohydrate diets elicit NK of ≥ 0.5 mmol/L BOHB.

Materials and Methods

Population

Seventy-seven participants, 25 males, 52 females (mean age: 39 years, range: 25 to 49; mean BMI 27 kg/m², range: 20 to 39) were recruited between the 7th and 19th of August 2017 and gave written, informed consent to participate in a 12-week, randomised, clinical intervention study. The study took place between the 11th of September and 10th of December 2017. Collection of data and analysis was performed at AUT Human Potential Centre, Auckland, New Zealand. This paper reports results for measures of dietary induction occurring in the first three weeks of the 12-week trial. Outcome measures are reported in a separate paper.

Inclusion and exclusion criteria

Participants were required to be healthy and between the ages of 25 and 49 years. Exclusion criteria were; underweight (< 18.5 BMI kg/m²), diagnosed with diabetes, diagnosed with any serious medical condition, having previously following a ketogenic diet, or being a current or former client of any of the researchers in clinical practice.

Ethical approval

The trial was registered by the Australia New Zealand Clinical Trial Registry. ACTRN12617000421336p. Ethics approval for this study was granted by the Southern Committee of the Health and Disability Ethics Committee of New Zealand. 17/STH/60

Dietary interventions and allocation

Participants completed baseline testing of blood measures and a lead-in week to identify habitual calorie intake and baseline morning, fasted BOHB levels. The study statistician randomised participants, stratified by gender, using a pre-prepared sequence, with investigators blinded to treatment allocation. Participants were assigned to the next treatment group according to their order of recruitment. The primary researcher responsible for initial statistical analysis was blinded to the treatment group allocation until the initial analysis had been completed. Participants were allocated to one of three low-carbohydrate diet plans, a VLCKD, a low-carbohydrate diet (LCD), and moderate-low-carbohydrate diet (MCD), which advised intakes of either 5%, 15%, or 25% of total energy (TE) derived from carbohydrate respectively.

Diet plans, which included macronutrient and calorie allocation and a sample menu plan, were individualised to the participant, with energy intake determined by the mean reported energy consumed per day in the lead-in dietary recording week. Participants were also provided a workshop to educate them on low-carbohydrate eating, meal planning, and how to use the mobile application and the blood-prick device. Advice was given to limit protein intake to 1.4 g/kg of body mass per day, consistent with the International Society of Sports Nutrition (ISSN) guidelines for optimal protein intake for performance. (173) We chose this as an appropriate protein intake that was not likely to unduly influence the study results because the study participants were healthy people, who may also be engaged in physical activity and

sports. Participants were advised to adhere as strictly as possible to the energy and macronutrient prescription for the first three weeks of the intervention that this paper describes. Comparisons of outcome measures between groups are reported in a separate paper. Usual exercise patterns were continued. Dietary intake was recorded by participants in a mobile application (Fat Secret) with the researchers able to obtain real-time entry on a partner mobile application (Fat Secret Pro). Results were monitored for safety and compliance by the primary researcher and research assistants tasked with data-monitoring. Compliance to the dietary allocation was monitored daily by a data monitoring team. Where non-compliance to the dietary allocation, especially for carbohydrate, was noticed, the participant was notified and offered support and advice. Figure 9 profiles the instructions for the dietary allocations over the 12-week study course.

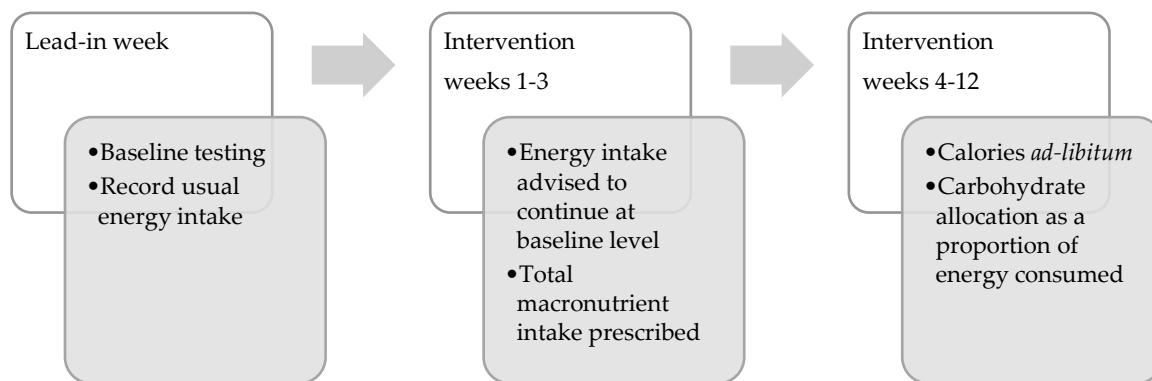


Figure 9. Flow-chart showing instructions for the dietary allocations

Participants were instructed to contact either the registered clinical nutritionist or the registered dietitian in the research team for any assistance during the study duration.

Blood ketone, and survey measures

Participants were provided with a 'Freestyle Optium Neo' finger-prick ketometer/glucometer (Abbott Industries) and were required to use the device to measure and record fasted BOHB daily upon waking. Participants were also instructed to complete a questionnaire including a keto-induction symptoms questionnaire (Symptom-Q) and a simplified 5-point scale indicator of mood state (Figure 10.) developed by the lead author. The Symptom-Q was based on symptoms observed in previous studies of ketogenic diets. One key question was asked ("In the past 24 hours to what extent have you experienced the following symptoms?") for 12

symptoms/effects: headache, constipation, diarrhoea, stomach or intestinal pain, intestinal bloating, change in breath odour, muscle cramps, muscle weakness, skin rash, difficulty concentrating, light-headedness, and craving for sugary or starchy foods. This survey has been previously used in our study comparing NK and symptoms and mood in a ketogenic diet including either medium chain triglyceride supplementation or control. (35)

Responses were reported on a 5-point Likert scale, and scored for analysis as, 0) Not at all; 1) Mild; 2) Moderate; 3) Severe; and 4) Intolerable, providing an overall sum of symptoms scores (SOSS) between 0 and 48 and individual symptoms scores of 0-4 for analysis.

15 → Overall, how do you feel today?*



Figure 10. 5-point mood disturbance scale

Statistical analyses

Effects of the dietary interventions on BOHB, SOSS resulting from the Symptom-Q and Mood-Q, were determined by the change in the mean of the various measures from baseline. The significance of these changes between groups was determined by ANOVA. Statistical significance was taken at the 5% two-sided alpha level. Further comparisons were made by undertaking multiple linear regression with adjustment made for variables recorded at baseline.

Results

A total of 283 people was assessed for eligibility with 206 excluded and 77 included for randomisation to the trial groups. The groups did not differ significantly at baseline (Table 5.) Two failed to comply with guidelines to submit baseline data and withdrew from the study (one male, one female). (Figure 11.)

Table 5. Baseline characteristics of study participants

	Treatment group			Total	Test	p-value
	MCD	LCD	VLCKD			
n	23	26	28	77		
Age mean (SD)	39.7 (6.1)	38.7 (7.7)	35.4 (7.1)	37.8 (7.2)	ANOVA	0.083
Gender (%)					Chi Sq	0.922
Female	16 (66.7)	17 (65.4)	19 (70.4)	52 (67.5)		
Male	8 (33.3)	9 (34.6)	8 (29.6)	25 (32.5)		
Ethnicity (%)					Chi Sq	0.634
Asian	2 (8.3)	1 (3.9)	1 (3.7)	4 (5.2)		
European	16 (66.7)	20 (76.9)	20 (74.1)	56 (72.7)		
Maori	5 (20.8)	3 (11.5)	6 (22.2)	14 (18.2)		
Indian	0 (0.0)	1 (3.9)	0 (0.0)	1 (1.3)		
Other ethnicity	1 (4.2)	0 (0.0)	0 (0.0)	1 (1.3)		
Pacific peoples	0 (0.0)	1 (3.9)	0 (0.0)	1 (1.3)		
Weight (kg) mean (SD)	80.0 (14.8)	82.92 (20.1)	78.7 (12.9)	80.5 (16.1)	ANOVA	0.628
Height (m) mean (SD)	1.72 (0.09)	1.72 (0.09)	1.72 (0.11)	1.72 (0.09)	ANOVA	0.980
BMI (kg/m ²) mean (SD)	26.9 (3.06)	27.9 (4.97)	26.5 (3.13)	27.1 (3.83)	ANOVA	0.396

SD: standard deviation. BMI: body mass index

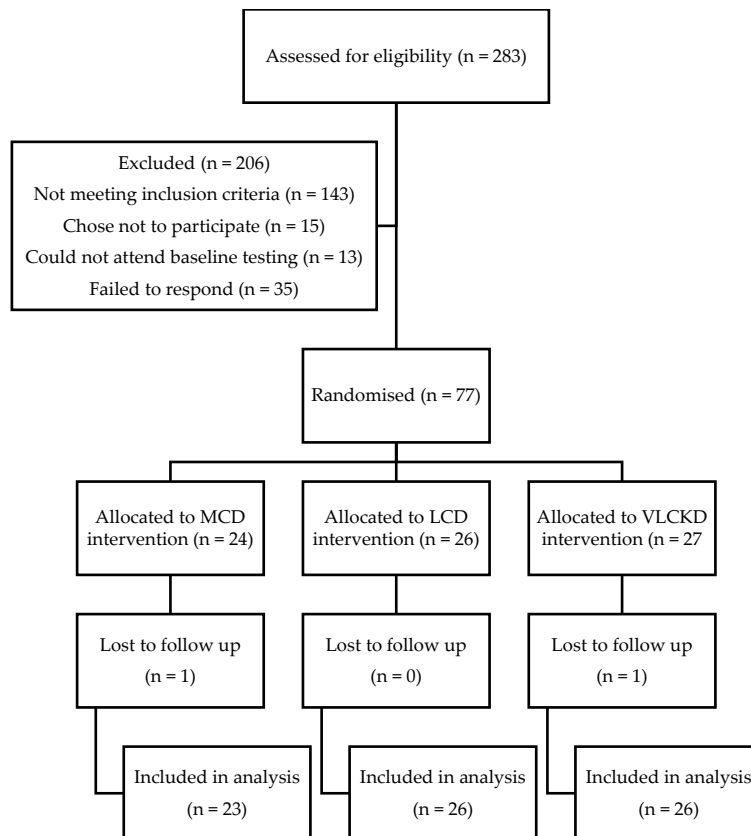


Figure 11. Flow-chart showing participant recruitment, randomisation, allocation, and lost-to-follow-up

Effects of carbohydrate restriction on BOHB

There was no difference in BOHB between groups at baseline with all groups exhibiting 0.1 mmol/L, 95% CI [0.1, 0.1]. Serum levels of BOHB rose in all groups appreciably with an overall change from baseline of 0.27 ± 0.32 , 0.41 ± 0.38 , and 0.62 ± 0.49 mmol/L for MCD, LCD, and VLCKD respectively ($p = 0.013$). All three diets resulted in ketosis with mean levels of BOHB consistent with NK on day-4 for the VLCKD group, and on day-5 for the LCD and MCD groups. The mean achievement of NK was maintained post-induction for both VLCKD and LCD groups, except day-20 for LCD) but was more sporadic for the MCD group with NK ≥ 0.5 mmol/L on five days. Only the VLCKD group exhibited 95% CI levels that were consistent at ≥ 0.5 mmol/L, on 14 of 17 days after the achievement of NK, while the other groups did not achieve this level of confidence. (Figure 12.)

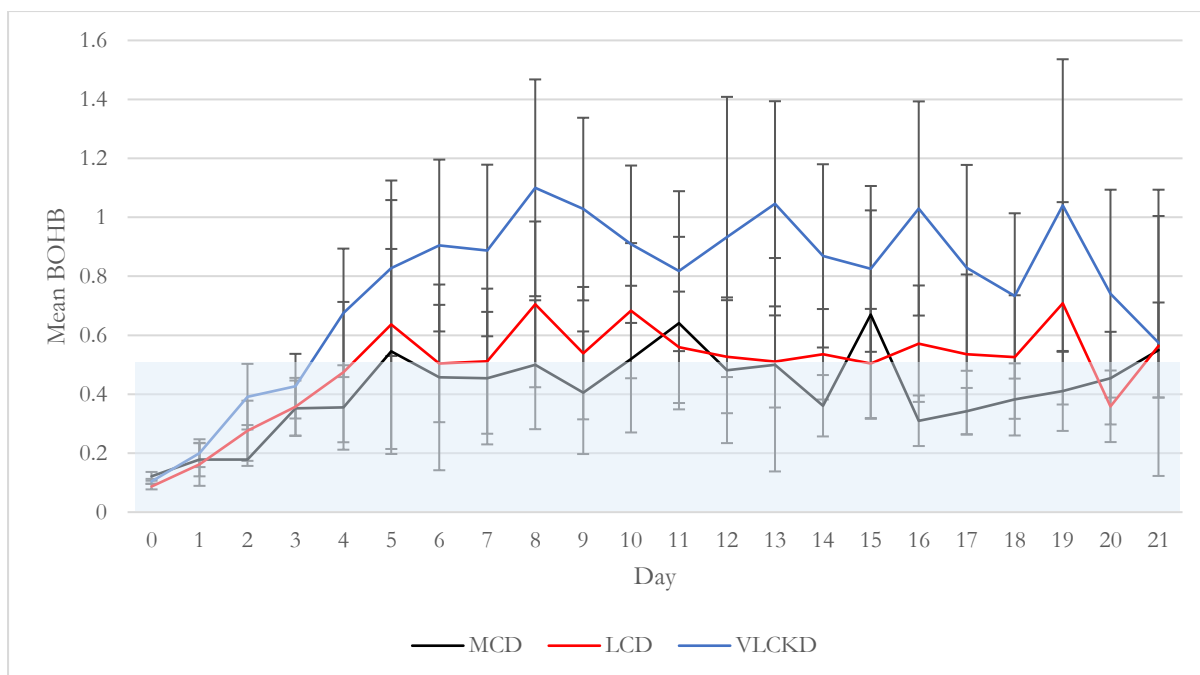


Figure 12. Mean BOHB (beta-hydroxybutyrate) by group by day
 Bars represent 95% CI. The shaded area represents readings under the threshold for nutritional ketosis. BOHB: beta-hydroxybutyrate

Symptoms of carbohydrate withdrawal

The mean SOSS scores differed at baseline and were; 3.94 ± 3.84 , 2.35 ± 2.78 , and 4.36 ± 3.75 for VLCKD, LCD, and MCD respectively ($p < 0.001$). Mean change in SOSS from baseline overall were 0.81 ± 2.84 ($p < 0.001$). We observed minor changes that were highest in the VLCKD group (1.49 ± 2.47), followed by LCD (0.65 ± 2.70), and MCD (0.18 ± 3.3). Between-group differences did not reach the threshold for statistical significance ($p = 0.264$). (Table 6.)

There were identical return-to-baseline SOSS scores for VLCKD and MCD on day 18, with the most rapid return to baseline seen in the LCD group on day 11. (Figure 13.) The magnitude of both symptoms of carbohydrate withdrawal and changes from baseline were small overall with the highest recorded value being 24 out of a maximum of 48. The highest mean value was 7.24 for VLC on day 4. Proportional changes from baseline were significant ($p < 0.001$). The proportional changes from baseline were similar for VLCKD and LCD, and overall, there was little substantive difference in changes in symptoms between groups (Figure 13.)

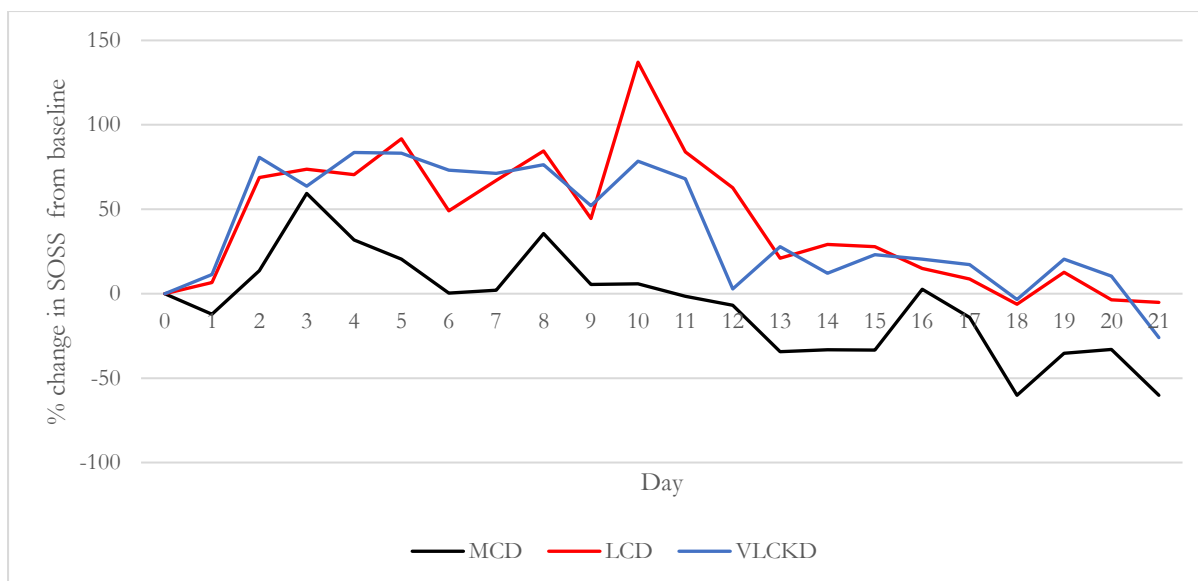


Figure 13. Percent change in mean SOSS (sum of symptom score) from baseline

Over the three-week study period there was a small, yet statistically significant overall increase in mean headache severity, constipation, diarrhoea, halitosis ('change in breath odour' / "nail polish breath"), muscle cramps and muscle weakness, and light-headedness. Intestinal bloating and craving for sugar and starch improved from baseline. Of these findings, only halitosis ($p = 0.039$) and muscle weakness ($p = 0.005$) differed significantly between the groups. Halitosis was highest in the VLCKD group, followed by LCD, and MCD. Muscle weakness worsened most overall in the VLCKD group, followed by MCD, and was least affected by the LCD intervention. All mean changes from baseline for BOHB, symptoms, and mood are presented in Table 6.

Dietary treatment and mood

At baseline there was a significant difference in mood disturbance scores; MCD: 2.37 ± 0.77 , LCD: 1.84 ± 0.70 , and VLCKD: 2.11 ± 0.91 ($p < 0.001$). Consistent improvement in mood (reduced mood disturbance) was demonstrated for the MCD and VLCKD groups, with mean change from baseline on all days of the study period, while the LCD group had some worsening of mean mood scores on 10 of 21 days (Figure 14.) Mood significantly improved from baseline overall, but there was no significant difference between groups ($p = 0.181$) (Table 6).

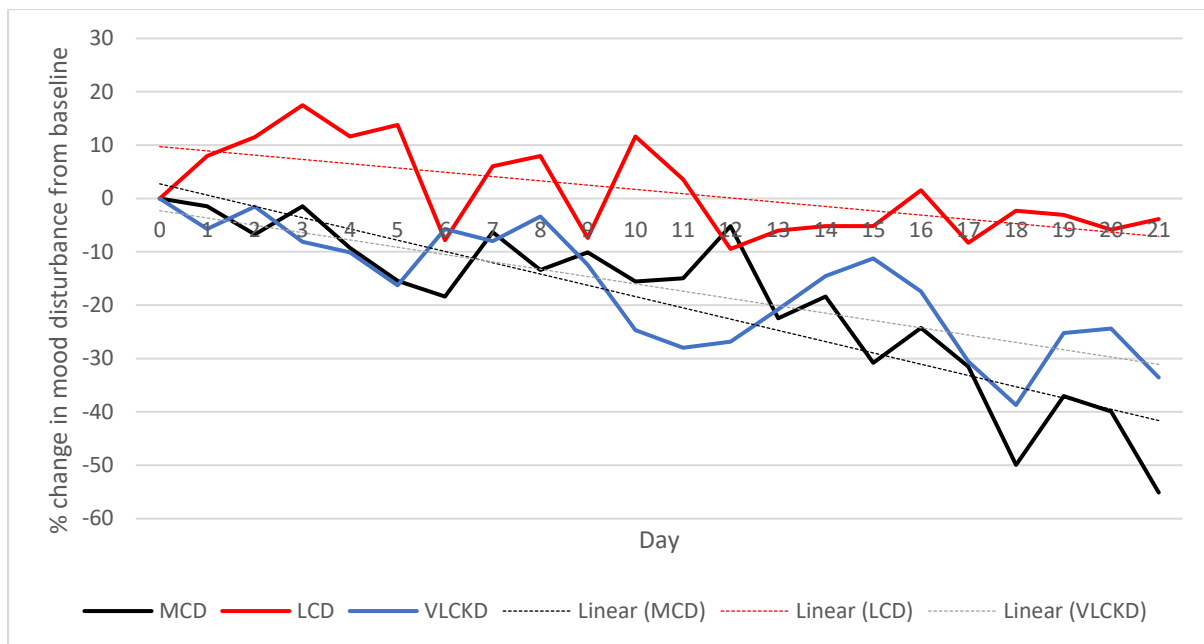


Figure 14. Percent change in mood disturbance by day, per group. Continuous line shows the mean change from baseline per day; the dashed line shows the linear trend. MCD: moderate-low carbohydrate diet; LCD: low-carbohydrate diet; VLCKD: very-low-carbohydrate ketogenic diet

Table 6. Overall mean and group mean changes from baseline for BOHB, symptoms scores and mood impression

Measure	Overall change† Mean change from baseline [95% CI]	Treatment group‡ Mean change from baseline [95% CI]		
		Moderate-low carbohydrate diet	Low carbohydrate diet	Very low carbohydrate ketogenic diet
BOHB (mmol/L)	0.44 [0.34, 0.54] $p < 0.001$	0.27 [0.13, 0.41] $p = 0.013$	0.41 [0.26, 0.57]	0.62 [0.42, 0.82]
Sum of symptoms score	0.81 [0.14, 1.47] $p = 0.018$	0.18 [-1.30, 1.65] $p = 0.264$	0.65 [-1.17, 0.33]	1.49 [0.50, 2.49]
Headache	0.11 [0.00, 0.22] $p = 0.042$	0.12 [-0.09, 0.32] $p = 0.893$	0.08 [-4.29, 1.91]	0.14 [0.01, 0.28]
Constipation	0.17 [0.07, 0.27] $p = 0.002$	0.12 [-0.10, 0.34] $p = 0.268$	0.09 [-0.03, 0.22]	0.28 [0.09, 0.47]
Diarrhoea	0.08 [0.00, 0.15] $p = 0.040$	0.02 [-0.12, 0.14] $p = 0.565$	0.09 [-0.00, 0.18]	0.11 [-0.05, 0.27]
Stomach or intestinal pain	0.04 [-0.05, 0.14] $p = 0.364$	0.09 [-0.14, 0.32] $p = 0.763$	0.00 [-0.12, 0.13]	0.04 [-0.13, 0.21]
Intestinal bloating	-0.19 [-0.34, -0.04] $p = 0.012$	-0.30 [-0.65, 0.06] $p = 0.670$	-0.14 [-0.29, 0.01]	-0.16 [-0.44, 0.13]
Change in breath odour/'nail polish breath'	0.29 [0.15, 0.42] $p < 0.001$	0.03 [-0.18, 0.25] $p = 0.039$	0.36 [0.13, 0.59]	0.43 [0.19, 0.67]
Muscle cramps	0.09 [0.03, 0.14] $p = 0.005$	0.10 [-0.03, 0.23] $p = 0.147$	0.01 [-0.07, 0.09]	0.14 [0.04, 0.24]
Muscle weakness	0.33 [0.23, 0.43] $p < 0.001$	0.30 [0.11, 0.49] $p = 0.005$	0.14 [0.04, 0.24]	0.54 [0.32, 0.75]
Skin rash	-0.02 [-0.07, 0.02] $p = 0.355$	-0.02 [-0.10, 0.06] $p = 0.71$	0.00 [-0.04, 0.04]	-0.04 [-0.15, -0.07]
Difficulty concentrating	-0.02 [-0.14, 0.11] $p = 0.790$	-0.04 [-0.26, 0.19] $p = 0.889$	0.03 [-0.16, 0.21]	-0.04 [-0.30, 0.22]
Light headedness	0.21 [0.11, 0.32] $p < 0.001$	0.16 [-0.04, 0.36] $p = 0.419$	0.16 [-0.04, 0.37]	0.31 [0.14, 0.48]
Craving for sugary or starchy foods	-0.28 [-0.50, -0.05] $p = 0.017$	-0.41 [-0.79, -0.04] $p = 0.708$	-0.18 [-0.59, 0.23]	-0.26 [-0.68, 0.17]
Overall mood impression (how do you feel today?)	-0.18 [-0.30, -0.05] $p = 0.007$	-0.30 [-0.56, -0.05] $p = 0.181$	-0.02 [-0.21, 0.16]	-0.22 [-0.46, 0.02]

BOHB: beta-hydroxybutyrate

Associations between BOHB, symptoms, and mood

In our regression analyses, BOHB was not a significant predictor of symptoms of carbohydrate withdrawal (Beta = 0.019, $p = 0.981$) or overall mood impression (Beta = 0.107, $p = 0.485$). Of the individual symptoms reported, BOHB was a significant predictor of only muscle weakness (Beta = 0.300, $p = 0.015$). However, symptoms of carbohydrate withdrawal were a highly significant predictor of mood (Beta = 0.114, $p < 0.001$)

We performed additional multiple regression analyses to determine any potential associations between baseline cardiometabolic measures and symptoms of carbohydrate withdrawal, and mood disturbance. There were no significant associations between baseline cardiometabolic measures and SOSS or mood disturbance.

Associations between changes in dietary components and mood and symptoms

Mean daily energy intake increased from baseline overall by 87 Kcal \pm 394 ($p = 0.089$). There was no significant between-group for the change in calories from baseline ($p = 0.404$). There was, however, a significant change in mean daily protein from baseline of 24.57 g \pm 36.21 ($p < 0.001$). This observed increase in protein did not differ significantly between groups ($p = 0.339$). There were no significant associations between the changes in mean daily carbohydrate, protein, or fat intake, or the change in proportional (%) macronutrient intakes, and SOSS or mood disturbance.

The change in calories from baseline approached the threshold for significance ($p = 0.087$) as a predictor of SOSS (B = -0.002) and was a significant predictor of mood disturbance (B = -0.0004, $p = 0.022$). For every 100 Kcal increase in total energy, there is a 1% improvement in mood or 0.3% improvement in SOSS. Conversely, reduced mood (i.e. increased mood disturbance score) was a significant predictor of under-eating (Beta -233.78, $p = 0.022$).

Discussion

Principal findings

All diets were functionally ketogenic based on the mean level of BOHB achieved per group but only mean levels of BOHB ≥ 0.5 mmol/L were demonstrated consistently at levels within the thresholds of 95% CI in the VLCKD group. Ketonaemia is proportionate to the degree of carbohydrate restriction. A 5% carbohydrate allocation is consistently ketogenic for almost all

participants, but a low-carbohydrate intervention of 25% or 15% carbohydrate can be ketogenic for some people. Therefore, NK can be achieved by individuals following less restrictive lower-carbohydrate diets, but this effect is not consistent and to ensure NK there should be a higher lipid-to-non-lipid ratio (i.e. < 15% TE derived from carbohydrate).

The adverse effects of carbohydrate withdrawal observed in the literature, headache, constipation, diarrhoea, halitosis, muscle cramps and weakness, and light-headedness were apparent in this study. While symptoms of carbohydrate withdrawal increased concomitant to the magnitude of carbohydrate restriction, the changes from baseline were small, and there was no significant difference between the intervention groups. Mean reported symptoms differed by less than 2 out of a possible score of 48 (range between groups; 3.00 - 4.95), and there is little clinically meaningful difference in adverse effects of carbohydrate withdrawal between diets differing in carbohydrate restriction. Of the symptoms that differed significantly between groups (halitosis and muscle weakness) the difference was also small; less than 0.4 between groups out of a possible 5-point scale. Conversely, intestinal bloating, and craving for sugary and starchy foods reduced significantly. There were also non-significant improvements in skin rash and reductions in concentration difficulties. Therefore, the symptoms of carbohydrate withdrawal commonly referred to as 'keto-flu' might in actuality mostly result from any appreciable carbohydrate restriction. The 'keto-flu' resulting from a VLCKD is limited to the symptoms of breath expression of ketone bodies and a minor increase in muscle weakness. This muscle weakness is likely to be a result of reduced fuel provision while adapting to greater use of fatty acids and ketone bodies for fuel, or due to the transient natriuresis, kaluresis and diuresis observed in early keto-adaptation. (70-73) Adverse effects need to be weighed up against the significant overall improvements in cravings for sugary and starchy foods and reductions in intestinal bloating. Improvements in cravings for sugary foods, in particular, could result in improved compliance to healthier long-term eating behaviours, and reductions in intestinal bloating suggest improvements in digestive function, gut-health, or perhaps microbiome status, hypotheses that future research could explore.

Mood improved significantly from baseline as a result of all dietary interventions but did not differ significantly between groups, and the magnitude overall was small. An improvement

in mood is a positive outcome, although it is unclear whether these improvements resulted from carbohydrate restriction, increased lipids or protein, or simply from dietary change.

There was a small but potentially meaningful association shown between energy intake and both mood and symptoms of carbohydrate withdrawal suggesting that the magnitude of symptoms experienced with dietary change and disturbance in mood could result from energy restriction rather than the magnitude of carbohydrate restriction.

Strengths and weaknesses of the study

This study was, to our knowledge, the first to specifically assess the impact of diets differing in carbohydrate allocation on the achievement of NK and symptoms of carbohydrate withdrawal and mood. There is a common perception that the adverse effects of carbohydrate withdrawal (sometimes called in common parlance 'keto flu') are significant barriers to the adoption and maintenance of a very-low-carbohydrate diet. It was a randomised trial, including food tracking with real-time researcher monitoring and feedback, along with advice and information provided to participants from a competent team with extensive experience in the prescription of LCDs and VLCKDs. As such, we believe it provides a valuable addition to the literature to help inform clinical practice.

The study did not include a control group with a higher carbohydrate allocation consistent with existing dietary guidelines of 45-65% of energy derived from carbohydrate (198) as the larger study in which this was embedded was not designed to compare low-carbohydrate diets with usual care protocols.

It is possible that the results may have been influenced by our chosen cohort, which was relatively healthy and absent diagnosed metabolic disorder. It is not inconceivable that those who might benefit most from a ketogenic diet, might also be those who suffer the worst symptoms of carbohydrate withdrawal and so, further studies in those with metabolic syndrome and diabetes are warranted.

Meanings and implications of the study

This study shows that NK of ≥ 0.5 mmol/L can be achieved with higher intakes of carbohydrate than are typically prescribed for the achievement of ketosis and some individuals will consistently achieve NK with 15-25% TE derived from carbohydrate without

the addition of known ketogenic agents such as MCTs. In this study, participants were advised to not consume MCT oil or coconut oil, which contains a high proportion of MCT, as MCTs have been demonstrated consistently to increase ketonaemia and may shorten time-to-NK. (35, 196) However, overall, NK is most effectively achieved by most people, within a lower carbohydrate allocation of 5% TE from carbohydrate.

There are only minor differences in mood and symptoms of carbohydrate withdrawal in healthy people from diets differing in carbohydrate allocation ranging from 5% to 25% TE from carbohydrate. Therefore, there appears to be little benefit in a more 'moderate' carbohydrate restriction to reduce symptoms of carbohydrate withdrawal (or 'keto-flu') or reduce mood disturbance when compared to a greater restriction in carbohydrate. Clinicians should, therefore, based on the results of this study, prescribe dietary carbohydrate according to need, rather than for the avoidance of adverse effects associated with carbohydrate withdrawal. Conversely, to mitigate the severity of carbohydrate withdrawal symptoms, in the absence of a defined clinical need for the patient to achieve ketosis, a more moderate carbohydrate restriction might be warranted.

Unanswered questions and directions for future research

It is unclear whether these results apply to those with metabolic disorders, and the warrants research to determine the effect of differing dietary allocation of carbohydrate on symptoms of carbohydrate withdrawal and mood for this population. Additional research with larger samples is also warranted to investigate the conclusions of this study further.

Conclusion

Diets differing in carbohydrate allocation between 5% and 25% TE from carbohydrate can be ketogenic and result in mean BOHB ≥ 0.5 mmol/L but this effect varies widely among individuals, and there is a clear effect on ketonaemia and ketosis with greater carbohydrate restriction. The only diet in this study that was consistently ketogenic was the VLCKD consisting of a carbohydrate allocation of 5% TE from carbohydrate. Symptoms of carbohydrate withdrawal increase with greater carbohydrate restriction, but this increase is small, and there is no meaningful difference between low-carbohydrate diets that contain 5-15% TE from carbohydrate, and a more moderate low-carbohydrate diet containing 25% TE from carbohydrate. Mood was similarly improved in all interventions by a small magnitude,

with no significant difference between interventions. A demonstrated association between reduced energy intake and mood disturbance, which also approached the threshold for significance for symptoms of carbohydrate withdrawal suggests that calorie sufficiency might be a better mitigator of mood disturbance and adverse effects of dietary change than carbohydrate restriction.

Chapter 6. Low-carbohydrate diets differing in carbohydrate restriction improve cardiometabolic and anthropometric markers in healthy adults.

This chapter comprises the following paper, published in *Peer J*.

Harvey CJdC, Schofield GM, Zinn C, Thornley S, Crofts C, Merien FLR. Low-carbohydrate diets differing in carbohydrate restriction improve cardiometabolic and anthropometric markers in healthy adults: A randomised clinical trial. *PeerJ* 2018 7, e6273. doi:10.7717/peerj.6273

Author contributions:

CJdCH: 82.5%, GMS: 5%, CZ: 5%, ST: 5%, CC: 1.25%, FLRM: 1.25%

Preface

The study described in the previous chapter demonstrated a small improvement in mood across all low-carbohydrate groups and only a trivial increase in symptoms of carbohydrate withdrawal concomitant to the magnitude of carbohydrate restriction. Because of the lack of meaningful differences in mood and symptoms between groups, it is quite possible that the purported 'keto-flu' is more likely to be a general response to any significant reduction in carbohydrate intake and to overall reduction in calories, whether self-instigated (i.e. as a result of the 'autoregulation' of energy intake often observed in studies of either lower-carbohydrate or higher-protein diets) or by intentional energy-restriction. Therefore, what has been described as 'keto-flu' might, in actuality be symptoms of any appreciable carbohydrate restriction *and* calorie restriction. Because of the trivial observed differences in the outcomes of mood and symptoms of carbohydrate withdrawal, dietary prescription is likely to be better based off of projected outcomes from differing diets, rather than primarily for the avoidance of symptoms of carbohydrate withdrawal. In the study contained within this chapter, cardiometabolic and anthropometric outcomes resulting from diets differing in the allocation

of carbohydrate was analysed in order to determine whether low-carbohydrate diets with greater carbohydrate restriction result in better cardiometabolic and anthropometric outcomes, an area that has not previously been well studied.

Introduction

Low-carbohydrate, high-fat (LCHF) and very low-carbohydrate ketogenic diets (VLCKD) are increasingly used for the management of a range of health conditions, including neurological disorders, obesity, diabetes, metabolic syndrome, and various cancers. (1-11) They are also used widely in the general population for weight-loss and maintenance, (12) with improved satiety and control of hunger frequently reported by those who adhere to these diets. (13-15) Despite the potential offered by LCHF and low-carbohydrate, high-protein (LCHP) diets, there is little evidence for the superiority of greater carbohydrate restriction compared to moderate. Systematic reviews show that despite greater weight- and fat-loss initially, over longer timeframes, when energy intake is restricted, there is little difference in outcomes for weight-loss, total and low-density lipoprotein cholesterol (LDL-c) concentrations between diets that are higher or lower in carbohydrate. (18-22) However, there are greater reductions in fasted glucose concentrations, (21) and greater improvements in high-density lipoprotein cholesterol (HDL-c) and glycated haemoglobin (HbA1c) with greater degrees of carbohydrate restriction. (22) Controversy exists about the nature of low-carbohydrate diets (LCD) and VLCKDs, (28) and definitions for LCDs range from 20-200 g of carbohydrate per day, (29, 30) or up to 40-45% of daily energy from carbohydrate. (31, 32) Definitions for VLCKDs are similarly vague. The accepted definition for nutritional ketosis (NK) in the clinical nutrition field has become the achievement of ≥ 0.5 mmol/L beta-hydroxybutyrate (BOHB), as the majority of people following a VLCKD achieve this level of blood ketones (33), and this threshold has been used by several studies as an indicator of entry into NK. (34, 35) Ketonaemia consistent with NK typically results from diets containing a 3:1 to 4:1 ratio of lipids to non-lipid macronutrients, or at least 75% of calories coming from lipids, very low carbohydrates (often less than 50 g) and low-to-moderate amounts of protein, (36, 37) or diets containing 60%-75% of calories from lipids that include a high proportion of medium chain triglycerides (MCTs). (38, 39) Studies report that adherence is difficult with extreme

carbohydrate restriction, i.e. < 50 g of carbohydrate per day, (22) but insulin-resistant (IR) people may be less likely to adhere to a low-fat, high-carbohydrate diet, compared to those who are more insulin-sensitive (IS). Adherence and weight-loss are similar between both IR and IS participants allocated to a less restrictive low-carbohydrate diet. (67)

Few studies directly compare very low-carbohydrate diets with less extreme carbohydrate-restricted diets. Johnston and colleagues compared the effects of a non-ketogenic low-carbohydrate diet (fat 30% of total energy (TE); carbohydrate 40% of TE) to a ketogenic, low-carbohydrate diet (fat 60% TE; carbohydrate 5%TE) in twenty adults over six weeks, finding that the diets were equally effective in reducing body weight and insulin resistance. (65)

Our hypothesis was that moderate carbohydrate restriction may be easier to maintain, and thus more effective than greater degrees of carbohydrate restriction. The aim of the present study, therefore, is to compare anthropometric and cardiometabolic outcomes between a VLCKD, LCD and moderate-low carbohydrate diet (MCD), containing 5%, 15%, and 25% TE from carbohydrate respectively, in healthy adults.

Materials and Methods

Population

Seventy-seven participants, 25 males, 52 females (mean age: 39 years, range: 25 to 49; mean BMI 27 kg/m², range: 20-39) were recruited between the 7th and 19th of August 2017 and gave written, informed consent to participate in this 12-week, randomised, clinical intervention study. The study took place between 11th September and 10th December 2017. Collection of data and analysis was performed at AUT's Human Potential Centre, Auckland, New Zealand.

Inclusion and exclusion criteria

Participants were required to be healthy and between the ages of 25 and 49 years. Exclusion criteria were; underweight (< 18.5 BMI kg/m²), diagnosed with diabetes, diagnosed with any serious medical condition, having previously following a ketogenic diet, or being a current or former client of any of the researchers in clinical practice.

Ethical approval

The trial was registered by the Australia New Zealand Clinical Trial Registry. (ACTRN12617000421336p). Ethics approval for this study was granted by the Southern Committee of the Health and Disability Ethics Committee of New Zealand. 17/STH/60

Dietary interventions and allocation

Participants completed baseline testing of blood and basic anthropometric measures and a lead-in dietary recording week to identify habitual calorie intake. Participants were randomised by the study statistician to one of three low-carbohydrate diet plans which advised intakes of either 5%, 15%, or 25% of TE from carbohydrate. The randomisation was stratified by gender, using a pre-prepared sequence, with investigators blinded to treatment allocation at baseline and follow-up. Participants were assigned to the next treatment group according to their order of recruitment. The primary researcher responsible for initial statistical analysis was blinded to the treatment group allocation until this analysis had been completed.

Diet plans, which included macronutrient and calorie allocation and a sample menu plan, were individualised to the participant, with energy intake determined by the mean reported energy consumed per day in the lead-in dietary recording week. Advice was given to limit protein intake to 1.4 g/kg/d (weight at baseline testing), consistent with the International Society of Sports Nutrition guidelines for optimal protein intake for performance. (173) This was chosen as an appropriate protein intake that was not likely to unduly influence the study results because the study participants were healthy people, who may also be engaged in physical activity and sports. Participants were advised to adhere as strictly as possible to the energy and macronutrient prescription for the first three weeks of the intervention. For the final nine weeks of the intervention, they were advised to eat *ad libitum* but to adhere as closely as possible to the carbohydrate energy limit for their treatment group as a percentage of their total energy intake. Usual exercise patterns were continued. Dietary intake was recorded by participants in a mobile application (Fat Secret) with the researchers able to obtain real-time entry on a partner mobile application (Fat Secret Pro). Results were monitored for safety and compliance by the primary researcher and research assistants tasked with data-monitoring. Compliance to the dietary allocation was monitored daily by a data monitoring team. Where

non-compliance to the dietary allocation, especially for carbohydrate, was noticed, the participant was notified and offered support and advice. Figure 15 profiles the instructions for the dietary allocations over the 13-week study course.

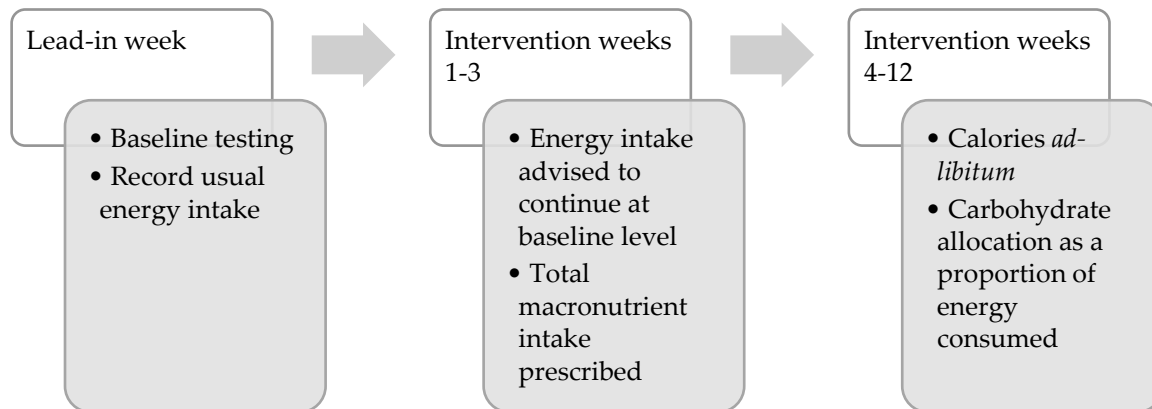


Figure 15. Flow-chart showing instructions for the dietary allocations

Participants were instructed to contact either the clinical nutritionist or the registered dietitian in the research team for any assistance during the study duration.

Anthropometry

The following measures were taken: height, weight, waist circumference at the narrowest point between the lowest rib and the iliac crest, and hip circumference at the widest point of the hips and buttocks. These measures were then used to derive body mass index (BMI), waist-hip ratio, and the waist-height ratio at baseline and during follow-up.

Blood measures

Following an overnight fast, blood samples were obtained from participants, before the first meal, via venipuncture by a certified phlebotomist from an antecubital vein and collected into PST Vacutainer tubes using lithium-heparin as the anticoagulant (Becton Dickinson). Within 15 minutes of collection, tubes were centrifuged at 1500 revolutions per minute for 10 min at +4°C, and plasma samples were transferred into clean polypropylene tubes and frozen at -80°C until analyses were conducted using specific diagnostics assays on a Roche Modular analyser (P800 and E170). Blood samples were analysed for total cholesterol (Total-c), LDL-c, HDL-c, triglycerides (TG), C-reactive protein (CRP), gamma-glutamyl transferase (GGT), alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP),

glucose and uric acid on the P800 module. Insulin and C-peptide concentrations were measured on the E170 module. All analytical biomarkers were measured at baseline and immediately following the 12-week intervention. The total duration of the assay for each analyte was less than 20 min based on the electrochemiluminescence principle (ruthenium-conjugated monoclonal antibodies) for the E170 module and specific enzyme assay methods for the P800 module. Quantitative results were determined via instrument-specific full point calibration curves and validated with specific controls. Additional information for analytes, lower limits of measurement, measuring range, and test principle can be found in appendix 1.

Statistical analyses

Effects of the dietary interventions on outcomes were determined for each participant by calculating the change in the various measures from baseline. The significance of these within-group changes from baseline was determined by a repeated measures t-test. Between-group variations were compared using ANOVA. A 5% two-sided alpha level was used to determine significance. Further comparisons were made by undertaking multiple linear regression with adjustment made for variables recorded at baseline. A sensitivity analysis of the results was carried out using stabilised inverse-probability of completing weights for the BMI change outcome to check whether these results were likely to have been different had the whole group returned for followed-up.

Results

A total of 283 people was assessed for eligibility with 206 excluded and 77 included for randomisation to the trial groups (Figure 16). Ten participants withdrew after they were randomised. Two failed to comply with guidelines to submit baseline data and withdrew from the study (one male, one female), and three females withdrew due to changes in personal circumstances, including two who became pregnant. A further five withdrew due to challenges arising from following the diets. The reasons for withdrawals were as follows: two female participants found the dietary allocation of carbohydrate too difficult to sustain (one each in the 5% and 15% allocation groups); one did not want to continue tracking with the food app; one felt that she could not maintain her sports performance on 15% total energy from carbohydrate; and one female in the 5% allocation group reported amenorrhea and

reductions in strength and power, despite improved mental clarity. A further 28 did not book for or failed to present for post-intervention measurements. This left 39 participants with follow-up results available for analysis.

There were no significant differences in baseline characteristics between completers and non-completers and no meaningful difference in the number of non-completers by group with 50%, 50%, and 48% of participants not completing post-intervention measures in the MCD, LCD, and VLCKD groups respectively. Mean baseline levels of TG were, however, 36% higher at baseline in those lost to follow-up compared to those who were not, even though the difference between the two distributions was not significant ($p = 0.08$). There was also no significant variation for age, gender, or ethnicity between the groups, in the participants analysed. At baseline, blood measures were all within reference ranges except for Total-c which had an overall mean of 5.31 mmol/L (SD = 1.29) for completers, and a significant between-group difference ($p = 0.005$).

Baseline characteristics of those included for analysis are presented in Table 7, by randomised treatment group.

Table 7. Baseline characteristics of study participants

	Treatment group			Total	Test	p-value
	MCD	LCD	VLCKD			
n	12	13	14	39		
Age mean (SD)	39.1 (6.6)	38.9 (8.3)	38.7 (7.1)	38.9 (7.1)	ANOVA	0.992
Gender (%)					Fisher's	0.198
Female	10 (83.3)	6 (46.2)	9 (64.3)	25 (64.1)		
Male	2 (16.67)	7 (53.85)	5 (35.71)	14 (35.9)		
Ethnicity (%)					Fisher's	0.733
Asian	1 (8.3)	0 (0.0)	1 (7.1)	2 (5.1)		
European	8 (66.7)	11 (84.6)	10 (71.4)	29 (74.4)		
Maori	2 (16.7)	1 (7.7)	3 (21.4)	6 (15.4)		
Other ethnicity	1 (8.3)	0 (0.0)	0 (0.0)	1 (2.6)		
Pacific peoples	0 (0.0)	1 (7.7)	0 (0.0)	1 (2.6)		
Total energy (Kcal) mean (SD)	1435 (293)	1567 (666)	1805 (857)	1603 (649)	ANOVA	0.378
Weight (kg) mean (SD)	76.3 (14.9)	90.4 (20.0)	76.8 (11.2)	81.2 (16.6)	ANOVA	0.046
Height (m) mean (SD)	1.70 (0.10)	1.76 (0.08)	1.74 (0.09)	1.73 (0.09)	ANOVA	0.245
BMI (kg/m ²) mean (SD)	26.4 (3.23)	29.1 (4.92)	25.5 (2.77)	27.0 (3.96)	ANOVA	0.050
Glucose (mmol/L) mean (SD)	5.54 (0.43)	5.38 (0.47)	5.44 (0.44)	5.45 (0.44)	ANOVA	0.673
Total cholesterol (mmol/L) mean (SD)	5.20 (1.3)	4.57 (0.61)	6.10 (1.37)	5.31 (1.29)	ANOVA	0.005
Triglyceride (mmol/L) mean (SD)	0.79 (0.2)	0.99 (0.36)	0.92 (0.22)	0.90 (0.27)	ANOVA	0.184
Insulin (pmol/L) mean (SD)	63.1 (37.3)	81.1 (39.4)	41.6 (17.6)	61.4 (35.8)	ANOVA	0.012

SD: standard deviation. BMI: body mass index

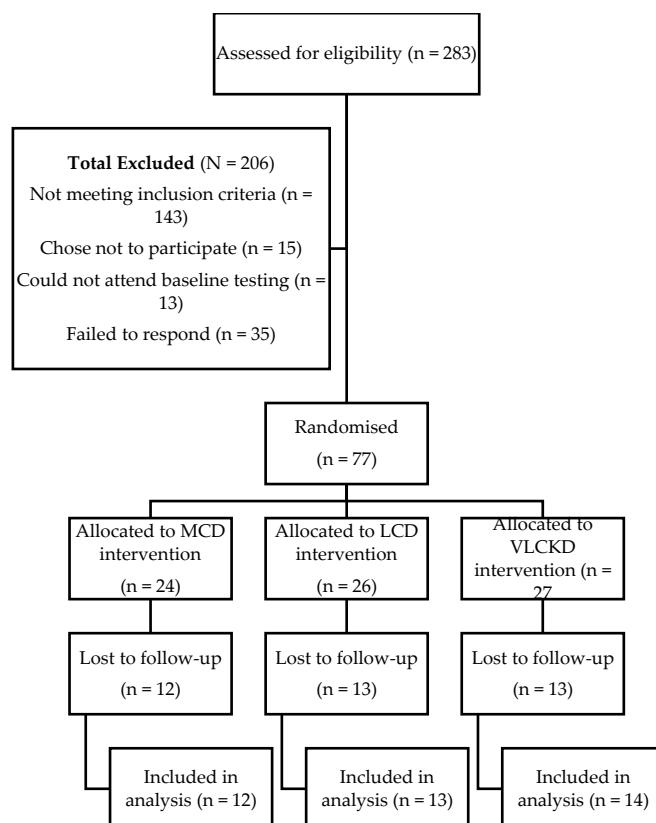


Figure 16. Flow-chart showing participant recruitment, randomisation, allocation, and lost-to-follow-up

Anthropometry

Mean weight and BMI at baseline differed between groups ($p = 0.046$ and 0.050 respectively). The LCD group had the highest starting BMI at baseline of 29.1 kg/m^2 ($SD = 4.9$), followed by MCD ($BMI = 26.4 \text{ kg/m}^2$, $SD = 3.2$). The lowest starting BMI was in the VLCKD group with a mean BMI of 25.5 kg/m^2 ($SD = 2.8$). Overall, there was a significant reduction in weight across all groups ($p < 0.001$). Mean weight loss increased with the magnitude of carbohydrate restriction, with 4.12 kg ($SD = 2.54$), 3.93 kg ($SD = 3.71$), and 2.97 kg ($SD = 3.25$) lost by the VLCKD, LCD, and MCD groups respectively. However, the differences in weight loss between these groups were not statistically significant ($p = 0.626$). Similarly, a highly significant change in BMI of -1.22 kg/m^2 ($SD = 1.03$, $p < 0.001$) was recorded overall. While the reduction in BMI was greater per magnitude of carbohydrate restriction, this difference was not significant ($p = 0.686$).

All dietary interventions led to reductions in both waist and hip girth. There was an overall reduction in waist measurement of 2.85 cm ($SD = 2.99$) and hip girth reduced by 3.43 cm ($SD = 4.67$, $p < 0.001$ for both measures). The reduction in waist measurement girth did not differ

significantly by group ($p = 0.99$) but the change in hip girth approached the threshold for significance ($p = 0.06$). There was a significant change overall to the waist-height ratio (-0.02 , $p < 0.001$) but no significant difference between groups and no significant overall change in the waist-hip ratio. All changes in measures, both overall and by group, with 95% confidence intervals are reported in Table 8.

Blood measures

This paper focuses on the key cardiometabolic outcome measures of Total-c, LDL-c, HDL-c, TG, CRP, glucose, and insulin. Liver enzymes and uric acid were included in the initial analysis as they are emerging markers of interest for metabolic syndrome and insulin resistance. (199, 200) One participant had GGT levels above the reference range upper limit of 60 U/L. This was reduced from baseline to completion; 143 U/L to 106 U/L. Another participant had baseline levels of ALT of 79 U/L which normalised to 30 U/L at completion (reference range upper limit, 45 U/L). Overall, there was no meaningful change in liver enzymes or uric acid and the differences between groups were not significant.

The most meaningful changes observed were for CRP and insulin. CRP was reduced in the MCD and LCD treatment groups overall by -3.90 mg/L (SD = 12.60), and -3.04 mg/L (SD = 3.90), respectively. There was a marginal increase in CRP in the VLCKD group of 0.14 mg/L (SD = 1.10) which we would not consider to be meaningful. While the overall change from baseline CRP approached the threshold for significance ($p = 0.074$), there was no difference between the groups ($p = 0.339$). While at baseline, no significant difference for CRP was present between groups ($p = 0.346$), there were several readings for CRP that were above the reference range upper limit of 5 mg/L. The highest reading of 46.9 mg/L was recorded in the MCD group and there were also three readings > 5 mg/L in the LCD group, with the highest maximal reading of 13 mg/L. Conversely, the maximal recorded value for CRP in the VLCKD group at baseline was 2.6 mg/L. On follow-up, all results were < 5 mg/L.

Insulin concentration was reduced overall by 13.6 pmol/L (SD = 24.8, $p < 0.001$). The greatest change occurred in the LCD group, followed by the VLCKD group, with the smallest change in the MCD group. The difference between groups, however, was not statistically significant ($p = 0.185$).

Statistically significant changes, albeit of a relatively small magnitude, occurred for Total-c, LDL-c, and HDL-c, which were all increased at completion vs baseline, and for TG which were reduced, with no significant variation between groups. No meaningful change from baseline was observed for fasted glucose. There was, however, a significant improvement in the TG-HDL-c ratio of -0.102 (SD = 0.220, $p = 0.006$). This improvement was increased with greater carbohydrate restriction with changes of -0.023 (SD = 0.158), -0.118 (SD = 0.291) and -0.154 (SD = 0.182), for MCD, LCD, and VLCKD respectively ($p = 0.308$).

Large proportional changes from baseline occurred for insulin, TG, Total-c, LDL-c, and HDL-c. Proportional increases from baseline for Total-c and LDL-c were greatest for LCD, followed by VLCKD, and MCD. There was no relative change from baseline for both TG and HDL-c in the MCD group. Improvements in HDL-c and TG occurred for the LCD group, with the greatest proportional change in the VLCKD group. There were relatively minor proportional changes for the remaining measures. (Figure 3.) All changes in reported measures, overall and by group, with 95% confidence intervals, are reported in Table 8.

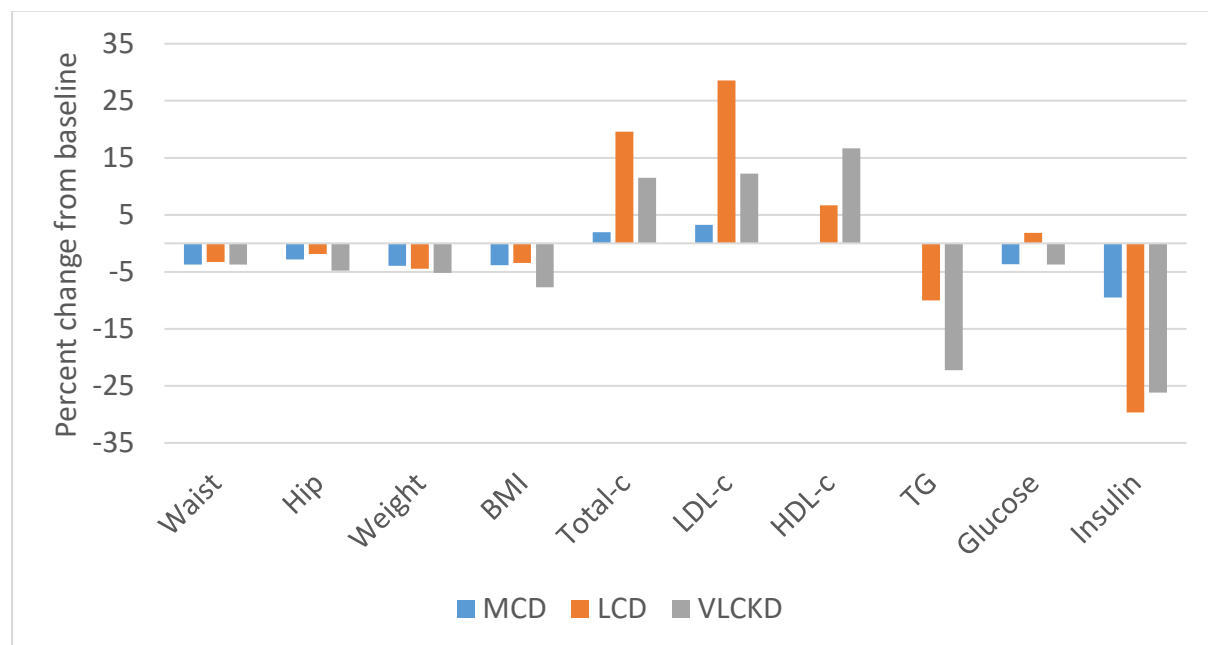


Figure 17. Percent change in outcome measures from baseline
MCD: moderately-low carbohydrate diet. LCD: low-carbohydrate diet. VLCKD: very-low-carbohydrate ketogenic diet. BMI: body mass index. LDL-c: low-density lipoprotein. HDL-c: high-density lipoprotein. TG: triglyceride

Table 8. Change in outcome measures

Measure	Overall change† Mean change from baseline [95% CI]	Treatment group‡ Mean change from baseline [95% CI]		
		Moderate-low carbohydrate diet	Low carbohydrate diet	Very low carbohydrate ketogenic diet
Weight (kg)	-3.70 [-4.72, -2.68] $p < 0.01$	-2.97 [-5.03, -0.90] $p = 0.63$	-3.93 [-6.17, -1.69]	-4.12 [5.58, -2.65]
Waist circumference (cm)	-2.85 [-3.82, -1.88] $p < 0.01$	-2.95 [-5.57, -0.33] $p = 0.99$	-2.80 [-4.62, -0.98]	-2.81 [-3.88, -1.75]
Hip circumference (cm)	-3.43 cm [-4.95, -1.92] $p < 0.01$	-3.56 [-5.00, -2.12] $p = 0.06$	-1.19 [-4.29, 1.91]	-5.40 [-8.34, -2.46]
Waist-height ratio	-0.02 [-0.02, -0.01] $p < 0.001$	-0.02 [-0.03, -0.002] $p = 0.98$	-0.02 [-0.03, -0.006]	-0.02 [-0.02, -0.01]
Waist-hip ratio	-0.003 [-0.016, 0.010] $p = 0.66$	-0.004 [-0.026, 0.018] $p = 0.16$	-0.017 [-0.046, 0.011]	0.011 [-0.008, 0.030]
BMI (kg/m ²)	-1.223 [-1.556, -0.889] $p < 0.001$	-1.031 [-1.757, -0.306] $p = 0.686$	-1.22 [-1.894, -0.546]	-1.39 [-1.899, -0.881]
Total cholesterol (mmol/L)	0.58 [0.11, 1.05] $p = 0.02$	0.08 [-0.57, 0.72] $p = 0.33$	0.94 [0.08, 1.80]	0.68 [-0.33, 1.69]
LDL-c (mmol/L)	0.49 [0.06, 0.92] $p = 0.03$	0.14 [-0.39, 0.67] $p = 0.47$	0.80 [-0.02, 1.62]	0.50 [-0.44, 1.44]
HDL-c (mmol/L)	0.11 [0.00, 0.23] $p = 0.05$	-0.05 [-0.33, 0.24] $p = 0.10$	0.13 [-0.02, 0.27]	0.24 [0.07, 0.42]
Triglycerides (mmol/L)	-0.12 [0.20, -0.02] $p = 0.02$	-0.04 [-0.22, 0.15] $p = 0.41$	-0.09 [-0.27, 0.09]	-0.18 [-0.32, -0.04]
TG-HDL-c ratio	-0.101 [-0.173, -0.030] $p = 0.006$	-0.023 [-0.123, 0.078] $p = 0.31$	-0.118 [-0.294, 0.058]	-0.154 [-0.259, -0.048]
Insulin (pmol/L)	-13.58 [-21.61, -5.56] $p < 0.01$	-6.45 [-23.38, 10.48] $p = 0.19$	-23.68 [-42.49, -4.86]	-10.33 [-17.03, -3.62]
Glucose (mmol/L)	-0.11 [-0.26, 0.04] $p = 0.14$	-0.22 [-0.55, 0.11] $p = 0.20$	0.08 [-0.19, 0.34]	-0.20 [-0.45, 0.04]
C-reactive protein (mg/L)	-2.16 [-4.55, 0.22] $p = 0.07$	-3.90 [-11.90, 4.10] $p = 0.34$	-3.04 [-5.39, -0.68]	0.14 [-0.50, 0.77]

†Mean change from baseline [95% CI]; p -value relates to repeated measures t -test. ‡Mean change from baseline [95% CI]; p -value relates to Anova comparing change from baseline within treatment group. BMI: body mass index. LDL-c: low-density lipoprotein cholesterol. HDL-c: high-density lipoprotein cholesterol

Adherence to diet

The individual mean daily energy intake per group, by week, is shown in Figure 18. A marginal increase in reported energy intake occurred during the first three weeks, during which time participants had been advised to maintain usual energy intake. After the first three weeks, participants had been advised to eat *ad libitum* but preserve the carbohydrate allocation as a percentage of total energy intake. In this phase, the pattern of increased energy over baseline was maintained over most weeks but eventually declined. By week 12 there was an overall reduction in mean energy compared to baseline of 66 Kcal, 95 Kcal, and 192 Kcal for MCD, LCD, and VLCKD respectively. So, overall there was a greater magnitude of energy increase initially with greater carbohydrate restriction, but over time this resulted in a greater reduction in total energy consumed commensurate with the magnitude of carbohydrate restriction. These changes from baseline were relatively small with the greatest magnitude of change from baseline, 12%, 10%, and 18% for MCD, LCD, and VLCKD respectively.

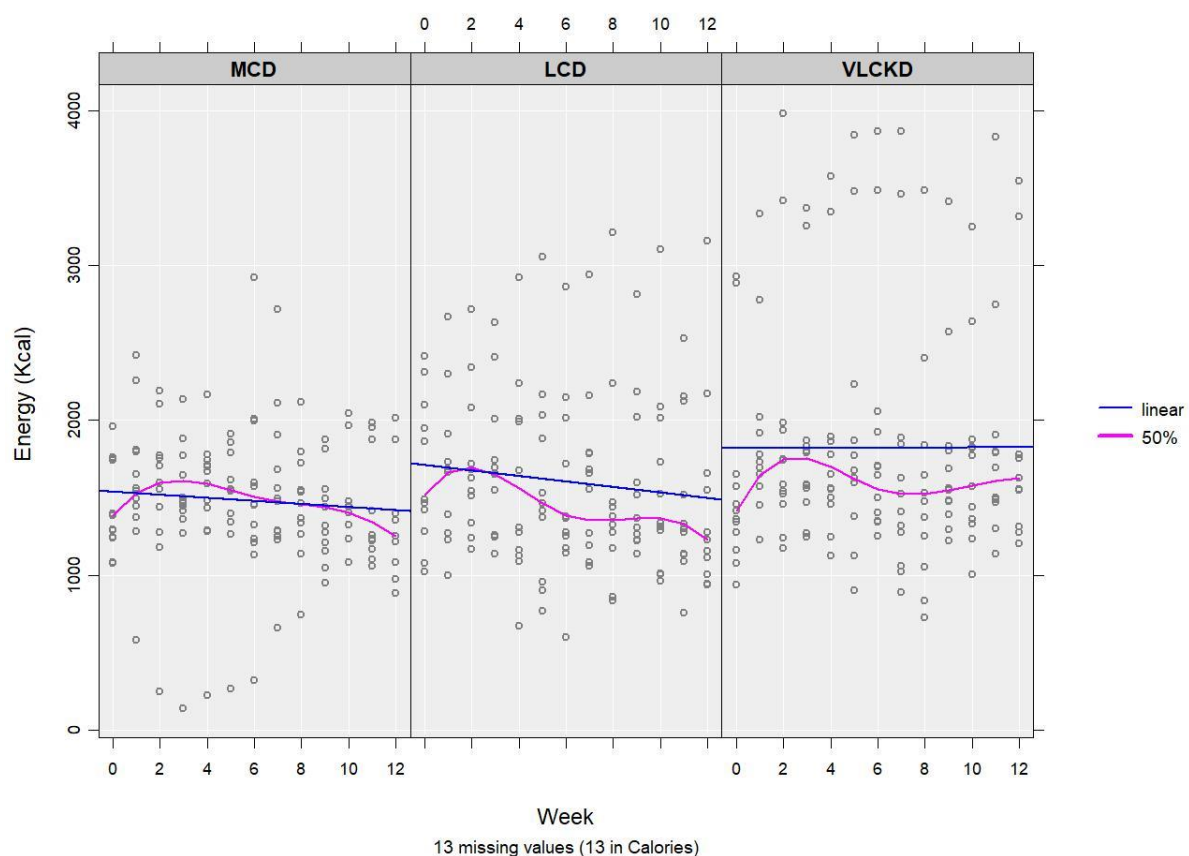


Figure 18. Mean reported daily energy intake (Kcal) per week by group over twelve weeks. The continuous line represents the 50th percentile, whereas the straight (linear) line is a linear regression. MCD: moderately-low-carbohydrate diet. LCD: low-carbohydrate diet. VLCKD: very-low-carbohydrate ketogenic diet

Over 12 weeks, carbohydrate intake by group was less than allocation for both MCD (22.5%, SD = 4.5%) and LCD (14.1%, SD = 3.2%) and higher than allocation for VLCKD (7.9%, SD = 4.9%). A linear trend was observed for reduction in carbohydrate intake as a proportion of TE for MCD relative to week (Beta = -0.137, $p = 0.24$). Conversely increased intake by week was observed for LCD (Beta = 0.096, $p = 0.24$), and VLCKD (Beta = 0.174, $p = 0.15$) but these trends were not significant within groups, or between group allocations ($p = 0.108$). Figure 19 shows the reported energy per participant, derived from carbohydrate per group, by week.

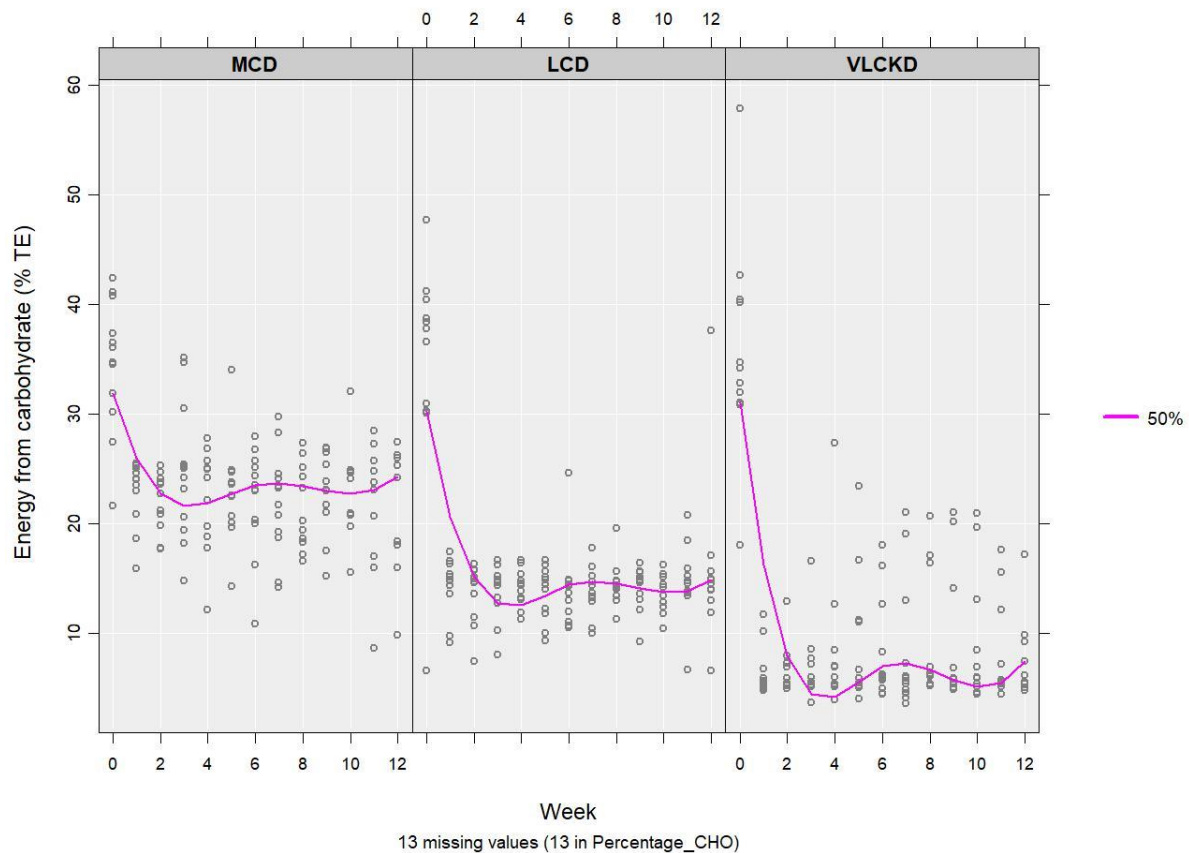


Figure 19. Reported carbohydrate intake as a percentage of TE. The continuous line represents the 50th percentile. MCD, moderately-low-carbohydrate diet; LCD, low-carbohydrate diet; VLCKD, very-low-carbohydrate ketogenic diet

Protein intake did not differ between the groups at baseline ($p = 0.299$). There was no significant variation between groups for average protein intake per day over the course of the study. Fat intake varied by group but was consistent with their total energy intake, and protein and carbohydrate allocations. Additional macronutrient data is presented in appendix 2.

Discussion

Principal findings

Overall, the results demonstrated that reduced carbohydrate diets have a positive effect on select markers of health. Despite a high number of participants who did not present for follow-up testing, in those included for analysis, low-carbohydrate diets were easily adhered to over a 12-week period. While there was little difference in the consistency of adherence between the different dietary interventions for calorie and macronutrient allocations overall, carbohydrate intake was more easily maintained in the MCD and LCD groups, as demonstrated by mean intakes lower than allocation, whereas mean intake of carbohydrate as a percentage of TE was higher than allocation in the VLCKD group. There was a marginal increase in energy intake from baseline, but this declined over the course of the study in all groups. Of interest was the relatively low-calorie intake recorded at baseline which might indicate a cohort focussed on weight loss or under-reporting of actual food intake.

Almost all participants began the study with measurements within the normal range. We would, therefore, not expect large changes for markers of health in a generally 'healthy' cohort. This was also a eucaloric intervention, designed to match habitual energy intake and was not designed as a 'weight loss' trial. Despite this, there were significant and clinically meaningful, albeit relatively small, improvements in weight, waist-height ratio, HDL-c, and TG. Of the changes in outcome measures that reached the threshold for significance, seven of nine were improved from baseline favourably (HDL-c, TG, insulin, weight, waist, hip, and BMI) while only Total-c and LDL-c increased by a small magnitude. Of particular interest, was the improvement in waist-height ratio, as this is a strong predictor of all-cause mortality. (201) We would also consider the significant improvements in HDL-c and TG to be clinically meaningful measures of interest when compared to relatively minor changes in Total-c or LDL-c. Of all the commonly measured biomarkers of cardiovascular risk, TG concentrations are most convincingly linked to incident cardiovascular disease. (82-84) Reductions in relative risk are seen at TG < 1.02 mmol/L, with every 1 mmol/L increase associated with a > 12% increase in risk, for both cardiovascular disease mortality and all-cause mortality. (84) Interestingly, in the current study, while mean TG levels were reduced in all groups at 12-weeks, only the VLCKD group showed an improvement in TG levels, with a reduction of 0.04

mmol/L at the upper limit of the 95% confidence intervals, compared to an increase of 0.16 mmol/L and 0.09 mmol/L for the MCD and LCD groups, respectively. This suggests that the higher the baseline TG, the greater the benefit of carbohydrate restriction. Our weighted regression re-analysis also showed that baseline TG affected the change in BMI relative to treatment group, suggesting the hypothesis that baseline lipids may predict outcomes from diets differing in carbohydrate allocation. This hypothesis will be investigated and reported in a separate paper. There is debate around the respective roles that Total-c, LDL-c, HDL-c, TG, and their interactions play with respect to mortality and morbidity outcomes. This warrants further investigation, especially in the context of reduced carbohydrate diets.

An additional sensitivity analysis was subsequently carried out which modelled the probability of completing the study, given baseline values of age, gender, weight, TG and glucose concentration using a logistic regression model. These values were then used in a re-analysis of the change in BMI at the end of the study with observations re-weighted by stabilised-inverse probability of treatment from the logistic model. This model showed a larger decrease in mean BMI, comparing the VLCKD to the MCD group (mean change from baseline: -0.59 kg/m²; 95% CI: 0.21 to -1.39). This difference from the unweighted analysis is likely to be due to different effects of the diets by baseline TG concentration. These changes will be explored further in a future analysis.

Several CRP readings were above the reference range of < 5 mg/L. The highest reading of 46.9 mg/L, recorded in the MCD group, was found, on subsequent investigation to be due to an unreported flu-like viral infection. At the conclusion of the study, all results for CRP were < 5 mg/L. This suggests a positive effect on systemic inflammation from low-carbohydrate diets overall, but high baseline results may have been due to undisclosed illness or another stressor.

Strengths and weaknesses of the study

This study is one of the first to compare eucaloric diets differing in the magnitude of carbohydrate restriction for anthropometric and cardiometabolic outcomes in healthy people. It was a randomised trial, including food tracking with real-time researcher monitoring and feedback, along with advice and information provided to participants from a competent team with extensive experience in the prescription of LCDs and VLCKDs. As such, we believe it provides a valuable addition to the literature to help inform clinical practice.

Our study was limited by small sample size and by the failure of 49% of participants to either complete the intervention or present for follow-up testing. This was expected, as high dropout rates are common in dietary studies. For example, a systematic review of low-carbohydrate diets vs low-fat, calorie-restricted diet interventions showed an overall attrition rate of 36%, with a higher rate of attrition in low-fat, high-carbohydrate interventions. (23) Few participants reported dropping out due to challenges with the diets and most dropouts were instead due to failure to present for testing rather than a failure to adhere to the diet, and these numbers were almost identical between the intervention groups. Participants who failed to present were asked to provide reasons for (not) doing so. Two participants responded, stating a clash with work and inability to attend due to parental responsibilities. It is therefore unclear whether there were other factors, outside of scheduling or other logistical challenges, that affected participants completing the study.

The final numbers included in our analysis due to attrition, therefore, lacked statistical power. With larger numbers, greater statistical significance may be detected. This will be of value to elucidate the impact of differing magnitudes of carbohydrate restriction on important markers of cardiometabolic health in which there was a between-group difference in change from baseline, for example, TG and HDL-c. The small sample size also highlights a potential problem of applying parametric tests i.e., whether or not the data collected fit the probability distributions associated with them. An alternative that does not rely on such assumptions is a randomisation test. Results from these tests in our study were very similar to those obtained from *t*-tests, for example, the *p*-value for the between-group differences in change from baseline Total-c was $p = 0.658$ which was very similar to the results of the ANOVA, $p = 0.686$.

The study also did not include a group with a higher carbohydrate allocation consistent with existing dietary guidelines of 45-65% of energy derived from carbohydrate, (198) (i.e., a true control group) and therefore, we cannot discount that higher-carbohydrate, lower-fat diets with an emphasis on high-quality food intake, a reduced preponderance of refined, energy-dense foods, nutrition counselling as available in this study, and the accountability of being involved in a study, could lead to similar beneficial results. In the recent DIETFITS study a higher- and lower-carbohydrate intervention, with nutritional counselling and an emphasis on 'quality' nutrition resulted in similar results for weight-loss over twelve months. (81)

However, in this study, there was a non-significant trend towards greater weight loss and statistically significant improvements in HDL-c and TG in the lower-carbohydrate group. In the present study, these were improved in a dose-dependent fashion per carbohydrate restriction. There is already a large body of evidence comparing low- to high-carbohydrate diets, and this study helps to instead differentiate between differing lower-carbohydrate diets and their benefits.

Meanings and implications of the study

The consistency of the improvements in important predictors of mortality suggest a beneficial effect of lower carbohydrate interventions overall, and similarly, towards greater improvement on the most meaningful markers of health, concomitant to the magnitude of carbohydrate restriction. This is of particular interest because the dietary interventions were not hypocaloric and were designed to match habitual energy intake. Yet, despite matching the calorie intake at baseline to the dietary prescription, meaningful anthropometric and blood measures of cardiometabolic health, were improved and trended towards greater (non-significant) improvements with greater carbohydrate restriction. However, the adherence to the carbohydrate allocation was more likely to be achieved in those on more moderate carbohydrate-restricted diets.

Unanswered questions and directions for future research

This study shows positive effects overall from reduced carbohydrate diets on select markers of health and further suggests a potential benefit from a greater magnitude of carbohydrate restriction, despite this greater carbohydrate restriction being more difficult to achieve. Additional research with larger sample sizes is warranted to investigate this further. Due to the large numbers that failed to present for follow-up testing, further investigation is warranted to ascertain factors associated with adherence to the diet.

Conclusion

Low-carbohydrate diets are beneficial for the improvement of anthropometric and blood markers of cardiometabolic health in healthy adults and are easily adhered to over 12-weeks. However, the greatest restriction of carbohydrate to 5% of TE may not be realistically achievable for this population. Our results demonstrate that non-hypocaloric, low-

carbohydrate diets, matched to habitual calorie intake, result in significant improvements in predictors of long-term health including weight, waist and hip girth, waist-to-height ratio, TG and HDL-c, which increase in magnitude with a greater degree of carbohydrate restriction. However, between-group differences typically did not reach thresholds for statistical significance, and further research with larger samples is required to investigate further, the effects of different degrees of carbohydrate restrictions on outcomes in healthy populations.

Chapter 7. Can baseline cardiometabolic markers predict the efficacy of carbohydrate restriction in healthy adults? A pilot study.

This chapter comprises the following paper, submitted to the *Journal of Holistic Performance*:

Harvey CJdC, Schofield GM, Zinn C, Thornley S. Can baseline cardiometabolic markers predict efficacy of carbohydrate restriction in healthy adults? A pilot study. JHolistPerform [Submitted for review]

Author contributions:

CJdCH: 85%, GMS: 5%, CZ: 5%, ST: 5%

Preface

The results presented in the previous chapter showed that while the consistency of adherence overall was similar between groups, the very-low-carbohydrate ketogenic diet (VLCKD) group did have more difficulty adhering to the carbohydrate allocation (5% of total energy intake), consuming on average ~8% TE from carbohydrate. Comparatively, in the low- or moderate-low-carbohydrate groups, mean carbohydrate intake was lower than allocation. Despite the relative inability to achieve the carbohydrate allocation in the VLCKD intervention, and while lacking statistical power because of the sample size and those lost to follow-up, there was a clear trend towards greater improvements in key cardiometabolic measures of health status resulting from a greater magnitude of carbohydrate restriction overall with seven of ten measures most improved by the lowest carbohydrate intervention. Key markers of health such as triglyceride (TG) concentration, high-density lipoprotein cholesterol (HDL-c) and body mass index showed greater improvements resulting from the VLCKD intervention when compared to more moderate restrictions of carbohydrate. There was also variability between individual results, and it was unclear which dietary interventions are best for any given individual due to these variations.

There are purported, but not well-described differences in outcomes dependent on health status, particularly insulin resistance or sensitivity, and we were interested to see whether related, key indicators of cardiometabolic health, including blood and anthropometric measures, are able to predict outcomes from differing dietary allocations of carbohydrate because if this hypothesis was supported, it would provide a valuable tool for the prescription of differing diets in clinical practice.

Introduction

Low-carbohydrate diets (LCDs) and VLCKDs are routinely used for the management of a range of health conditions, including neurological disorders, obesity, diabetes and metabolic syndrome, and various cancers. (1-11) They are also used widely for a range of outcomes in the general population including weight loss and maintenance (12) and improved satiety. (13-15) Despite the potential offered by LCDs, and the common use of these diets, there is little evidence for the superiority of greater carbohydrate restriction compared to moderate restriction, and how benefits might play out for people of different metabolic status.

Systematic reviews show that, despite the greater loss of weight and fat initially from LCDs, over longer timeframes, when calories are equally restricted, there is little difference in outcomes for weight loss, and total or low-density lipoprotein cholesterol (LDL-c). (18-22) However, there is a larger glucose-lowering effect, (21) and greater improvements in HDL-c and glycated haemoglobin (HbA1c) resulting from greater carbohydrate restriction. (22)

Those with greater insulin resistance might adhere better to a low-carbohydrate vs higher-carbohydrate diet, (67) but studies also show that adherence is more difficult with extreme carbohydrate restriction, i.e. < 50 g of carbohydrate per day (22). Therefore, while there is overall little difference between diets containing greater or lesser amounts of carbohydrate, over time there are individuals who are likely to benefit from a greater carbohydrate restriction.

Currently, though, there are few studies that have explored the indicative value of baseline markers to outcomes achieved from differing diets. Several indicators have been proposed. For example, blood type is used by some practitioners as a way to determine food choices for

individuals based on unproven allergic responses to lectins in foods, (202) but no effects of blood-type on the effectiveness of, or outcomes from, any diet have been observed. (203, 204)

Relative insulin homeostasis has also been investigated as a predictor of outcomes from diet and it has previously been demonstrated that those with above median insulin response after an oral glucose challenge (i.e. those more insulin resistant) lose more weight from a lower-carbohydrate diet, while those with below median insulin responses (more insulin sensitive) lose more weight from a higher-carbohydrate, lower-fat diet. (77-79, 205) A pilot trial to investigate these effects in an ad-libitum diet over six-months found increased weight loss resulting from low-carbohydrate diets in insulin-resistant participants and improved weight loss resulting from low-fat diets for insulin sensitive participants. There were also non-significant improvements in HDL-c, TG, fasting glucose and insulin, and blood pressure resulting from the low-carbohydrate diet versus the higher-carbohydrate diet in those more insulin resistant. In those more insulin sensitive, the low-carbohydrate diet improved HDL-c and TG more than that of the low-fat diet, whereas the low-fat diet resulted in improved fasted insulin and glucose. (80) A recent study by Gardner and colleagues demonstrated no significant difference in weight loss over 12 months between a moderate carbohydrate diet with 48% of total energy (TE) from carbohydrate versus a lower carbohydrate diet (30% TE) but significant improvements in HDL-c and TG in the lower-carbohydrate diet group. (81) However, baseline gene markers and insulin homeostasis were not associated with outcomes in either diet group in this study.

We hypothesised that blood measures associated with cardiometabolic health can predict anthropometric and cardiometabolic outcomes relative to diets differing in carbohydrate restriction. The present pilot study aimed compared baseline anthropometric and blood measures of cardiometabolic health, to changes in these markers, in individuals, relative to a twelve-week dietary intervention differing in the magnitude of carbohydrate restriction.

Materials and Methods

Population

Seventy-seven participants, 25 males, 52 females (mean age: 39 years, range: 25 to 49; mean BMI 27 kg/m², range: 20-39) were recruited between the 7th and 19th of August 2017 and gave written, informed consent to participate in a 12-week, randomised, clinical intervention study.

The study took place between the 11th of September and 10th of December 2017. Collection of data and analysis was performed at AUT Human Potential Centre, Auckland, New Zealand.

Inclusion and exclusion criteria

Participants were required to be healthy and between the ages of 25 and 49 years. Exclusion criteria were; underweight (<18.5 BMI kg/m²), diagnosed with diabetes, diagnosed with any serious medical condition, having previously following a ketogenic diet, or current or former clients of any of the researchers in clinical practice.

Ethical approval

The trial was registered by the Australia New Zealand Clinical Trial Registry. ACTRN12617000421336p. Ethics approval for this study was granted by the Southern Committee of the Health and Disability Ethics Committee of New Zealand. 17/STH/60

Dietary interventions and allocation

Participants completed baseline testing of blood and basic anthropometric measures and a lead-in dietary recording week to identify habitual calorie intake. Participants were randomised by the study statistician to one of three low-carbohydrate diet plans which advised intakes of either 5%, 15%, or 25% of TE from carbohydrate. The randomisation was stratified by gender, using a pre-prepared sequence, with investigators blinded to treatment allocation at baseline and follow-up. Participants were assigned to the next treatment group according to their order of recruitment. The primary researcher responsible for initial statistical analysis was blinded to the treatment group allocation until this analysis had been completed.

Diet plans were individualised per participant, with calories determined by the mean calories consumed per day in the lead-in week. Protein was controlled at 1.4 g/kg of body mass per day, consistent with the International Society of Sports Nutrition (ISSN) guidelines for optimal protein intake for performance. (173) Participants were advised to adhere as strictly as possible to the energy and macronutrient prescription for the first three weeks of the intervention. For the final nine weeks of the intervention, they were advised to eat *ad libitum* but to adhere as closely as possible to the carbohydrate energy limit for their treatment group as a percentage of their total energy intake (Figure 20.) Usual exercise patterns were

continued. Dietary intake was recorded by participants in a mobile application (Fat Secret) with the researchers able to obtain real-time entry on a partner mobile application (Fat Secret Pro). Results were monitored for safety and compliance by the primary researcher and research assistants tasked with data-monitoring. Compliance to the dietary allocation was monitored daily by a data monitoring team. Where non-compliance to the dietary allocation, especially for carbohydrate, was noticed, the participant was notified and offered support and advice. Figure 1 profiles the instructions for the dietary allocations over the 13-week study course. Participants were instructed to contact either the clinical nutritionist or the registered dietitian in the research team for any assistance during the study duration.

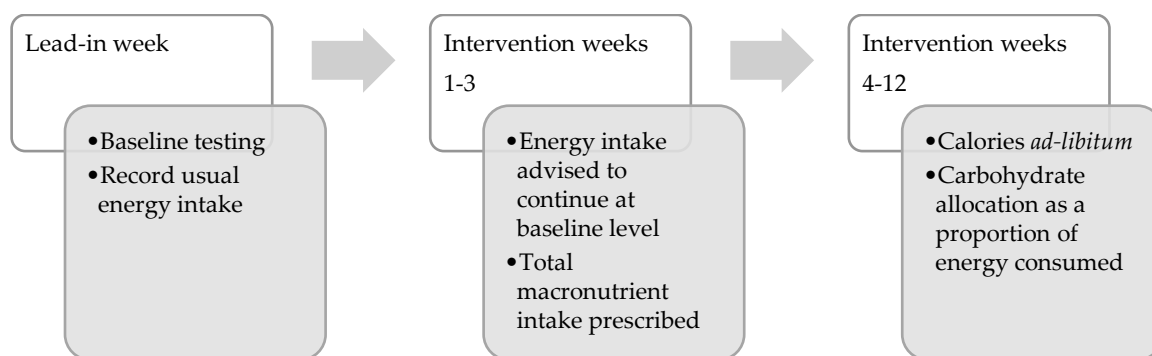


Figure 20. Participant flow of dietary interventions

Blood measures

Following an overnight fast, blood samples were obtained from participants, before the first meal, via venipuncture by a certified phlebotomist from an antecubital vein and collected into PST Vacutainer tubes using lithium-heparin as the anticoagulant (Becton Dickinson). Within 15 minutes of collection, tubes were centrifuged at 1500 revolutions per minute for 10 min at +4°C, and plasma samples were transferred into clean polypropylene tubes and frozen at -80°C until analyses were conducted using specific diagnostics assays on a Roche Modular analyser (P800 and E170). Blood samples were analysed for total cholesterol (Total-c), LDL-c, HDL-c, TG, C-reactive protein (CRP), gamma-glutamyl transferase (GGT), alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), glucose and uric acid on the P800 module. Insulin and C-peptide concentrations were measured on the E170 module. All analytical biomarkers were measured at baseline and immediately following the 12-week intervention. The total duration of the assay for each

analyte was less than 20 min based on the electrochemiluminescence principle (ruthenium-conjugated monoclonal antibodies) for the E170 module and specific enzyme assay methods for the P800 module. Quantitative results were determined via instrument-specific full point calibration curves and validated with specific controls.

Anthropometry

The following measures were taken: height, weight, waist circumference at the narrowest point between the lowest rib and the iliac crest, and hip circumference at the widest point of the hips and buttocks. These measures were additionally used to derive body mass index (BMI) at baseline and during follow-up.

Statistical analyses

The association between baseline blood and anthropometric measures and the changes in these measures were made by undertaking multiple linear regression for the baseline measure and treatment group as independent variables with the change in outcome measures as dependent variables. The hypothesis that the linear relationship between baseline measures and outcome change varied by group was tested for by including an interaction term in the model and evaluating the p -value from a likelihood ratio test. Whether or not this was statistically significant, the results of the model including the interaction term are presented, to explore the hypothesis of whether the baseline-to-change association varied by degree of carbohydrate restriction (treatment group), since the numbers of participants in each group are small and the likelihood ratio may be under-powered. A p -value of less than 0.05 was used as the threshold for significance. Beta coefficients from regression models therefore represent the mean change in outcome associated with a one unit increase in baseline measure.

Results

A total of 283 people was assessed for eligibility with 206 excluded and 77 included for randomisation to the trial groups. Baseline characteristics of study participants are shown in Table 9.

Ten participants withdrew after they were randomised. Two failed to comply with guidelines to submit baseline data and withdrew from the study (one male, one female), and three females withdrew due to changes in personal circumstances, including two who became

pregnant. A further five withdrew due to challenges arising from following the diets: two female participants found the dietary allocation of carbohydrate too difficult to sustain (one each in the 5% and 15% allocation groups). One did not want to use the food app; one felt that she could not maintain her sports performance on 15% TE from carbohydrate; and one female in the 5% allocation group reported amenorrhea and reduced strength and power, despite improved mental clarity. A further 28 failed to present for post-intervention measurements. This left 39 participants with follow-up results available for analysis. (Figure 21.)

There were no significant differences in baseline characteristics between completers and non-completers and no meaningful difference in the number of non-completers by group with 50%, 50%, and 48% of participants not completing post-intervention measures in the MCD, LCD, and VLCKD groups respectively. Mean baseline levels of TG were, however, 36% higher at baseline in those lost to follow-up compared to those who were not, even though the difference between the two distributions was not significant ($p = 0.08$). There was also no significant variation for age, gender, or ethnicity between the groups, in the participants analysed. At baseline, blood measures were all within reference ranges except for Total-c which had an overall mean of 5.31 mmol/L (SD = 1.29) for completers, and a significant between-group difference ($p = 0.005$).

Table 9. Baseline characteristics of study participants

	Treatment group				Test	p-value
	MCD (n=12)	LCD (n=13)	VLCKD (n=14)	Total (n=39)		
Age mean (SD)	39.1 (6.6)	38.9 (8.3)	38.7 (7.1)	38.9 (7.1)	ANOVA	0.992
Gender (%)					Fisher's	0.198
Female	10 (83.3)	6 (46.2)	9 (64.3)	25 (64.1)		
Male	2 (16.67)	7 (53.85)	5 (35.71)	14 (35.9)		
Ethnicity (%)					Fisher's	0.733
Asian	1 (8.3)	0 (0.0)	1 (7.1)	2 (5.1)		
European	8 (66.7)	11 (84.6)	10 (71.4)	29 (74.4)		
Maori	2 (16.7)	1 (7.7)	3 (21.4)	6 (15.4)		
Pacific	0 (0.0)	1 (7.7)	0 (0.0)	1 (2.6)		
Other ethnicity	1 (8.3)	0 (0.0)	0 (0.0)	1 (2.6)		
Total energy (Kcal) mean (SD)	1435 (293)	1567 (666)	1805 (857)	1603 (649)	ANOVA	0.378
Weight (kg) mean (SD)	76.3 (14.9)	90.4 (20.0)	76.8 (11.2)	81.2 (16.6)	ANOVA	0.046
Height (m) mean (SD)	1.70 (0.10)	1.76 (0.08)	1.74 (0.09)	1.73 (0.09)	ANOVA	0.245
BMI (kg/m ²) mean (SD)	26.4 (3.23)	29.1 (4.92)	25.5 (2.77)	27.0 (3.96)	ANOVA	0.050
Glucose (mmol/L) mean (SD)	5.54 (0.43)	5.38 (0.47)	5.44 (0.44)	5.45 (0.44)	ANOVA	0.673
Total cholesterol (mmol/L) mean (SD)	5.20 (1.3)	4.57 (0.61)	6.10 (1.37)	5.31 (1.29)	ANOVA	0.005
Triglyceride (mmol/L) mean (SD)	0.79 (0.2)	0.99 (0.36)	0.92 (0.22)	0.90 (0.27)	ANOVA	0.184
Insulin (pmol/L) mean (SD)	63.1 (37.3)	81.1 (39.4)	41.6 (17.6)	61.4 (35.8)	ANOVA	0.012

SD: standard deviation. BMI: body mass index

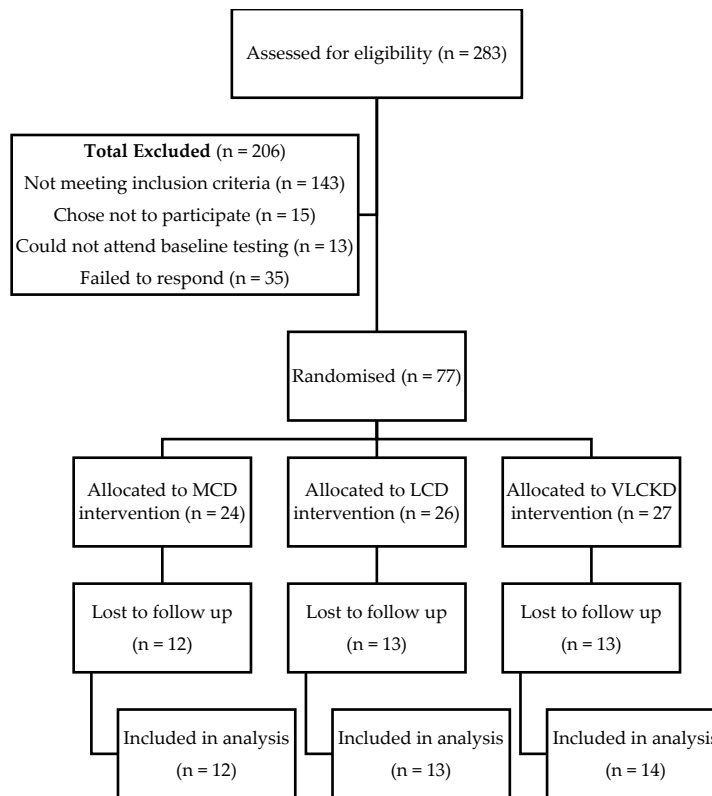


Figure 21. Flow-chart showing participant recruitment, randomisation, allocation and lost-to-follow-up

Associations between baseline and change in outcome measures

Overall, several measures showed significant associations between baseline and change in outcome, including HDL-c, glucose, and weight and hip measurements. Of these measures, the higher the subject's baseline measure, the greater the reduction in HDL-c, which is a less favourable outcome (Figure 22). However, the higher the baseline glucose, weight, and hip, the greater the reduction (favourable outcomes). All results are presented in Tables 10 and 11. There was a trend towards greater improvements to more adverse baseline measures from greater carbohydrate restriction. Seven of 11 blood and anthropometric measures showed the strongest association between baseline and greatest improvement or least worsening in outcome measure, in the VLCKD intervention compared to more moderate carbohydrate restriction.

Only HDL-c reached the threshold for significance between groups, with every 1 mmol/L higher HDL-c recorded at baseline associated with a 0.5 and 0.3 mmol/L decrease in HDL-c for MCD and LCD respectively, and a 0.4 mmol/L increase for VLCKD (Figure 23.) These results were also significant, within-group, for MCD and VLCKD (Table 10.)

Within-group changes were significant in the VLCKD group for glucose (Beta = -0.589, $p = 0.020$) and change in hip measurement (Beta = -0.418, $p = 0.002$).

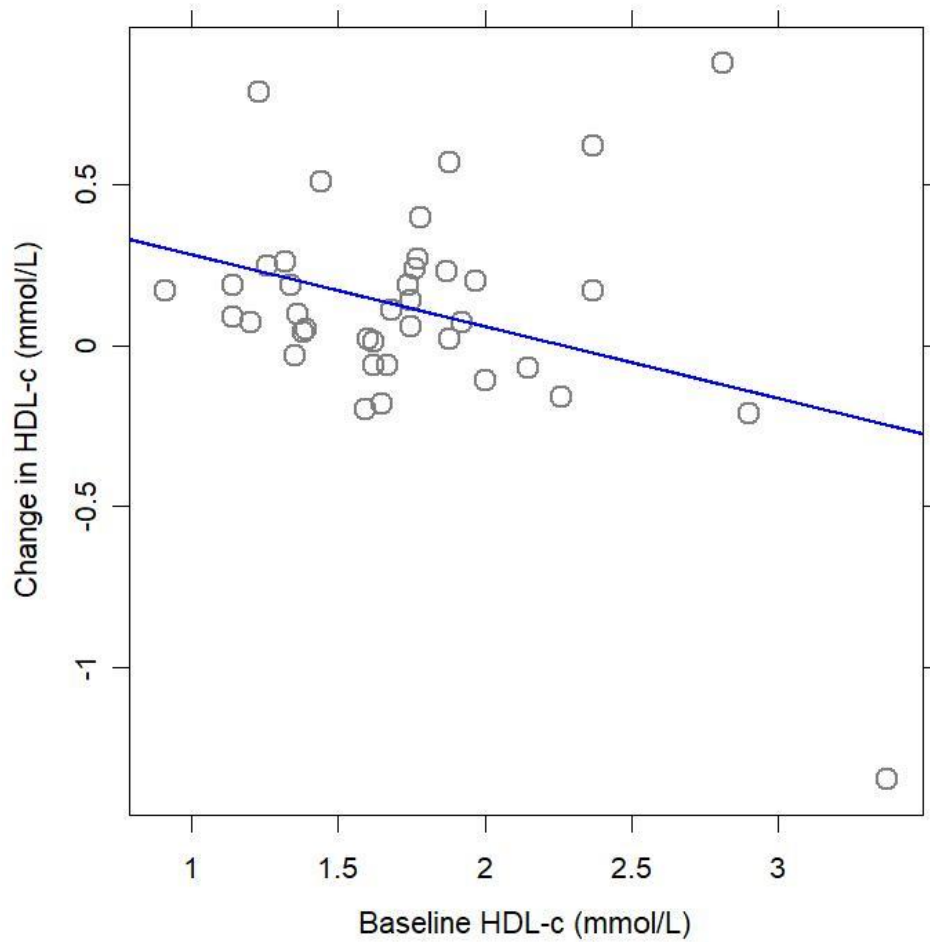


Figure 22. Baseline HDL-c vs change in HDL-c
The blue line shows the linear regression. HDL-c: high-density lipoprotein cholesterol

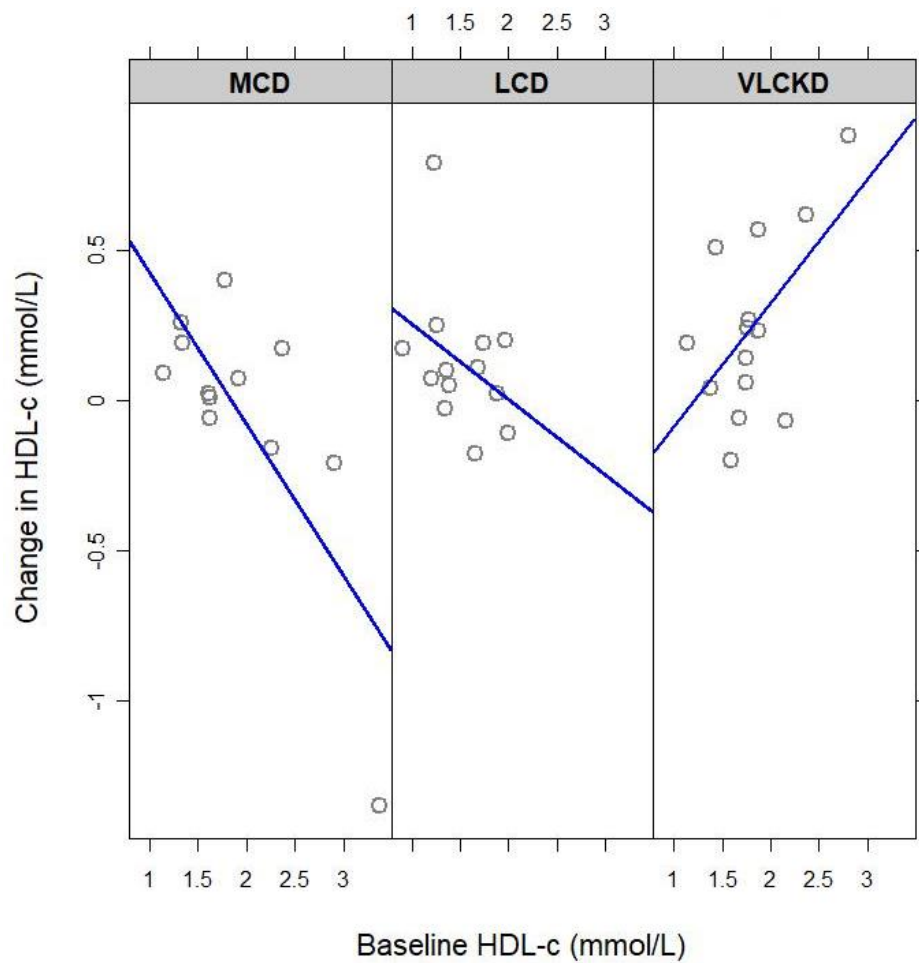


Figure 23. Baseline HDL-c vs change in HDL-c by group
The blue line shows the linear regression. HDL-c: high-density lipoprotein cholesterol; MCD: Moderate-low carbohydrate diet; LCD: Low-carbohydrate diet; VLCKD: Very-low-carbohydrate ketogenic diet

Table 10. Association between baseline blood measures and magnitude of change over twelve weeks by group

Baseline association to 12-week Δ	Treatment group Beta coefficient and p -value		
	Moderate-Low Carbohydrate	Low Carbohydrate	Very Low Carbohydrate
Total cholesterol (mmol/L)	Overall $\beta = -0.224^\dagger$; $p = 0.225$ -0.257 $p = 0.30$ $p = 0.887^\ddagger$	-0.578 $p = 0.41$	-0.209 $p = 0.58$
LDL-c (mmol/L)	Overall $\beta = -0.262^\dagger$; $p = 0.173$ 0.042 $p = 0.86$ $p = 0.360^\ddagger$	-0.866 $p = 0.27$	-0.532 $p = 0.17$
HDL-c (mmol/L)	Overall $\beta = -0.224^\dagger$; $p = 0.042^{**}$ -0.505 $p = 0.004^{**}$ $p = 0.0006^\ddagger$ **	-0.252 $p = 0.23$	0.413 $p = 0.034^{**}$
Triglycerides (mmol/L)	Overall $\beta = -0.001^\dagger$; $p = 0.99$ 0.715 $p = 0.108$ $p = 0.135^\ddagger$	0.027 $p = 0.92$	-0.389 $p = 0.21$
Insulin (pmol/L)	Overall $\beta = -0.024^\dagger$; $p = 0.83$ 0.261 $p = 0.242$ $p = 0.394^\ddagger$	-0.052 $p = 0.830$	-0.215 $p = 0.26$
C-peptide (nmol/L)	Overall $\beta = -0.144^\dagger$; $p = 0.160$ 0.083 $p = 0.64$ $p = 0.448^\ddagger$	-0.122 $p = 0.62$	-0.346 $p = 0.069^*$
Glucose (mmol/L)	Overall $\beta = -0.447^\dagger$; $p = 0.008^{**}$ -0.301 $p = 0.43$ $p = 0.745^\ddagger$	-0.353 $p = 0.21$	-0.589 $p = 0.020^{**}$

* = $p < 0.1$; ** = $p < 0.05$

† β refers to the beta coefficient of the overall linear regression between measure at baseline and change in outcome measure.

‡ This p -value relates to a regression model of the baseline measure and treatment group as independent variables and change in the outcome as dependent variables. The p -value relates to the interaction term, testing for a significant difference in the baseline-change in outcome by treatment group.

Table 11. Association between baseline anthropometric measures and magnitude of change over twelve weeks by group

Baseline association to 12-week Δ	Treatment group Beta coefficient and p -value		
	Moderate-Low Carbohydrate	Low Carbohydrate	Very Low Carbohydrate
BMI	Overall $\beta = -0.031^\dagger$; $p = 0.47$		
	-0.058 $p = 0.62$ $p = 0.610^\dagger$	-0.004 $p = 0.96$	-0.123 $p = 0.16$
Weight (kg)	Overall $\beta = -0.051^\dagger$; $p = 0.095^*$		
	-0.077 $p = 0.26$ $p = 0.900^\dagger$	-0.053 $p = 0.34$	-0.030 $p = 0.65$
Waist (cm)	Overall $\beta = 0.012^\dagger$; $p = 0.79$		
	0.118 $p = 0.42$ $p = 0.508^\dagger$	-0.004 $p = 0.96$	-0.061 $p = 0.44$
Hip (cm)	Overall $\beta = -0.260^\dagger$; $p = 0.003^{**}$		
	-0.130 $p = 0.23$ $p = 0.351^\dagger$	0.271 $p = 0.12$	-0.418 $p = 0.002^{**}$

* = $p < 0.1$; ** = $p < 0.05$

† β refers to the beta coefficient of the overall linear regression between measure at baseline and change in outcome measure.

‡ This p -value relates to a regression model of the baseline measure and treatment group as independent variables and change in the outcome as dependent variables. The p -value relates to the interaction term, testing for a significant difference in the baseline-change in outcome by treatment group.

Discussion

Principal findings

There were significant, greater overall improvements in cardiometabolic health markers occurred in those with more adverse measures at baseline. This association was more exaggerated in those who were allocated to the more restricted carbohydrate interventions. This suggests that those wanting to improve HDL-c, blood glucose, weight, and hip measures especially benefit most from a reduced carbohydrate dietary intervention. There is also an overall trend towards the improvement of cardiometabolic measures of health relative to carbohydrate allocation and that those with poorer baseline markers of health, might improve these most effectively with greater reductions in carbohydrate, while those with 'better' baseline markers could benefit more from a lesser carbohydrate restriction. Of 11 measures, 7 were most improved relative to baseline by the VLCKD intervention and although these variables are not independent, if these effects were random, we would expect ~ 3-4 of 11 of these outcomes to show the greatest improvements resulting from VLCKD. In the only

measure to reach between-group statistical significance, poorer HDL at baseline was most improved by MCD, followed by LCD and VLCKD. However, the greatest magnitude of improvements was observed in the VLCKD group and the number of participants to have worsened outcomes for HDL-c were identical between the three groups.

Strengths and weaknesses of the study

The present study is one of the first to compare diets differing in the magnitude of carbohydrate restriction for cardiometabolic outcomes in healthy people and to investigate the possible predictive value of baseline blood measures for 'best-fit' to dietary prescription. As such, it provides an important addition to the literature to help inform clinical practice.

Our study was limited by relatively small sample size and by withdrawals. The sample size of 39 is likely to be too small to justify the use at this time of baseline cardiometabolic markers in isolation for the prescription of diets differing in carbohydrate content. However, the trend towards greater improvements resulting from very-low-carbohydrate diets for those with 'worse' baseline measures of cardiometabolic health suggests both their predictive, clinical use and the need for further research in this area.

The magnitude of any associations between baseline markers and changes could have also been affected by our chosen cohort as this was, according to our inclusion and exclusion criteria, a healthy cohort, absent from metabolic or other health conditions. Almost all participants began the study with anthropometric and blood measurements within the normal range. We would, therefore, not expect large changes for markers of health in a generally 'healthy' cohort. This was also a eucaloric intervention, designed to match habitual energy intake and was not designed as a 'weight loss' trial. The study also did not include a control group containing higher carbohydrate allocation consistent with existing dietary guidelines (i.e. 45-65 % of energy derived from carbohydrate) (206) and so, we cannot completely discount that higher-carbohydrate, lower-fat diets might exhibit differences to the trends shown in this study.

Meanings and implications of the study

The trend towards greater improvements in outcomes from lower-carbohydrate diets when compared to cardiometabolic measures at baseline suggest a potential role for the

cardiometabolic profile as a predictor of efficacy of diets differing in carbohydrate content. Or, that the 'better' the baseline cardiometabolic markers, the more 'carbohydrate tolerant' someone might be.

Unanswered questions and directions for future research

This study suggests that those with 'worse' baseline measures of cardiometabolic health benefit most from low-carbohydrate diets overall and that there might be additional benefits resulting from larger reductions in carbohydrate for those with poorer cardiometabolic markers. However, in the most significant finding a moderately carbohydrate-restricted diet most improved the worst baseline measures for HDL-c. However, due to the small sample size and results that failed to reach the threshold for statistical significance, additional research is warranted to validate this hypothesis.

Conclusion

Overall, there is a consistent association between baseline markers of cardiometabolic health and changes in these markers relative to the amount of carbohydrate included in the diet. However, low HDL-c might be improved most by a moderate restriction of carbohydrate to ~25% of TE when compared to greater carbohydrate restriction. Because most results were not significant due to the small sample size and preliminary nature of this study, further research is required with larger cohorts to investigate this hypothesis further.

Chapter 8. Can a 'carbohydrate tolerance questionnaire' predict outcomes from diets differing in carbohydrate content? A pilot study.

This chapter comprises the following paper, submitted to the *Journal of Holistic Performance*:

Harvey CJdC, Schofield GM, Zinn C, Thornley S. Can a 'carbohydrate tolerance questionnaire' predict outcomes from diets differing in carbohydrate content? A pilot study. JHolistPerform [Submitted for review]

Author contributions:

CJdCH: 85%, GMS: 5%, CZ: 5%, ST: 5%

Preface

The results of the previous pilot study suggest that there might be some predictive value from key cardiometabolic markers for the allocation of differing lower-carbohydrate diets to individuals. There was a trend towards the 'worseness' of measures at baseline indicating the use of greater carbohydrate restriction, with the exception of high-density lipoprotein cholesterol (HDL-c). Overall, the trend was towards poorer baseline measures being indicative of the use of a lower-carbohydrate vs more moderate carbohydrate regimen, but this needs to be investigated further.

While blood measures provide a relatively accessible and cost-effective method for helping to determine dietary allocation, they may not always be immediately available to a nutrition practitioner and they require either patient-funded blood testing or referral to the patient's medical practitioner for subsequent referral of testing. This increases the burden of both monetary and time cost to the patient. There are, however, common signs observed in the clinical consultation process that might indicate an individual's 'carbohydrate tolerance' to and thereby aid the prescription of the most appropriate diet ancillary to or in the absence of further testing. This paper explores whether a 'carbohydrate tolerance questionnaire' of

commonly observed signs that might relate to an individual's carbohydrate tolerance, could predict outcomes from diets differing in carbohydrate allocation to help clinicians determine the dietary allocation of carbohydrate.

Introduction

Low-carbohydrate diets (LCDs) and very low-carbohydrate ketogenic diets (VLCKDs) are routinely used for the management of a range of health conditions, including neurological disorders, obesity, diabetes, other conditions on the spectrum of metabolic syndrome, and various cancers. (1-11) They are also used widely in the general population to achieve weight-loss and maintenance, (12) improve satiety, and reduce hunger. (13-15) Despite the potential offered by LCDs, and the common use of these diets by the general public, there is little evidence for the superiority of greater carbohydrate restriction compared to more moderate restriction both overall, and for whom greater restriction might be more effective.

Furthermore, there is little research available to support the use of tools to guide the degree of carbohydrate restriction for individual patients. For example, while it has been suggested that a 'metabolic type' with a physiological preference to oxidation of protein, carbohydrate or a 'mixed type' can be indicated by a simple dietary and lifestyle questionnaire, (207) a pilot trial of rugby players in New Zealand found that test results did not match up with laboratory analysis of fat and carbohydrate oxidation rates. (208) To our knowledge, there is also no accepted or validated questionnaire that might indicate the usefulness of diets differing in carbohydrate restriction for the improvement of anthropometric or cardiometabolic measures of health.

The present pilot study aimed to evaluate changes in cardiometabolic and anthropometric measures, and mood and symptoms of carbohydrate withdrawal, resulting from a twelve-week dietary intervention differing in the magnitude of carbohydrate restriction, relative to baseline scoring on a carbohydrate tolerance questionnaire (CTQ). We hypothesised that those with a higher 'carbohydrate intolerance score' (CIS) at baseline would benefit more from greater carbohydrate restriction.

Materials and Methods

Population

Seventy-seven participants, 25 males, 52 females (mean age: 39 years, range: 25 to 49; mean BMI 27 kg/m², range: 20-39) were recruited between the 7th and 19th of August 2017 and gave written, informed consent to participate in this 12-week, randomised, clinical intervention study. The study took place between 11th September and 10th December 2017. Collection of data and analysis was performed at AUT's Human Potential Centre, Auckland, New Zealand.

Inclusion and exclusion criteria

Participants were required to be healthy and between the ages of 25 and 49 years. Exclusion criteria were; underweight (< 18.5 BMI kg/m²), diagnosed with diabetes, diagnosed with any serious medical condition, having previously following a ketogenic diet, or being a current or former client of any of the researchers in clinical practice.

Ethical approval

The trial was registered by the Australia New Zealand Clinical Trial Registry. (ACTRN12617000421336p). Ethics approval for this study was granted by the Southern Committee of the Health and Disability Ethics Committee of New Zealand. 17/STH/60

Dietary interventions and allocation

Participants completed baseline testing of blood and basic anthropometric measures and a lead-in dietary recording week to identify habitual calorie intake. The study statistician prepared a randomised sequence to one of three low-carbohydrate diet plans which advised intakes of either 5%, 15%, or 25% of total energy (TE) from carbohydrate. The randomisation was stratified by gender, with investigators blinded to treatment allocation at both baseline and follow-up. Participants were assigned to the next treatment group according to their order of recruitment. The primary researcher responsible for initial statistical analysis was blinded to the treatment group allocation until this analysis had been completed.

Diet plans, which included macronutrient and calorie allocation and a sample menu plan, were individualised to the participant, with energy intake determined by the mean reported energy consumed per day in the lead-in dietary recording week. Advice was given to limit

protein intake to 1.4 g/kg body mass, per day (weight at baseline testing), consistent with the International Society of Sports Nutrition guidelines for optimal protein intake for performance. (173) Participants were advised to adhere as strictly as possible to the energy and macronutrient prescription for the first three weeks of the intervention. For the final nine weeks of the intervention, they were advised to eat *ad libitum* but to adhere as closely as possible to the carbohydrate energy limit for their treatment group as a percentage of their TE. Usual exercise patterns were continued. Dietary intake was recorded by participants in a mobile application (Fat Secret) with the researchers able to obtain real-time entry on a partner mobile application (Fat Secret Pro). Results were monitored for safety and compliance by the primary researcher and research assistants tasked with data-monitoring. Figure 24 profiles the instructions for the dietary allocations over the 13-week study course.

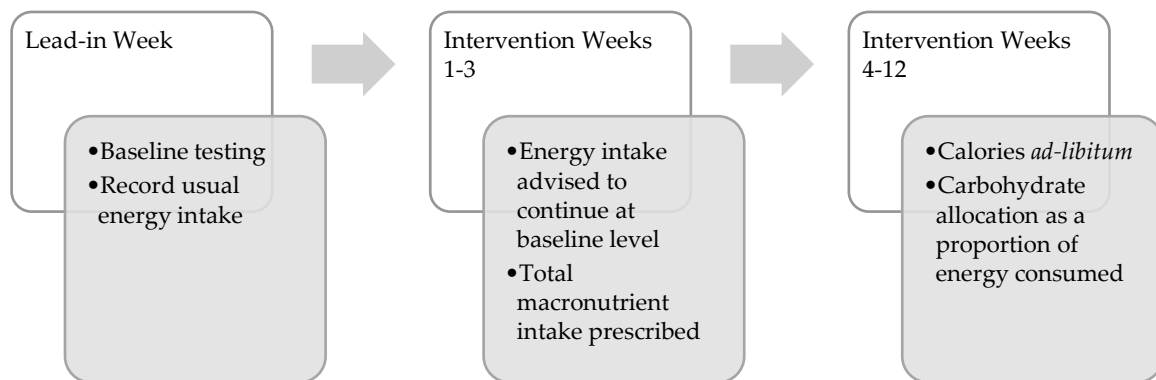


Figure 24. Flow-chart showing instructions for the dietary allocations

Participants were instructed to contact either the clinical nutritionist or the registered dietitian in the research team for any assistance during the study duration.

Carbohydrate Tolerance Questionnaire

The CTQ was compiled from qualitative indicators of expected carbohydrate ‘tolerance’ from the experience of the authors, with additional input from industry colleagues. The questionnaire was put through a 4-stage process of content analysis and peer review with a cohort of academics and experienced nutrition practitioners. This involved the creation of the questionnaire by the primary and tertiary authors, followed by feedback and additions from the rest of the research team, additional peer-review by two additional practitioner-researchers, and final adjustment by the research team. The CTQ included the following statements: *When I gain weight, I tend to put it on my tummy / around my middle, If I don’t eat*

regularly / every few hours I suffer energy 'crashes', or mood / mental disturbance [i.e. 'hangry'], I crave sweet and/or starchy foods often, I snack on sugary or starchy food to relieve headaches/irritability/ craving/excessive hunger; ranked on a 5-point Likert scale (Not at all, Seldom, Occasionally, Often, Almost always). These results were ranked from 1 to 5 and added to create a combined CIS out of a total possible score of 20. The greater the CIS score, the greater the expected carbohydrate 'intolerance'. The CTQ was administered to participants at baseline.

Anthropometry

The following measures were taken: height (m), weight (kg), waist circumference (cm) at the narrowest point between the lowest rib and the iliac crest, and hip circumference (cm) at the widest point of the hips and buttocks. These measures were then used to derive BMI, waist-hip ratio, and the waist-height ratio. Records were taken at both baseline and at the end of follow-up.


Blood measures

Following an overnight fast, blood samples were obtained from participants, before the first meal, via venipuncture by a certified phlebotomist from an antecubital vein and collected into PST Vacutainer tubes using lithium-heparin as the anticoagulant (Becton Dickinson). Within 15 minutes of collection, tubes were centrifuged at 1500 revolutions per minute for 10 min at +4°C, and plasma samples were transferred into clean polypropylene tubes and frozen at -80°C until analyses were conducted using specific diagnostics assays on a Roche Modular analyser (P800 and E170). Blood samples were analysed for total cholesterol (Total-c), low-density lipoprotein cholesterol (LDL-c), HDL-c, triglycerides (TG), C-reactive protein (CRP), gamma-glutamyl transferase (GGT), alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), glucose and uric acid on the P800 module. Insulin and C-peptide concentrations were measured on the E170 module. All analytical biomarkers were measured at baseline and immediately following the 12-week intervention. The total duration of the assay for each analyte was less than 20 min based on the electrochemiluminescence principle (ruthenium-conjugated monoclonal antibodies) for the E170 module and specific enzyme assay methods for the P800 module. Quantitative results were determined via instrument-specific full point calibration curves and validated with specific controls.

Mood and symptoms questionnaires

Participants were instructed to complete a questionnaire including one relating to keto-induction symptoms (Symptom-Q) and a simplified 5-point scale indicator of mood state (Figure 25) developed by the lead author. The Symptom-Q was developed based on published reports of symptoms observed in the early phase of subjects starting a ketogenic diet. One question was asked (“In the past 24 hours to what extent have you experienced the following symptoms?”) for any of: headache, constipation, diarrhoea, stomach or intestinal pain, intestinal bloating, change in breath odour, muscle cramps, muscle weakness, skin rash, difficulty concentrating, light-headedness, and craving for sugary or starchy foods. These responses were reported on a 5-point Likert scale, and scored as 0) Not at all, 1) Mild, 2) Moderate, 3) Severe, and 4) Intolerable. Individual symptoms scores were added to form an overall sum of symptoms scores (SOSS) between 0 and 48 for analysis.

15 → Overall, how do you feel today?*



1	2	3	4	5
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Figure 25. 5-point mood disturbance scale

Statistical analyses

The association between CIS and the change from baseline in anthropometric and cardiometabolic markers, and mood and symptoms of carbohydrate withdrawal, were made by undertaking multiple linear regression for the CIS and treatment group as independent variables with the change in outcome measures as dependent variables. The hypothesis that the linear relationship between CIS and outcome change varied by group, was tested for by

including an interaction term in the model and evaluating the p -value from a likelihood ratio test. Whether or not this was statistically significant, the results of the model including the interaction term are presented, to explore the hypothesis of whether the CIS-outcome association varied by degree of carbohydrate restriction (treatment group), since the numbers of participants in each group are small, and the likelihood ratio may be under-powered. A p -value of less than 0.05 was used as the threshold for significance. Beta coefficients from regression models, therefore, represent the mean change in outcome associated with a one unit increase in CIS.

Results

A total of 283 people was assessed for eligibility with 206 excluded and 77 included for randomisation to the trial groups (Figure 26). Ten participants withdrew after they were randomised. Two failed to comply with guidelines to submit baseline data and withdrew from the study (one male, one female), and three females withdrew due to changes in personal circumstances, including two who became pregnant. A further five withdrew due to challenges arising from following the diets: two female participants found the dietary allocation of carbohydrate too difficult to sustain (one each in the 5% and 15% allocation groups). One did not want to use the food app; one felt that she could not maintain her sports performance on 15% total energy from carbohydrate; and one female in the 5% allocation group reported amenorrhea and reduced strength and power, despite improved mental clarity. A further 28 failed to present for post-intervention measurements. This left 39 participants with follow-up results available for analysis.

There were no significant differences in baseline characteristics between completers and non-completers and no meaningful difference in the number of non-completers by group with 50%, 50%, and 48% of participants not completing post-intervention measures in the MCD, LCD, and VLCKD groups, respectively. The CIS did not differ significantly between groups ($p = 0.129$) but did differ between individuals at baseline, as did all subscales, suggesting that the measures used could show validity (all results $p < 0.001$).

Mean baseline levels of TG were, however, 36% higher at baseline in those lost to follow-up compared to those who were not, even though the difference between the two distributions was not significant ($p = 0.08$). There was also no significant variation for age, gender, or

ethnicity between the groups, in the participants analysed. At baseline, blood measures were all within reference ranges except for Total cholesterol (Total-c) which had an overall mean of 5.31 mmol/L (SD = 1.29) for completers, and a significant between-group difference ($p = 0.005$).

Baseline characteristics of those included for analysis are presented in Table 12, by randomised treatment group.

Table 12. Baseline characteristics of study participants

	Treatment group			Total (n=39)	Test	p-value
	MCD (n=12)	LCD (n=13)	VLCKD (n=14)			
Age mean (SD)	39.1 (6.6)	38.9 (8.3)	38.7 (7.1)	38.9 (7.1)	ANOVA	0.992
Gender (%)					Fisher's	0.198
Female	10 (83.3)	6 (46.2)	9 (64.3)	25 (64.1)		
Male	2 (16.67)	7 (53.85)	5 (35.71)	14 (35.9)		
Ethnicity (%)					Fisher's	0.733
Asian	1 (8.3)	0 (0.0)	1 (7.1)	2 (5.1)		
European	8 (66.7)	11 (84.6)	10 (71.4)	29 (74.4)		
Maori	2 (16.7)	1 (7.7)	3 (21.4)	6 (15.4)		
Pacific	0 (0.0)	1 (7.7)	0 (0.0)	1 (2.6)		
Other ethnicity	1 (8.3)	0 (0.0)	0 (0.0)	1 (2.6)		
Carbohydrate Intolerance Score mean (SD)	9.2 (2.3)	9.6 (2.8)	11.5 (3.8)	10.2 (3.2)	ANOVA	0.129
Total energy (Kcal) mean (SD)	1435 (293)	1567 (666)	1805 (857)	1603 (649)	ANOVA	0.378
Weight (kg) mean (SD)	76.3 (14.9)	90.4 (20.0)	76.8 (11.2)	81.2 (16.6)	ANOVA	0.046
Height (m) mean (SD)	1.70 (0.10)	1.76 (0.08)	1.74 (0.09)	1.73 (0.09)	ANOVA	0.245
BMI (kg/m ²) mean (SD)	26.4 (3.23)	29.1 (4.92)	25.5 (2.77)	27.0 (3.96)	ANOVA	0.050
Glucose (mmol/L) mean (SD)	5.54 (0.43)	5.38 (0.47)	5.44 (0.44)	5.45 (0.44)	ANOVA	0.673
Total cholesterol (mmol/L) mean (SD)	5.20 (1.3)	4.57 (0.61)	6.10 (1.37)	5.31 (1.29)	ANOVA	0.005
Triglyceride (mmol/L) mean (SD)	0.79 (0.2)	0.99 (0.36)	0.92 (0.22)	0.90 (0.27)	ANOVA	0.184
Insulin (pmol/L) mean (SD)	63.1 (37.3)	81.1 (39.4)	41.6 (17.6)	61.4 (35.8)	ANOVA	0.012

SD: standard deviation. BMI: body mass index.

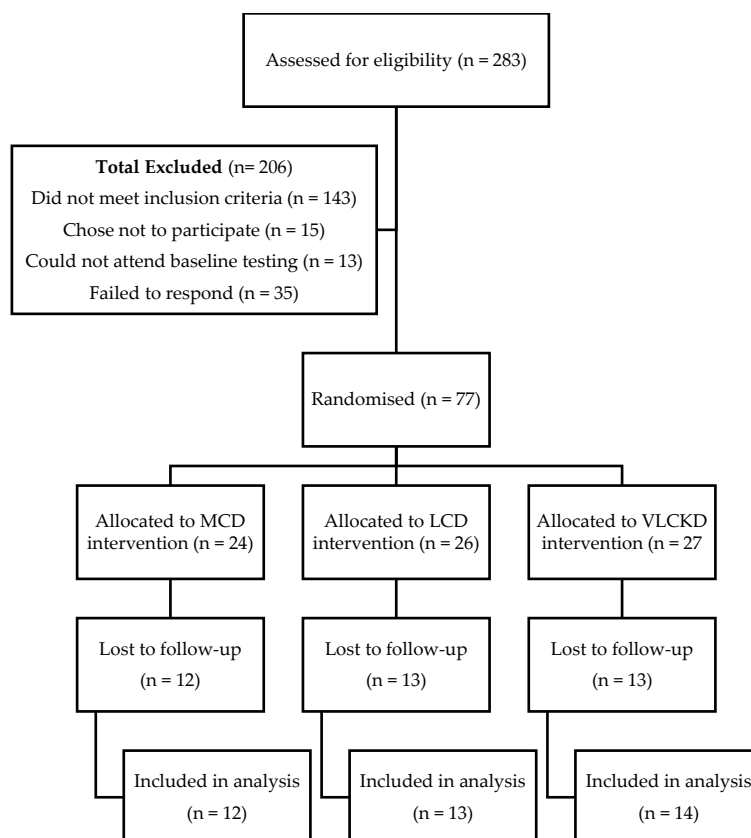


Figure 26. Flow-chart showing participant recruitment, randomisation, allocation, and loss-to-follow-up

Predictive value of CIS to change in outcome measures

The outcome measures which were most convincingly associated with CIS were for change in TG (Beta = -0.025, $p = 0.073$) and change in HDL-c (Beta = 0.029, $p = 0.106$). This means that higher CIS scores at baseline were associated with more beneficial changes in TG and HDL-c. On average, people with higher CIS had less marked improvements in BMI at the end of follow-up (Figure 27) and the association between CIS and change in BMI differed significantly between interventions ($p = 0.007$) with relative carbohydrate intolerance associated with improvements in BMI in the MCD group only (a result that was also significant within that group) as shown in Figure 28. The other interventions did not reach within-group thresholds for significance. Reduction in TG relative to CIS approached the threshold for significance in the VLCKD group only. Results are presented in Table 13. In subscale analysis the only measure to show meaningful and significant results was the interaction for *I snack on sugary or starchy food to relieve headaches/irritability/ craving/excessive hunger* and TG, with an increase in this subscale score related to a greater improvement in TG

(Beta = -0.0834, $p = 0.050$). This subscale approached the threshold for significance for HDL-c with higher scores related to an improvement in HDL-c (Beta = 0.088, $p = 0.100$). However, this interaction did not differ significantly within or between the intervention groups.

Table 13. Association between CIS and change in key outcome measures over twelve weeks by group

Outcome measures*	Treatment group Beta-coefficient and p -value		
	Moderate-Low Carbohydrate	Low Carbohydrate	Very Low Carbohydrate
Total cholesterol	Overall $\beta = -0.017$; $p = 0.82^{\dagger}$ 0.034 $p = 0.81$ p for interaction = 0.891 \ddagger	-0.012 $p = 0.94$	-0.068 $p = 0.62$
LDL cholesterol (mmol/L)	Overall $\beta = -0.045$; $p = 0.51^{\dagger}$ 0.016 $p = 0.89$ p for interaction = 0.882 \ddagger	-0.045 $p = 0.77$	-0.086 $p = 0.49$
HDL cholesterol (mmol/L)	Overall $\beta = -0.029$; $p = 0.11^{\dagger}$ 0.047 $p = 0.44$ p for interaction = 0.773 \ddagger	0.007 $p = 0.79$	0.018 $p = 0.45$
Triglycerides (mmol/L)	Overall $\beta = -0.025$; $p = 0.073^{\dagger}$ -0.060 $p = 0.12$ p for interaction = 0.103 \ddagger	0.027 $p = 0.40$	-0.034 $p = 0.055$
BMI (kg/m ²)	Overall $\beta = -0.026$; $p = 0.627^{\dagger}$ -0.309 $p = 0.032$ p for interaction = 0.007 \ddagger	0.213 $p = 0.061$	0.073 $p = 0.275$

* All measures are change from baseline. BMI: body mass index. LDL: low-density lipoprotein. HDL: high-density lipoprotein

\dagger β refers to the beta coefficient of the overall linear regression between carbohydrate intolerance score (CIS) and change in outcome measures.

\ddagger This p -value relates to a regression model of CIS and treatment group as independent variables and change in the outcome as dependent variables. The p -value relates to the interaction term, testing for a significant difference in the CIS-change in outcome by treatment group.

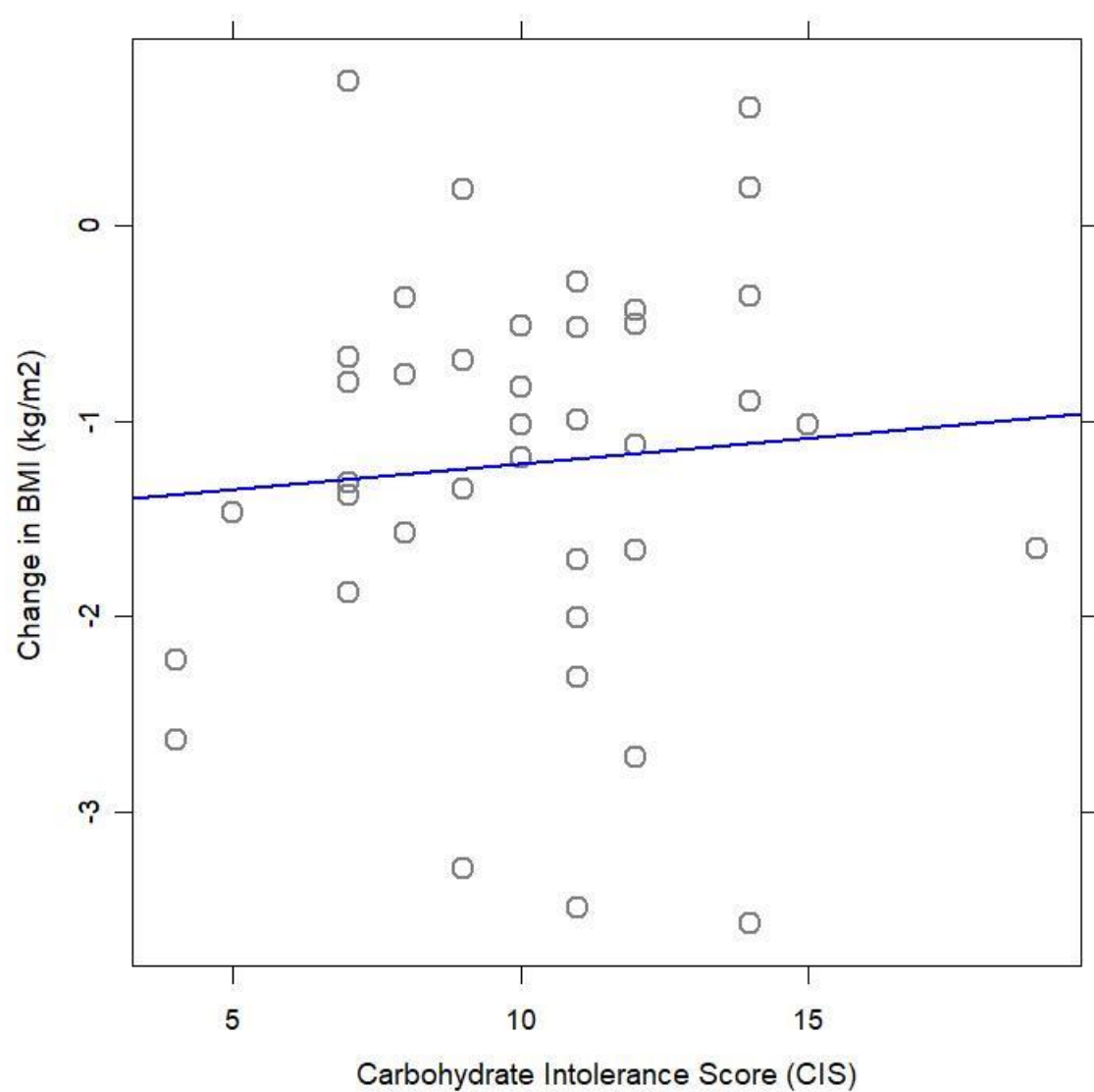


Figure 27. Scatter plot illustrating the relationship between body mass index (BMI) and carbohydrate intolerance score (CIS) at baseline
The blue line is the linear regression

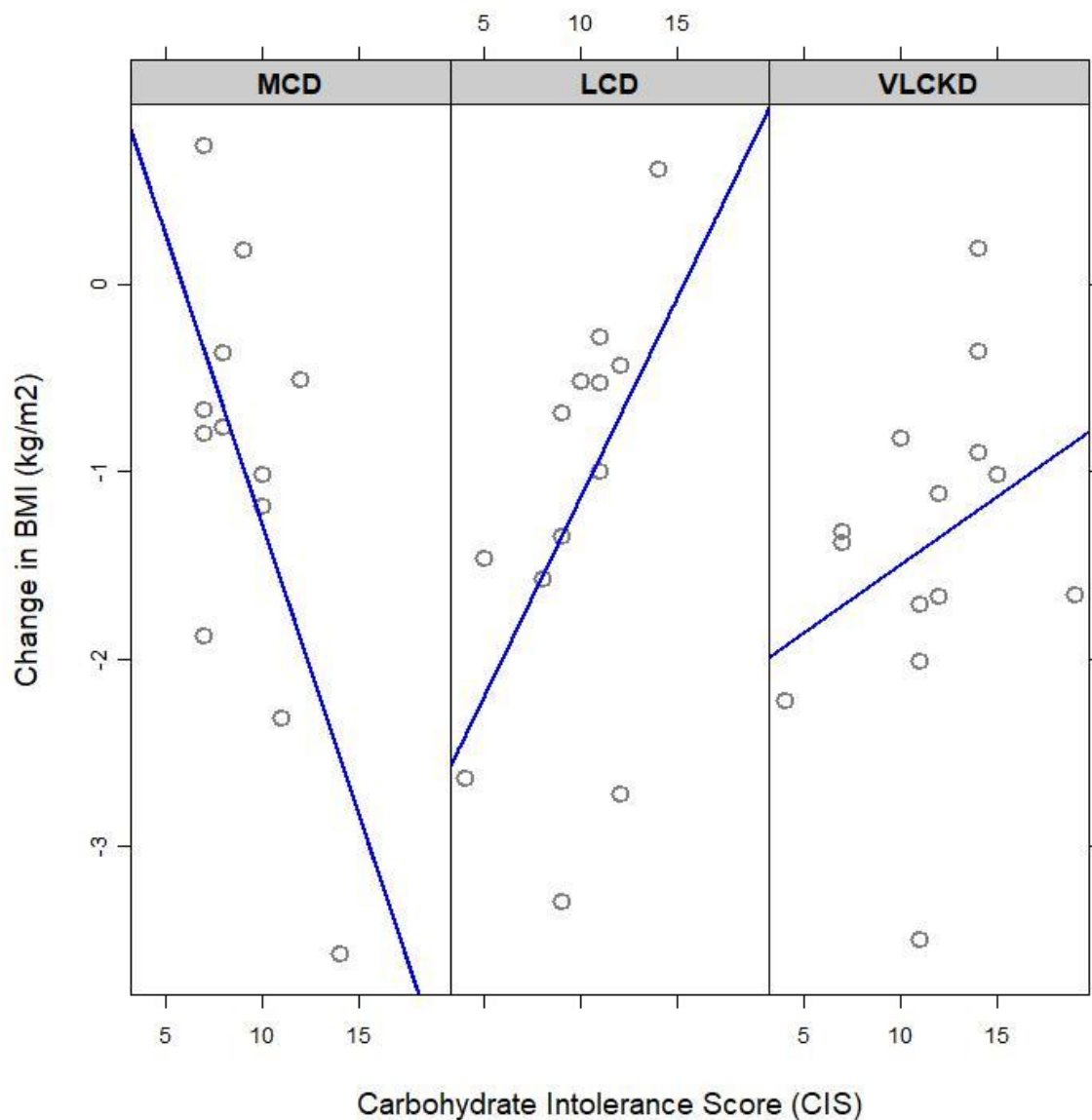


Figure 28. Scatter plot of the change in body mass index (BMI) and carbohydrate intolerance score (CIS) at baseline, by intervention group

MCD: Moderate-low carbohydrate diet. LCD: Low-carbohydrate diet. VLCKD: Very-low-carbohydrate ketogenic diet. Blue lines are the linear regressions.

Associations for symptoms and mood

A higher baseline CIS was associated with greater symptoms of carbohydrate withdrawal (Beta = 0.214) and mood (Beta = 0.044), although neither was statistically significant ($p = 0.084$ and 0.060 respectively). Between-group differences were not statistically significant, and the only significant within-group association was for a trivial increase in mood disturbance associated with greater CIS in the VLCKD intervention. An increase in symptoms of

carbohydrate withdrawal related to the severity of carbohydrate intolerance at baseline also approached the threshold for significance in the VLCKD group. (Table 14).

Table 14. Association between CIS and change in symptoms and mood

Outcome measures*	Treatment group Beta-coefficient and <i>p</i> -value		
	Moderate-Low Carbohydrate	Low Carbohydrate	Very Low Carbohydrate
Symptoms of carbohydrate withdrawal	Overall $\beta = 0.214$; $p = 0.084$ † 0.070 $p = 0.85$ p for interaction = 0.880‡	0.162 $p = 0.49$	0.242 $p = 0.096$
Mood disturbance	Overall $\beta = 0.044$; $p = 0.060$ † -0.015 $p = 0.81$ p for interaction = 0.185‡	0.008 $p = 0.82$	0.083 $p = 0.014$

* All measures are change from baseline.

† β refers to the beta coefficient of the overall linear regression between carbohydrate intolerance score (CIS) and change in outcome measures.

‡ This *p*-value relates to a regression model of CIS and treatment group as independent variables and change in the outcome as dependent variables. The *p*-value relates to the interaction term, testing for a significant difference in the CIS-change in outcome by treatment group.

Discussion

Principal findings

We believed that the higher the CIS, the greater the benefit to health outcomes from greater magnitudes of carbohydrate restriction. Overall, these findings suggest that a CIS might be beneficial to indicate the magnitude of carbohydrate restriction most beneficial for improvements in key outcome measures of health. The CTQ created for this study is useful for identifying people that are likely to benefit from lower-carbohydrate interventions for improvements in their important cardiometabolic markers of HDL-c and TG. People with high scores on this questionnaire are also more likely to suffer most from symptoms resulting from carbohydrate withdrawal and mood disturbance when beginning a carbohydrate-restricted diet. However, the CTQ was not useful for distinguishing between different levels of carbohydrate-restriction diets for outcome measures, except for BMI. In this case, a higher CIS was associated with greater improvements from a moderately low-carbohydrate intervention when compared to one that is more restrictive. Therefore, based on these preliminary results, those with a higher CIS might be best allocated to a moderately restricted low carbohydrate diet, initially, rather than one that is more heavily restrictive.

Interestingly, in a secondary analysis, we found that this might have resulted from greater relative changes in carbohydrate intake as a proportion of TE by group. While there was a higher intake of carbohydrate overall in the MCD group, those with a higher CIS were more likely to have had a greater relative reduction in carbohydrate as a percentage of daily energy intake (Beta = -1.052, $p = 0.20$) when compared to positive associations between change in carbohydrate intake and CIS in the LCD and VLCKD groups. Similarly, greater improvements in TG and HDL-c relative to CIS were seen in the MCD group and might be attributable to the association between CIS and change in carbohydrate intake independent of the absolute magnitude of carbohydrate intake.

Strengths and weaknesses of the study

To our knowledge, this pilot study is the first to compare commonly purported signs of *carbohydrate intolerance* and the effect this might have on outcomes from differing low-carbohydrate diets.

It was a randomised trial, including regular food tracking, along with real-time researcher monitoring and feedback and advice and information provided to participants from a competent team with extensive experience in the prescription of LCDs and VLCKDs. As such, we believe it provides a valuable addition to the literature to help inform clinical practice.

Our study was limited by small sample size and by 49% of participants not completing the intervention or presenting for follow-up testing. This was expected, as high dropout rates are common in dietary studies. A systematic review of low-carbohydrate diets vs low-fat, calorie-restricted diet interventions showed an overall attrition rate of 36%, with a higher rate of attrition in low-fat, high-carbohydrate interventions. (23) Despite high drop-out rates being common in dietary studies, few participants in this study reported dropping out due to challenges with the diets allocated, and most dropouts were instead due to failure to present for testing rather than a failure to adhere to the diet. These numbers were almost identical between the intervention groups. Participants who failed to present were asked to provide reasons for (not) doing so. Two participants responded, stating a clash with work and inability to attend due to parental responsibilities. It is therefore unclear whether there were other factors, outside of scheduling or other logistical challenges, that affected participants

completing the study. The final numbers included in our analysis due to attrition, therefore, resulted in a lack of statistical power.

The study did not include a group with a higher carbohydrate allocation consistent with existing dietary guidelines of 45-65% of energy derived from carbohydrate. (198) Because of the possible predictive value of baseline CIS on mood, symptoms, TG, HDL-c, and BMI changes resulting from these low-carbohydrate diet allocations overall, a comparison with a higher-carbohydrate, lower-fat diet, might provide a better evaluation of the predictive value of carbohydrate 'tolerance' questionnaires.

Meanings and practical implications of the study

The key research question of this pilot study was whether relative carbohydrate intolerance, as indicated by a CTQ born of clinical experience, could predict anthropometric, cardiometabolic, and subjective mood and symptoms outcomes from differing magnitudes of carbohydrate restriction. There is a likely predictive value of greater *carbohydrate intolerance* on mood disturbance, symptoms of carbohydrate withdrawal, and TG, HDL-c, and BMI. This could provide the clinician with valuable information to tailor dietary prescriptions more effectively for the client. Additionally, it could allow the practitioner to provide more information about changes in mood and symptoms of carbohydrate withdrawal that might occur, in anticipation of starting a lower-carbohydrate diet.

Unanswered questions and directions for future research

Clinical experience and several of this study's findings suggest that a higher CIS might indicate the use of a low-carbohydrate diet overall and a moderately carbohydrate-restricted diet to improve BMI, TG, and HDL-c. However, this might be a result of the magnitude of relative carbohydrate restriction, irrespective of the absolute carbohydrate intake; research with larger numbers of participants and a higher-carbohydrate comparison group is necessary to further explore this hypothesis. While the questionnaire was subjected to some content analysis and peer-review by experienced nutrition practitioners and researchers, it has not yet been validated and should it be utilised in future research, it will need to undergo more thorough validity and reliability testing.

Conclusion

Our findings demonstrate that those with higher CIS are more likely to benefit from low-carbohydrate diets for the improvement of triglyceride concentrations. Subjects with higher scores are also more likely to experience mood disturbance and symptoms of carbohydrate withdrawal. The questionnaire might also be useful to allocate those with the highest CIS to a more moderately restricted plan to mitigate symptoms of carbohydrate withdrawal and effects on mood and to potentially offer greater improvements in BMI. However, at this time and contrary to our hypothesis, due to the lack of clear between-group significance, it is unclear whether it can accurately predict the efficacy of dietary allocations for the individual. To investigate this hypothesis further, additional research, with larger sample sizes, and a higher-carbohydrate control-group is required.

Chapter 9. Conclusions and Practical Implications

The work undertaken for this doctoral thesis sought to answer questions relevant to the understanding of the achievement of clinically relevant nutritional ketosis (NK). It explores the symptoms of keto-induction that have become known by the possible misnomer of *keto-flu*, how they might be mitigated, and how the experiences and application of differing lower-carbohydrate diets relate to outcomes overall and for individuals. Understanding more about these areas is important to extend the body of academic knowledge in this area of nutrition science which is escalating in interest from health professionals, consumers and academics. It also has a valuable role, informing clinical practice and the application of more individualised diets, and the translation of this knowledge to improving public health outcomes.

There is a growing awareness and rising use of low-carbohydrate diets for health conditions, most especially metabolic syndrome and related disorders, and increasing interest in their use for cancer treatment. They are also becoming extremely popular in the mainstream. For example, at the time of writing, four of five *Amazon* best-sellers in the 'Diet and Weight-loss' category were based on low-carbohydrate diet principles and a google search for "Low-Carbohydrate Diet" returned over 44,000,000 results. Despite this mainstream popularity, many important areas within this area suffer from a lack of relevant research findings and translation into clinical practice and public health guidelines.

Summary of findings

In exploring the overarching questions, this thesis took a circuitous route. Firstly, it explores nutritional ketosis and ketonaemia and what ketogenic diets, in fact, are. There is debate over what constitutes a 'low-carbohydrate' diet, and somewhat surprisingly, almost no definitive evidence to support the *lingua franca* definition of NK that has become standard in the field of clinical nutrition. Because of this lack of definition, time-to-NK has not been adequately studied, nor have the commonly reported set of adverse effects associated with ketogenic diets that has become known in the mainstream as the 'keto-flu'. In fact, at the time of writing, an internet search for 'keto flu' results in nearly half a million results and yet, a search in the scientific literature for this and related terms only returned five results, two of which have

been published this year, subsequent to the three papers addressing this topic which are included in this thesis. This means that there is a large body of anecdotal evidence being used to guide and support the use of low-carbohydrate and ketogenic diets by the many people following them, without a strong evidential basis from properly conducted research. This could prove detrimental to outcomes if there is not further thorough investigation of these topics.

The research featured at the beginning of this thesis focussed on understanding more about these critical areas. There are, in the mainstream, many purported ketogenic supplements and the review which comprises Chapter 2 was the first to synthesise the extant research on these. It is clear that medium chain triglycerides (MCTs) are demonstrably ketogenic and so, MCTs are a supplement which one could be reasonably confident would elicit increased ketonaemia when compared to a control oil rich in long-chain fatty acids. This effect was used in the follow-up randomised, controlled study (RCT), to elucidate whether greater ketonaemia would result in lessening of symptoms and improvements in mood. This study was the first to directly evaluate the time taken to achieve NK in a 'classic' 4:1 ketogenic diet and to evaluate the symptoms of keto-flu in the keto-induction phase. Furthermore, it was the first study to directly compare the use of MCT oil in comparison to control in a classic ketogenic diet as previous research had solely focussed on the use of MCT in modified ketogenic diets with lesser carbohydrate restriction vs classic ketogenic protocols.

The initial results showed an association between ketonaemia and reduced mood disturbance and a possible effect of MCTs and ketonaemia on symptoms of carbohydrate withdrawal. Overall, symptoms of carbohydrate withdrawal exhibited by those following either a very-low-carbohydrate diet (VLCKD), with, or without MCT, was minimal but the MCT group, with a larger elevation in beta-hydroxybutyrate (BOHB) levels, had fewer symptoms (with the exception of gastrointestinal symptoms) likely to result from the MCT oil itself. These results also showed that the so-called 'keto-flu' is a relatively minor event, typically only lasting a few days for most participants and providing for only limited negative effects.

The lived experience of the diet was also a positive one for the participants included in the qualitative analysis and the purported negative effects of VLCKDs are trivial but varied considerably between individuals.

The subsequent RCT sought to clarify these initial findings by comparing diets differing in carbohydrate allocation. While there is a large and growing body of research comparing the effects of higher-carbohydrate 'best-practice' or 'usual care' diets to a variety of low-carbohydrate and ketogenic diets, there are few which compare low-carbohydrate diets differing in the allocation of energy from carbohydrate. To truly move towards better prescription of diets to the individual and achieve the best outcomes both on an individual level and for public health, this is a crucial area of exploration.

Based on our previous results, the 'keto-flu' phenomenon appears to be; a) self-limiting, b) trivial, and c) transient, and so, we sought to determine whether the keto-flu was, in actuality, a symptom profile associated with a ketogenic diet or whether it was a result of any significant magnitude of carbohydrate withdrawal overall. In this follow-up study, while ketonaemia and the achievement of NK differed between the groups, non-ketogenic low-carbohydrate diets did elicit NK. In fact, the mean BOHB was consistent with NK in the 15% total energy (TE) from carbohydrate intervention, while some participants in the 25% carbohydrate allocation achieved NK sporadically. Mean NK was achieved on day 3, 4, and 5 for 5%, 15%, and 25% TE from carbohydrate interventions respectively, which was consistent with our earlier VLCKD study. This shows that the achievement of NK varies between individuals and between differing diets but also confirms that the only diet to result in ketosis consistently and for almost all subjects is one containing < 15% TE from carbohydrate. Ketonaemia was, as expected, commensurate to the degree of carbohydrate restriction but in contrast to our earlier findings, BOHB was not significantly associated with either symptoms of carbohydrate withdrawal or mood. Similarly, there was no significant difference between the interventions, for mood, or symptoms of carbohydrate withdrawal but there *was* a significant association between reduction in calories from baseline and mood disturbance and this was also a likely predictor of symptoms of carbohydrate withdrawal.

These findings suggested, for the first time, that symptoms of carbohydrate withdrawal are unlikely to be primarily 'keto-flu', as despite being marginally higher in the VLCKD, they are not significantly associated with a VLCKD or the magnitude of ketonaemia, but instead are significantly associated with relative reductions in energy intake alongside restriction of carbohydrate. The clinical implication of this is that reduced carbohydrate dietary

interventions should be allocated based on what will provide the best results for the client and what the client will be able to adhere to, not prescription to mitigate the symptoms of carbohydrate withdrawal. It further demonstrated that energy sufficiency is likely to be the major affecter of adverse effects. This provides important clinical applications for the dietary prescription of energy by clinicians and is a reminder of the importance of energy balance overall for health and performance. In retrospect though, given the equivocal effect of ketonaemia on mood and symptoms of carbohydrate withdrawal in a non-supplemented diet in this subsequent study, the trend towards improved mood and reduced symptoms of carbohydrate withdrawal observed in the first RCT might have been due to the use of MCTs providing a 'boost' to ketone levels. After reaching NK after day-3, the mean BOHB in the VLCKD group in the second RCT was 0.9 mmol/L but the mean BOHB in the MCT-supplemented group in the first RCT was 1.4 mmol/L. Clinically, this is a large difference in ketonaemia, and it provides justification for the potential use of MCT to mitigate adverse effects arising from VLCKDs. The implication that diet should be allocated to individuals based on outcomes likely to be achieved (notwithstanding the probable benefit from increased ketonaemia from supplementation), provides a compelling rationale for the analysis of overall outcomes achieved by those following differing carbohydrate-restricted diets.

The cardiometabolic and anthropometric outcomes and adherence resulting from the three, differing low-carbohydrate diets were analysed. Overall, the reduced carbohydrate dietary interventions resulted in improvements in key cardiometabolic indicators of health status. The population studied was one that was healthy and free from metabolic disorders; hence the baseline measurements were within normal ranges. Furthermore, due to the use of a eucaloric intervention in which participants attempted to meet habitual calorie targets we did not expect pronounced anthropometric or cardiometabolic changes. Despite this, there were meaningful improvements in weight, waist-height ratio, high-density lipoprotein cholesterol (HDL-c) and triglycerides (TG). We considered the improvements in TG to be particularly interesting due to the strength of the association between TG levels and cardiovascular and all-cause mortality. There was a consistent trend towards greater improvements in these key markers resulting from a greater restriction of carbohydrate; with seven of ten measures most improved by the VLCKD intervention. If these results were due to chance, we would expect

only three to be most improved by VLCKD (notwithstanding that these measures are not truly independent).

Consistent with the existing body of literature, many participants did not present for follow-up testing, but in those included for analysis, LCDs were easily adhered to and there was little difference in adherence between the differing dietary interventions. Intakes of carbohydrate were relatively consistent with the dietary allocation across the study period but those on the lowest carbohydrate intervention on average consumed more carbohydrate than allocation (8% compared to the allocation of 5%) whereas those on the more moderate low-carbohydrate diets had mean intakes slightly less than allocation. There was a marginal increase in energy intake from baseline, but this declined over the course of the study in all groups. Of interest was the relatively low-calorie intake recorded at baseline which might have indicated a cohort focussed on weight loss, under-reporting of food intake, either in the lead-in week, or overall, or a group that was actually undereating.

Because of trivial differences in mood and symptoms between allocations, and the trend towards greater improvements in outcome measures resulting from greater carbohydrate restriction, these results could, at this time, be interpreted that low-carbohydrate interventions are effective for improvements in predictors of all-cause and cardiovascular disease mortality in the short term, consistent with the extant literature, *and that a greater degree of carbohydrate restriction offers greater benefits than a more moderate carbohydrate restriction overall.*

There was a consistent trend (albeit results not always meeting the threshold for statistical significance) towards improved results from greater restriction of carbohydrate but due to the variability in observed results and our desire to translate this work into clinical application and further research, we explored the predictive value of baseline measures of cardiometabolic health on outcomes. This showed that there is a consistent trend towards poorer baseline measures of health predicting better outcomes from greater reductions in carbohydrate, with the exception of HDL-c, which was improved most, relative to adverse baseline measures, by a more moderate carbohydrate restriction to 25% of TE. However, the small sample size and large numbers of participants who did not present for follow up testing affected the statistical power of this finding and so, further research with larger numbers is needed. If this research is conducted and the hypothesis that those with poorer measures of

cardiometabolic health benefit more from greater carbohydrate restriction is confirmed, this would provide a powerful tool for the allocation of diet to the individual, something that is currently lacking in clinical nutrition. It could also provide a template for a return to a larger compendium of foods for those for whom a VLCKD is indicated as their cardiometabolic status resumes as a result of an appropriate nutritional strategy.

A tool that would be invaluable for the allocation of diet to individuals is a validated and credible questionnaire of 'carbohydrate tolerance'. While blood and anthropometric measures are relatively cost-effective and easily attained, they are not always immediately available to the nutrition practitioner and there is a delay (for blood testing) between the referral for testing, receipt of tests, and the ability to prescribe dietary interventions based on the results. Thus, a questionnaire would be a valuable in-clinic tool. With input from our colleagues in clinical practice, we developed a set of signs that we commonly observe or that are commonly reported to us in clinical practice that might be associated with 'carbohydrate tolerance'. The questionnaire developed results in a 'carbohydrate intolerance score' (CIS). Our findings demonstrated that those with higher CIS are more likely to benefit from low-carbohydrate diets for the improvement of triglyceride concentrations. While those with higher scores are more likely to experience mood disturbance and symptoms of carbohydrate withdrawal. Therefore, the questionnaire might be useful to allocate those with the highest CIS to a more moderate diet plan to mitigate symptoms of carbohydrate withdrawal and any effects on mood. However, overall, the results were unclear, and the questionnaire needs to be refined and undergo comprehensive validation testing to determine whether it has any value for the prediction of carbohydrate allocation. We intend to undertake this further work and are currently awaiting approval from the Health and Disability Ethics Commission.

Original and substantive contributions to the literature

As clinicians, we have the responsibility of finding the most effective intervention for the individual and this takes precedence over either mainstream or media attention to particular diets, or indeed to any prevailing guidelines for purported best-practice. Notwithstanding that best-practice guidelines *should* provide, if truly evidence-based, a starting point for the prescription of nutrition for most people, most of the time, no one is *the perfect mean* and many people show significant variation in their responses to dietary interventions and dietary

prescription must be adjusted to meet the needs of the individual. Unfortunately, in clinical nutrition, there is limited data on the achievement of NK—overall, between diets, and between individuals, and on the effects of keto-induction and carbohydrate withdrawal. There is also limited data on the differences in outcomes resulting from different magnitudes of carbohydrate restriction and more importantly, for whom a greater or lesser allocation of carbohydrate is most effective.

There have been many comparisons between LCDs and usual care, high-carbohydrate, low-fat diets but, thus far, only one study comparing differing low-carbohydrate diets. In this study by Johnson et al., (65) the results between groups were equivocal. However, the ‘ketogenic’ diet contained only 60% TE from lipids, which based on the extant literature would not be considered ketogenic without the addition of MCTs. This diet resulted in a modest 0.6 mmol/L increase in serum BOHB at week-2 and only a 0.2 mmol/L increase in BOHB from baseline at the conclusion of the study (week-6). Conversely, the non-ketogenic, low-carbohydrate diet consisted of a relatively modest reduction in carbohydrate to 40% of TE. Therefore, while an important study in the context of the literature, it doesn’t adequately address the variation in outcomes between differing, low-carbohydrate diets, overall and per individual.

There are also few studies on dietary interventions, and particularly LCDs, in healthy people. Much of the research into lower-carbohydrate interventions (aside from the early research on the ketogenic diet for epilepsy) has focussed on weight-loss and the treatment of metabolic disorders and there is a growing body of research on the ketogenic diet for other conditions like cancer, neurodegenerative disorders, and metabolic syndrome, and lower-carbohydrate strategies for fat-adaptation conducive to sports performance. However, there is a dearth of RCTs on healthy populations for the purposes of disease prevention. While one would expect greater magnitudes of change in those with obesity or metabolic disorder, along with resultant improvements in statistical power, the research in this thesis adds important evidence to the extant literature by looking instead at the use of varying lower-carbohydrate diet strategies in healthy people for the improvement of markers associated with future health outcomes.

There are many challenges to translating the findings within the existing literature to clinical practice. Firstly, there is not yet a clear consensus for the definition of nutritionally induced

ketosis nor even for what constitutes a low-carbohydrate diet. Previous to this thesis, and papers herein, the published literature was limited to a post-hoc analysis of the BOHB levels achieved by those following a VLCKD with < 50 g of carbohydrate per day, (33) and to a study in which this level was used as a cut-off for entry into 'ketosis', (34) and the subjective opinions of clinicians and researchers, like those presented by Phinney and Volek. (183) Other research has not defined NK and has used various methods of measurement and levels of either ketonuria or ketonaemia, to determine ketosis and time-to-NK. (94-98) The papers presented in this thesis add to this body of knowledge and provide the first defined time-points for entry into NK as defined by the accepted threshold of ≥ 0.5 mmol/L BOHB.

Most likely because of the lack of clear definitions for NK, there had never been a synthesis of the research on ketogenic supplements prior to the paper presented in this thesis. There had also never been a comparison of the effects of MCT supplementation between equivalent 'classic' ketogenic diets of a 4:1 lipid to non-lipid ratio as previous research had been focussed on comparing classic ketogenic diets with modified (lipid reduced) ketogenic diets containing MCTs for the purpose of seizure control in people with epilepsy. (39, 145, 146) Thus, the RCT presented in this thesis, (35) provides the first 'like-for-like' comparison of the true effects of MCTs on ketonaemia and symptoms of keto-induction and mood.

Definitions for LCDs are similarly vague and can range from a post-hoc definition based on existing guidelines, through to what is purported to result in metabolic changes of fat- and keto-adaptation. (28-32) Therefore, there is little homogeneity in the diets explored in low-carbohydrate research. Some of the larger and more methodologically robust studies, such as the recent DIETFITS study by Gardner et al., (81) while showing a small yet significant benefit towards the lower-carbohydrate diet (and yet concluding no meaningful difference), suffer from criticism because they compare relatively small differences in carbohydrate allocation between the lower- and higher-carbohydrate intervention groups. Thus, this thesis provides a novel and unique exploration into a relatively unresearched area.

Adherence to LCDs is often questioned. Notwithstanding that this mainstream contention is not supported by the evidence, in which LCDs result in better adherence than low-fat diets, (67) the results presented in this thesis show that the overall experience of LCDs and VLCKDs is positive and that these diets are easily adhered to (for those who completed the study).

While there were many participants in the second trial who did not present for follow-up testing, few of those reported dropping out due to any difficulties with the diet and instead failure-to-show was typically due to logistical or scheduling difficulties. Notwithstanding the limitation that logistical difficulties may have been used as an excuse for not adhering by some participants. While the mean intake of carbohydrate in the VLCKD group in this study was above allocation (8% vs 5% allocation), the consistency of carbohydrate intake by group was practically identical, with a variance of less than 2% carbohydrate intake as a proportion of TE within 95% confidence limits for all groups. This shows that while the 5% carbohydrate allocation was not achievable, the dietary prescription of a VLCKD with 5% TE from carbohydrate enabled a lower overall carbohydrate intake and this resulted in a trend towards greater results when compared to more moderate carbohydrate restriction. This has important implications for clinical practice, and while very low dietary allocation of carbohydrate may not be able to be adhered to it can translate to a greater reduction in carbohydrate and consistent adherence over time, that also provides the most beneficial outcomes.

Finally, the predictive value of baseline measures on the best-fit diet for an individual is hotly debated. While the majority of studies hint at insulin homeostasis being a predictor of dietary outcomes from either lower- or higher-carbohydrate diets, (77, 79, 80) the DIETFITS findings cast some doubt on this hypothesis, (81) while the recent study on increased energy expenditure related to baseline insulin homeostasis by Ebbeling et al., (90) suggest that the carbohydrate-insulin interaction is a modifier of dietary outcomes. An area that hasn't been investigated is the predictive value for dietary prescription provided by standard, accessible blood and anthropometric measures related to both insulin resistance and overall cardiometabolic health. This thesis provides additional data to inform clinical practice of the value of these measures for the allocation of diet to individuals.

Clinical applications

The findings presented in this thesis suggest the following practical applications:

1. MCTs result in primary ketonaemia and increased BOHB levels due to enhanced ketogenesis. Over a 20-day period, this resulted in a difference of 0.6 mmol/L BOHB between an MCT supplemented VLCKD vs control.

2. The use of MCTs results in reduced symptoms of carbohydrate withdrawal overall, although this was non-significant (-0.021 SOSS, $p = 0.768$), and improved mood (-1.12 POMS-TMDS, $p = 0.046$) but increased symptoms of GI distress at a dosage of 30 ml taken three times per day.
3. Shorter chain fats (such as butyric acid) are likely to be even more ketogenic. While this hypothesis requires further research both 2 and 4 g of butyric acid have been demonstrated to be more ketogenic than either 5 or 10 g of an 8-carbon-chain fatty acid. This suggests greater ketogenic potential from shorter vs longer fatty-acid chains.
4. There is a trivial increase in symptoms of carbohydrate withdrawal with greater carbohydrate restriction but there is no significant association between diet or ketonaemia with these symptoms. The addition of MCTs, with a concomitant increase in BOHB, might modify these effects.
5. There is a significant effect of calorie reduction on mood (Beta = -0.002 , $p = 0.022$) and a plausible effect on symptoms of carbohydrate withdrawal (Beta = -0.002 , $p = 0.087$).
6. Overall, greater carbohydrate restriction results in the greatest magnitude of improvements in some measures of cardiometabolic health in healthy adults over 12-weeks.
7. Baseline cardiometabolic measures show promise as clinical tools for the prescription of diets to the individual.
8. It is unclear whether a carbohydrate tolerance questionnaire can, at this time, predict likely cardiometabolic outcomes from differing carbohydrate-restricted diets.

Based on these results the most promising for translation to clinical practice is that a cardiometabolic panel of standard blood measures including HDL-c and TG, and BMI could help to guide carbohydrate allocation to individuals, with poorer baseline scores indicative of the need for greater carbohydrate restriction. These tests are easily acquired by the clinician and if the hypothesis that they can predict the type of diet most beneficial to the client is supported, would, for the first time, provide a relatively simple and cost-effective means of individualising diet. We suspect that with further refinement of a carbohydrate tolerance questionnaire and further validity testing, especially when compared to blood and other predictive markers, this could also result in a valuable clinical tool for the prescription of diet.

There is also a strong implication from this work that supplements such as MCTs which support ketogenesis can aid the introduction of VLCKDs and support their maintenance, especially by improving mood in those following the diet.

Finally, the combined results show that low-carbohydrate and VLCKDs are easily adhered to and typically result in positive results.

Strengths and limitations of this work

The studies presented in this thesis were randomised trials, with support in real-time from experienced clinicians. They provided dietary prescriptions in the form of nutrition plans and advice that was consistent with those that would be provided in a clinical setting, along with (in the second RCT), real-time application-based food tracking. Thus, they were valuable to help inform the clinical application of low-carbohydrate and ketogenic diets.

Both studies were limited by participant numbers which reduced statistical power. This is common in nutrition studies, in which budgetary and infrastructural constraints limit sample sizes, which was a consideration in the first RCT. In the second RCT, a larger cohort was recruited but the study was limited by many participants who did not present for follow-up testing. While high dropouts of around 36% are common in nutrition studies of a similar nature, (23) the failure to complete rate in this RCT was 49%. However, most dropouts were likely due to failure to present for testing rather than a failure to adhere to the diet, and these numbers were almost identical between the intervention groups. The small sample sizes highlight a problem of applying parametric tests i.e., whether or not the data collected fit the probability distributions associated with them. This also increases the equivocacy of the findings, even when, as was observed in these studies, there are clear trends towards a conclusion (of, for example, the superiority of a VLCKD for outcomes, and for the predictive value of baseline measures for dietary allocation). The inclusion criteria also meant that there were, in both RCTs, a healthy cohort, absent from disease and metabolic disorders. As one would not expect large magnitudes of change for outcome measures that were within normal ranges at baseline, this further reduced the statistical power and strength of the findings. While the literature shows that the greatest benefit from lower-carbohydrate diets is likely to be for those with metabolic syndrome and with obesity, it was important to do this work as there are few randomised clinical studies that evaluate the use of LCDs in healthy people and

performing this, despite smaller expected magnitudes of change, has important implications for translation to public health outcomes.

In the second intervention study, a potential limitation might be the confounding effect of weight-loss. Despite participants eating as close as possible to habitual calorie intake, weight-loss was observed in all groups and this was highest in the VLCKD group in which most other measures were also most improved. While the results were presented as individual outcome measures, they are not independent and thus, weight-loss will have impacted the findings. However, the design of the study specifically did not seek to control for weight-loss but instead for energy intake because this was not a weight-loss study. There have been, to our knowledge, no studies comparing LCDs differing in carbohydrate restriction, in healthy people, and consuming their habitual energy intake. Thus, the findings are important despite the potential confounding of weight-loss.

There are challenges with self-reporting of food intake. We cannot be certain that the participants accurately recorded their food intake. While accuracy was strived for, we recognised the limitations of self-reporting, and the food-tracking application but as a translational study, the intention to follow dietary prescriptions and best-effort application were more important than absolute accuracy. The improvements in outcomes resulting from the *application* of diet are more important for the translation of this research to a clinical setting are more important than from diet per se.

The duration of the studies offers limitations. For the purposes of measuring keto-induction and symptoms and mood within this timeframe, the allocated time of twenty days was more than sufficient, as time-to-NK is typically 3-4 days. However, 12-weeks, while of a relatively long duration when compared to similar intervention studies, may not be long enough to accurately suggest longer-term outcomes or longer-term adherence.

The studies also did not include groups with a higher carbohydrate allocation consistent with existing dietary guidelines of 45-65% of energy derived from carbohydrate, (198) There is, however, already a large body of evidence comparing low- to high-carbohydrate diets, and this thesis helps to instead differentiate between differing lower-carbohydrate diets and supplemental strategies and their respective benefits.

Implications for future research

The papers presented in this thesis provide a valuable exploration of low-carbohydrate diets in clinical practice. There are many unanswered questions and most specifically, and for the greatest benefit to patients, further exploration of the role of predictive markers for the allocation of diet is required.

Firstly, additional research to determine the true effect of MCTs, or other ketogenic supplements and the dose-effect of ketonaemia on symptoms of carbohydrate withdrawal and mood will be beneficial. In the first RCT there was a likely benefit of ketonaemia on mood and symptoms of carbohydrate withdrawal that wasn't apparent in the second RCT. The magnitude of increase in BOHB was much higher in the first study as a result of MCT supplementation and therefore further research should focus on evaluating the dose-effect of blood BOHB levels to mood and symptoms of carbohydrate withdrawal, or if in fact, the supplement itself was responsible for improvements.

Additional research is also required with a larger sample and greater support for logistical considerations to ensure larger numbers present for follow-up to help support or refute the hypothesis that baseline cardiometabolic markers like TG and HDL-c, and BMI offer value as predictors of the desired dietary prescription for individuals. Within this context of larger studies, the predictive value of baseline insulin homeostasis for dietary prescription, a hypothesis which has conflicting results in the extant research, could also be further examined. Similarly, additional research with a larger sample is required to test the hypothesis that a 'Carbohydrate Tolerance Questionnaire' offers value for also helping to predict dietary allocation. There also needs to be a reliability study of the questionnaire and possible refinement in order to then further test validity. This further research is currently pending ethics approval.

There are many other important questions hinted at by the research presented in this thesis. While we focussed on MCTs as a supplemental method to increase ketonaemia due to the amount of supporting evidence, other supplements offer promise for increasing ketonaemia and the hypothetical benefits of that. Shorter-chain fatty acids are likely to be more ketogenic than MCTs (127) due to greater uptake by the hepatic portal vein and distribution to the liver for ketogenesis but this effect might be limited by direct uptake intestinal microbiota and

epithelia. Future research should focus on more thoroughly evaluating ketonaemia resulting from shorter-chain fatty acid supplementation and how this modifies mood and symptoms of carbohydrate withdrawal in a VLCKD. Similarly, exogenous ketones offer promise for increasing ketonaemia markedly and rapidly but result in inhibition, albeit modest, of endogenous ketogenesis, (196) and research evaluating the effects of ketone supplementation on mood and symptoms of carbohydrate withdrawal would also be valuable.

There also needs to be further research comparing different carbohydrate allocations. As presented earlier in this work, many comparative studies suffer from methodologies that reduce their conclusiveness, such as comparing relatively modest differences in carbohydrate or fat content of the diet. More research, comparing a range of dietary strategies will more adequately be able to address how different dosages of macronutrients promote beneficial changes overall. There is for example extraordinary debate on which diet, overall, is ‘best’ within the framework of indicators of cardiometabolic risk such as weight, adiposity, BMI, and blood measures. The evidence suggests that LCDs are more effective for these outcomes and more research will either further support these findings or refute them.

The *elephant in the room* is adherence and compliance to diet. While LCDs do typically result in better outcomes than low-fat diets (LFDs) the benefits narrow over time and there are considerable benefits from any diet that is able to control energy intake or relative energy availability either by restriction, macronutrient modification—such as higher-protein diets in which there is less available adipose-generating energy quotient, or lower-carbohydrate improve satiety, or diets with fewer refined and processed foods. So being able to adhere to any quality diet is a key, yet often overlooked topic in dietary debates that focus on macronutrient composition of diets. While LCDs are easier to adhere to overall than LFDs, sub-group analysis shows that this is relevant in those more insulin resistant. (67) There are many other factors that could be relevant to an individual’s ability to adhere to a dietary prescription, ranging from other health status, through to cultural, behavioural and psychosocial considerations. Physiological drivers of satiety and hunger are likely to be important for adherence and compliance to diet, but psychological and emotional drivers may be just as, if not more important. In summary, the best diet for someone’s physiology will be moot if they cannot stick to it.

Finally, to understand this topic in a more comprehensive manner, more qualitative research needs to be conducted to increase the scope of our understanding of people's perceptions of diet and how different diets might improve their life-outcomes in ways that are missed by standard, quantitative methods. To paraphrase the words from the supervisor of this thesis, Professor Grant Schofield; "At the end of the day, the most important thing for most people is how they *feel*". How someone feels is not only important for their sense of wellness, satisfaction, health, and happiness in the moment, but will also likely affect adherence to a diet that will help them reduce their risk of future ill-health, and thus, has important implications for societal health.

Appendix 1. Analytes and methods of measurement

Analyte	Lower limit of measurement*	Measuring range	Test principle
Total cholesterol	0.1 mmol/L	0.1-20.7 mmol/L	Enzymatic colorimetric test
LDL-c	0.078 mmol/L	0.078–14.2 mmol/L	Homogeneous enzymatic colorimetric assay
HDL-c	0.08 mmol/L	0.08–3.10 mmol/L	Homogeneous enzymatic colorimetric assay
TG	0.05 mmol/L	0.05-11.3 mmol/L	Enzymatic colorimetric test
CRP	2.9 nmol/L	2.9-3333 nmol/L	Particle-enhanced immunoturbidimetric assay
GGT	3 U/L	3-1,200 U/L	Enzymatic colorimetric test
ALT	5 U/L	5-700 U/L	Enzymatic colorimetric test
AST	5 U/L	5-700 U/L	Enzymatic colorimetric test
ALP	3 U/L	3-1200 U/L	Enzymatic colorimetric test
Glucose	0.11 mmol/L	0.11-41.6 mmol/L	Enzymatic colorimetric test
Uric acid	11.9 µmol/L	11.9-1487 µmol/L	Enzymatic colorimetric test
Insulin	1.39 pmol/L	1.39-6945 pmol/L	Electrochemoluminescence
C-peptide	0.003 mmol/L	0.003-13.3 nmol/L	Electrochemoluminescence

* Functional sensitivity. It represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying two or three standard deviations above that of the lowest standard. Method comparisons, limitations and specific performance data can be found on www.e-labdoc.roche.com

Appendix 2. Supplement: Change in calories from baseline and average protein and carbohydrate per day, by week.

		Baseline	Wk 1	2	3	4	5	6	7	8	9	10	11	12
MCD 25% CHO	Δ Kcal	1435 [1249, 1621]	166.2 [- 29.16, 361.7] <i>p</i> = 0.0879	100.2 [- 126.0, 326.5] <i>p</i> = 0.35033	24.0 [- 219.4, 267.4] <i>p</i> = 0.83216	70.25 [- 146.92, 287.4] <i>p</i> = 0.4913	60.67 [- 189.8, 311.1] <i>p</i> = 0.6046	83.42 [- 222.4, 389.3] <i>p</i> = 0.5605	120.25 [-143.0, 383.5] <i>p</i> = 0.336387271	30.25 [-97.41, 157.9] <i>p</i> = 0.612328423	-91.09 [-370.76, 188.6] <i>P</i> = 0.484650243	86.33 [-140.55, - 313.2] <i>P</i> = 0.405808951	-13.1 [-255.75, - 229.5] <i>P</i> = 0.905480891	-66.33 [-305.0, 172.4] <i>P</i> = 0.539547211
CHO		125.4 [101.14, 149.6]	91.02 [74.58, 107.46]	83.88 [67.54, 100.21]	84.77 [67.77, 101.78]	82.43 [64.86, 100.00]	87.19 [65.40, 108.99]	80.98 [61.24, 100.73]	83.86 [64.20, 103.52]	79.33 [66.14, 92.52]	77.48 [66.25, 88.72]	84.51 [71.02, 97.99]	76.54 [58.26, 94.82]	69.42 [52.7, 86.14]
PRO		71.18 [61.60, 80.75]	92.95 [80.18, 105.7]	90.07 [73.61, 106.5]	90.48 [68.09, 112.9]	83.87 [67.81, 99.92]	85.42 [71.49, 99.35]	81.41 [65.10, 97.71]	85.75 [69.09, 102.4]	86.22 [70.09, 102.3]	81.09 [61.43, 100.7]	85.15 [60.74, 109.6]	82.31 [62.84, 101.8]	78.08 [59.19, 96.98]
LCD 15% CHO	Δ Kcal	1567 [1119, 2015]	121.8 [- 183.33, 427.0] <i>P</i> = 0.3946	157.0 [- 174.0, 488.0] <i>P</i> = 0.31545	159.5 [- 202.77, 521.9] <i>P</i> = 0.34966	21.27 [-286.71, 329.3] <i>P</i> = 0.8807	26.82 [- 379.71, 433.3] <i>P</i> = 0.8861	-27.55 [- 428.7, 373.6] <i>P</i> = 0.8814	85.27 [-221.0, 391.5] <i>P</i> = 0.548891902	-50.73 [- 457.5, 356.0] <i>P</i> = 0.786778148	38.36 [- 320.23, 397.0] <i>P</i> = 0.816402643	12.27 [- 391.55, 416.1] <i>P</i> = 0.947346012	-81.0 [- 529.17, 367.2] <i>P</i> = 0.695639782	-95.18 [- 474.9, 284.6] <i>P</i> = 0.588800417
CHO		148.6 [100.28, 197.0]	61.27 [46.13, 76.4]	59.45 [44.62, 74.29]	59.56 [45.39, 73.74]	56.93 [38.95, 74.90]	55.36 [37.18, 73.54]	51.34 [37.52, 65.17]	57.76 [41.47, 74.05]	55.13 [37.48, 72.77]	57.28 [41.92, 72.64]	54.86 [37.33, 72.39]	55.42 [37.67, 73.18]	56.12 [35.18, 77.06]
PRO		78.88 [51.72, 106.04]	110.78 [95.33, 126.2]	105.47 [89.92, 121.0]	104.21 [91.57, 116.9]	93.04 [73.53, 112.56]	93.65 [73.79, 113.52]	90.52 [68.55, 112.49]	97.10 [79.68, 114.5]	88.68 [65.87, 111.5]	91.10 [75.40, 106.8]	97.27 [79.01, 115.5]	91.19 [75.45, 106.9]	89.25 [63.32, 115.17]
VLCDKD 5% CHO	Δ Kcal	1805 [1261, 2350]	234.4 [50.44, 418.4] <i>P</i> = 0.0171	330.6 [123.2, 538.0] <i>P</i> = 0.00489	244.0 [67.42, 420.6] <i>P</i> = 0.01122	263.5 [90.75, 436.2] <i>P</i> = 0.0064	314.08 [78.09, 550.1] <i>P</i> = 0.0137	325.75 [113.8, 537.7] <i>P</i> = 0.00061	156.5 [-127.3, 440.3] <i>P</i> = 0.25025703	21.91 [- 231.41, 275.2] <i>P</i> = 0.851048183	138.17 [- 59.84, 336.2] <i>P</i> = 0.152823159	141.17 [- 82.04, 364.4] <i>P</i> = 0.191421546	243.4 [-13.04, 499.8] <i>P</i> = 0.06054516	-191.9 [- 134.3, 518.1] <i>P</i> = 0.215999515
CHO		145.5 [96.28, 194.8]	29.4 [19.95, 38.84]	31.21 [20.13, 42.30]	31.45 [20.72, 42.18]	35.70 [22.04, 49.36]	41.49 [21.16, 61.83]	41.49 [18.18, 64.80]	35.47 [16.45, 54.50]	34.94 [17.78, 52.11]	39.66 [17.67, 61.65]	39.90 [17.96, 61.85]	39.41 [18.03, 60.79]	40.05 [12.23, 67.87]
PRO		92.99 [66.92, 119.07]	114.88 [94.75, 135.0]	113.32 [89.83, 136.8]	109.28 [89.22, 129.3]	111.7 [86.44, 136.96]	116.58 [92.92, 140.25]	108.06 [86.46, 129.65]	106.41 [77.13, 135.7]	100.87 [71.21, 130.5]	103.52 [79.75, 127.3]	106.21 [81.34, 131.1]	115.63 [91.75, 139.5]	110.27 [83.24, 137.30]
<i>p</i> value between group variation (ANOVA)	Δ Kcal	0.37757	0.74609	0.34437	0.42478	0.24666	0.27447	0.20713	0.92726	0.88321	0.44575	0.78454	0.27853	0.32629
	CHO		<i>P</i> < 0.001	<i>P</i> < 0.001	<i>P</i> < 0.001	<i>P</i> < 0.001	<i>P</i> = 0.004	<i>P</i> = 0.009	<i>P</i> = 0.001	<i>P</i> < 0.001	<i>P</i> = 0.006	<i>P</i> = 0.004	0.018854	<i>P</i> = 0.14822
	PRO	<i>P</i> = 0.2985		<i>P</i> = 0.16186	<i>P</i> = 0.29025	<i>P</i> = 0.11193	<i>P</i> = 0.045624 (MLCD v VLCDKD, <i>p</i> = 0.043)	<i>P</i> = 0.11716	<i>P</i> = 0.34792	<i>P</i> = 0.57465	<i>P</i> = 0.23176	<i>P</i> = 0.37193	<i>P</i> = 0.036736 (MLCD v VLCDKD, <i>p</i> = 0.037)	<i>P</i> = 0.1384

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