Low-carbohydrate diets differing in carbohydrate restriction improve cardiometabolic and anthropometric markers in healthy adults: A randomised clinical trial

Cliff J. d. C. Harvey[1], Grant M. Schofield[1], Caryn Zinn[1],
Simon J. Thornley[1], Catherine Crofts[1] and Fabrice L. R. Merien[2]

[1] Human Potential Centre, Auckland University of Technology, Auckland, New Zealand
[2] AUT-Roche Diagnostics Laboratory, School of Science, Auckland University of Technology, Auckland, New Zealand

ABSTRACT

Background: Low-carbohydrate, high-fat (LCHF) diets are useful for treating a range of health conditions, but there is little research evaluating the degree of carbohydrate restriction on outcome measures. This study compares anthropometric and cardiometabolic outcomes between differing carbohydrate-restricted diets.

Objective: Our hypothesis was that moderate carbohydrate restriction is easier to maintain and more effective for improving cardiometabolic health markers than greater restriction.

Design: A total of 77 healthy participants were randomised to a very low-carbohydrate ketogenic diet (VLCKD), low-carbohydrate diet (LCD), or moderate-low carbohydrate diet (MCD), containing 5%, 15% and 25% total energy from carbohydrate, respectively, for 12-weeks. Anthropometric and metabolic health measures were taken at baseline and at 12 weeks. Using ANOVA, both within and between-group outcomes were analysed.

Results: Of 77 participants, 39 (51%) completed the study. In these completers overall, significant reductions in weight and body mass index occurred ((mean change) 3.7 kg/m^2; 95% confidence limits (CL): 3.8, 1.8), along with increases in high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, (0.49 mmol/L; 95% CL; 0.06, 0.92; $p = 0.03$), and total cholesterol concentrations (0.11 mmol/L; 95% CL; 0.00, 0.23; $p = 0.05$). Triglyceride (TG) levels were reduced by 0.12 mmol/L (95% CL; -0.20, 0.02; $p = 0.02$). No significant changes occurred between groups. The largest improvements in high density lipoprotein cholesterol (HDL-c) and TG and anthropometric changes occurred for the VLCKD group.

Conclusions: Low-carbohydrate, high-fat diets have a positive effect on markers of health. Adherence to the allocation of carbohydrate was more easily achieved in MCD, and LCD groups compared to VLCKD and there were comparable improvements in weight loss and waist circumference and greater improvements in HDL-c and TG with greater carbohydrate restriction.

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 (*Castro et al., 2015*; *Henderson et al., 2006*; *Keene, 2006*; *Kulak & Polotsky, 2013*; *Lefevre & Aronson, 2000*; *Levy et al., 2012*; *Maalouf, Rho & Mattson, 2009*; *Neal et al., 2008*; *Paoli et al., 2013*; *Sumithran & Proietto, 2008*; *Varshneya et al., 2015*). They are also used widely in the general population for weight-loss and maintenance, (*Bueno et al., 2013*) with improved satiety and control of hunger frequently reported by those who adhere to these diets (*Johnstone et al., 2008*; *McClernon et al., 2007*; *Paoli et al., 2015*). Despite the potential offered by LCHF and low-carbohydrate, high-protein 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 liproprotein cholesterol (LDL-c) concentrations between diets that are higher or lower in carbohydrate (*Hernández Alcantara, Jiménez Cruz & Bacardí Gascón, 2015*; *Huntriss, Campbell & Bedwell, 2017*; *Naude et al., 2014*; *Snorgaard et al., 2017*; *Van Wyk, Davis & Davies, 2016*). However, there are greater reductions in fasted glucose concentrations (*Snorgaard et al., 2017*), and greater improvements in high density lipoprotein cholesterol (HDL-c) and glycated haemoglobin (HbA1c) with greater degrees of carbohydrate restriction (*Huntriss, Campbell & Bedwell, 2017*). Controversy exists about the nature of low-carbohydrate diets (LCD) and VLCKDs (*Wood & Fernandez, 2009*), and definitions for LCDs range from 20 to 200 g of carbohydrate per day (*Last & Wilson, 2006*; *Westman et al., 2007*), or up to 40–45% of daily energy from carbohydrate (*Hu et al., 2012*; *Wheeler et al., 2012*). 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 ß- hydroxybutyrate, as the majority of people following a VLCKD achieve this level of blood ketones (*Gibson et al., 2015*), and this threshold has been used by several studies as an indicator of entry into NK (*Guerci et al., 2003*; *Harvey et al., 2018*). Ketonaemia consistent with NK typically results from diets containing a 3:1–4:1 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 (*Livingston, Pauli & Pruce, 1977*; *Livingstone, 1972*), or diets containing 60–75% of calories from lipids that include a high proportion of medium chain triglycerides (*Huttenlocher, Wilbourn & Signore, 1971*; *Huttenlocher, 1976*). Studies report that adherence is difficult with extreme carbohydrate restriction, that is, <50 g of carbohydrate per day (*Huntriss, Campbell & Bedwell, 2017*), 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 LCD (*McClain et al., 2013*).

Few studies directly compare very LCDs with less extreme carbohydrate-restricted diets. Johnstone and colleagues compared the effects of a non-ketogenic LCD (fat 30% of total energy (TE); carbohydrate 40% of TE) to a ketogenic, LCD (fat 60% TE; carbohydrate 5%TE) in 20 adults over 6 weeks, finding that the diets were equally effective in reducing body weight and insulin resistance (*Johnston et al., 2006*).

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
A total of 77 participants, 25 males, 52 females (mean age: 39 years, range: 25–49; mean body mass index (BMI) 27 kg/m^2, 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/m^2), 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 LCD 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.
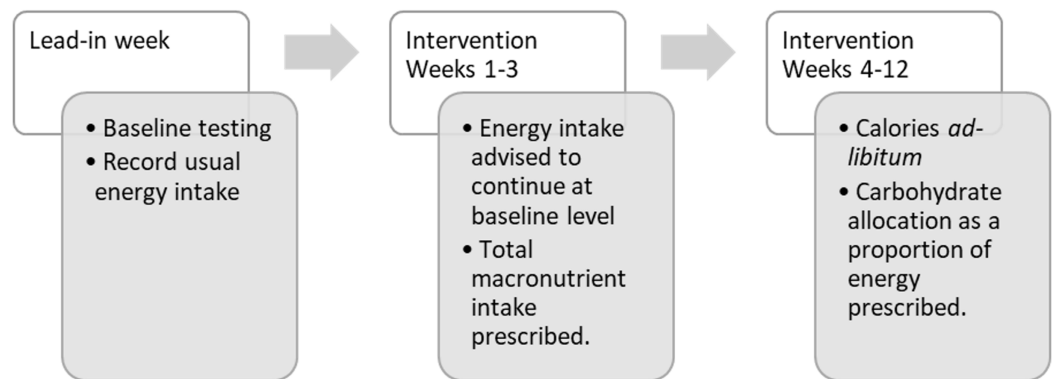
Figure 1 Flow of participants with dietary allocations during the study period.
Full-size ◮ DOI: 10.7717/peerj.6273/fig-1

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/day (weight at baseline testing), consistent with International Society of Sports Nutrition guidelines for optimal protein intake for performance (*Campbell et al., 2007*). 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 3 weeks of the intervention. For the final 9 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 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 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.

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 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 plasma separation tubes (PST) Vacutainer tubes using lithium-heparin as the anticoagulant (Becton Dickinson, Franklin Lakes, NJ, USA). Within 15 min of collection, tubes were centrifuged at $1,500 \times g$ 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, 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 paired t-test. All 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 were assessed for eligibility with 206 excluded and 77 included for randomisation to the trial groups (Fig. 2). A total of 10 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% TE from carbohydrate; and one female in the
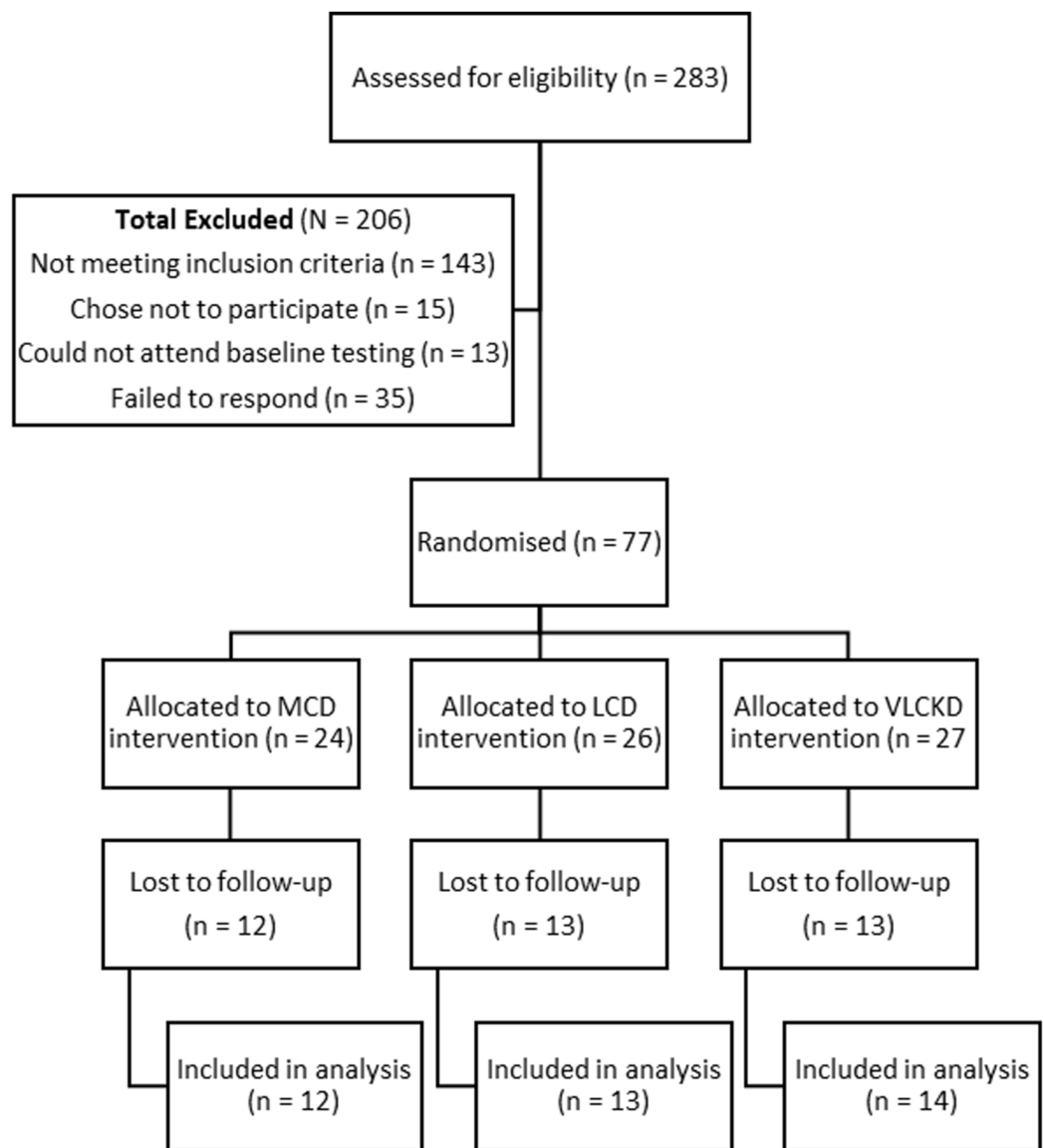
Figure 2 Participants included for participation, randomisation, allocation, and lost to follow up.
Full-size ⬛ DOI: 10.7717/peerj.6273/fig-2

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

Table 1 Baseline characteristics of study participants.

	Treatment group			Total	Test	p-value
	MCD	LCD	VLCKD			
	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)	1,435 (293)	1,567 (666)	1,805 (857)	1,603 (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

Note:
SD, standard deviation; BMI, body mass index.

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 1, by randomised treatment group.

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 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 ($p = 0.686$).

Table 2 Change in outcome measures, overall, and by group.

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

Notes:
[†] 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.

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 2.

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 (*Babio et al., 2015*; *Ballestri et al., 2016*). One participant had GGT levels above the reference range upper limit of 60 U/L. This was reduced from baseline to completion; 143 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 five 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 MLC 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 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. (Fig. 3.) All changes in reported measures, overall and by group, with 95% confidence intervals, are reported in Table 2.
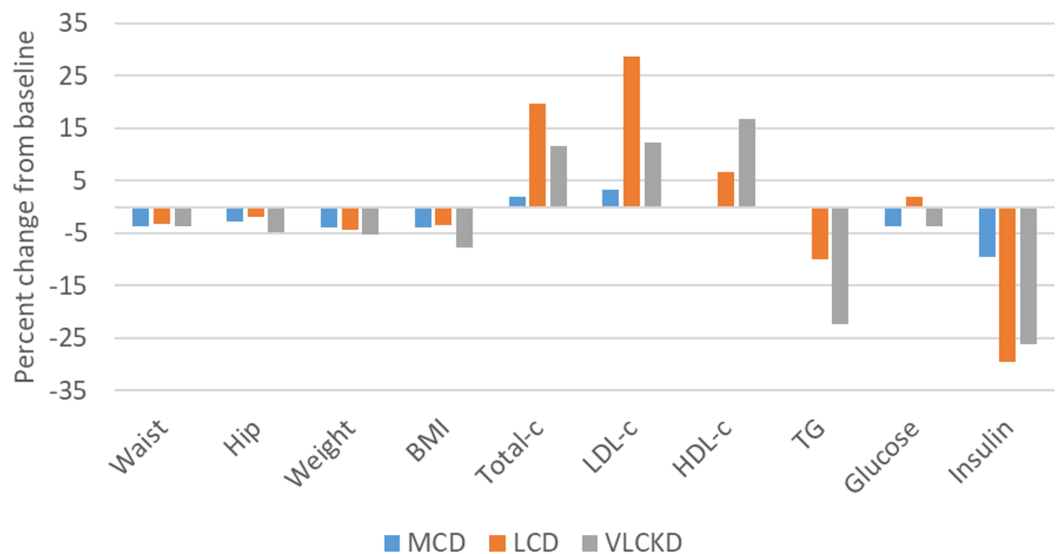
Figure 3 Percentage change from baseline in cardiometabolic and athropometric outcome measures.
Full-size 🖼 DOI: 10.7717/peerj.6273/fig-3

Adherence to diet

The individual mean daily energy intake per group, by week, is shown in Fig. 4. A marginal increase in reported energy intake occurred during the first 3 weeks, during which time participants had been advised to maintain usual energy intake. After the first 3 weeks, participants had been advised to eat ad libitum but preserve the carbohydrate allocation as a percentage of TE 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, 95, 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 TE 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.

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 5 shows the reported energy per participant, derived from carbohydrate per group, by week.

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 TE intake, and protein and carbohydrate allocations.
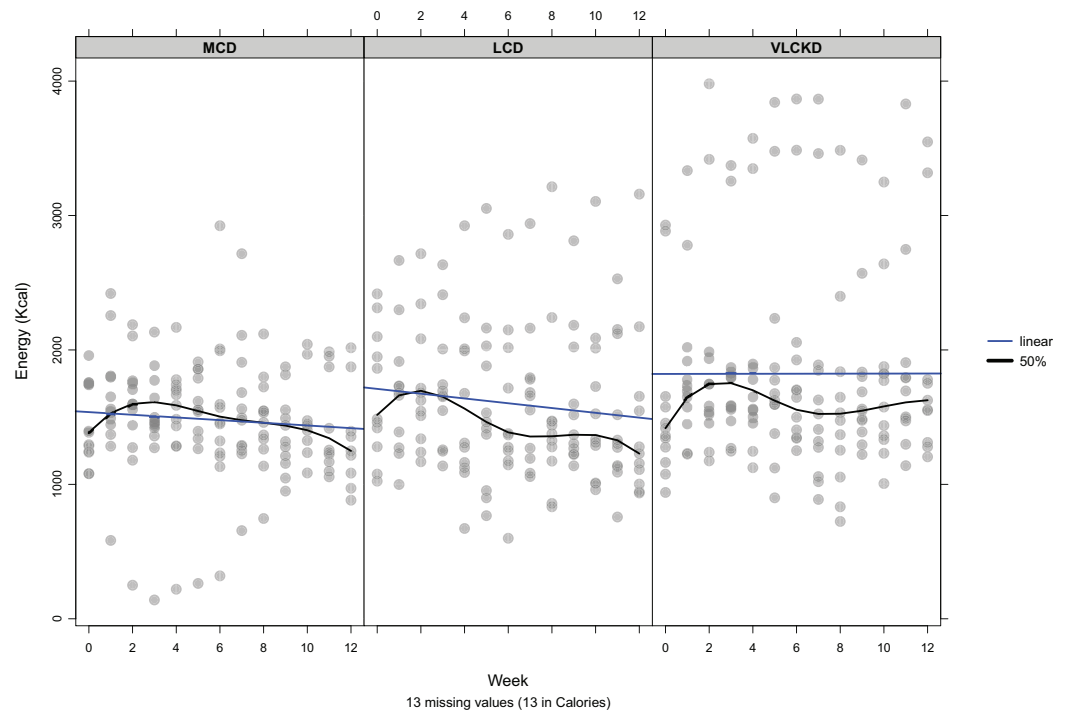
Figure 4 Mean daily energy intake by week per participant. The blue line indicates the linear trend. Black line indicates the 50th percentile. Full-size 🖼 DOI: 10.7717/peerj.6273/fig-4
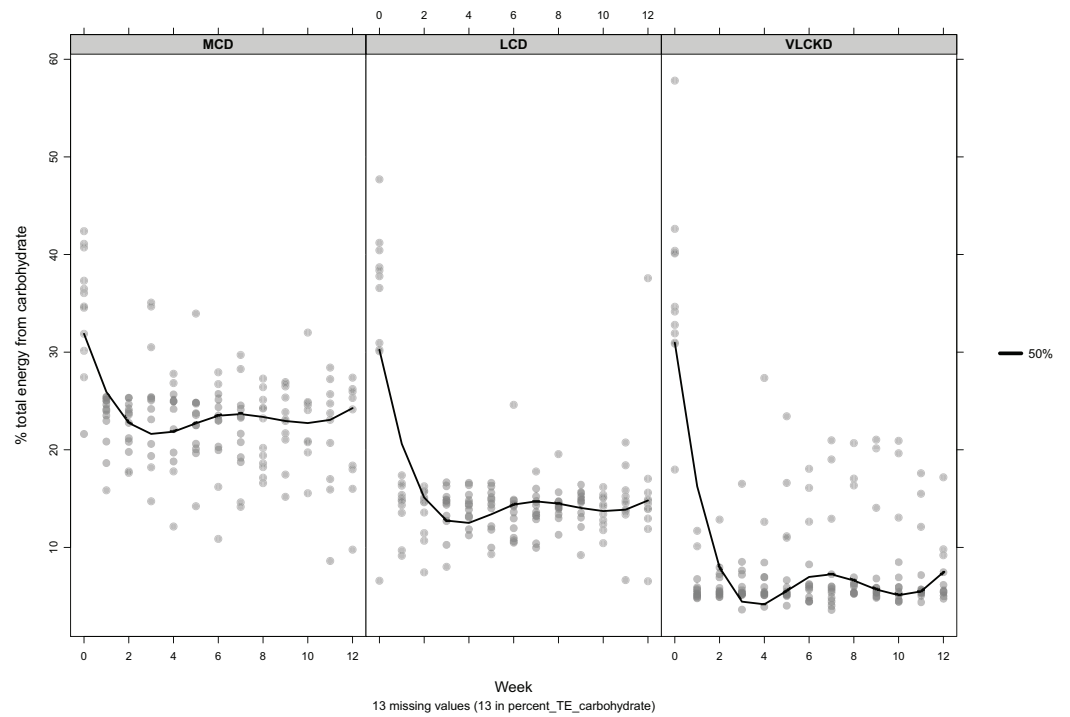

Figure 5 Mean percent of total energy derived from carbohydrate by participant, per week The black line indicates the 50th percentile. Full-size 🖼 DOI: 10.7717/peerj.6273/fig-5

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, LCDs were easily adhered to over a 12-week period. While there was little difference in the consistency of adherence between the differing 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 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. 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, 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. (*Ashwell et al., 2014*) 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 (*Harcombe et al., 2015*; *Liu et al., 2013*; *Ravnskov et al., 2016*). Reductions in relative risk are seen at TG <1.02 mmol/L, with every one mmol/L increase associated with a >12% increase in risk, for both cardiovascular disease mortality and all-cause mortality (*Liu et al., 2013*). 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 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/m^2; 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 LCDs 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 LCDs 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 (*Hession et al., 2009*). 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 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, that is, 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 45–75% of energy derived from carbohydrate (*Buyken et al., 2018*), (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 12 months (*Gardner et al., 2018*). 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, LCDs, 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.

APPENDIX 1 ASSAY PERFORMANCE

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	3–1,200 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	11.9–1,487 µmol/L	Enzymatic colorimetric test
Insulin	1.39 pmol/L	1.39–6,945 pmol/L	Electrochemoluminescence
C-peptide	0.003 mmol/L	0.003–13.3 nmol/L	Electrochemoluminescence

Note:
* 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.

ADDITIONAL INFORMATION AND DECLARATIONS

Competing Interests
The authors declare that they have no competing interests.

Author Contributions
- Cliff J. d. C. Harvey conceived and designed the experiments, performed the experiments, analysed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, approved the final draft.
- Grant M. Schofield conceived and designed the experiments, prepared figures and/or tables, authored or reviewed drafts of the paper, approved the final draft.
- Caryn Zinn conceived and designed the experiments, prepared figures and/or tables, authored or reviewed drafts of the paper, approved the final draft.
- Simon J. Thornley conceived and designed the experiments, analysed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, approved the final draft.
- Catherine Crofts conceived and designed the experiments, performed the experiments, authored or reviewed drafts of the paper, approved the final draft.
- Fabrice L. R. Merien performed the experiments, analysed the data, contributed reagents/materials/analysis tools, authored or reviewed drafts of the paper, approved the final draft.

Human Ethics
The following information was supplied relating to ethical approvals (i.e. approving body and any reference numbers):
Ethics approval for this study was granted by the Southern Committee of the Health and Disability Ethics Committee of New Zealand (17/STH/60).

Data Availability
The following information was supplied regarding data availability:
Raw data for baseline and outcome measures is available in the Supplementary Dataset.

Clinical Trial Registration
The following information was supplied regarding Clinical Trial registration:
The trial was registered by the Australia New Zealand Clinical Trial Registry. (ACTRN12617000421336p).

Supplemental Information

Supplemental information for this article can be found online at http://dx.doi.org/10.7717/peerj.6273#supplemental-information.

REFERENCES

Ashwell M, Mayhew L, Richardson J, Rickayzen B. 2014. Waist-to-height ratio is more predictive of years of life lost than body mass index. *PLOS ONE* **9(9)**:e103483 DOI 10.1371/journal.pone.0103483.

Babio N, Martínez-González MA, Estruch R, Wärnberg J, Recondo J, Ortega-Calvo M, Serra-Majem L, Corella D, Fitó M, Ros E, Becerra-Tomás N, Basora J, Salas-Salvadó J. 2015. Associations between serum uric acid concentrations and metabolic syndrome and its components in the PREDIMED study. *Nutrition, Metabolism and Cardiovascular Diseases* **25(2)**:173–180 DOI 10.1016/j.numecd.2014.10.006.

Ballestri S, Zona S, Targher G, Romagnoli D, Baldelli E, Nascimbeni F, Roverato A, Guaraldi G, Lonardo A. 2016. Nonalcoholic fatty liver disease is associated with an almost twofold increased risk of incident type 2 diabetes and metabolic syndrome. Evidence from a systematic review and meta-analysis. *Journal of Gastroenterology and Hepatology* **31(5)**:936–944 DOI 10.1111/jgh.13264.

Bueno NB, De Melo ISV, De Oliveira SL, Da Rocha Ataide T. 2013. Very-low-carbohydrate ketogenic diet v. low-fat diet for long-term weight loss: a meta-analysis of randomised controlled trials. *British Journal of Nutrition* **110(7)**:1178–1187 DOI 10.1017/S0007114513000548.

Buyken AE, Mela DJ, Dussort P, Johnson IT, MacDonald IA, Stowell JD, Brouns FJ. 2018. Dietary carbohydrates: A review of international recommendations and the methods used to derive them. *European Journal of Clinical Nutrition* **72(12)**:1625–1643 DOI 10.1038/s41430-017-0035-4.

Campbell B, Kreider RB, Ziegenfuss T, La Bounty P, Roberts M, Burke D, Landis J, Lopez H, Antonio J. 2007. International society of sports nutrition position stand: protein and exercise. *Journal of the International Society of Sports Nutrition* **4(1)**:8 DOI 10.1186/1550-2783-4-8.

Castro K, Faccioli LS, Baronio D, Gottfried C, Perry IS, Dos Santos Riesgo R. 2015. Effect of a ketogenic diet on autism spectrum disorder: a systematic review. *Research in Autism Spectrum Disorders* **20**:31–38 DOI 10.1016/j.rasd.2015.08.005.

Gardner CD, Trepanowski JF, Del Gobbo LC, Hauser ME, Rigdon J, Ionnidis JPA, Desai M, King AC. 2018. Effect of low-fat vs low-carbohydrate diet on 12-month weight loss in overweight adults and the association with genotype pattern or insulin secretion: the dietfits randomized clinical trial. *JAMA* **319(7)**:667–679 DOI 10.1001/jama.2018.0245.

Gibson AA, Seimon RV, Lee CMY, Ayre J, Franklin J, Markovic TP, Caterson ID, Sainsbury A. 2015. Do ketogenic diets really suppress appetite? A systematic review and meta-analysis. *Obesity Reviews* **16(1)**:64–76 DOI 10.1111/obr.12230.

Guerci B, Benichou M, Floriot M, Bohme P, Fougnot S, Franck P, Drouin P. 2003. Accuracy of an electrochemical sensor for measuring capillary blood ketones by fingerstick samples during metabolic deterioration after continuous subcutaneous insulin infusion interruption in type 1 diabetic patients. *Diabetes Care* **26(4)**:1137–1141 DOI 10.2337/diacare.26.4.1137.

Harcombe Z, Baker JS, Cooper SM, Davies B, Sculthorpe N, DiNicolantonio JJ, Grace F. 2015. Evidence from randomised controlled trials did not support the introduction of dietary fat guidelines in 1977 and 1983: a systematic review and meta-analysis. *Open Heart* **2(1)**:e000196 DOI 10.1136/openhrt-2014-000196.

Harvey CJdC, Schofield GM, Williden M, McQuillan JA. 2018. The effect of medium chain triglycerides on time to nutritional ketosis and symptoms of keto-induction in healthy adults: a randomised controlled clinical trial. *Journal of Nutrition and Metabolism* **2018**:1–9 DOI 10.1155/2018/2630565.

Henderson CB, Filloux FM, Alder SC, Lyon JL, Caplin DA. 2006. Efficacy of the ketogenic diet as a treatment option for epilepsy: meta-analysis. *Journal of Child Neurology* **21**:193–198.

Hernández Alcantara G, Jiménez Cruz A, Bacardí Gascón M. 2015. Effect of low carbohydrate diets on weight loss and glycosilated hemoglobin in people with type 2 diabetes: systematic review. *Nutricion hospitalaria* **32(5)**:1960–1966 DOI 10.3305/nh.2015.32.5.9695.

Hession M, Rolland C, Kulkarni U, Wise A, Broom J. 2009. Systematic review of randomized controlled trials of low-carbohydrate vs. low-fat/low-calorie diets in the management of obesity and its comorbidities. *Obesity Reviews* **10(1)**:36–50 DOI 10.1111/j.1467-789X.2008.00518.x.

Hu T, Mills KT, Yao L, Demanelis K, Eloustaz M, Yancy WS, Kelly TN, He J, Bazzano LA. 2012. Effects of low-carbohydrate diets versus low-fat diets on metabolic risk factors: a meta-analysis of randomized controlled clinical trials. *American Journal of Epidemiology* **176(Suppl_7)**:S44–S54 DOI 10.1093/aje/kws264.

Huntriss R, Campbell M, Bedwell C. 2017. The interpretation and effect of a low-carbohydrate diet in the management of type 2 diabetes: a systematic review and meta-analysis of randomised controlled trials. *European Journal of Clinical Nutrition* **72(3)**:311–325 DOI 10.1038/s41430-017-0019-4.

Huttenlocher PR. 1976. Ketonemia and seizures: metabolic and anticonvulsant effects of two ketogenic diets in childhood epilepsy. *Pediatric Research* **10(5)**:536–540 DOI 10.1203/00006450-197605000-00006.

Huttenlocher PR, Wilbourn AJ, Signore JM. 1971. Medium-chain triglycerides as a therapy for intractable childhood epilepsy. *Neurology* **21(11)**:1097–1097 DOI 10.1212/wnl.21.11.1097.

Johnston CS, Tjonn SL, Swan PD, White A, Hutchins H, Sears B. 2006. Ketogenic low-carbohydrate diets have no metabolic advantage over nonketogenic low-carbohydrate diets. *American Journal of Clinical Nutrition* **83(5)**:1055–1061 DOI 10.1093/ajcn/83.5.1055.

Johnstone AM, Horgan GW, Murison SD, Bremner DM, Lobley GE. 2008. Effects of a high-protein ketogenic diet on hunger, appetite, and weight loss in obese men feeding ad libitum. *American Journal of Clinical Nutrition* **87(41)**:44–55 DOI 10.1093/ajcn/87.1.44.

Keene DL. 2006. A systematic review of the use of the ketogenic diet in childhood epilepsy. *Pediatric Neurology* **35(1)**:1–5 DOI 10.1016/j.pediatrneurol.2006.01.005.

Kulak D, Polotsky AJ. 2013. Should the ketogenic diet be considered for enhancing fertility? *Maturitas* **74(1)**:10–13 DOI 10.1016/j.maturitas.2012.10.003.

Last AR, Wilson SA. 2006. Low-carbohydrate diets. *American Family Physician* **73**:1951–1958.

Lefevre F, Aronson N. 2000. Ketogenic diet for the treatment of refractory epilepsy in children: a systematic review of efficacy. *Pediatrics* **105(4)**:e46 DOI 10.1542/peds.105.4.e46.

Levy RG, Cooper PN, Giri P, Weston J. 2012. Ketogenic diet and other dietary treatments for epilepsy. *Cochrane database of systematic reviews* **2**:CD001903 DOI 10.1002/14651858.CD001903.pub3.

Liu J, Zeng F-F, Liu Z-M, Zhang C-X, Ling W-h, Chen Y-M. 2013. Effects of blood triglycerides on cardiovascular and all-cause mortality: a systematic review and meta-analysis of 61 prospective studies. *Lipids in Health and Disease* **12(1)**:159 DOI 10.1186/1476-511X-12-159.

Livingston S, Pauli LL, Pruce I. 1977. Ketogenic diet in treatment of childhood epilepsy. *Developmental Medicine and Child Neurology* **19**:833–834.

Livingstone S. 1972. Comprehensive management of epilepsy in infancy, childhood and adolescence. *Archives of Disease in Childhood* **47(255)**:842 DOI 10.1136/adc.47.255.842.

Maalouf M, Rho JM, Mattson MP. 2009. The neuroprotective properties of calorie restriction, the ketogenic diet, and ketone bodies. *Brain Research Reviews* **59(2)**:293–315 DOI 10.1016/j.brainresrev.2008.09.002.

McClain AD, Otten JJ, Hekler EB, Gardner CD. 2013. Adherence to a low-fat vs. low-carbohydrate diet differs by insulin resistance status. *Diabetes, Obesity and Metabolism* **15(1)**:87–90 DOI 10.1111/j.1463-1326.2012.01668.x.

McClernon FJ, Yancy WS Jr, Eberstein JA, Atkins RC, Westman EC. 2007. The effects of a low-carbohydrate ketogenic diet and a low-fat diet on mood, hunger, and other self-reported symptoms*. *Obesity (Silver Spring)* **15(1)**:182 DOI 10.1038/oby.2007.516.

Naude CE, Schoonees A, Senekal M, Young T, Garner P, Volmink J. 2014. Low carbohydrate versus isoenergetic balanced diets for reducing weight and cardiovascular risk: a systematic review and meta-analysis. *PLOS ONE* **9(7)**:e100652 DOI 10.1371/journal.pone.0100652.

Neal EG, Chaffe H, Schwartz RH, Lawson MS, Edwards N, Fitzsimmons G, Whitney A, Cross JH. 2008. The ketogenic diet for the treatment of childhood epilepsy: a randomised controlled trial. *Lancet Neurology* **7(6)**:500–506 DOI 10.1016/S1474-4422(08)70092-9.

Paoli A, Bosco G, Camporesi E, Mangar D. 2015. Ketosis, ketogenic diet and food intake control: a complex relationship. *Frontiers in Psychology* **6**:27 DOI 10.3389/fpsyg.2015.00027.

Paoli A, Rubini A, Volek J, Grimaldi K. 2013. Beyond weight loss: a review of the therapeutic uses of very-low-carbohydrate (ketogenic) diets. *European Journal of Clinical Nutrition* **67(8)**:789–796 DOI 10.1038/ejcn.2013.116.

Ravnskov U, Diamond DM, Hama R, Hamazaki T, Hammarskjold B, Hynes N, Kendrick M, Langsjoen PH, Malhotra A, Mascitelli L, McCully KS, Ogushi Y, Okuyama H, Rosch PJ, Schersten T, Sultan S, Sundberg R. 2016. Lack of an association or an inverse association between low-density-lipoprotein cholesterol and mortality in the elderly: a systematic review. *BMJ Open* **6(6)**:e010401 DOI 10.1136/bmjopen-2015-010401.

Snorgaard O, Poulsen GM, Andersen HK, Astrup A. 2017. Systematic review and meta-analysis of dietary carbohydrate restriction in patients with type 2 diabetes. *BMJ Open Diabetes Research & Care* **5(1)**:e000354 DOI 10.1136/bmjdrc-2016-000354.

Sumithran P, Proietto J. 2008. Ketogenic diets for weight loss: a review of their principles, safety and efficacy. *Obesity Research & Clinical Practice* **2(1)**:1–13 DOI 10.1016/j.orcp.2007.11.003.

Van Wyk HJ, Davis RE, Davies JS. 2016. A critical review of low-carbohydrate diets in people with Type 2 diabetes. *Diabetic Medicine* **33(2)**:148–157 DOI 10.1111/dme.12964.

Varshneya K, Carico C, Ortega A, Patil CG. 2015. The efficacy of ketogenic diet and associated hypoglycemia as an adjuvant therapy for high-grade gliomas: a review of the literature. *Cureus* **7**:e251 DOI 10.7759/cureus.251.

Westman EC, Feinman RD, Mavropoulos JC, Vernon MC, Volek JS, Wortman JA, Yancy WS, Phinney SD. 2007. Low-carbohydrate nutrition and metabolism. *American Journal of Clinical Nutrition* **86**:276–284.

Wheeler ML, Dunbar SA, Jaacks LM, Karmally W, Mayer-Davis EJ, Wylie-Rosett J, Yancy WS. 2012. Macronutrients, food groups, and eating patterns in the management of diabetes: a systematic review of the literature, 2010. *Diabetes Care* **35(2)**:434–445 DOI 10.2337/dc11-2216.

Wood RJ, Fernandez ML. 2009. Carbohydrate-restricted versus low-glycemic-index diets for the treatment of insulin resistance and metabolic syndrome. *Nutrition Reviews* **67(3)**:179–183 DOI 10.1111/j.1753-4887.2009.00186.x.