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Complete List of Authors:	Jenkins, David; St. Michael's Hospital, Clinical Nutrition & Risk Factor Modification Center; University of Toronto, Nutritional Sciences Wong, Julia; St. Michael's Hospital, Clinical Nutrition & Risk Factor Modification Center Kendall, Cyril; St. Michael's Hospital, Clinical Nutrition & Risk Factor Modification Center Esfahani, Amin; St. Michael's Hospital, Clinical Nutrition & Risk Factor Modification Center Ng, Vivian; St. Michael's Hospital, Clinical Nutrition & Risk Factor Modification Center Leong, Tracy; University of Toronto, Nutritional Sciences Faulkner, Dorothea; St. Michael's Hospital, Clinical Nutrition & Risk Factor Modification Center Vidgen, Ed; St. Michael's Hospital, Clinical Nutrition & Risk Factor Modification Center Paul, Gregory; Solae LLC, Mukherjea, Ratna; Solae LLC, Krul, Elaine; Solae LLC, Singer, William; St. Michael's Hospital, Medicine
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Six Months of a Vegan Low-Carbohydrate ("Eco-Atkins") Diet Improves Cardiovascular Risk Factors and Body Weight in Hyperlipidemic Adults: A Randomized Controlled Trial

David JA Jenkins, MD[1-5] Julia MW Wong, PhD[1,4] Cyril WC Kendall, PhD[1,4] Amin Esfahani, MSc[1,4] Vivian WY Ng, RD[1,4] Tracy CK Leong, BASc[1,4] Dorothea A Faulkner, PhD[1,4] Ed Vidgen, BSc[1,4] Gregory Paul, PhD[6] Ratna Mukherjea, PhD[6] Elaine S. Krul, PhD[6] William Singer, MD[1,2,4,5]

[1]Clinical Nutrition & Risk Factor Modification Center, St. Michael's Hospital, Toronto, Ontario, Canada; [2]Department of Medicine, Division of Endocrinology and Metabolism, [3]Li Ka Shing Knowledge Institute, St. Michael's Hospital, Toronto, Ontario, Canada; Departments of [4]Nutritional Sciences, [5]Medicine, Faculty of Medicine, University of Toronto, Toronto, Ontario, Canada; [6]Solae LLC, St. Louis, Missouri, USA

JMWW current affiliation is the New Balance Foundation Obesity Prevention Center, Boston Children's Hospital, Boston, MA, USA, and Department of Pediatrics, Harvard Medical School, Boston, MA, USA.

AE current affiliation is New York Medical College, School of Medicine, Valhalla, NY, USA.

Address correspondence and reprint requests to David JA Jenkins, Clinical Nutrition and Risk Factor Modification Center, St. Michael's Hospital, 61 Queen St. East, Toronto, Ontario, CANADA, M5C 2T2. Phone: (416) 978-4752; Fax: (416) 978-5310; EM: cyril.kendall@utoronto.ca

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Contributions

Conception and design - Jenkins, Wong, Kendall, Faulkner, Paul, Mukherjea, Krul, Singer

Acquisition of data - Jenkins, Wong, Kendall, Esfahani, Ng, Leong

Analysis and interpretation of data – Jenkins, Wong, Kendall, Vidgen

Drafting of the manuscript – Jenkins, Wong

Critical revision of the manuscript for important intellectual content – Jenkins, Wong, Kendall, Esfahani, Ng, Leong, Faulkner, Vidgen, Paul, Mukherjea, Krul, Singer

Statistical analysis - Vidgen

Obtaining funding – Jenkins, Kendall, Wong

Administrative, technical, or material support – Wong, Kendall, Esfahani, Ng, Leong, Faulkner

Supervision – Jenkins, Kendall, Wong, Singer

No additional contributions - Paul, Mukherjea, Krul

Abstract

Objective: Low-carbohydrate diets may be useful for weight loss. Diets high in vegetable proteins and oils may reduce the risk of coronary heart disease (CHD). The main objective was to determine the longer term effect of a diet that was both low-carbohydrate and plant-based on weight loss and LDL-C.

Design, Setting, Participants: A parallel design study of 39 overweight hyperlipidemic men and postmenopausal women conducted at a Canadian university-affiliated hospital nutrition research center from April 2005 to November 2006.

Intervention: Participants were advised to consume either a low-carbohydrate vegan diet or a high-carbohydrate lacto-ovo vegetarian diet for six-months after completing one-month metabolic (all foods provided) versions of these diets. The prescribed macronutrient intakes for the low- and high-carbohydrate diets were: 26% and 58% of energy from carbohydrate, 31% and 16% from protein and 43% and 25% from fat, respectively.

Primary Outcome: Change in body weight.

Results: Twenty-three participants (50% test, 68% control) completed the six-month ad libitum study. The approximate 4kg weight loss on the metabolic study was increased to -6.9kg on low-carbohydrate and -5.8kg on high-carbohydrate six-month ad libitum treatments (treatment difference [95% CI]: -1.1kg [-2.1, 0.0], P=0.047). The relative LDL-C and triglyceride reductions were also greater on the low-carbohydrate treatment (treatment difference [95% CI]: -0.49mmol/L [-0.70, -0.28], P<0.001 and -0.34mmol/L [-0.57, -0.11], P=0.005, respectively), as were the TC:HDL-C and apolipoprotein B:A1 ratios (-0.57 [-0.83, -0.32], P<0.001 and -0.05 [-0.09, -0.02], P=0.003, respectively).

Conclusions: A self-selected low-carbohydrate vegan diet, containing increased protein and fat from gluten and soy products, nuts, and vegetable oils, had lipid lowering advantages over a high-carbohydrate, low-fat weight loss diet, thus improving heart disease risk factors.

Trial Registration: clinicaltrials.gov (http://www.clinicaltrials.gov/), #NCT00256516

Abstract Word Count: 273 (up to 300 allowed)

Article Summary

Article Focus

- Low-carbohydrate diets may be useful for weight loss. Diets high in vegetable proteins and oils may reduce the risk of coronary heart disease (CHD).
- The objective of the randomized clinical trial was to determine the longer term effect of a diet that was both low-carbohydrate and plant-based on weight loss and LDL-C.
- We have already reported the effect of this dietary strategy in producing a difference of 8% in LDL-C reduction between calorie-restricted diets (60% of estimated calorie requirements) when all food was provided. We now report findings after these same participants continued on their respective diets for an additional 6 months, under self-selected conditions, in order to gain insights into the real life effectiveness of this diet.

Key Messages

- By comparison with the high-carbohydrate diet, consumption of the low-carbohydrate diet containing vegetable proteins and oils was also associated with significantly reduced concentrations of LDL-C. This LDL-C reduction has not been reported for other low-carbohydrate diet studies in which a large part of the protein and fat originated from animal sources and where increases in LDL-C were seen.
- The present study also demonstrated that consumption of a low-carbohydrate vegan diet resulted in modestly greater body weight reductions compared to a high-carbohydrate diet (7% versus 6% reductions, respectively) over a six-month ad libitum period.

- The sustained reduction in LDL-C, associated with only a small incremental weight loss on the 6-month self-selected diet, is a potentially important attribute of the diet in reducing long-term CHD risk

Strengths and Limitations of this Study

The study weaknesses include the relatively small sample size and the high dropout rate. Nevertheless, high dropout rates have been reported in similar dietary studies and it is noteworthy that attrition rates were low in the metabolic study when all food was provided [1]. Food availability and preparation may therefore be important factors. For those who did complete the study, however, there were benefits in weight loss and LDL-C reduction, an additional 2% advantage in body weight reduction compared to the high-carbohydrate diet and a 13% drop in LDL-C for participants consuming a more plant-based low-carbohydrate diet. The study's strength is that the prescribed hypocaloric diet was self-selected, meaning the results are more in line with what can be expected under free-living conditions. The breadth of application of the plant-based low-carbohydrate diet, however, remains to be determined, but it may provide an option for some individuals for whom LDL-C reduction is an equal concern to weight loss. If low-carbohydrate dietary options become more generally available the number of individuals who will benefit is likely to increase.

Introduction

Many popular weight loss diets emphasize carbohydrate restriction (Atkins, Eddies, South Beach, Zone). Their success is determined by the level of compliance with the prescribed diets [2-7]. However, a high content of animal products, rich in saturated fat and cholesterol, may make conventional low-carbohydrate diets less appropriate for those with hypercholesterolemia [3 8]. Even during active weight loss, these high saturated fat diets, may raise serum LDL-C above baseline [3 8] and there is concern that if such diets continue to be eaten when weight loss has ceased, a more atherogenic blood lipid profile may result [9]. These concerns have prompted exploration of other weight loss strategies, but only modest reductions in LDL-C have been observed [10].

By contrast vegan diets significantly lower LDL-C [11]. Trials of vegan and vegetarian diets also reduce progression of coronary heart disease (CHD) [12] and improve diabetes control [13]. Plant food components such as vegetable proteins, vegetable oils, nuts and viscous fibers, reduce serum lipids in many studies [14-19] and may increase flow mediated vasodilatation [20-23]. Nuts, fiber and vegetarian diets in general, all reduce CHD and diabetes in cohort studies [24-29]. Finally, in cohort studies, low-carbohydrate diets, high in vegetable oils and proteins as opposed to animal products, reduce CHD events and diabetes incidence in women [30 31], while lower red meat intake reduces total, cardiovascular and cancer mortality [32]. Most recently a large randomized controlled trial confirmed the effect of nuts and increased vegetable oil (olive oil) intake in reducing cardiovascular events in the context of a Mediterranean diet [33].

In view of the apparent success of low-carbohydrate diets for weight loss and the demonstration that relatively high-carbohydrate vegetarian and vegan diets, and diets low in animal products, lower CHD risk factors [34-37], we designed a diet that combined both vegan and low-

carbohydrate elements to determine whether such a diet captured both the weight loss and CHD risk reduction advantages. We have already reported the effect of this dietary strategy in producing a difference of 8% in LDL-C reduction between calorie-restricted diets (60% of estimated calorie requirements) when all food was provided [1]. We now report findings after these same participants continued on their respective diets for an additional 6 months, under self-selected conditions, in order to gain insights into the real life effectiveness of this diet. The results of the metabolic (all foods provided) study have been reported previously and had demonstrated a CHD risk factor advantage, but with no greater weight loss than the control diet [1].

Methods

Participants

Forty-seven overweight participants, recruited by newspaper advertisement and hospital clinic notices, undertook the one-month metabolic first phase of the study (Figure 1) that has been previously reported [1]. On completion of this phase, thirty-nine participants (19 control and 20 test participants) continued for an ad libitum six-month study (Table 1). The study was conducted at a Canadian university-affiliated hospital nutrition research center from April 2005 to November 2006. All participants had high normal to raised LDL-C levels (>3.4mmol/L at diagnosis) and a body mass index > 27 kg/m^2. Details of the eligibility criteria have been previously reported [1]. After recruitment, 11/39 participants discontinued lipid lowering medications at least two weeks prior to starting and for the study duration (Table 1).

Study Protocol

The intervention was a randomized parallel study stratified by sex in which participants were randomized to either low- or high-carbohydrate, calorie-reduced diets. The first month was the previously reported metabolically controlled study [1]. For the following six-months, participants continued on the diet to which they had been assigned as a self-selected (ad libitum) diet. Anthropometric, blood pressure and blood lipid measurements were repeated at monthly intervals. Insulin and HbA1c were measured at baseline and at the start and end of the ad libitum treatment. Percentage body fat was measured at baseline and end of the ad libitum treatment by bioelectrical impedance (Quantum II; RJL Systems, Clinton Township, Michigan). Seven-day diet and exercise histories were recorded in the week prior to each visit and discussed with the dietitian to enhance adherence. Alterations in exercise were allowed and recorded.

The Ethics Committees of St. Michael's Hospital and the University of Toronto, and the Therapeutic Products Directorate of Health Canada approved the study. Written informed consent was obtained from the participants. The study's clinical trial registration number was #NCT00256516.

Diets

As with the previous metabolic study, participants were encouraged to eat only 60% of the estimated caloric requirements to maintain a stable body weight [38-40]. The prescribed test diet was a low-carbohydrate vegan diet containing 26% of calories from carbohydrate, 31% of calories from vegetable proteins and 43% from fat (primarily vegetable oils). The control, high-carbohydrate diet (58% carbohydrate, 16% protein and 25% fat) emphasized whole wheat cereals and cereal fiber. Details of the diets have been published previously [1]. Carbohydrate sources on the low-carbohydrate diet featured viscous fiber-containing foods (such as oats and

barley) and low-starch vegetables (emphasizing okra and eggplant) for the relatively limited amount of carbohydrate allowed. Participants were able to purchase at the research center the "no" starch high protein nut bread and three of the seitan (wheat gluten) products used in the study which were not available in Canada.

Self-taring electronic scales (My Weigh Scales, Vancouver, BC or Tanita Corporation, Arlington Heights, IL) were provided to all participants and they were instructed to weigh all food items while recording the seven-day food dairy in the week prior to clinic visits. Adherence was assessed from the completed seven-day food records. Neither the dietitians nor participants could be blinded, but equal emphasis was placed on the potential importance for health of both diets. The analytical technicians were blinded to diet allocation, as was the statistician, up to analysis of the primary outcome. Participants were offered no financial compensation for participation in the study.

Analyses

The analytical techniques have been reported previously [1]. Serum was analyzed according to the Lipid Research Clinics protocol in the J. Alick Little Lipid Research Laboratory [35] and LDL-C (in mmol/L) was calculated by the method of Friedewald et al. [1]. The methods for analyzing apolipoproteins A1 and B, high sensitivity C-reactive protein (hs-CRP), blood glucose, insulin, HbA1c, and homeostasis model assessment – insulin resistance model (HOMA-IR) have been described previously [1]. Exercise data were calculated as metabolic equivalents (METs) [41]. The absolute 10-year CHD risk score was calculated using the Framingham risk equation [42].

Diets were assessed for macronutrients, fatty acids, cholesterol and fiber using a computer program based on the USDA database [43] and developed in our laboratory to allow the addition of the macronutrient content of study foods obtained from food labels or directly from food manufacturers.

Adherence with the three principal cholesterol-lowering components [vegetable proteins (soy and gluten), nuts, and viscous fibers] of the low-carbohydrate diet was estimated from the 7-day food records by applying 33.3% adherence factor to the recorded intake for each of the three main components. The sum of the three components if consumed as prescribed would equal 100% adherence.

Statistical Analyses

Results are expressed as means ± SEM or 95% confidence intervals (CIs). Time zero was used as the baseline and refers to the pre-metabolic study baseline [1]. Treatment differences in physical and biochemical measures were assessed using all available data and a repeated measures mixed model accounting for time of assessment (SAS 9.2) [44] in the Tables (Table 2 and 3) and the Results. The response variable was change from baseline, with diet and week as fixed effects and subject ID nested in diet. There was no adjustment for baseline. Any participant who started the ad libitum treatment was included in the analysis (N=39).

Multiple imputation (taking the mean of 5 sets of randomly imputed values) was used to present baseline and treatment values in the Tables (2 and 3) and Figures (2 and 3) by generating data for those who dropped out or had missing values [44].

Results

Compliance with the major dietary components [vegetable proteins (soy and gluten), nuts, and viscous fibers] was 33.6% or one-third of that prescribed during the metabolic phase (Table 2). Saturated fat intakes were similar on both treatments whereas intake of monounsaturated fats, vegetable proteins, and soy protein were significantly higher on the low-carbohydrate diet (Table 2). Available carbohydrate intake was significantly lower on the low-carbohydrate diet (Table 2). The dropout rate was 35% (7/20) on the low-carbohydrate and 26% (5/19) on the high-carbohydrate (Figure 1). Three participants were withdrawn by the study physician due to failure to attain LDL-C targets on the low-carbohydrate diet (mean LDL-C = 5.24mmol/L) and one subject on the high-carbohydrate diet (LDL-C = 7.78mmol/L). Participants on the low-carbohydrate diet tended to have larger reductions in body weight over time (Figure 2). The weight loss from baseline to the end of the 6-month ad libitum treatment was -6.9kg [95% CI, -7.7, -6.1] on the low-carbohydrate and –5.8kg [95% CI, -6.6, -5.1] on the control diet with a significant difference between groups (treatment difference [95% CI]: -1.1kg [-2.1, 0.0]; P=0.047) (Table 3). The final reduction in BMI was also greater on the low-carbohydrate versus high-carbohydrate diet (treatment difference [95% CI]: -0.4kg/m^2 [-0.8, 0.0]; P=0.039) (Table 3). There was a relative increase in recorded exercise by the high-carbohydrate diet participants, whereas there was no relative change in the low-carbohydrate participants (treatment difference [95% CI]: -9.3 [-16.4, -2.2] METs; P=0.012), but this was not reflected in a greater weight loss (Table 3). There were no treatment differences in percent body fat, waist circumference or satiety (Table 3).

Lipids

At the end of the study, the reduction on the low-carbohydrate versus high-carbohydrate diet was greater for LDL-C (treatment difference [95% CI]: -0.49mmol/L [-0.70, -0.28]; P<0.001, for TC (-0.62mmol/L [-0.86, -0.37]; P<0.001, for TC:HDL-C -0.57 [-0.83, -0.32]; P<0.001, for LDL-C:HDL-C (-0.42 [-0.60, -0.24]; P<0.001, and for triglycerides (-0.34mmol/L [-0.57, -0.11]; P=0.005). No treatment difference was seen in HDL-C (Table 3). Values for LDL-C and the TC:HDL-C ratio were consistently lower in participants on the low-carbohydrate diet throughout the study while HDL-C values were not different from baseline (Figure 3 A-C).

Apolipoproteins

ApoB and the ApoB:A1 ratio were reduced more on the low- versus the high-carbohydrate diet at the end of the study (treatment different [95% CI]: -0.11g/L [-0.16, -0.06]; P<0.001 and -0.05 [-0.09, -0.02]; P=0.003, respectively) (Table 3). No significant difference between the diets was observed for ApoA1 concentrations. Figure 3D and 3F show that the low-carbohydrate diet resulted in lower apoB and ApoB:ApoA1 ratio relative to baseline over the course of the study.

C-Reactive Protein, HbA1c, Blood Glucose, Serum Insulin, Insulin Resistance and Blood Pressure

Both treatments reduced hs-CRP with no difference between treatments (Table 3). HbA1c, fasting blood glucose, insulin, and insulin resistance (calculated using the HOMA model) fell similarly on both treatments during the course of the study (Table 3). Systolic and diastolic blood pressure decreased similarly with no treatment differences (Table 3).

Calculated CHD Risk

The low-carbohydrate diet significantly reduced the calculated 10-year CHD risk relative to the high-carbohydrate diet (2% [-2, -1]; P<0.001) (Table 3).

Adverse Events

No serious adverse events or events that involved hospitalisation occurred during the study.

Discussion

The present study demonstrated that consumption of a low-carbohydrate vegan diet resulted in modestly greater body weight reductions compared to a high-carbohydrate diet (7% versus 6% reductions, respectively) over a six-month ad libitum period. These reductions were similar to those reported for low-carbohydrate "Atkins-like" diets[2 3 6 10]. However by comparison with the high-carbohydrate diet, consumption of the low-carbohydrate diet containing vegetable proteins and oils was also associated with significantly reduced concentrations of LDL-C. This LDL-C reduction has not been reported for other low-carbohydrate diet studies in which a large part of the protein and fat originated from animal sources and where increases in LDL-C were seen [2-6 8]. The sustained reduction in LDL-C, associated with only a small incremental weight loss on the 6-month self-selected diet, is a potentially important attribute of the diet in reducing long-term CHD risk [45 46]. Furthermore, as seen in the present study, a low-carbohydrate diet, in which vegetable fat and protein options were encouraged, demonstrated a larger reduction in the TC:HDL-C ratio than that reported at 6 months in weight loss studies employing either a Mediterranean or a high-carbohydrate diet [10].

The majority of studies undertaken to date have been 6 months to one year in duration [2-6 47] with more recent studies of up to 2 years [2 8] and, as with the present study, a number of these

studies had a high dropout rate [2 3 5 47]. However, the high dropout rate in the present study did not prevent identification of significant LDL-C and body weight differences in the intent-to-treat analysis (using all available data). The completer data therefore demonstrated an even larger treatment difference in LDL-C of -0.60mmol/L [-0.84, -0.36] favoring the test treatment (P<0.001). Those on the low-carbohydrate diet showed overall adherence to the major dietary components [vegetable proteins (soy and gluten), nuts, and viscous fibers] at 33.6% of that provided during the metabolic phase [1]. This adherence is similar to the 43.3% seen with the dietary portfolio in the comparison of the metabolic one month [35] and the ad libitium six month studies [48]. In this comparison also just under half the LDL-C reduction (13-14%) seen on the ad libitium compared to the metabolic study [35].

The effect of low-carbohydrate diets on CHD events has not been assessed in randomized controlled trials. Nevertheless, low-carbohydrate diets high in vegetable proteins and oils have been associated with a 30% reduced CHD risk and an 18% reduced incidence of diabetes in cohort studies [30 31]. The median interquantile difference in these studies between the first and 10th decile for vegetable protein and monounsaturated fat (MUFA) intakes, as a marker of increased vegetable oil consumption, was 1.4% and 9.3% expressed as a percentage of total caloric intake [30]. These figures compared to 8.2% and 4.6% in our studies as the relative increase from baseline on the Eco-Atkins diet compared to the control diet. The increases in MUFA were therefore seen in both studies. Recently a Spanish Mediterranean diet emphasizing increased nut or olive oil consumption, increasing monounsaturated fat intake by 2-3%, has been shown to significantly reduce cardiovascular events also by approximately 30% [33]. These data provide consistent support for the view that the Eco-Atkins approach would reduce CHD risk in the long term.

The present diet, while lowering LDL-C by 9%, did not result in any significant depression of HDL-C. Lowering LDL-C while maintaining HDL-C would be expected to reduce CHD risk [45 46]. Similarly, reductions in ApoB and the ApoB:A1 ratio were also observed in the present study. These findings further support the potential CHD benefit that this weight loss diet may have [49-51]. It has also been claimed that apolipoproteins may be stronger predictors of CHD events than conventional lipid variables [52-54].

In contrast to the metabolic study, the reductions in systolic and diastolic blood pressure were not significant between the diets. Similarly, hs-CRP was unchanged between treatments, however, the level was significantly reduced with the low-carbohydrate diet compared to baseline. Studies have shown that hs-CRP tended to be lowest on the diets containing the highest proportion of carbohydrate [5]. Low glycemic index and low glycemic load diets have also been associated with lower hs-CRP concentrations [55 56]. These advantages of the higher carbohydrate diet may have reduced any hs-CRP difference between the two diets in the present study.

Soy-containing foods as well as nuts have cholesterol lowering effects [15 17 18 57 58] and may explain the present results on LDL-C. Viscous fiber in low starch vegetables and β-glucan in oats and barley may also contribute to the overall cholesterol lowering effect of the diet [9 14 45]. Furthermore, nuts and high fiber food consumption have been associated with lower body weight [59].

The study weaknesses include the relatively small sample size and the high dropout rate. Nevertheless, high dropout rates have been reported in similar dietary studies and it is noteworthy that attrition rates were low in the metabolic study when all food was provided [1]. Food availability and preparation may therefore be important factors. For those who did complete the study, however, there were benefits in weight loss and LDL-C reduction, an

additional 2% advantage in body weight reduction compared to the high-carbohydrate diet and a 13% drop in LDL-C for participants consuming a more plant-based low-carbohydrate diet.

The study's strength is that the prescribed hypocaloric diet was self-selected, meaning the results are more in line with what can be expected under free-living conditions. The breadth of application of the plant-based low-carbohydrate diet, however, remains to be determined, but it may provide an option for some individuals for whom LDL-C reduction is an equal concern to weight loss. If low-carbohydrate dietary options become more generally available the number of individuals who will benefit is likely to increase.

We conclude that a weight-reducing diet which reduced carbohydrate in exchange for increased intakes of vegetable sources of protein, such as gluten, soy and nuts, together with vegetable oils offer an opportunity to improve both LDL-C and body weight, both being risk factors for CHD. Further human trials are warranted to evaluate low-carbohydrate diets, including more plant-based low-carbohydrate diets, on CHD risk factors and ultimately on CHD.

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Dr. Jenkins, together with those responsible for analysis and interpretation of data, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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Role of the Sponsors

None of the funding organizations or sponsors played any significant role in the design and conduct of the study, in the collection, management, analysis, and interpretation of the data, or in the preparation, or approval of the manuscript. However, the named co-authors from Solae LLC reviewed the manuscript.

Disclosures

Dr. Jenkins has served on the Scientific Advisory Board of Sanitarium Company, Agri-Culture and Agri-Food Canada (AAFC), Canadian Agriculture Policy Institute (CAPI), California Strawberry Commission, Loblaw Supermarket, Herbal Life International, Nutritional Fundamental for Health, Pacific Health Laboratories, Metagenics, Bayer Consumer Care, Orafti,

Dean Foods, Kellogg's, Quaker Oats, Procter & Gamble, Coca-Cola, NuVal Griffin Hospital, Abbott, Pulse Canada, Saskatchewan Pulse Growers, and Canola Council of Canada; received honoraria for scientific advice from Sanitarium Company, Orafti, the Almond Board of California, the American Peanut Council, International Tree Nut Council Nutrition Research and Education Foundation and the Peanut Institute, Herbal Life International, Pacific Health Laboratories, Nutritional Fundamental for Health, Barilla, Metagenics, Bayer Consumer Care, Unilever Canada and Netherlands, Solae LLC, Oldways, Kellogg's, Quaker Oats, Procter & Gamble, Coca-Cola, NuVal Griffin Hospital, Abbott, Canola Council of Canada, Dean Foods, California Strawberry Commission, Haine Celestial, Pepsi, and Alpro Foundation; has been on the speakers panel for the Almond Board of California; received research grants from Saskatchewan Pulse Growers, the Agricultural Bioproducts Innovation Program (ABIP) through the Pulse Research Network (PURENet), Advanced Food Materials Network (AFMNet), Loblaw, Unilever, Barilla, Almond Board of California, Coca-Cola, Solae LLC, Haine Celestial, Sanitarium Company, Orafti, International Tree Nut Council Nutrition Research and Education Foundation and the Peanut Institute, the Canola and Flax Councils of Canada, Calorie Control Council, Canadian Institutes of Health Research, Canada Foundation for Innovation, and the Ontario Research Fund; and received travel support to meetings from the Solae LLC, Sanitarium Company, Orafti, AFMNet, Coca-Cola, The Canola and Flax Councils of Canada, Oldways Preservation Trust, Kellogg's, Quaker Oats, Griffin Hospital, Abbott Laboratories, Dean Foods, the California Strawberry Commission, American Peanut Council, Herbal Life International, Nutritional Fundamental for Health, Metagenics, Bayer Consumer Care, AAFC, CAPI, Pepsi, Almond Board of California, Unilever, Alpro Foundation, International Tree Nut Council, Barilla, Pulse Canada, and the Saskatchewan Pulse Growers. Dr Jenkins' wife is a director of

Glycemic Index Laboratories, Toronto, Ontario, Canada. Dr. Kendall reported being on speakers bureaus for Almond Board of California, Solae LLC, and Unilever; and receiving research grants from CIHR, Unilever, Solae LLC, Loblaw Brands Ltd, International Tree Nut Council, and Almond Board of California. Mr. Vidgen has received partial salary funding from research grants provided by Unilever, Loblaws, and the Almond Board of California. Drs. Paul, Mukherjea, and Krul are employees of Solae, LLC.

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Figure Legends

Figure 1: Patient Flow Diagram.

Figure 2: Weight loss during the study on both diets.

Figure 3: Mean (A) LDL-C, (B) HDL-C, (C) TC:HDL-C, (D) apoplipoprotein B (apoB) and (E) apolipoprotein A1 (apoA1), (F) ApoB:ApoA1 ratio between the two treatments.

Table 1: Baseline Characteristics for Those Who Started the 6-Month Self-Selected Diets (n=39)

	High-carbohydrate (n=19)	Low-Carbohydrate (n=20)
Age (y)	55.3 ± 1.8	57.6 ± 1.4
Males/Females	6/13	9/11
Body Weight, kg	85.4 [79.3, 91.6]	83.7 [78.5, 89.0]
Body Mass Index, kg/m^2	31.1 [29.9, 32.4]	31.1 [29.8, 32.4]
Blood Pressure, mm Hg		
Systolic	122 [116, 128]	128 [123, 132]
Diastolic	75 [72, 79]	77 [74, 80]
Cholesterol, mmol/L		
Total	6.75 [6.28, 7.21]	6.76 [6.21, 7.31]
LDL-C	4.40 [3.99, 4.82]	4.53 4.14, 4.93]
HDL-C	1.36 [1.22, 1.50]	1.21 [1.06, 1.36]
Triglycerides, mmol/L	2.16 [1.62, 2.70]	2.23 [1.65, 2.80]
Ratios		
TC:HDL-C	5.17 [4.54, 5.80]	5.81 [5.20, 6.41]
LDL-C: HDL-C	3.35 [2.95, 3.75]	3.89 [3.49, 4.29]
Medications		
Lipid lowering (prior to start of study)	4	7
Blood pressure	3	6
Diabetes	0	0
Thyroid	2	1

Values represent mean ± SEM or 95% confidence intervals (CIs).

No significant differences between treatments at baseline assessed by two sample t-test (two-tailed).

Table 2: Nutritional Profiles on the High and Low Carbohydrate Diets (n=39)

	High Carbohydrate		Low Carbohydrate			
	Week 0[b]	Ad Libitum[b]	Week 0[b]	Ad Libitum[b]	Between-Treatment Difference[c]	P-value[d]
Calories (kcal)	1598 [1421, 1775]	1347 [1140, 1553]	1840 [1550, 2130]	1388 [1234, 1541]	-248 [-391, -106]	0.001
% of Total Calories						
Available Carbohydrate	46.3 [42.2, 50.4]	53.9 [50.2, 57.5]	43.8 [40.2, 47.4]	39.6 [35.7, 43.6]	-10.5 [-13.6, -7.5]	< 0.001
Protein	20.6 [18.7, 22.5]	18.4 [17.4, 19.5]	20.1 [18.0, 22.2]	22.7 [20.1, 25.4]	5.9 [4.3, 7.5]	< 0.001
Vegetable Protein	5.6 [5.0, 6.1]	6.7 [6.1, 7.3]	5.7 [5.3, 6.1]	15.0 [11.7, 18.2]	8.2 [6.5, 9.9]	< 0.001
Soy Protein	0 [0, 0]	0.2 [0.1, 0.2]	0 [0, 0]	4.7 [2.7, 6.8]	3.6 [2.9, 4.4]	< 0.001
Fat	30.8 [27.3, 34.4]	27.5 [24.6, 30.4]	34.4 [31.4, 37.5]	36.0 [31.5, 40.5]	5.2 [2.6, 7.7]	< 0.001
Saturated	10.8 [9.1, 12.6]	7.6 [6.2, 8.9]	11.8 [10.3, 13.3]	7.5 [6.6, 8.4]	-0.4 [-1.4, 0.6]	0.401
Monounsaturated	12.3 [10.7, 13.8]	10.4 [9.3, 11.6]	13.0 [11.9, 14.2]	14.8 [13.1, 16.6]	4.6 [3.1, 6.1]	< 0.001
Polyunsaturated*	5.2 [4.6, 5.8]	6.3 [5.4, 7.2]	6.6 [5.5, 7.8]	8.4 [7.5, 9.4]	0.4 [-0.5, 1.4]	0.4
Alcohol	2.2 [0.3, 4.2]	1.9 [0.7, 3.2]	1.6 [0.0, 3.3]	1.1 [0.1, 2.1]	-0.5 [-1.3, 0.2]	0.160
Dietary Fibre (g/1000 kcal)	10.9 [9.2, 12.5]	18.2 [15.2, 21.1]	12.1 [9.9, 14.4]	21.3 [18.8, 23.8]	1.5 [-0.5, 3.5]	0.127
Dietary Cholesterol (mg/1000 kcal)	149 [129, 169]	87 [61, 113]	157 [136, 177]	117 [44, 189]	11 [-22, 23]	0.954
Adherence with "Eco-Atkins" Components[a]						
Viscous Fiber (out of 33.3%)	---	---	---	14.0 [9.4, 18.6]	---	---
Vegetable Protein (soy and gluten) (out of 33.3%)	---	---	---	14.7 [10.3, 19.1]	---	---
Nuts (out of 33.3%)	---	---	---	6.3 [3.3, 9.3]	---	---
Total Adherence (out of 100%)	---	---	---	33.6 [22.1, 45.2]	---	---

Values represent mean ± 95% confidence intervals (CIs).

[a]Adherence represents the mean percentage intake of the prescribed intake of the 3 cholesterol-lowering components [viscous fiber, vegetable protein (soy and gluten), nuts] by expressing the recorded intake for each component as 33.3%. The sume of the 3 components if consumed as prescribed would equal 100% adherence.

[b]Values represent multiple imputation (taking the mean of 5 sets of randomly imputed values) to generate data for those who dropped out or had missing values.

[c]Between Treatment Difference = Change from baseline between the two diets using all available data.

[d]P-values assessed using all available data and a repeated measures mixed model accounting for time of assessment. The response variable was change from baseline, with diet and week as fixed effects and subject ID nested in diet. There was no adjustment for baseline.

*Significantly different betweeen treatments at baseline assessed by two sample t-test (two tailed), P=0.025.

Table 3: Effect of high and low carbohydrate diets on body weight, blood lipids, apolipoproteins and 10-yr CHD risk (n=39)

	High Carbohydrate		Low Carbohydrate			
	Week 0[a]	Ad Libitum[a]	Week 0[a]	Ad Libitum[a]	Between Treatment Difference[b]	P-value[c]
Body Weight, kg	85.4 [79.3, 91.6]	80.4 [74.2, 86.6]	83.7 [78.5, 89.0]	76.9 [71.9, 81.9]	-1.1 [-2.1, 0.0]	0.047
BMI	31.1 [29.9, 32.4]	29.2 [27.9, 30.5]	31.1 [29.8, 32.4]	28.7 [27.3, 30.1]	-0.4 [-0.8, 0.0]	0.039
Body Fat, %	38.9 [34.0, 43.8]	35.0 [30.7, 39.2]	35.6 [30.1, 41.1]	31.4 [26.1, 36.6]	-1.7 [-4.0, 0.7]	0.161
Waist Circumference (cm)	102.8 [99.4, 106.2]	97.4 [93.1, 101.6]	99.8 [96.1, 103.5]	93.7 [89.8, 97.7]	0.1 [-1.1, 1.3]	0.861
Fasting Glucose (mmol/L)	5.2 [4.9, 5.4]	4.6 [4.5, 4.7]	5.2 [5.0, 5.4]	4.6 [4.4, 4.9]	0.1 [-0.1, 0.2]	0.447
Fasting Insulin (pmol/L)	50.0 [38.3, 61.7]	36.4 [27.5, 45.4]	47.3 [36.9, 57.6]	33.3 [22.8, 43.9]	-0.6 [-9.1, 8.0]	0.898
HOMA-IR	1.65 [1.17, 2.13]	1.11 [0.81, 1.41]	1.53 [1.19, 1.88]	0.99 [0.68, 1.30]	0.01 [-0.30, 0.33]	0.937
Satiety (-4 to 4)	1.0 [0.7, 1.4]	0.9 [0.7, 1.2]	1.2 [0.8, 1.7]	1.1 [0.8, 1.4]	-0.1 [-0.4, 0.2]	0.440
Exercise, METs	17.4 [12.4, 22.4]	25.8 [21.1, 30.6]	24.0 [12.9, 35.0]	23.9 [15.3, 32.6]	-9.3 [-16.4, -2-2]	0.012
Cholesterol, mmol/L[†]						
Total	6.75 [6.28, 7.21]	6.49 [5.97, 7.02]	6.76 [6.21, 7.31]	6.10 [5.67, 6.53]	-0.62 [-0.86, -0.37]	<0.001
LDL-C	4.40 [3.99, 4.82]	4.40 [3.91, 4.90]	4.53 [4.14, 4.93]	4.06 [3.71, 4.42]	-0.49 [-0.70, -0.28]	<0.001
HDL-C	1.36 [1.22, 1.50]	1.35 [1.22, 1.48]	1.21 [1.06, 1.36]	1.25 [1.10, 1.39]	0.03 [-0.02, 0.07]	0.245
Triglycerides	2.16 [1.62, 2.70]	1.71 [1.35, 2.07]	2.23 [1.65, 2.80]	1.50 [1.22, 1.77]	-0.34 [-0.57, -0.11]	0.005
Ratios						
Tchol:HDL-C	5.17 [4.54, 5.80]	4.92 [4.49, 5.34]	5.81 [5.20, 6.41]	5.13 [4.65, 5.62]	-0.57 [-0.83, -0.32]	<0.001
LDL-C:HDL-C	3.35 [2.95, 3.75]	3.34 [3.00, 3.68]	3.89 [3.49, 4.29]	3.48 [3.06, 3.90]	-0.42 [-0.60, -0.24]	<0.002
Apolipoproteins, g/L[‡]						
Apo A1	1.69 [1.60, 1.78]	1.69 [1.60, 1.77]	1.57 [1.45, 1.69]	1.57 [1.46, 1.67]	-0.02 [-0.06, 0.02]	0.316
Apo B	1.38 [1.26, 1.50]	1.23 [1.13, 1.33]	1.42 [1.30, 1.54]	1.20 [1.10, 1.31]	-0.11 [-0.16, -0.06]	<0.001
Apo B: Apo A1	0.83 [0.74, 0.91]	0.74 [0.68, 0.80]	0.92 [0.84, 0.99]	0.78 [0.70, 0.86]	-0.05 [-0.09, -0.02]	0.003
hs-CRP, mg/dL	2.1 [1.0, 3.3]	1.9 [1.3, 2.4]	3.0 [1.5, 4.5]	2.6 [1.0, 4.1]	-0.4 [-0.9, 0.1]	0.082
Blood Pressure, mmHg						
Systolic	122 [116, 128]	118 [114, 122]	128 [123, 132]	123 [119, 128]	-2 [-5, 2]	0.356
Diastolic	75 [72, 79]	74 [71, 77]	77 [74, 80]	76 [71, 80]	-1 [-3, 1]	0.288
10-yr CHD risk (%)*	8 [6, 9]	7 [6, 9]	12 [9, 14]	9 [7, 11]	-2 [-2, -1]	<0.001

Values represent mean ± 95% confidence intervals (CIs).

[†]To convert total cholesterol, LDL-C, and HDL-C to mg/dL, divide by 0.0259; to convert triglycerides to mg/dL, divide by 0.0113.

[‡]To convert apolipoprotein A1 and B to mg/dL, multiply by 100.

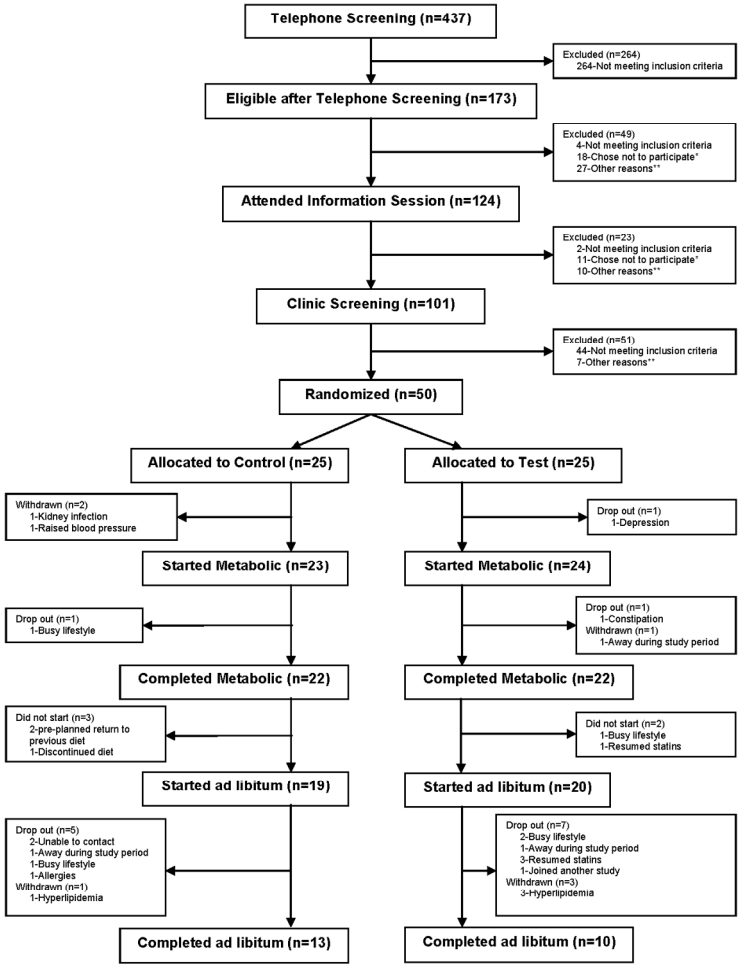
[a]Values represent multiple imputation (taking the mean of 5 sets of randomly imputed values) to generate data for those who dropped out or had missing values.

[b]Between Treatment Difference = Change from baseline between the two diets using all available data.

[c]P-values assessed using all available data and a repeated measures mixed model accounting for time of assessment. The response variable was change from baseline, with diet and week as fixed effects and subject ID nested in diet. There was no adjustment for baseline.

[*]Significantly different betweeen treatments at baseline assessed by two sample t-test (two tailed), P=0.007.

Figure 1



*Chose not to participate (29): busy lifestyle (13), not interested (6), study too demanding (3), currently on another diet (2), no compensation (2), work-related (2), dislike prepackaged foods (1)

**Other reasons (44): unable to contact (19), unable to come to clinic (13), away (5), throat surgery (1), bowel resection (1), high potassium and BP (1), high potassium (1), raised liver function tests (1), not interested (1), medical insurance issue (1)

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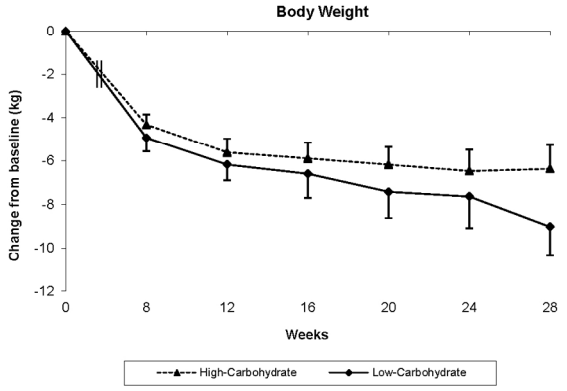

Figure 2: Weight loss during the study on both diets. Values represent mean ± SEM of the change from baseline using multiple imputation (taking the mean of 5 sets of randomly imputed values) to generate data for those who dropped out or had missing values.

The change in weight was significantly reduced (P=0.047) on the low versus the high carbohydrate diet using all available data in the repeated measures mixed model analysis.

215x279mm (200 x 200 DPI)

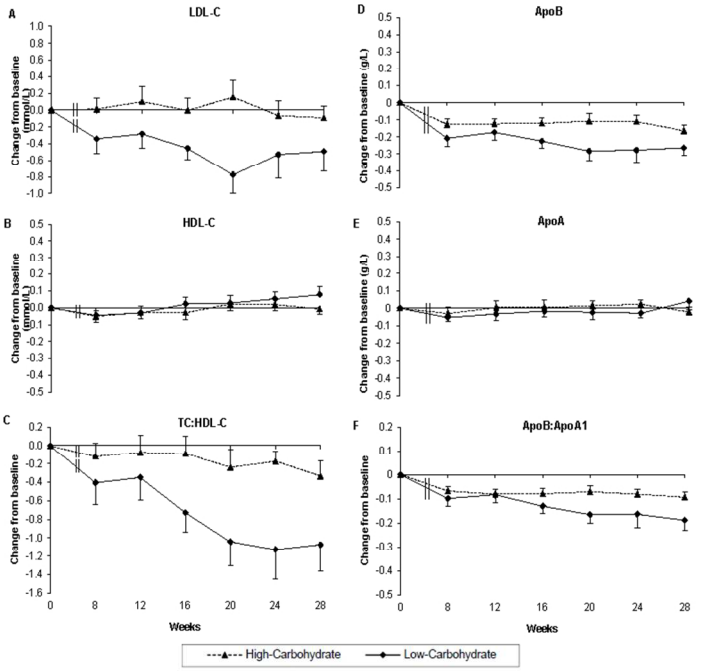

Figure 3:Change in (A) LDL-C, (B) HDL-C, (C) TC:HDL-C, (D) Apolipoprotein B (apoB), (E) Apolipoprotein A1 (apoA1), (F) ApoB:ApoA1 ratio between the two treatments. Values represent mean ± SEM of the change from baseline using multiple imputation (taking the mean of 5 sets of randomly imputed values) to generate data for those who dropped out or had missing values.

Significant treatment differences were seen for LDL-C (P<0.001),apo B (P<0.001) and the ratios TC:HDL-C (P<0.001) and apoB:apoA1 (p=0.003) using all available data in the repeated measures mixed model analysis.

279x215mm (102 x 114 DPI)

CONSORT 2010 checklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	1
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	5-6
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	7-8
	2b	Specific objectives or hypotheses	8
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	9
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	NA
Participants	4a	Eligibility criteria for participants	8, also previously published from results of metabolic phase
	4b	Settings and locations where the data were collected	8
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	9-10
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	10-11
	6b	Any changes to trial outcomes after the trial commenced, with reasons	NA
Sample size	7a	How sample size was determined	Continuation with ad libitum phase, metabolic phase published

	7b	When applicable, explanation of any interim analyses and stopping guidelines	NA
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	Continuation with ad libitum phase, randomized metabolic phase published
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	Continuation with ad libitum phase, randomized metabolic phase published
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	Continuation with ad libitum phase, randomized metabolic phase published
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	Continuation with ad libitum phase, randomized metabolic phase published
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those assessing outcomes) and how	10
	11b	If relevant, description of the similarity of interventions	NA

Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	11
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	11
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	Figure 1, CONSORT Diagram
	13b	For each group, losses and exclusions after randomisation, together with reasons	Figure 1, CONSORT Diagram
Recruitment	14a	Dates defining the periods of recruitment and follow-up	8
	14b	Why the trial ended or was stopped	NA
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	Table 1
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	11
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	12-13, Table 3, Figure 2 & 3
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	Relative effect sizes are given in Results 12-13 and Tables 2 & 3. The absolute differences from each treatment can be derived from Table 2 & 3 and Figures 2 & 3.
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	12, 13

Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	14
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	16
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	14,15
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	14-16
Other information			
Registration	23	Registration number and name of trial registry	2
Protocol	24	Where the full trial protocol can be accessed, if available	2
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	2-3, (repeated 18)

*We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.

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Six Months of a Vegan Low-Carbohydrate ("Eco-Atkins") Diet Improves Cardiovascular Risk Factors and Body Weight in Hyperlipidemic Adults: A Randomized Controlled Trial

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Complete List of Authors:	Jenkins, David; University of Toronto, Nutritional Sciences; St. Michael's Hospital, Clinical Nutrition & Risk Factor Modification Center Wong, Julia; University of Toronto, Nutritional Sciences; St. Michael's Hospital, Clinical Nutrition & Risk Factor Modification Center Kendall, Cyril; University of Toronto, Nutritional Sciences; St. Michael's Hospital, Clinical Nutrition & Risk Factor Modification Center Esfahani, Amin; University of Toronto, Nutritional Sciences; St. Michael's Hospital, Clinical Nutrition & Risk Factor Modification Center Ng, Vivian; University of Toronto, Nutritional Sciences; St. Michael's Hospital, Clinical Nutrition & Risk Factor Modification Center Leong, Tracy; University of Toronto, Nutritional Sciences; St. Michael's Hospital, Clinical Nutrition & Risk Factor Modification Center Faulkner, Dorothea; University of Toronto, Nutritional Sciences; St. Michael's Hospital, Clinical Nutrition & Risk Factor Modification Center Vidgen, Ed; University of Toronto, Nutritional Sciences; St. Michael's Hospital, Clinical Nutrition & Risk Factor Modification Center Paul, Gregory; Solae LLC, Mukherjea, Ratna; Solae LLC, Krul, Elaine; Solae LLC, Singer, William; St. Michael's Hospital, Medicine
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Six Months of a Vegan Low-Carbohydrate ("Eco-Atkins") Diet Improves Cardiovascular Risk Factors and Body Weight in Hyperlipidemic Adults: A Randomized Controlled Trial

David JA Jenkins, MD[1-5] Julia MW Wong, PhD[1,3] Cyril WC Kendall, PhD[1,3] Amin Esfahani, MSc[1,3] Vivian WY Ng, RD[1,3] Tracy CK Leong, BASc[1,3] Dorothea A Faulkner, PhD[1,3] Ed Vidgen, BSc[1,3] Gregory Paul, PhD[6] Ratna Mukherjea, PhD[6] Elaine S. Krul, PhD[6] William Singer, MD[1-4]

Departments of [1]Nutritional Sciences, [2]Medicine, Faculty of Medicine, University of Toronto, Toronto, Ontario, Canada; [3]Clinical Nutrition & Risk Factor Modification Center, St. Michael's Hospital, Toronto, Ontario, Canada; [4]Department of Medicine, Division of Endocrinology and Metabolism, [5]Li Ka Shing Knowledge Institute, St. Michael's Hospital, Toronto, Ontario, Canada; [6]Solae LLC, St. Louis, Missouri, USA

JMWW current affiliation is the New Balance Foundation Obesity Prevention Center, Boston Children's Hospital, Boston, MA, USA, and Department of Pediatrics, Harvard Medical School, Boston, MA, USA.

AE current affiliation is New York Medical College, School of Medicine, Valhalla, NY, USA.

Address correspondence and reprint requests to David JA Jenkins, Clinical Nutrition and Risk Factor Modification Center, St. Michael's Hospital, 61 Queen St. East, Toronto, Ontario, CANADA, M5C 2T2. Phone: (416) 978-4752; Fax: (416) 978-5310; EM: cyril.kendall@utoronto.ca

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Contributions

Conception and design - Jenkins, Wong, Kendall, Faulkner, Paul, Mukherjea, Krul, Singer

Acquisition of data - Jenkins, Wong, Kendall, Esfahani, Ng, Leong

Analysis and interpretation of data – Jenkins, Wong, Kendall, Vidgen

Drafting of the manuscript – Jenkins, Wong

Critical revision of the manuscript for important intellectual content – Jenkins, Wong, Kendall, Esfahani, Ng, Leong, Faulkner, Vidgen, Paul, Mukherjea, Krul, Singer

Statistical analysis - Vidgen

Obtaining funding – Jenkins, Kendall, Wong

Administrative, technical, or material support – Wong, Kendall, Esfahani, Ng, Leong, Faulkner

Supervision – Jenkins, Kendall, Wong, Singer

No additional contributions - Paul, Mukherjea, Krul

Abstract

Objective: Low-carbohydrate diets may be useful for weight loss. Diets high in vegetable proteins and oils may reduce the risk of coronary heart disease (CHD). The main objective was to determine the longer term effect of a diet that was both low-carbohydrate and plant-based on weight loss and LDL-C.

Design, Setting, Participants: A parallel design study of 39 overweight hyperlipidemic men and postmenopausal women conducted at a Canadian university-affiliated hospital nutrition research center from April 2005 to November 2006.

Intervention: Participants were advised to consume either a low-carbohydrate vegan diet or a high-carbohydrate lacto-ovo vegetarian diet for six-months after completing one-month metabolic (all foods provided) versions of these diets. The prescribed macronutrient intakes for the low- and high-carbohydrate diets were: 26% and 58% of energy from carbohydrate, 31% and 16% from protein and 43% and 25% from fat, respectively.

Primary Outcome: Change in body weight.

Results: Twenty-three participants (50% test, 68% control) completed the six-month ad libitum study. The approximate 4kg weight loss on the metabolic study was increased to -6.9kg on low-carbohydrate and -5.8kg on high-carbohydrate six-month ad libitum treatments (treatment difference [95% CI]: -1.1kg [-2.1, 0.0], P=0.047). The relative LDL-C and triglyceride reductions were also greater on the low-carbohydrate treatment (treatment difference [95% CI]: -0.49mmol/L [-0.70, -0.28], P<0.001 and -0.34mmol/L [-0.57, -0.11], P=0.005, respectively), as were the TC:HDL-C and apolipoprotein B:A1 ratios (-0.57 [-0.83, -0.32], P<0.001 and -0.05 [-0.09, -0.02], P=0.003, respectively).

Conclusions: A self-selected low-carbohydrate vegan diet, containing increased protein and fat from gluten and soy products, nuts, and vegetable oils, had lipid lowering advantages over a high-carbohydrate, low-fat weight loss diet, thus improving heart disease risk factors.

Trial Registration: clinicaltrials.gov (http://www.clinicaltrials.gov/), #NCT00256516

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Article Summary

Article Focus

- Low-carbohydrate diets may be useful for weight loss. Diets high in vegetable proteins and oils may reduce the risk of coronary heart disease (CHD).
- The objective of the randomized clinical trial was to determine the longer term effect of a diet that was both low-carbohydrate and plant-based on weight loss and LDL-C.
- We have already reported the effect of this dietary strategy in producing a difference of 8% in LDL-C reduction between calorie-restricted diets (60% of estimated calorie requirements) when all food was provided. We now report findings after these same participants continued on their respective diets for an additional 6 months, under self-selected conditions, in order to gain insights into the real life effectiveness of this diet.

Key Messages

- By comparison with the high-carbohydrate diet, consumption of the low-carbohydrate diet containing vegetable proteins and oils was also associated with significantly reduced concentrations of LDL-C. This LDL-C reduction has not been reported for other low-carbohydrate diet studies in which a large part of the protein and fat originated from animal sources.
- The present study also demonstrated that consumption of a low-carbohydrate vegan diet resulted in modestly greater body weight reductions compared to a high-carbohydrate diet (7% versus 6% reductions, respectively) over a six-month ad libitum period.

- The sustained reduction in LDL-C, associated with only a small incremental weight loss on the 6-month self-selected diet, is a potentially important attribute of the diet in reducing long-term CHD risk

Strengths and Limitations of this Study

The study weaknesses include the relatively small sample size and the high dropout rate. Nevertheless, high dropout rates have been reported in similar dietary studies and it is noteworthy that attrition rates were low in the metabolic study when all food was provided [1]. Food availability and preparation may therefore be important factors. For those who did complete the study, however, there were benefits in weight loss and LDL-C reduction, an additional 2% advantage in body weight reduction compared to the high-carbohydrate diet and a 13% drop in LDL-C for participants consuming a more plant-based low-carbohydrate diet. The study's strength is that the prescribed hypocaloric diet was self-selected, meaning the results are more in line with what can be expected under free-living conditions. The breadth of application of the plant-based low-carbohydrate diet, however, remains to be determined, but it may provide an option for some individuals for whom LDL-C reduction is an equal concern to weight loss. If low-carbohydrate dietary options become more generally available the number of individuals who will benefit is likely to increase.

Introduction

Many popular weight loss diets emphasize carbohydrate restriction (Atkins, Eddies, South Beach, Zone). Their success is determined by the level of compliance with the prescribed diets [2-7]. However, a high content of animal products, rich in saturated fat and cholesterol, may make conventional low-carbohydrate diets less appropriate for those with hypercholesterolemia [3 8]. Even during active weight loss, these high saturated fat diets do not lower serum LDL-C below baseline [3 8] and there is concern that if such diets continue to be eaten when weight loss has ceased, a more atherogenic blood lipid profile may result [9]. These concerns have prompted exploration of other weight loss strategies, but only modest reductions in LDL-C have been observed [10].

By contrast vegan diets significantly lower LDL-C [11]. Trials of vegan and vegetarian diets also reduce progression of coronary heart disease (CHD) [12] and improve diabetes control [13]. Plant food components such as vegetable proteins, vegetable oils, nuts and viscous fibers, reduce serum lipids in many studies [14-19] and may increase flow mediated vasodilatation [20-23]. Nuts, fiber and vegetarian diets in general, all reduce CHD and diabetes in cohort studies [24-29]. Finally, in cohort studies, low-carbohydrate diets, high in vegetable oils and proteins as opposed to animal products, reduce CHD events and diabetes incidence in women [30 31], while lower red meat intake reduces total, cardiovascular and cancer mortality [32]. Most recently a large randomized controlled trial confirmed the effect of nuts and increased vegetable oil (olive oil) intake in reducing cardiovascular events in the context of a Mediterranean diet [33].

In view of the apparent success of low-carbohydrate diets for weight loss and the demonstration that relatively high-carbohydrate vegetarian and vegan diets, and diets low in animal products, lower CHD risk factors [34-37], we designed a diet that combined both vegan and low-

carbohydrate elements to determine whether such a diet captured both the weight loss and CHD risk reduction advantages. We have already reported the effect of this dietary strategy in producing a difference of 8% in LDL-C reduction between calorie-restricted diets (60% of estimated calorie requirements) when all food was provided [1]. We now report findings after these same participants continued on their respective diets for an additional 6 months, under self-selected conditions, in order to gain insights into the real life effectiveness of this diet. The results of the metabolic (all foods provided) study have been reported previously and had demonstrated a CHD risk factor advantage, but with no greater weight loss than the control diet [1].

Methods

Participants

Forty-seven overweight participants, recruited by newspaper advertisement and hospital clinic notices, undertook the one-month metabolic first phase of the study (Figure 1) that has been previously reported [1]. At the start of the study, participants were given the option to participate in both the metabolic and ad libitum phases or only the metabolic phase. On completion of the metabolic phase, thirty-nine participants (19 control and 20 test participants) continued for an ad libitum six-month study (Table 1). The study was conducted at a Canadian university-affiliated hospital nutrition research center from April 2005 to November 2006. All participants had high normal to raised LDL-C levels (>3.4mmol/L at diagnosis) and a body mass index > 27 kg/m^2. Details of the eligibility criteria have been previously reported [1]. After recruitment, the 11/39 participants who were taking lipid lowering medications discontinued their medications at least two weeks prior to starting and for the study duration (Table 1).

Study Protocol

The intervention was a randomized parallel study stratified by sex in which participants were randomized to either low- or high-carbohydrate, calorie-reduced diets. The first month was the previously reported metabolically controlled study [1]. For the following six-months, participants continued on the diet to which they had been assigned as a self-selected (ad libitum) diet. Anthropometric, blood pressure and blood lipid measurements were repeated at monthly intervals. Insulin and HbA1c were measured at baseline and at the start and end of the ad libitum treatment. Percentage body fat was measured at baseline and end of the ad libitum treatment by bioelectrical impedance (Quantum II; RJL Systems, Clinton Township, Michigan). Seven-day diet and exercise histories were recorded in the week prior to each monthly visit. These histories were reviewed and discussed with the dietitian and appropriate dietary counselling was provided to enhance adherence. The overall feeling of satiety for the previous week was assessed at each study visit using a 9-point bipolar semantic scale, where −4 was extremely hungry, 0 was neutral, and +4 was uncomfortably full [1 35]. No exercise advise was given during the study, but alterations in exercise were allowed and recorded.

The Ethics Committees of St. Michael's Hospital and the University of Toronto, and the Therapeutic Products Directorate of Health Canada approved the study. Written informed consent was obtained from the participants. The study's clinical trial registration number was #NCT00256516.

Diets

As with the previous metabolic study, participants were encouraged to eat only 60% of their estimated caloric requirements in order to continue the body weight reduction started on their metabolic phase [38-40]. The prescribed test diet was a low-carbohydrate vegan diet containing 26% of calories from carbohydrate, 31% of calories from vegetable proteins and 43% from fat (primarily vegetable oils). Carbohydrate sources on the low-carbohydrate diet featured viscous fiber-containing foods (such as oats and barley) and low-starch vegetables (emphasizing okra and eggplant) for the relatively limited amount of carbohydrate allowed. The vegetable proteins were prescribed as gluten (54.8% of total protein), soy (23.0%), fruits and vegetables (8.7%), nuts (7.5%), and cereals (6.0%). Gluten was contained in the nut bread and wheat gluten (also called "seitan") products. Soy protein was present in the form of burgers, veggie bacon, deli slices, breakfast links, tofu, and soy milks. Nuts included almonds, cashews, hazelnuts, macadamia, pecans, and pistachios. The fat sources were nuts (43.6% of total fat), vegetable oils (24.4%), soy products (18.5%), avocado (7.1%), cereals (2.7%), fruits and vegetables (2.3%), and seitan products (1.4%). Participants were able to purchase at the research center the "no" starch high protein nut bread and three of the seitan (wheat gluten) products used in the study which were not available in Canada. The control, high-carbohydrate lacto-ovo vegetarian diet (58% carbohydrate, 16% protein and 25% fat) emphasized whole wheat cereals and cereal fiber, as well as low-fat or skim milk dairy products and liquid egg substitute to reduce saturated fat and cholesterol intakes. These diets have been published previously [1]. Participants were given a copy of the menu plans that outlined the food items and amounts prescribed during the metabolic phase. This served as a reference during the ad libitum phase. Furthermore, participants were given an exchange list of the items prescribed on the menu plan. The goal was to enhance adherence.

Self-taring electronic scales (My Weigh Scales, Vancouver, BC or Tanita Corporation, Arlington Heights, IL) were provided to all participants and they were instructed to weigh all food items while recording the seven-day food diary in the week prior to monthly clinic visits. Adherence to the three principal cholesterol-lowering components [vegetable proteins (soy and gluten), nuts, and viscous fibers] of the low-carbohydrate diet was assessed from the completed monthly seven-day food records. The amount of each component provided during the metabolic phase remained the same as that prescribed during the ad libitum phase.

Neither the dietitians nor participants could be blinded, but equal emphasis was placed on the potential importance for health of both diets. The analytical technicians were blinded to diet allocation, as was the statistician, up to analysis of the primary outcome. Participants were offered no financial compensation for participation in the study.

Analyses

The analytical techniques have been reported previously [1]. Serum was analyzed in the J. Alick Little Lipid Research Laboratory [35] and LDL-C (in mmol/L) was calculated by the method of Friedewald et al. [1]. The methods for analyzing apolipoproteins A1 and B, high sensitivity C-reactive protein (hs-CRP), blood glucose, insulin, HbA1c, and homeostasis model assessment – insulin resistance model (HOMA-IR) have been described previously [1]. Exercise data were calculated as metabolic equivalents (METs) [41]. The absolute 10-year CHD risk score was calculated using the Framingham risk equation [42].

Diets were assessed for macronutrients, fatty acids, cholesterol and fiber using a computer program based on the USDA database [43] and developed in our laboratory to allow the addition of the macronutrient content of study foods obtained from food labels or directly from food

manufacturers. The nutritional profiles of the diets were calculated from the 7-day food records completed once a month throughout the study and mean intakes are presented.

Adherence with the three principal cholesterol-lowering components [vegetable proteins (soy and gluten), nuts, and viscous fibers] of the low-carbohydrate diet was estimated from the 7-day food records by applying 33.3% adherence factor to the recorded intake for each of the three main components. The sum of the three components if consumed as prescribed would equal 100% adherence.

Statistical Analyses

Results are expressed as means ± SEM or 95% confidence intervals (CIs). Time zero was used as the baseline and refers to the pre-metabolic study baseline [1]. Treatment differences in physical and biochemical measures were assessed using all available data and a repeated measures mixed model accounting for time of assessment (SAS 9.2) [44] in the Tables (Table 2 and 3) and the Results. The response variable was change from baseline, with diet and week as fixed effects and subject ID nested in diet. There was no adjustment for baseline. Any participant who started the ad libitum treatment was included in the analysis (N=39). The completer analysis included the 23 participants who completed the study.

Multiple imputation (taking the mean of 5 sets of randomly imputed values) was used to present baseline and treatment values in the Tables (2 and 3) and Figures (2 and 3) by generating data for those who dropped out or had missing values [44].

Results

Compliance with the major dietary components [vegetable proteins (soy and gluten), nuts, and viscous fibers] was 33.6% or one-third of that prescribed during the metabolic phase (Table 2). Saturated fat intakes were similar on both treatments whereas intake of monounsaturated fats, vegetable proteins, and soy protein were significantly higher on the low-carbohydrate diet (Table 2). Available carbohydrate intake was significantly lower on the low-carbohydrate diet (Table 2). The attrition rate was 50% (10/20) on the low-carbohydrate and 32% (6/19) on the high-carbohydrate (Figure 1), this equates to a total attrition rate of 41% (16/39). The number of participants who did not complete the study (including dropouts and withdrawals) did not differ between treatments. Three participants were withdrawn by the study physician due to failure to attain LDL-C targets on the low-carbohydrate diet (mean LDL-C = 5.24mmol/L) and one subject on the high-carbohydrate diet (LDL-C = 7.78mmol/L). Participants on the low-carbohydrate diet tended to have larger reductions in body weight over time (Figure 2). The weight loss from baseline to the end of the 6-month ad libitum treatment was -6.9kg [95% CI, -7.7, -6.1] on the low-carbohydrate and –5.8kg [95% CI, -6.6, -5.1] on the control diet with a significant difference between groups (treatment difference [95% CI]: -1.1kg [-2.1, 0.0]; P=0.047) (Table 3). The final reduction in BMI was also greater on the low-carbohydrate versus high-carbohydrate diet (treatment difference [95% CI]: -0.4kg/m^2 [-0.8, 0.0]; P=0.039) (Table 3). Among the completers, there were numerically larger differences between treatments for both body weight and BMI (treatment difference [95% CI]: -1.8 kg [-3.0, -0.6]; P=0.0041 and -0.7 kg/m^2 [-1.1, -0.2]; P=0.0039, respectively).

There was a relative increase in recorded exercise by the high-carbohydrate diet participants, whereas there was no relative change in the low-carbohydrate participants (treatment difference [95% CI]: -9.3 [-16.4, -2.2] METs; P=0.012), but this was not reflected in a greater weight loss

(Table 3). There were no treatment differences in percent body fat, waist circumference or satiety (Table 3).

Lipids

At the end of the study, the reduction on the low-carbohydrate versus high-carbohydrate diet was greater for LDL-C (treatment difference [95% CI]: -0.49mmol/L [-0.70, -0.28]; P<0.001, for TC (-0.62mmol/L [-0.86, -0.37]; P<0.001, for TC:HDL-C -0.57 [-0.83, -0.32]; P<0.001, for LDL-C:HDL-C (-0.42 [-0.60, -0.24]; P<0.001, and for triglycerides (-0.34mmol/L [-0.57, -0.11]; P=0.005). No treatment difference was seen in HDL-C (Table 3). A similar pattern was observed in the completers. The treatment difference was numerically larger for LDL-C (-0.60mmol/L [-0.84, -0.36]; P<0.0001), TC (-0.73mmol/L [-1.00, -0.45]; P<0.0001), TC:HDL-C (-0.68 [-0.97, -0.39]; P<0.0001), and LDL-C:HDL-C (-0.53 [-0.73, -0.32]; P<0.0001). Values for LDL-C and the TC:HDL-C ratio were consistently lower in participants on the low-carbohydrate diet throughout the study while HDL-C values were not different from baseline (Figure 3 A-C).

Apolipoproteins

ApoB and the ApoB:A1 ratio were reduced more on the low- versus the high-carbohydrate diet at the end of the study (treatment different [95% CI]: -0.11g/L [-0.16, -0.06]; P<0.001 and -0.05 [-0.09, -0.02]; P=0.003, respectively) (Table 3). No significant difference between the diets was observed for ApoA1 concentrations. The pattern of change in the apolipoproteins in the completers reflected the changes seen in the whole group. Figure 3D and 3F show that the low-carbohydrate diet resulted in lower apoB and ApoB:ApoA1 ratios relative to baseline over the course of the study.

C-Reactive Protein, HbA1c, Blood Glucose, Serum Insulin, Insulin Resistance and Blood Pressure

Both treatments reduced hs-CRP with no difference between treatments (Table 3). HbA1c, fasting blood glucose, insulin, and insulin resistance (calculated using the HOMA model) fell similarly on both treatments during the course of the study (Table 3). Systolic and diastolic blood pressure decreased similarly with no treatment differences (Table 3). The completers also failed to show a difference between treatments.

Calculated CHD Risk

The low-carbohydrate diet significantly reduced the calculated 10-year CHD risk relative to the high-carbohydrate diet (2% [-2, -1]; P<0.001) (Table 3). A reduced CHD risk on the low-carbohydrate diet was also observed in the completers (2% [-3, -1]; P<0.001).

Adverse Events

No serious adverse events or events that involved hospitalisation occurred during the study.

Discussion

The present study demonstrated that consumption of a low-carbohydrate vegan diet resulted in a modestly greater body weight reduction compared to a high-carbohydrate diet (7% versus 6% reductions, respectively) over a six-month ad libitum period. These reductions were similar to those reported for low-carbohydrate "Atkins-like" diets[2 3 6 10]. However by comparison with the high-carbohydrate diet, consumption of the low-carbohydrate diet containing vegetable

proteins and oils was also associated with significantly reduced concentrations of LDL-C. This LDL-C reduction has not been reported for other low-carbohydrate diet studies in which a large part of the protein and fat originated from animal sources and in which no significant LDL-C reductions were seen [2-6 8]. The sustained reduction in LDL-C, associated with only a small incremental weight loss on the 6-month self-selected diet, is a potentially important attribute of the diet in reducing long-term CHD risk [45 46]. Furthermore, as seen in the present study, a low-carbohydrate diet, in which vegetable fat and protein options were encouraged, demonstrated a larger reduction in the TC:HDL-C ratio than that reported at 6 months in weight loss studies employing either a Mediterranean or a high-carbohydrate diet [10].

The majority of studies undertaken to date have been 6 months to one year in duration [2-6 47] with more recent studies of up to 2 years [2 8] and, as with the present study, a number of these studies had a high dropout rate [2 3 5 47]. The high dropout rate in the present study did not prevent identification of significant LDL-C and body weight differences in the intent-to-treat analysis (using all available data). However, the completer data demonstrated an even larger treatment difference in LDL-C favoring the low-carbohydrate treatment . Those on the low-carbohydrate diet showed overall adherence to the major dietary components [vegetable proteins (soy and gluten), nuts, and viscous fibers] at 33.6% of that provided during the metabolic phase [1]. This adherence is similar to the 43.3% seen with the dietary portfolio in the comparison of the metabolic one month [35] and the ad libitum six month studies [48]. In this comparison also just under half the LDL-C reduction (13-14%) seen on the ad libitum compared to the metabolic study [35].

The effect of low-carbohydrate diets on CHD events has not been assessed in randomized controlled trials. Nevertheless, low-carbohydrate diets high in vegetable proteins and oils have

been associated with a 30% reduced CHD risk and an 18% reduced incidence of diabetes in cohort studies [30 31]. The median interquantile difference in these studies between the first and 10th decile for vegetable protein and monounsaturated fat (MUFA) intakes, as a marker of increased vegetable oil consumption, was 1.4% and 9.3% expressed as a percentage of total caloric intake [30]. These figures compare with a 8.2% and a 4.6% relative increase in vegetable protein and oil consumption from baseline on the Eco-Atkins diet compared to the control diet. The increases in MUFA were therefore seen in both studies. Recently a Spanish Mediterranean diet emphasizing increased nut or olive oil consumption, increasing monounsaturated fat intake by 2-3%, has been shown to significantly reduce cardiovascular events also by approximately 30% [33]. These data provide consistent support for the view that the Eco-Atkins approach would reduce CHD risk in the long term.

The present diet, while lowering LDL-C by 9%, did not result in any significant depression of HDL-C. Lowering LDL-C while maintaining HDL-C would be expected to reduce CHD risk [45 46]. Similarly, reductions in ApoB and the ApoB:A1 ratio were also observed in the present study. These findings further support the potential CHD benefit that this weight loss diet may have [49-51]. It has also been claimed that apolipoproteins may be stronger predictors of CHD events than conventional lipid variables [52-54].

In contrast to the metabolic study, the reductions in systolic and diastolic blood pressure were not significant between the low- and high-carbohydrate diets. Similarly, hs-CRP was unchanged between treatments, however, the level was significantly reduced with the low-carbohydrate diet compared to baseline. Studies have shown that hs-CRP tended to be lowest on the diets containing the highest proportion of carbohydrate [5]. Low glycemic index and low glycemic load diets have also been associated with lower hs-CRP concentrations [55 56]. These

advantages of the higher carbohydrate diet may have reduced any hs-CRP difference between the two diets in the present study.

Soy-containing foods as well as nuts have cholesterol lowering effects [15 17 18 57 58] and may explain the reduction in LDL-C. Viscous fiber in low starch vegetables and β-glucan in oats and barley may also have contributed to the overall cholesterol lowering effect of the diet [9 14 45]. Furthermore, nuts and high fiber food consumption have been associated with lower body weight [59].

The study weaknesses include the relatively small sample size and the high dropout rate. Nevertheless, high dropout rates have been reported in similar dietary studies and it is noteworthy that attrition rates were low in the metabolic study when all food was provided [1]. Food availability and preparation may therefore be important factors. Future studies will need to focus on strategies to increase and maintain adherence, especially to the cholesterol lowering components, which all bear US FDA health claims for cardiovascular disease risk reduction. Furthermore, collaboration with food industry may be helpful in addressing concerns of availability, variety, and ease of preparation. In retrospect, a simplified one page eating plan for breakfast, lunch, and dinner with a number of options and amounts for each meal, as we have used in our dietary portfolio studies, might also be helpful [48]. For those who did complete the study, however, there were benefits in weight loss and LDL-C reduction, an additional 2% advantage in body weight reduction compared to the high-carbohydrate diet and a 13% drop in LDL-C for participants consuming a more plant-based low-carbohydrate diet. Unfortunately it was not possible to predict who would complete the diet based on pre-study data or changes observed during the metabolic phase.

The study's strength is that the prescribed hypocaloric diet was self-selected, meaning the results are more in line with what can be expected under free-living conditions. The breadth of application of the plant-based low-carbohydrate diet, however, remains to be determined, but it may provide an option for some individuals for whom LDL-C reduction is an equal concern to weight loss. If low-carbohydrate dietary options become more generally available the number of individuals who will benefit is likely to increase.

We conclude that a weight loss diet which reduced carbohydrate in exchange for increased intakes of vegetable sources of protein, such as gluten, soy and nuts, together with vegetable oils offers an opportunity to improve both LDL-C and body weight, both being risk factors for CHD. Further trials are warranted to evaluate low-carbohydrate diets, including more plant-based low-carbohydrate diets, on CHD risk factors and ultimately on CHD.

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Dr. Jenkins, together with those responsible for analysis and interpretation of data, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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Role of the Sponsors

None of the funding organizations or sponsors played any significant role in the design and conduct of the study, in the collection, management, analysis, and interpretation of the data, or in the preparation, or approval of the manuscript. However, the named co-authors from Solae LLC reviewed the manuscript.

Disclosures

Dr. Jenkins has served on the Scientific Advisory Board of Sanitarium Company, Agri-Culture and Agri-Food Canada (AAFC), Canadian Agriculture Policy Institute (CAPI), California Strawberry Commission, Loblaw Supermarket, Herbal Life International, Nutritional Fundamental for Health, Pacific Health Laboratories, Metagenics, Bayer Consumer Care, Orafti,

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Figure Legends

Figure 1: Patient Flow Diagram.

Figure 2: Weight loss during the study on both diets.

Figure 3: Mean (A) LDL-C, (B) HDL-C, (C) TC:HDL-C, (D) apoplipoprotein B (apoB) and (E) apolipoprotein A1 (apoA1), (F) ApoB:ApoA1 ratio between the two treatments during the metabolic and ad libitum phases.

Table 1: Baseline Characteristics for Those Who Started the 6-Month Self-Selected Diets (n=39)

	High-carbohydrate (n=19)	Low-Carbohydrate (n=20)
Age (y)	55.3 ± 1.8	57.6 ± 1.4
Males/Females	6/13	9/11
Body Weight, kg	85.4 [79.3, 91.6]	83.7 [78.5, 89.0]
Body Mass Index, kg/m^2	31.1 [29.9, 32.4]	31.1 [29.8, 32.4]
Blood Pressure, mm Hg		
Systolic	122 [116, 128]	128 [123, 132]
Diastolic	75 [72, 79]	77 [74, 80]
Cholesterol, mmol/L		
Total	6.75 [6.28, 7.21]	6.76 [6.21, 7.31]
LDL-C	4.40 [3.99, 4.82]	4.53 4.14, 4.93]
HDL-C	1.36 [1.22, 1.50]	1.21 [1.06, 1.36]
Triglycerides, mmol/L	2.16 [1.62, 2.70]	2.23 [1.65, 2.80]
Ratios		
TC:HDL-C	5.17 [4.54, 5.80]	5.81 [5.20, 6.41]
LDL-C: HDL-C	3.35 [2.95, 3.75]	3.89 [3.49, 4.29]
Medications		
Lipid lowering (prior to start of study)	4	7
Blood pressure	3	6
Diabetes	0	0
Thyroid	2	1

Values represent mean ± SEM or 95% confidence intervals (CIs).

No significant differences between treatments at baseline assessed by two sample t-test (two-tailed).

Table 2: Nutritional Profiles on the High and Low Carbohydrate Diets (n=39)

	High Carbohydrate		Low Carbohydrate			
	Week 0[b]	Ad Libitum[b]	Week 0[b]	Ad Libitum[b]	Between-Treatment Difference[c]	P-value[d]
Calories (kcal)	1598 [1421, 1775]	1347 [1140, 1553]	1840 [1550, 2130]	1388 [1234, 1541]	-248 [-391, -106]	0.001
% of Total Calories						
Available Carbohydrate	46.3 [42.2, 50.4]	53.9 [50.2, 57.5]	43.8 [40.2, 47.4]	39.6 [35.7, 43.6]	-10.5 [-13.6, -7.5]	< 0.001
Protein	20.6 [18.7, 22.5]	18.4 [17.4, 19.5]	20.1 [18.0, 22.2]	22.7 [20.1, 25.4]	5.9 [4.3, 7.5]	< 0.001
Vegetable Protein	5.6 [5.0, 6.1]	6.7 [6.1, 7.3]	5.7 [5.3, 6.1]	15.0 [11.7, 18.2]	8.2 [6.5, 9.9]	< 0.001
Soy Protein	0 [0, 0]	0.2 [0.1, 0.2]	0 [0, 0]	4.7 [2.7, 6.8]	3.6 [2.9, 4.4]	< 0.001
Fat	30.8 [27.3, 34.4]	27.5 [24.6, 30.4]	34.4 [31.4, 37.5]	36.0 [31.5, 40.5]	5.2 [2.6, 7.7]	< 0.001
Saturated	10.8 [9.1, 12.6]	7.6 [6.2, 8.9]	11.8 [10.3, 13.3]	7.5 [6.6, 8.4]	-0.4 [-1.4, 0.6]	0.401
Monounsaturated	12.3 [10.7, 13.8]	10.4 [9.3, 11.6]	13.0 [11.9, 14.2]	14.8 [13.1, 16.6]	4.6 [3.1, 6.1]	< 0.001
Polyunsaturated*	5.2 [4.6, 5.8]	6.3 [5.4, 7.2]	6.6 [5.5, 7.8]	8.4 [7.5, 9.4]	0.4 [-0.5, 1.4]	0.4
Alcohol	2.2 [0.3, 4.2]	1.9 [0.7, 3.2]	1.6 [0.0, 3.3]	1.1 [0.1, 2.1]	-0.5 [-1.3, 0.2]	0.160
Dietary Fibre (g/1000 kcal)	10.9 [9.2, 12.5]	18.2 [15.2, 21.1]	12.1 [9.9, 14.4]	21.3 [18.8, 23.8]	1.5 [-0.5, 3.5]	0.127
Dietary Cholesterol (mg/1000 kcal)	149 [129, 169]	87 [61, 113]	157 [136, 177]	117 [44, 189]	11 [-22, 23]	0.954
Adherence with "Eco-Atkins" Components[a]						
Viscous Fiber (out of 33.3%)	---	---	---	14.0 [9.4, 18.6]	---	---
Vegetable Protein (soy and gluten) (out of 33.3%)	---	---	---	14.7 [10.3, 19.1]	---	---
Nuts (out of 33.3%)	---	---	---	6.3 [3.3, 9.3]	---	---
Total Adherence (out of 100%)	---	---	---	33.6 [22.1, 45.2]	---	---

Values represent mean ± 95% confidence intervals (CIs).

[a]Adherence represents the mean percentage intake of the prescribed intake of the 3 cholesterol-lowering components [viscous fiber, vegetable protein (soy and gluten), nuts] by expressing the recorded intake for each component as 33.3%. The sume of the 3 components if consumed as prescribed would equal 100% adherence.

[b]Values represent multiple imputation (taking the mean of 5 sets of randomly imputed values) to generate data for those who dropped out or had missing values.

[c]Between Treatment Difference = Change from baseline between the two diets using all available data.

[d]P-values assessed using all available data and a repeated measures mixed model accounting for time of assessment. The response variable was change from baseline, with diet and week as fixed effects and subject ID nested in diet. There was no adjustment for baseline.

*Significantly different betweeen treatments at baseline assessed by two sample t-test (two tailed), P=0.025.

Table 3: Effect of high and low carbohydrate diets on body weight, blood lipids, apolipoproteins and 10-yr CHD risk (n=39)

	High Carbohydrate		Low Carbohydrate			
	Week 0[a]	Ad Libitum[a]	Week 0[a]	Ad Libitum[a]	Between Treatment Difference[b]	P-value[c]
Body Weight, kg	85.4 [79.3, 91.6]	80.4 [74.2, 86.6]	83.7 [78.5, 89.0]	76.9 [71.9, 81.9]	-1.1 [-2.1, 0.0]	0.047
BMI	31.1 [29.9, 32.4]	29.2 [27.9, 30.5]	31.1 [29.8, 32.4]	28.7 [27.3, 30.1]	-0.4 [-0.8, 0.0]	0.039
Body Fat, %	38.9 [34.0, 43.8]	35.0 [30.7, 39.2]	35.6 [30.1, 41.1]	31.4 [26.1, 36.6]	-1.7 [-4.0, 0.7]	0.161
Waist Circumference (cm)	102.8 [99.4, 106.2]	97.4 [93.1, 101.6]	99.8 [96.1, 103.5]	93.7 [89.8, 97.7]	0.1 [-1.1, 1.3]	0.861
Fasting Glucose	5.2 [4.9, 5.4]	4.6 [4.5, 4.7]	5.2 [5.0, 5.4]	4.6 [4.4, 4.9]	0.1 [-0.1, 0.2]	0.447
HbA1c (%)	5.2 [5.0, 5.4]	5.2 [5.0, 5.3]	5.3 [5.0, 5.5]	5.2 [5.0, 5.4]	0.0 [-0.2, 0.1]	0.852
Fasting Insulin	50.0 [38.3, 61.7]	36.4 [27.5, 45.4]	47.3 [36.9, 57.6]	33.3 [22.8, 43.9]	-0.6 [-9.1, 8.0]	0.898
HOMA-IR	1.65 [1.17, 2.13]	1.11 [0.81, 1.41]	1.53 [1.19, 1.88]	0.99 [0.68, 1.30]	0.01 [-0.30, 0.33]	0.937
Satiety (-4 to 4)	1.0 [0.7, 1.4]	0.9 [0.7, 1.2]	1.2 [0.8, 1.7]	1.1 [0.8, 1.4]	-0.1 [-0.4, 0.2]	0.440
Exercise, METs	17.4 [12.4, 22.4]	25.8 [21.1, 30.6]	24.0 [12.9, 35.0]	23.9 [15.3, 32.6]	-9.3 [-16.4, -2-2]	0.012
Cholesterol, mmol/L[†]						
Total	6.75 [6.28, 7.21]	6.49 [5.97, 7.02]	6.76 [6.21, 7.31]	6.10 [5.67, 6.53]	-0.62 [-0.86, -0.37]	<0.001
LDL-C	4.40 [3.99, 4.82]	4.40 [3.91, 4.90]	4.53 [4.14, 4.93]	4.06 [3.71, 4.42]	-0.49 [-0.70, -0.28]	<0.001
HDL-C	1.36 [1.22, 1.50]	1.35 [1.22, 1.48]	1.21 [1.06, 1.36]	1.25 [1.10, 1.39]	0.03 [-0.02, 0.07]	0.245
Triglycerides	2.16 [1.62, 2.70]	1.71 [1.35, 2.07]	2.23 [1.65, 2.80]	1.50 [1.22, 1.77]	-0.34 [-0.57, -0.11]	0.005
Ratios						
Tchol:HDL-C	5.17 [4.54, 5.80]	4.92 [4.49, 5.34]	5.81 [5.20, 6.41]	5.13 [4.65, 5.62]	-0.57 [-0.83, -0.32]	<0.001
LDL-C:HDL-C	3.35 [2.95, 3.75]	3.34 [3.00, 3.68]	3.89 [3.49, 4.29]	3.48 [3.06, 3.90]	-0.42 [-0.60, -0.24]	<0.002
Apolipoproteins, g/L[‡]						
Apo A1	1.69 [1.60, 1.78]	1.69 [1.60, 1.77]	1.57 [1.45, 1.69]	1.57 [1.46, 1.67]	-0.02 [-0.06, 0.02]	0.316
Apo B	1.38 [1.26, 1.50]	1.23 [1.13, 1.33]	1.42 [1.30, 1.54]	1.20 [1.10, 1.31]	-0.11 [-0.16, -0.06]	<0.001
Apo B: Apo A1	0.83 [0.74, 0.91]	0.74 [0.68, 0.80]	0.92 [0.84, 0.99]	0.78 [0.70, 0.86]	-0.05 [-0.09, -0.02]	0.003
hs-CRP, mg/dL	2.1 [1.0, 3.3]	1.9 [1.3, 2.4]	3.0 [1.5, 4.5]	2.6 [1.0, 4.1]	-0.4 [-0.9, 0.1]	0.082
Blood Pressure, mmHg						
Systolic	122 [116, 128]	118 [114, 122]	128 [123, 132]	123 [119, 128]	-2 [-5, 2]	0.356
Diastolic	75 [72, 79]	74 [71, 77]	77 [74, 80]	76 [71, 80]	-1 [-3, 1]	0.288
10-yr CHD risk (%)*	8 [6, 9]	7 [6, 9]	12 [9, 14]	9 [7, 11]	-2 [-2, -1]	<0.001

Values represent mean ± 95% confidence intervals (CIs).
†To convert total cholesterol, LDL-C, and HDL-C to mg/dL, divide by 0.0259; to convert triglycerides to mg/dL, divide by 0.0113.
‡To convert apolipoprotein A1 and B to mg/dL, multiply by 100.
[a]Values represent multiple imputation (taking the mean of 5 sets of randomly imputed values) to generate data for those who dropped out or had missing values.
[b]Between Treatment Difference = Change from baseline between the two diets using all available data.
[c]P-values assessed using all available data and a repeated measures mixed model accounting for time of assessment. The response variable was change from baseline, with diet and week as fixed effects and subject ID nested in diet. There was no adjustment for baseline.
*Significantly different betweeen treatments at baseline assessed by two sample t-test (two tailed), P=0.007.

Six Months of a Vegan Low-Carbohydrate ("Eco-Atkins") Diet Improves Cardiovascular Risk Factors and Body Weight in Hyperlipidemic Adults: A Randomized Controlled Trial

David JA Jenkins, MD[1-5] Julia MW Wong, PhD[1,3] Cyril WC Kendall, PhD[1,3] Amin Esfahani, MSc[1,3] Vivian WY Ng, RD[1,3] Tracy CK Leong, BASc[1,3] Dorothea A Faulkner, PhD[1,3] Ed Vidgen, BSc[1,3] Gregory Paul, PhD[6] Ratna Mukherjea, PhD[6] Elaine S. Krul, PhD[6] William Singer, MD[1-4]

Departments of [1]Nutritional Sciences, [2]Medicine, Faculty of Medicine, University of Toronto, Toronto, Ontario, Canada; [3]Clinical Nutrition & Risk Factor Modification Center, St. Michael's Hospital, Toronto, Ontario, Canada; [4]Department of Medicine, Division of Endocrinology and Metabolism, [5]Li Ka Shing Knowledge Institute, St. Michael's Hospital, Toronto, Ontario, Canada; [6]Solae LLC, St. Louis, Missouri, USA

JMWW current affiliation is the New Balance Foundation Obesity Prevention Center, Boston Children's Hospital, Boston, MA, USA, and Department of Pediatrics, Harvard Medical School, Boston, MA, USA.

AE current affiliation is New York Medical College, School of Medicine, Valhalla, NY, USA.

Address correspondence and reprint requests to David JA Jenkins, Clinical Nutrition and Risk Factor Modification Center, St. Michael's Hospital, 61 Queen St. East, Toronto, Ontario, CANADA, M5C 2T2. Phone: (416) 978-4752; Fax: (416) 978-5310; EM: cyril.kendall@utoronto.ca

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Contributions

Conception and design - Jenkins, Wong, Kendall, Faulkner, Paul, Mukherjea, Krul, Singer

Acquisition of data - Jenkins, Wong, Kendall, Esfahani, Ng, Leong

Analysis and interpretation of data – Jenkins, Wong, Kendall, Vidgen

Drafting of the manuscript – Jenkins, Wong

Critical revision of the manuscript for important intellectual content – Jenkins, Wong, Kendall, Esfahani, Ng, Leong, Faulkner, Vidgen, Paul, Mukherjea, Krul, Singer

Statistical analysis - Vidgen

Obtaining funding – Jenkins, Kendall, Wong

Administrative, technical, or material support – Wong, Kendall, Esfahani, Ng, Leong, Faulkner

Supervision – Jenkins, Kendall, Wong, Singer

No additional contributions - Paul, Mukherjea, Krul

Abstract

Objective: Low-carbohydrate diets may be useful for weight loss. Diets high in vegetable proteins and oils may reduce the risk of coronary heart disease (CHD). The main objective was to determine the longer term effect of a diet that was both low-carbohydrate and plant-based on weight loss and LDL-C.

Design, Setting, Participants: A parallel design study of 39 overweight hyperlipidemic men and postmenopausal women conducted at a Canadian university-affiliated hospital nutrition research center from April 2005 to November 2006.

Intervention: Participants were advised to consume either a low-carbohydrate vegan diet or a high-carbohydrate lacto-ovo vegetarian diet for six-months after completing one-month metabolic (all foods provided) versions of these diets. The prescribed macronutrient intakes for the low- and high-carbohydrate diets were: 26% and 58% of energy from carbohydrate, 31% and 16% from protein and 43% and 25% from fat, respectively.

Primary Outcome: Change in body weight.

Results: Twenty-three participants (50% test, 68% control) completed the six-month ad libitum study. The approximate 4kg weight loss on the metabolic study was increased to -6.9kg on low-carbohydrate and -5.8kg on high-carbohydrate six-month ad libitum treatments (treatment difference [95% CI]: -1.1kg [-2.1, 0.0], P=0.047). The relative LDL-C and triglyceride reductions were also greater on the low-carbohydrate treatment (treatment difference [95% CI]: -0.49mmol/L [-0.70, -0.28], P<0.001 and -0.34mmol/L [-0.57, -0.11], P=0.005, respectively), as were the TC:HDL-C and apolipoprotein B:A1 ratios (-0.57 [-0.83, -0.32], P<0.001 and -0.05 [-0.09, -0.02], P=0.003, respectively).

Conclusions: A self-selected low-carbohydrate vegan diet, containing increased protein and fat from gluten and soy products, nuts, and vegetable oils, had lipid lowering advantages over a high-carbohydrate, low-fat weight loss diet, thus improving heart disease risk factors.

Trial Registration: clinicaltrials.gov (http://www.clinicaltrials.gov/), #NCT00256516

Abstract Word Count: 273 (up to 300 allowed)

Article Summary

Article Focus

- Low-carbohydrate diets may be useful for weight loss. Diets high in vegetable proteins and oils may reduce the risk of coronary heart disease (CHD).
- The objective of the randomized clinical trial was to determine the longer term effect of a diet that was both low-carbohydrate and plant-based on weight loss and LDL-C.
- We have already reported the effect of this dietary strategy in producing a difference of 8% in LDL-C reduction between calorie-restricted diets (60% of estimated calorie requirements) when all food was provided. We now report findings after these same participants continued on their respective diets for an additional 6 months, under self-selected conditions, in order to gain insights into the real life effectiveness of this diet.

Key Messages

- By comparison with the high-carbohydrate diet, consumption of the low-carbohydrate diet containing vegetable proteins and oils was also associated with significantly reduced concentrations of LDL-C. This LDL-C reduction has not been reported for other low-carbohydrate diet studies in which a large part of the protein and fat originated from animal sources. ~~and where increases in LDL-C were seen.~~
- The present study also demonstrated that consumption of a low-carbohydrate vegan diet resulted in modestly greater body weight reductions compared to a high-carbohydrate diet (7% versus 6% reductions, respectively) over a six-month ad libitum period.

- The sustained reduction in LDL-C, associated with only a small incremental weight loss on the 6-month self-selected diet, is a potentially important attribute of the diet in reducing long-term CHD risk

Strengths and Limitations of this Study

The study weaknesses include the relatively small sample size and the high dropout rate. Nevertheless, high dropout rates have been reported in similar dietary studies and it is noteworthy that attrition rates were low in the metabolic study when all food was provided [1]. Food availability and preparation may therefore be important factors. For those who did complete the study, however, there were benefits in weight loss and LDL-C reduction, an additional 2% advantage in body weight reduction compared to the high-carbohydrate diet and a 13% drop in LDL-C for participants consuming a more plant-based low-carbohydrate diet. The study's strength is that the prescribed hypocaloric diet was self-selected, meaning the results are more in line with what can be expected under free-living conditions. The breadth of application of the plant-based low-carbohydrate diet, however, remains to be determined, but it may provide an option for some individuals for whom LDL-C reduction is an equal concern to weight loss. If low-carbohydrate dietary options become more generally available the number of individuals who will benefit is likely to increase.

Introduction

Many popular weight loss diets emphasize carbohydrate restriction (Atkins, Eddies, South Beach, Zone). Their success is determined by the level of compliance with the prescribed diets [2-7]. However, a high content of animal products, rich in saturated fat and cholesterol, may make conventional low-carbohydrate diets less appropriate for those with hypercholesterolemia [3 8]. Even during active weight loss, these high saturated fat diets do not lower~~, may raise~~ serum LDL-C below ~~above~~ baseline [3 8] and there is concern that if such diets continue to be eaten when weight loss has ceased, a more atherogenic blood lipid profile may result [9]. These concerns have prompted exploration of other weight loss strategies, but only modest reductions in LDL-C have been observed [10].

By contrast vegan diets significantly lower LDL-C [11]. Trials of vegan and vegetarian diets also reduce progression of coronary heart disease (CHD) [12] and improve diabetes control [13]. Plant food components such as vegetable proteins, vegetable oils, nuts and viscous fibers, reduce serum lipids in many studies [14-19] and may increase flow mediated vasodilatation [20-23]. Nuts, fiber and vegetarian diets in general, all reduce CHD and diabetes in cohort studies [24-29]. Finally, in cohort studies, low-carbohydrate diets, high in vegetable oils and proteins as opposed to animal products, reduce CHD events and diabetes incidence in women [30 31], while lower red meat intake reduces total, cardiovascular and cancer mortality [32]. Most recently a large randomized controlled trial confirmed the effect of nuts and increased vegetable oil (olive oil) intake in reducing cardiovascular events in the context of a Mediterranean diet [33].

In view of the apparent success of low-carbohydrate diets for weight loss and the demonstration that relatively high-carbohydrate vegetarian and vegan diets, and diets low in animal products, lower CHD risk factors [34-37], we designed a diet that combined both vegan and low-

carbohydrate elements to determine whether such a diet captured both the weight loss and CHD risk reduction advantages. We have already reported the effect of this dietary strategy in producing a difference of 8% in LDL-C reduction between calorie-restricted diets (60% of estimated calorie requirements) when all food was provided [1]. We now report findings after these same participants continued on their respective diets for an additional 6 months, under self-selected conditions, in order to gain insights into the real life effectiveness of this diet. The results of the metabolic (all foods provided) study have been reported previously and had demonstrated a CHD risk factor advantage, but with no greater weight loss than the control diet [1].

Methods

Participants

Forty-seven overweight participants, recruited by newspaper advertisement and hospital clinic notices, undertook the one-month metabolic first phase of the study (Figure 1) that has been previously reported [1]. At the start of the study, participants were given the option to participate in both the metabolic and ad libitum phases or only the metabolic phase. On completion of the metabolic~~this~~ phase, thirty-nine participants (19 control and 20 test participants) continued for an ad libitum six-month study (Table 1). The study was conducted at a Canadian university-affiliated hospital nutrition research center from April 2005 to November 2006. All participants had high normal to raised LDL-C levels (>3.4mmol/L at diagnosis) and a body mass index > 27 kg/m^2. Details of the eligibility criteria have been previously reported [1]. After recruitment, the 11/39 participants who were taking lipid lowering medications discontinued their ~~lipid lowering~~ medications at least two weeks prior to starting and for the study duration (Table 1).

Study Protocol

The intervention was a randomized parallel study stratified by sex in which participants were randomized to either low- or high-carbohydrate, calorie-reduced diets. The first month was the previously reported metabolically controlled study [1]. For the following six-months, participants continued on the diet to which they had been assigned as a self-selected (ad libitum) diet. Anthropometric, blood pressure and blood lipid measurements were repeated at monthly intervals. Insulin and HbA1c were measured at baseline and at the start and end of the ad libitum treatment. Percentage body fat was measured at baseline and end of the ad libitum treatment by bioelectrical impedance (Quantum II; RJL Systems, Clinton Township, Michigan). Seven-day diet and exercise histories were recorded in the week prior to each monthly visit. These histories were reviewed and discussed with the dietitian and appropriate dietary counselling was provided to enhance adherence. The overall feeling of satiety for the previous week was assessed at each study visit using a 9-point bipolar semantic scale, where −4 was extremely hungry, 0 was neutral, and +4 was uncomfortably full [1 35]. No exercise advise was given during the study, ~~There was no prescription related to exercise where~~but ~~A~~alterations in exercise were allowed and recorded. The Ethics Committees of St. Michael's Hospital and the University of Toronto, and the Therapeutic Products Directorate of Health Canada approved the study. Written informed consent was obtained from the participants. The study's clinical trial registration number was #NCT00256516.

Diets

As with the previous metabolic study, participants were encouraged to eat only 60% of their estimated caloric requirements in order to continue the body weight reduction started on their metabolic phase [38-40]. The prescribed test diet was a low-carbohydrate vegan diet containing 26% of calories from carbohydrate, 31% of calories from vegetable proteins and 43% from fat (primarily vegetable oils). Carbohydrate sources on the low-carbohydrate diet featured viscous fiber-containing foods (such as oats and barley) and low-starch vegetables (emphasizing okra and eggplant) for the relatively limited amount of carbohydrate allowed. The vegetable proteins were prescribed as gluten (54.8% of total protein), soy (23.0%), fruits and vegetables (8.7%), nuts (7.5%), and cereals (6.0%). Gluten was contained in the nut bread and wheat gluten (also called "seitan") products. Soy protein was present in the form of burgers, veggie bacon, deli slices, breakfast links, tofu, and soy milks. Nuts included almonds, cashews, hazelnuts, macadamia, pecans, and pistachios. The fat sources were nuts (43.6% of total fat), vegetable oils (24.4%), soy products (18.5%), avocado (7.1%), cereals (2.7%), fruits and vegetables (2.3%), and seitan products (1.4%). Participants were able to purchase at the research center the "no" starch high protein nut bread and three of the seitan (wheat gluten) products used in the study which were not available in Canada. The control, high-carbohydrate lacto-ovo vegetarian diet (58% carbohydrate, 16% protein and 25% fat) emphasized whole wheat cereals and cereal fiber, as well as low-fat or skim milk dairy products and liquid egg substitute to reduce saturated fat and cholesterol intakes. These diets have been published previously [1]. Participants were given a copy of the menu plans that outlined the food items and amounts prescribed during the metabolic phase. This served as a reference during the ad libitum phase. Furthermore, participants were given an exchange list of the items prescribed on the menu plan. The goal was to enhance adherence.

Self-taring electronic scales (My Weigh Scales, Vancouver, BC or Tanita Corporation, Arlington Heights, IL) were provided to all participants and they were instructed to weigh all food items while recording the seven-day food d~~a~~iary in the week prior to monthly clinic visits. Adherence to the three principal cholesterol-lowering components [vegetable proteins (soy and gluten), nuts, and viscous fibers] of the low-carbohydrate diet was assessed from the completed monthly seven-day food records. The amount of each component provided during the metabolic phase remained the same as that prescribed during the ad libitum phase.

Neither the dietitians nor participants could be blinded, but equal emphasis was placed on the potential importance for health of both diets. The analytical technicians were blinded to diet allocation, as was the statistician, up to analysis of the primary outcome. Participants were offered no financial compensation for participation in the study.

Analyses

The analytical techniques have been reported previously [1]. Serum was analyzed ~~according to the Lipid Research Clinics protocol~~ in the J. Alick Little Lipid Research Laboratory [35] and LDL-C (in mmol/L) was calculated by the method of Friedewald et al. [1]. The methods for analyzing apolipoproteins A1 and B, high sensitivity C-reactive protein (hs-CRP), blood glucose, insulin, HbA1c, and homeostasis model assessment – insulin resistance model (HOMA-IR) have been described previously [1]. Exercise data were calculated as metabolic equivalents (METs) [41]. The absolute 10-year CHD risk score was calculated using the Framingham risk equation [42].

Diets were assessed for macronutrients, fatty acids, cholesterol and fiber using a computer program based on the USDA database [43] and developed in our laboratory to allow the addition

of the macronutrient content of study foods obtained from food labels or directly from food manufacturers. The nutritional profiles of the diets were calculated from the 7-day food records completed once a month throughout the study and mean intakes are presented.

Adherence with the three principal cholesterol-lowering components [vegetable proteins (soy and gluten), nuts, and viscous fibers] of the low-carbohydrate diet was estimated from the 7-day food records by applying 33.3% adherence factor to the recorded intake for each of the three main components. The sum of the three components if consumed as prescribed would equal 100% adherence.

Statistical Analyses

Results are expressed as means ± SEM or 95% confidence intervals (CIs). Time zero was used as the baseline and refers to the pre-metabolic study baseline [1]. Treatment differences in physical and biochemical measures were assessed using all available data and a repeated measures mixed model accounting for time of assessment (SAS 9.2) [44] in the Tables (Table 2 and 3) and the Results. The response variable was change from baseline, with diet and week as fixed effects and subject ID nested in diet. There was no adjustment for baseline. Any participant who started the ad libitum treatment was included in the analysis (N=39). The completer analysis included the 23 participants who completed the study.

Multiple imputation (taking the mean of 5 sets of randomly imputed values) was used to present baseline and treatment values in the Tables (2 and 3) and Figures (2 and 3) by generating data for those who dropped out or had missing values [44].

Results

Compliance with the major dietary components [vegetable proteins (soy and gluten), nuts, and viscous fibers] was 33.6% or one-third of that prescribed during the metabolic phase (Table 2). Saturated fat intakes were similar on both treatments whereas intake of monounsaturated fats, vegetable proteins, and soy protein were significantly higher on the low-carbohydrate diet (Table 2). Available carbohydrate intake was significantly lower on the low-carbohydrate diet (Table 2).

The attrition~~dropout~~ rate was 50~~35~~% (~~7~~10/20) on the low-carbohydrate and 32~~26~~% (65/~~1~~19) on the high-carbohydrate (Figure 1), this equates to a total attrition~~dropout~~ rate of 43~~1~~% (16/39). The number of participants who did not complete the study (including dropouts and withdrawals) did not differ between treatments. Three participants were withdrawn by the study physician due to failure to attain LDL-C targets on the low-carbohydrate diet (mean LDL-C = 5.24mmol/L) and one subject on the high-carbohydrate diet (LDL-C = 7.78mmol/L). Participants on the low-carbohydrate diet tended to have larger reductions in body weight over time (Figure 2). The weight loss from baseline to the end of the 6-month ad libitum treatment was -6.9kg [95% CI, -7.7, -6.1] on the low-carbohydrate and –5.8kg [95% CI, -6.6, -5.1] on the control diet with a significant difference between groups (treatment difference [95% CI]: -1.1kg [-2.1, 0.0]; P=0.047) (Table 3). The final reduction in BMI was also greater on the low-carbohydrate versus high-carbohydrate diet (treatment difference [95% CI]: -0.4kg/m^2 [-0.8, 0.0]; P=0.039) (Table 3). Among the completers, there were numerically larger differences between treatments for both body weight and BMI (treatment difference [95% CI]: -1.8 kg [-3.0, -0.6]; P=0.0041 and -0.7 kg/m^2 [-1.1, -0.2]; P=0.0039, respectively).

There was a relative increase in recorded exercise by the high-carbohydrate diet participants, whereas there was no relative change in the low-carbohydrate participants (treatment difference [95% CI]: -9.3 [-16.4, -2.2] METs; P=0.012), but this was not reflected in a greater weight loss

(Table 3). There were no treatment differences in percent body fat, waist circumference or satiety (Table 3).

Lipids

At the end of the study, the reduction on the low-carbohydrate versus high-carbohydrate diet was greater for LDL-C (treatment difference [95% CI]: -0.49mmol/L [-0.70, -0.28]; P<0.001, for TC (-0.62mmol/L [-0.86, -0.37]; P<0.001, for TC:HDL-C -0.57 [-0.83, -0.32]; P<0.001, for LDL-C:HDL-C (-0.42 [-0.60, -0.24]; P<0.001, and for triglycerides (-0.34mmol/L [-0.57, -0.11]; P=0.005). No treatment difference was seen in HDL-C (Table 3). A similar pattern was observed in the completers. The treatment difference was numerically larger for LDL-C (-0.60mmol/L [-0.84, -0.36]; P<0.0001), TC (-0.73mmol/L [-1.00, -0.45]; P<0.0001), TC:HDL-C (-0.68 [-0.97, -0.39]; P<0.0001), and LDL-C:HDL-C (-0.53 [-0.73, -0.32]; P<0.0001). Values for LDL-C and the TC:HDL-C ratio were consistently lower in participants on the low-carbohydrate diet throughout the study while HDL-C values were not different from baseline (Figure 3 A-C).

Apolipoproteins

ApoB and the ApoB:A1 ratio were reduced more on the low- versus the high-carbohydrate diet at the end of the study (treatment different [95% CI]: -0.11g/L [-0.16, -0.06]; P<0.001 and -0.05 [-0.09, -0.02]; P=0.003, respectively) (Table 3). No significant difference between the diets was observed for ApoA1 concentrations. The pattern of change in the apolipoproteins in the completers reflected the changes seen in the whole group. Figure 3D and 3F show that the low-carbohydrate diet resulted in lower apoB and ApoB:ApoA1 ratios relative to baseline over the course of the study.

C-Reactive Protein, HbA1c, Blood Glucose, Serum Insulin, Insulin Resistance and Blood Pressure

Both treatments reduced hs-CRP with no difference between treatments (Table 3). HbA1c, fasting blood glucose, insulin, and insulin resistance (calculated using the HOMA model) fell similarly on both treatments during the course of the study (Table 3). Systolic and diastolic blood pressure decreased similarly with no treatment differences (Table 3). The completers also failed to show a difference between treatments.

Calculated CHD Risk

The low-carbohydrate diet significantly reduced the calculated 10-year CHD risk relative to the high-carbohydrate diet (2% [-2, -1]; P<0.001) (Table 3). A reduced CHD risk on the low-carbohydrate diet was also observed in the completers (2% [-3, -1]; P<0.001).

Adverse Events

No serious adverse events or events that involved hospitalisation occurred during the study.

Discussion

The present study demonstrated that consumption of a low-carbohydrate vegan diet resulted in a modestly greater body weight reduction compared to a high-carbohydrate diet (7% versus 6% reductions, respectively) over a six-month ad libitum period. These reductions were similar to those reported for low-carbohydrate "Atkins-like" diets[2 3 6 10]. However by comparison with the high-carbohydrate diet, consumption of the low-carbohydrate diet containing vegetable

proteins and oils was also associated with significantly reduced concentrations of LDL-C. This LDL-C reduction has not been reported for other low-carbohydrate diet studies in which a large part of the protein and fat originated from animal sources and in which no significant LDL-C reductions were seen ~~where increases in LDL-C were seen~~ [2-6 8]. The sustained reduction in LDL-C, associated with only a small incremental weight loss on the 6-month self-selected diet, is a potentially important attribute of the diet in reducing long-term CHD risk [45 46]. Furthermore, as seen in the present study, a low-carbohydrate diet, in which vegetable fat and protein options were encouraged, demonstrated a larger reduction in the TC:HDL-C ratio than that reported at 6 months in weight loss studies employing either a Mediterranean or a high-carbohydrate diet [10]. The majority of studies undertaken to date have been 6 months to one year in duration [2-6 47] with more recent studies of up to 2 years [2 8] and, as with the present study, a number of these studies had a high dropout rate [2 3 5 47]. The high dropout rate in the present study did not prevent identification of significant LDL-C and body weight differences in the intent-to-treat analysis (using all available data). However, the completer data demonstrated an even larger treatment difference in LDL-C ~~of -0.60mmol/L [-0.84, -0.36]~~ favoring the low-carbohydrate~~test~~ treatment ~~(P<0.001)~~. Those on the low-carbohydrate diet showed overall adherence to the major dietary components [vegetable proteins (soy and gluten), nuts, and viscous fibers] at 33.6% of that provided during the metabolic phase [1]. This adherence is similar to the 43.3% seen with the dietary portfolio in the comparison of the metabolic one month [35] and the ad libit~~i~~um six month studies [48]. In this comparison also just under half the LDL-C reduction (13-14%) seen on the ad libit~~i~~um compared to the metabolic study [35].

The effect of low-carbohydrate diets on CHD events has not been assessed in randomized controlled trials. Nevertheless, low-carbohydrate diets high in vegetable proteins and oils have

been associated with a 30% reduced CHD risk and an 18% reduced incidence of diabetes in cohort studies [30 31]. The median interquantile difference in these studies between the first and 10th decile for vegetable protein and monounsaturated fat (MUFA) intakes, as a marker of increased vegetable oil consumption, was 1.4% and 9.3% expressed as a percentage of total caloric intake [30]. These figures compare with a 8.2% and a 4.6% relative increase in vegetable protein and oil consumption from baseline on the Eco-Atkins diet compared to the control diet. The increases in MUFA were therefore seen in both studies. Recently a Spanish Mediterranean diet emphasizing increased nut or olive oil consumption, increasing monounsaturated fat intake by 2-3%, has been shown to significantly reduce cardiovascular events also by approximately 30% [33]. These data provide consistent support for the view that the Eco-Atkins approach would reduce CHD risk in the long term.

The present diet, while lowering LDL-C by 9%, did not result in any significant depression of HDL-C. Lowering LDL-C while maintaining HDL-C would be expected to reduce CHD risk [45 46]. Similarly, reductions in ApoB and the ApoB:A1 ratio were also observed in the present study. These findings further support the potential CHD benefit that this weight loss diet may have [49-51]. It has also been claimed that apolipoproteins may be stronger predictors of CHD events than conventional lipid variables [52-54].

In contrast to the metabolic study, the reductions in systolic and diastolic blood pressure were not significant between the low- and high-carbohydrate diets. Similarly, hs-CRP was unchanged between treatments, however, the level was significantly reduced with the low-carbohydrate diet compared to baseline. Studies have shown that hs-CRP tended to be lowest on the diets containing the highest proportion of carbohydrate [5]. Low glycemic index and low glycemic load diets have also been associated with lower hs-CRP concentrations [55 56]. These

advantages of the higher carbohydrate diet may have reduced any hs-CRP difference between the two diets in the present study.

Soy-containing foods as well as nuts have cholesterol lowering effects [15 17 18 57 58] and may explain the reduction in LDL-C. Viscous fiber in low starch vegetables and β-glucan in oats and barley may also have contributed to the overall cholesterol lowering effect of the diet [9 14 45]. Furthermore, nuts and high fiber food consumption have been associated with lower body weight [59].

The study weaknesses include the relatively small sample size and the high dropout rate. Nevertheless, high dropout rates have been reported in similar dietary studies and it is noteworthy that attrition rates were low in the metabolic study when all food was provided [1]. Food availability and preparation may therefore be important factors. Future studies will need to focus on strategies to increase and maintain adherence, especially to the cholesterol lowering components, which all bear US FDA health claims for cardiovascular disease risk reduction. Furthermore, collaboration with food industry may be helpful in addressing concerns of availability, variety, and ease of preparation. In retrospect, a simplified one page eating plan for breakfast, lunch, and dinner with a number of options and amounts for each meal, as we have used in our dietary portfolio studies, might also be helpful [48]. For those who did complete the study, however, there were benefits in weight loss and LDL-C reduction, an additional 2% advantage in body weight reduction compared to the high-carbohydrate diet and a 13% drop in LDL-C for participants consuming a more plant-based low-carbohydrate diet. Unfortunately it was not possible to predict who would complete the diet based on pre-study data or changes observed during the metabolic phase.

The study's strength is that the prescribed hypocaloric diet was self-selected, meaning the results are more in line with what can be expected under free-living conditions. The breadth of application of the plant-based low-carbohydrate diet, however, remains to be determined, but it may provide an option for some individuals for whom LDL-C reduction is an equal concern to weight loss. If low-carbohydrate dietary options become more generally available the number of individuals who will benefit is likely to increase.

We conclude that a weight loss diet which reduced carbohydrate in exchange for increased intakes of vegetable sources of protein, such as gluten, soy and nuts, together with vegetable oils offers an opportunity to improve both LDL-C and body weight, both being risk factors for CHD. Further trials are warranted to evaluate low-carbohydrate diets, including more plant-based low-carbohydrate diets, on CHD risk factors and ultimately on CHD.

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Dr. Jenkins, together with those responsible for analysis and interpretation of data, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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None of the funding organizations or sponsors played any significant role in the design and conduct of the study, in the collection, management, analysis, and interpretation of the data, or in the preparation, or approval of the manuscript. However, the named co-authors from Solae LLC reviewed the manuscript.

Disclosures

Dr. Jenkins has served on the Scientific Advisory Board of Sanitarium Company, Agri-Culture and Agri-Food Canada (AAFC), Canadian Agriculture Policy Institute (CAPI), California Strawberry Commission, Loblaw Supermarket, Herbal Life International, Nutritional Fundamental for Health, Pacific Health Laboratories, Metagenics, Bayer Consumer Care, Orafti,

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Figure Legends

Figure 1: Patient Flow Diagram.

Figure 2: Weight loss during the study on both diets.

Figure 3: Mean (A) LDL-C, (B) HDL-C, (C) TC:HDL-C, (D) apoplipoprotein B (apoB) and (E) apolipoprotein A1 (apoA1), (F) ApoB:ApoA1 ratio between the two treatments during the metabolic and ad libitum phases.

Table 1: Baseline Characteristics for Those Who Started the 6-Month Self-Selected Diets (n=39)

	High-carbohydrate (n=19)	Low-Carbohydrate (n=20)
Age (y)	55.3 ± 1.8	57.6 ± 1.4
Males/Females	6/13	9/11
Body Weight, kg	85.4 [79.3, 91.6]	83.7 [78.5, 89.0]
Body Mass Index, kg/m^2	31.1 [29.9, 32.4]	31.1 [29.8, 32.4]
Blood Pressure, mm Hg		
Systolic	122 [116, 128]	128 [123, 132]
Diastolic	75 [72, 79]	77 [74, 80]
Cholesterol, mmol/L		
Total	6.75 [6.28, 7.21]	6.76 [6.21, 7.31]
LDL-C	4.40 [3.99, 4.82]	4.53 4.14, 4.93]
HDL-C	1.36 [1.22, 1.50]	1.21 [1.06, 1.36]
Triglycerides, mmol/L	2.16 [1.62, 2.70]	2.23 [1.65, 2.80]
Ratios		
TC:HDL-C	5.17 [4.54, 5.80]	5.81 [5.20, 6.41]
LDL-C: HDL-C	3.35 [2.95, 3.75]	3.89 [3.49, 4.29]
Medications		
Lipid lowering (prior to start of study)	4	7
Blood pressure	3	6
Diabetes	0	0
Thyroid	2	1

Values represent mean ± SEM or 95% confidence intervals (CIs).

No significant differences between treatments at baseline assessed by two sample t-test (two-tailed).

Table 2: Nutritional Profiles on the High and Low Carbohydrate Diets (n=39)

	High Carbohydrate		Low Carbohydrate			
	Week 0[b]	Ad Libitum[b]	Week 0[b]	Ad Libitum[b]	Between-Treatment Difference[c]	P-value[d]
Calories (kcal)	1598 [1421, 1775]	1347 [1140, 1553]	1840 [1550, 2130]	1388 [1234, 1541]	-248 [-391, -106]	0.001
% of Total Calories						
Available Carbohydrate	46.3 [42.2, 50.4]	53.9 [50.2, 57.5]	43.8 [40.2, 47.4]	39.6 [35.7, 43.6]	-10.5 [-13.6, -7.5]	< 0.001
Protein	20.6 [18.7, 22.5]	18.4 [17.4, 19.5]	20.1 [18.0, 22.2]	22.7 [20.1, 25.4]	5.9 [4.3, 7.5]	< 0.001
Vegetable Protein	5.6 [5.0, 6.1]	6.7 [6.1, 7.3]	5.7 [5.3, 6.1]	15.0 [11.7, 18.2]	8.2 [6.5, 9.9]	< 0.001
Soy Protein	0 [0, 0]	0.2 [0.1, 0.2]	0 [0, 0]	4.7 [2.7, 6.8]	3.6 [2.9, 4.4]	< 0.001
Fat	30.8 [27.3, 34.4]	27.5 [24.6, 30.4]	34.4 [31.4, 37.5]	36.0 [31.5, 40.5]	5.2 [2.6, 7.7]	< 0.001
Saturated	10.8 [9.1, 12.6]	7.6 [6.2, 8.9]	11.8 [10.3, 13.3]	7.5 [6.6, 8.4]	-0.4 [-1.4, 0.6]	0.401
Monounsaturated	12.3 [10.7, 13.8]	10.4 [9.3, 11.6]	13.0 [11.9, 14.2]	14.8 [13.1, 16.6]	4.6 [3.1, 6.1]	< 0.001
Polyunsaturated*	5.2 [4.6, 5.8]	6.3 [5.4, 7.2]	6.6 [5.5, 7.8]	8.4 [7.5, 9.4]	0.4 [-0.5, 1.4]	0.4
Alcohol	2.2 [0.3, 4.2]	1.9 [0.7, 3.2]	1.6 [0.0, 3.3]	1.1 [0.1, 2.1]	-0.5 [-1.3, 0.2]	0.160
Dietary Fibre (g/1000 kcal)	10.9 [9.2, 12.5]	18.2 [15.2, 21.1]	12.1 [9.9, 14.4]	21.3 [18.8, 23.8]	1.5 [-0.5, 3.5]	0.127
Dietary Cholesterol (mg/1000 kcal)	149 [129, 169]	87 [61, 113]	157 [136, 177]	117 [44, 189]	11 [-22, 23]	0.954
Adherence with "Eco-Atkins" Components[a]						
Viscous Fiber (out of 33.3%)	---	---	---	14.0 [9.4, 18.6]	---	---
Vegetable Protein (soy and gluten) (out of 33.3%)	---	---	---	14.7 [10.3, 19.1]	---	---
Nuts (out of 33.3%)	---	---	---	6.3 [3.3, 9.3]	---	---
Total Adherence (out of 100%)	---	---	---	33.6 [22.1, 45.2]	---	---

Values represent mean ± 95% confidence intervals (CIs).

[a]Adherence represents the mean percentage intake of the prescribed intake of the 3 cholesterol-lowering components [viscous fiber, vegetable protein (soy and gluten), nuts] by expressing the recorded intake for each component as 33.3%. The sume of the 3 components if consumed as prescribed would equal 100% adherence.

[b]Values represent multiple imputation (taking the mean of 5 sets of randomly imputed values) to generate data for those who dropped out or had missing values.

[c]Between Treatment Difference = Change from baseline between the two diets using all available data.

[d]P-values assessed using all available data and a repeated measures mixed model accounting for time of assessment. The response variable was change from baseline, with diet and week as fixed effects and subject ID nested in diet. There was no adjustment for baseline.

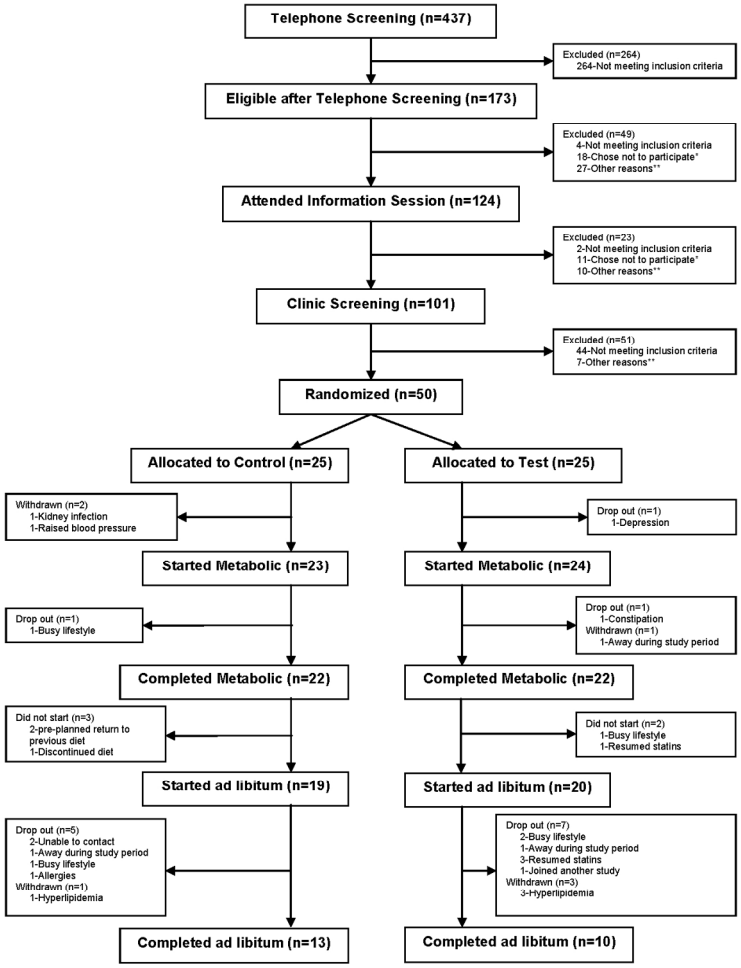
*Significantly different betweeen treatments at baseline assessed by two sample t-test (two tailed), P=0.025.

Table 3: Effect of high and low carbohydrate diets on body weight, blood lipids, apolipoproteins and 10-yr CHD risk (n=39)

	High Carbohydrate		Low Carbohydrate			
	Week 0[a]	Ad Libitum[a]	Week 0[a]	Ad Libitum[a]	Between Treatment Difference[b]	P-value[c]
Body Weight, kg	85.4 [79.3, 91.6]	80.4 [74.2, 86.6]	83.7 [78.5, 89.0]	76.9 [71.9, 81.9]	-1.1 [-2.1, 0.0]	0.047
BMI	31.1 [29.9, 32.4]	29.2 [27.9, 30.5]	31.1 [29.8, 32.4]	28.7 [27.3, 30.1]	-0.4 [-0.8, 0.0]	0.039
Body Fat, %	38.9 [34.0, 43.8]	35.0 [30.7, 39.2]	35.6 [30.1, 41.1]	31.4 [26.1, 36.6]	-1.7 [-4.0, 0.7]	0.161
Waist Circumference (cm)	102.8 [99.4, 106.2]	97.4 [93.1, 101.6]	99.8 [96.1, 103.5]	93.7 [89.8, 97.7]	0.1 [-1.1, 1.3]	0.861
Fasting Glucose	5.2 [4.9, 5.4]	4.6 [4.5, 4.7]	5.2 [5.0, 5.4]	4.6 [4.4, 4.9]	0.1 [-0.1, 0.2]	0.447
HbA1c (%)	5.2 [5.0, 5.4]	5.2 [5.0, 5.3]	5.3 [5.0, 5.5]	5.2 [5.0, 5.4]	0.0 [-0.2, 0.1]	0.852
Fasting Insulin	50.0 [38.3, 61.7]	36.4 [27.5, 45.4]	47.3 [36.9, 57.6]	33.3 [22.8, 43.9]	-0.6 [-9.1, 8.0]	0.898
HOMA-IR	1.65 [1.17, 2.13]	1.11 [0.81, 1.41]	1.53 [1.19, 1.88]	0.99 [0.68, 1.30]	0.01 [-0.30, 0.33]	0.937
Satiety (-4 to 4)	1.0 [0.7, 1.4]	0.9 [0.7, 1.2]	1.2 [0.8, 1.7]	1.1 [0.8, 1.4]	-0.1 [-0.4, 0.2]	0.440
Exercise, METs	17.4 [12.4, 22.4]	25.8 [21.1, 30.6]	24.0 [12.9, 35.0]	23.9 [15.3, 32.6]	-9.3 [-16.4, -2-2]	0.012
Cholesterol, mmol/L[†]						
Total	6.75 [6.28, 7.21]	6.49 [5.97, 7.02]	6.76 [6.21, 7.31]	6.10 [5.67, 6.53]	-0.62 [-0.86, -0.37]	<0.001
LDL-C	4.40 [3.99, 4.82]	4.40 [3.91, 4.90]	4.53 [4.14, 4.93]	4.06 [3.71, 4.42]	-0.49 [-0.70, -0.28]	<0.001
HDL-C	1.36 [1.22, 1.50]	1.35 [1.22, 1.48]	1.21 [1.06, 1.36]	1.25 [1.10, 1.39]	0.03 [-0.02, 0.07]	0.245
Triglycerides	2.16 [1.62, 2.70]	1.71 [1.35, 2.07]	2.23 [1.65, 2.80]	1.50 [1.22, 1.77]	-0.34 [-0.57, -0.11]	0.005
Ratios						
Tchol:HDL-C	5.17 [4.54, 5.80]	4.92 [4.49, 5.34]	5.81 [5.20, 6.41]	5.13 [4.65, 5.62]	-0.57 [-0.83, -0.32]	<0.001
LDL-C:HDL-C	3.35 [2.95, 3.75]	3.34 [3.00, 3.68]	3.89 [3.49, 4.29]	3.48 [3.06, 3.90]	-0.42 [-0.60, -0.24]	<0.002
Apolipoproteins, g/L[‡]						
Apo A1	1.69 [1.60, 1.78]	1.69 [1.60, 1.77]	1.57 [1.45, 1.69]	1.57 [1.46, 1.67]	-0.02 [-0.06, 0.02]	0.316
Apo B	1.38 [1.26, 1.50]	1.23 [1.13, 1.33]	1.42 [1.30, 1.54]	1.20 [1.10, 1.31]	-0.11 [-0.16, -0.06]	<0.001
Apo B: Apo A1	0.83 [0.74, 0.91]	0.74 [0.68, 0.80]	0.92 [0.84, 0.99]	0.78 [0.70, 0.86]	-0.05 [-0.09, -0.02]	0.003
hs-CRP, mg/dL	2.1 [1.0, 3.3]	1.9 [1.3, 2.4]	3.0 [1.5, 4.5]	2.6 [1.0, 4.1]	-0.4 [-0.9, 0.1]	0.082
Blood Pressure, mmHg						
Systolic	122 [116, 128]	118 [114, 122]	128 [123, 132]	123 [119, 128]	-2 [-5, 2]	0.356
Diastolic	75 [72, 79]	74 [71, 77]	77 [74, 80]	76 [71, 80]	-1 [-3, 1]	0.288
10-yr CHD risk (%)*	8 [6, 9]	7 [6, 9]	12 [9, 14]	9 [7, 11]	-2 [-2, -1]	<0.001

Values represent mean ± 95% confidence intervals (CIs).											
[†]To convert total cholesterol, LDL-C, and HDL-C to mg/dL, divide by 0.0259; to convert triglycerides to mg/dL, divide by 0.0113.											
[‡]To convert apolipoprotein A1 and B to mg/dL, multiply by 100.											
[a]Values represent multiple imputation (taking the mean of 5 sets of randomly imputed values) to generate data for those who dropped out or had missing values.											
[b]Between Treatment Difference = Change from baseline between the two diets using all available data.											
[c]P-values assessed using all available data and a repeated measures mixed model accounting for time of assessment. The response variable was change from baseline, with diet and week as fixed effects and subject ID nested in diet. There was no adjustment for baseline.											
[*]Significantly different betweeen treatments at baseline assessed by two sample t-test (two tailed), P=0.007.											

Figure 1



*Chose not to participate (29): busy lifestyle (13), not interested (6), study too demanding (3), currently on another diet (2), no compensation (2), work-related (2), dislike prepackaged foods (1)

**Other reasons (44): unable to contact (19), unable to come to clinic (13), away (5), throat surgery (1), bowel resection (1), high potassium and BP (1), high potassium (1), raised liver function tests (1), not interested (1), medical insurance issue (1)

215x279mm (200 x 200 DPI)

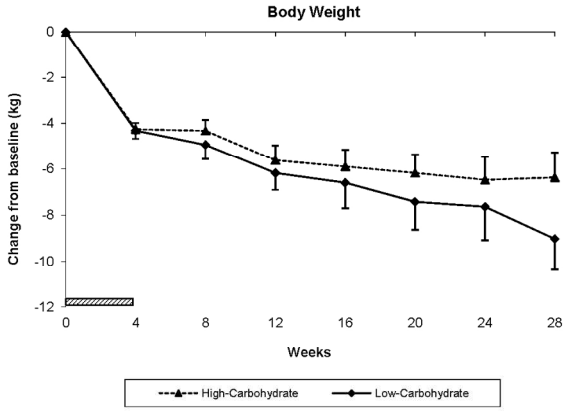


Figure 2: Weight loss during the study on both diets. Values represent mean ± SEM of the change from baseline during the metabolic and ad libitum phases, using multiple imputation (taking the mean of 5 sets of randomly imputed values) to generate data for those who dropped out or had missing values on the ad libitum phase.

The change in weight during the ad libitum phase was significantly reduced (P=0.047) on the low versus the high carbohydrate diet using all available data in the repeated measures mixed model analysis.

Represents the metabolic phase.

215x279mm (200 x 200 DPI)

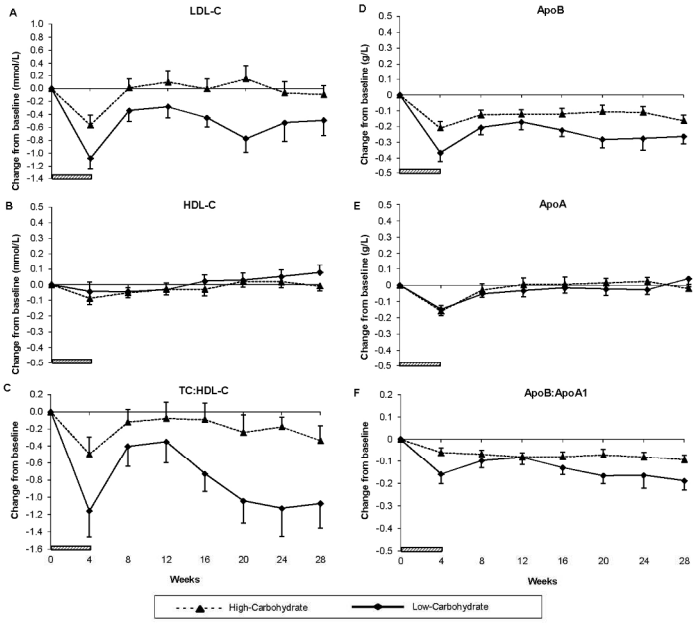


Figure 3:Change in (A) LDL-C, (B) HDL-C, (C) TC:HDL-C, (D) Apolipoprotein B (apoB), (E) Apolipoprotein A1 (apoA1), (F) ApoB:ApoA1 ratio between the two treatments during the metabolic and ad libitum phases. Values represent mean ± SEM of the change from baseline using multiple imputation (taking the mean of 5 sets of randomly imputed values) to generate data for those who dropped out or had missing values for the ad libitum phase.

Significant treatment differences were seen for LDL-C (P<0.001) apo B (P<0.001) and the ratios TC:HDL-C (P<0.001) and apoB:apoA1 (p=0.003) using all available data in the repeated measures mixed model analysis during the ad libitum phase.

Represents the metabolic phase.

279x215mm (200 x 200 DPI)

CONSORT 2010 checklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	1
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	5-6
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	7-8
	2b	Specific objectives or hypotheses	8
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	9
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	NA
Participants	4a	Eligibility criteria for participants	8, also previously published from results of metabolic phase
	4b	Settings and locations where the data were collected	8
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	9-1~~0~~1
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	1~~0~~1-12~~1~~
	6b	Any changes to trial outcomes after the trial commenced, with reasons	NA
Sample size	7a	How sample size was determined	Continuation with ad libitum phase, metabolic phase published

	7b	When applicable, explanation of any interim analyses and stopping guidelines	NA
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	Continuation with ad libitum phase, randomized metabolic phase published
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	Continuation with ad libitum phase, randomized metabolic phase published
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	Continuation with ad libitum phase, randomized metabolic phase published
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	Continuation with ad libitum phase, randomized metabolic phase published
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those assessing outcomes) and how	11~~0~~
	11b	If relevant, description of the similarity of interventions	NA

Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	~~1~~12
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	12~~1~~
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	Figure 1, CONSORT Diagram
	13b	For each group, losses and exclusions after randomisation, together with reasons	Figure 1, CONSORT Diagram
Recruitment	14a	Dates defining the periods of recruitment and follow-up	8
	14b	Why the trial ended or was stopped	NA
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	Table 1
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	12~~1~~
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	13~~2~~-15~~3~~, Table 3, Figure 2 & 3
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	Relative effect sizes are given in Results 13~~2~~-15~~3~~ and Tables 2 & 3. The absolute differences from each treatment can be derived from Table 2 & 3 and Figures 2 & 3.
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	13-15~~2, 13~~

Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	154
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	186
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	15-194,15
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	145-1916
Other information			
Registration	23	Registration number and name of trial registry	2
Protocol	24	Where the full trial protocol can be accessed, if available	2
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	2-3, (repeated 18) 20

*We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.

BMJ open

Effect of a Six Months Vegan Low-Carbohydrate ("Eco-Atkins") Diet on Cardiovascular Risk Factors and Body Weight in Hyperlipidemic Adults: A Randomized Controlled Trial

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Effect of a Six Month Vegan Low-Carbohydrate ("Eco-Atkins") Diet on Cardiovascular Risk Factors and Body Weight in Hyperlipidemic Adults: A Randomized Controlled Trial

David JA Jenkins, MD[1-5] Julia MW Wong, PhD[1,3] Cyril WC Kendall, PhD[1,3] Amin Esfahani, MSc[1,3] Vivian WY Ng, RD[1,3] Tracy CK Leong, BASc[1,3] Dorothea A Faulkner, PhD[1,3] Ed Vidgen, BSc[1,3] Gregory Paul, PhD[6] Ratna Mukherjea, PhD[6] Elaine S. Krul, PhD[6] William Singer, MD[1-4]

Departments of [1]Nutritional Sciences, [2]Medicine, Faculty of Medicine, University of Toronto, Toronto, Ontario, Canada; [3]Clinical Nutrition & Risk Factor Modification Center, St. Michael's Hospital, Toronto, Ontario, Canada; [4]Department of Medicine, Division of Endocrinology and Metabolism, [5]Li Ka Shing Knowledge Institute, St. Michael's Hospital, Toronto, Ontario, Canada; [6]Solae LLC, St. Louis, Missouri, USA

JMWW current affiliation is the New Balance Foundation Obesity Prevention Center, Boston Children's Hospital, Boston, MA, USA, and Department of Pediatrics, Harvard Medical School, Boston, MA, USA.

AE current affiliation is New York Medical College, School of Medicine, Valhalla, NY, USA.

Address correspondence and reprint requests to David JA Jenkins, Clinical Nutrition and Risk Factor Modification Center, St. Michael's Hospital, 61 Queen St. East, Toronto, Ontario, CANADA, M5C 2T2. Phone: (416) 978-4752; Fax: (416) 978-5310; EM: cyril.kendall@utoronto.ca

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Number of Tables: 3

Number of Figures: 3

Number of References: 59

Running Title: Weight loss in hyperlipidemia on a vegan diet

Trial Registration: #NCT00256516

Keywords: weight loss, vegetable proteins, nuts, soy, vegan diet, hyperlipidemia

Abstract Word Count: 273 (up to 300 allowed)

Abstract

Objective: Low-carbohydrate diets may be useful for weight loss. Diets high in vegetable proteins and oils may reduce the risk of coronary heart disease (CHD). The main objective was to determine the longer term effect of a diet that was both low-carbohydrate and plant-based on weight loss and LDL-C.

Design, Setting, Participants: A parallel design study of 39 overweight hyperlipidemic men and postmenopausal women conducted at a Canadian university-affiliated hospital nutrition research center from April 2005 to November 2006.

Intervention: Participants were advised to consume either a low-carbohydrate vegan diet or a high-carbohydrate lacto-ovo vegetarian diet for six-months after completing one-month metabolic (all foods provided) versions of these diets. The prescribed macronutrient intakes for the low- and high-carbohydrate diets were: 26% and 58% of energy from carbohydrate, 31% and 16% from protein and 43% and 25% from fat, respectively.

Primary Outcome: Change in body weight.

Results: Twenty-three participants (50% test, 68% control) completed the six-month ad libitum study. The approximate 4kg weight loss on the metabolic study was increased to -6.9kg on low-carbohydrate and -5.8kg on high-carbohydrate six-month ad libitum treatments (treatment difference [95% CI]: -1.1kg [-2.1, 0.0], P=0.047). The relative LDL-C and triglyceride reductions were also greater on the low-carbohydrate treatment (treatment difference [95% CI]: -0.49mmol/L [-0.70, -0.28], P<0.001 and -0.34mmol/L [-0.57, -0.11], P=0.005, respectively), as were the TC:HDL-C and apolipoprotein B:A1 ratios (-0.57 [-0.83, -0.32], P<0.001 and -0.05 [-0.09, -0.02], P=0.003, respectively).

Conclusions: A self-selected low-carbohydrate vegan diet, containing increased protein and fat from gluten and soy products, nuts, and vegetable oils, had lipid lowering advantages over a high-carbohydrate, low-fat weight loss diet, thus improving heart disease risk factors.

Trial Registration: clinicaltrials.gov (http://www.clinicaltrials.gov/), #NCT00256516

Article Summary

Article Focus

- Low-carbohydrate diets may be useful for weight loss. Diets high in vegetable proteins and oils may reduce the risk of coronary heart disease (CHD).
- The objective of the randomized clinical trial was to determine the longer term effect of a diet that was both low-carbohydrate and plant-based on weight loss and LDL-C.
- We have already reported the effect of this dietary strategy in producing a difference of 8% in LDL-C reduction between calorie-restricted diets (60% of estimated calorie requirements) when all food was provided. We now report findings after these same participants continued on their respective diets for an additional 6 months, under self-selected conditions, in order to gain insights into the real life effectiveness of this diet.

Key Messages

- By comparison with the high-carbohydrate diet, consumption of the low-carbohydrate diet containing vegetable proteins and oils was also associated with significantly reduced concentrations of LDL-C. This LDL-C reduction has not been reported for other low-carbohydrate diet studies in which a large part of the protein and fat originated from animal sources.
- The present study also demonstrated that consumption of a low-carbohydrate vegan diet resulted in modestly greater body weight reductions compared to a high-carbohydrate diet (7% versus 6% reductions, respectively) over a six-month ad libitum period.

- The sustained reduction in LDL-C, associated with a small incremental weight loss on the 6-month self-selected diet, is a potentially important attribute of the diet in reducing long-term CHD risk

Strengths and Limitations of this Study

The study weaknesses include the relatively small sample size and the high dropout rate. Nevertheless, it is noteworthy that attrition rates were low in the metabolic study when all food was provided [1]. Food availability and preparation may therefore be important factors. For those who did complete the study, however, there were benefits in weight loss and LDL-C reduction, an additional 2% advantage in body weight reduction compared to the high-carbohydrate diet and a 13% drop in LDL-C for participants consuming a more plant-based low-carbohydrate diet. The study's strength is that the prescribed hypocaloric diet was self-selected, meaning the results are more in line with what can be expected under free-living conditions. The breadth of application of the plant-based low-carbohydrate diet, however, remains to be determined, but it may provide an option for some individuals for whom LDL-C reduction is an equal concern to weight loss. If low-carbohydrate dietary options become more generally available the number of individuals who will benefit is likely to increase.

Introduction

Many popular weight loss diets emphasize carbohydrate restriction (Atkins, Eddies, South Beach, Zone). Their success is determined by the level of compliance with the prescribed diets [2-7]. However, a high content of animal products, rich in saturated fat and cholesterol, may make conventional low-carbohydrate diets less appropriate for those with hypercholesterolemia [3 8]. Even during active weight loss, these high saturated fat diets do not lower serum LDL-C below baseline [3 8] and there is concern that if such diets continue to be eaten when weight loss has ceased, a more atherogenic blood lipid profile may result [9]. These concerns have prompted exploration of other weight loss strategies, but only modest reductions in LDL-C have been observed [10].

By contrast vegan diets significantly lower LDL-C [11]. Trials of vegan and vegetarian diets also reduce progression of coronary heart disease (CHD) [12] and improve diabetes control [13]. Plant food components such as vegetable proteins, vegetable oils, nuts and viscous fibers, reduce serum lipids in many studies [14-19] and may increase flow mediated vasodilatation [20-23]. Nuts, fiber and vegetarian diets in general, all reduce CHD and diabetes in cohort studies [24-29]. Finally, in cohort studies, low-carbohydrate diets, high in vegetable oils and proteins as opposed to animal products, reduce CHD events and diabetes incidence in women [30 31], while lower red meat intake reduces total, cardiovascular and cancer mortality [32]. Most recently a large randomized controlled trial confirmed the effect of nuts and increased vegetable oil (olive oil) intake in reducing cardiovascular events in the context of a Mediterranean diet [33].

In view of the apparent success of low-carbohydrate diets for weight loss and the demonstration that relatively high-carbohydrate vegetarian and vegan diets, and diets low in animal products, lower CHD risk factors [34-37], we designed a diet that combined both vegan and low-

carbohydrate elements to determine whether such a diet captured both the weight loss and CHD risk reduction advantages. We have already reported the effect of this dietary strategy in producing a difference of 8% in LDL-C reduction between calorie-restricted diets (60% of estimated calorie requirements) when all food was provided [1]. We now report findings after these same participants continued on their respective diets for an additional 6 months, under self-selected conditions, in order to gain insights into the real life effectiveness of this diet. The results of the metabolic (all foods provided) study have been reported previously and had demonstrated a CHD risk factor advantage, but with no greater weight loss than the control diet [1].

Methods

Participants

Forty-seven overweight participants, recruited by newspaper advertisement and hospital clinic notices, undertook the one-month metabolic first phase of the study (Figure 1) that has been previously reported [1]. At the start of the study, participants were given the option to participate in both the metabolic and ad libitum phases or only the metabolic phase. On completion of the metabolic phase, thirty-nine participants (19 control and 20 test participants) continued for an ad libitum six-month study and their data (n=39) were used in the final analysis (Table 1). The study was conducted at a Canadian university-affiliated hospital nutrition research center from April 2005 to November 2006. All participants had high normal to raised LDL-C levels (>3.4mmol/L at diagnosis) and a body mass index > 27 kg/m^2. Details of the eligibility criteria have been previously reported [1]. After recruitment, the 11/39 participants who were taking

lipid lowering medications discontinued their medications at least two weeks prior to starting and for the study duration (Table 1).

Study Protocol

The intervention was a randomized parallel study stratified by sex in which participants were randomized to either low- or high-carbohydrate, calorie-reduced diets. The first month was the previously reported metabolically controlled study [1]. For the following six-months, participants continued on the diet to which they had been assigned as a self-selected (ad libitum) diet. Anthropometric, blood pressure and blood lipid measurements were repeated at monthly intervals. Insulin and HbA1c were measured at baseline and at the start and end of the ad libitum treatment. Percentage body fat was measured at baseline and end of the ad libitum treatment by bioelectrical impedance (Quantum II; RJL Systems, Clinton Township, Michigan). Seven-day diet and exercise histories were recorded in the week prior to each monthly visit. These histories were reviewed and discussed with the dietitian and appropriate dietary counselling was provided to enhance adherence. The overall feeling of satiety for the previous week was assessed at each study visit using a 9-point bipolar semantic scale, where −4 was extremely hungry, 0 was neutral, and +4 was uncomfortably full [1 35]. No exercise advise was given during the study, but alterations in exercise were allowed and recorded.

The Ethics Committees of St. Michael's Hospital and the University of Toronto, and the Therapeutic Products Directorate of Health Canada approved the study. Written informed consent was obtained from the participants. The study's clinical trial registration number was #NCT00256516.

Diets

As with the previous metabolic study, participants were encouraged to eat only 60% of their estimated caloric requirements in order to continue the body weight reduction started on their metabolic phase [38-40]. The prescribed test diet was a low-carbohydrate vegan diet containing 26% of calories from carbohydrate, 31% of calories from vegetable proteins and 43% from fat (primarily vegetable oils). Carbohydrate sources on the low-carbohydrate diet featured viscous fiber-containing foods (such as oats and barley) and low-starch vegetables (emphasizing okra and eggplant) for the relatively limited amount of carbohydrate allowed. The vegetable proteins were prescribed as gluten (54.8% of total protein), soy (23.0%), fruits and vegetables (8.7%), nuts (7.5%), and cereals (6.0%). Gluten was contained in the nut bread and wheat gluten (also called "seitan") products. Soy protein was present in the form of burgers, deli slices, breakfast links, veggie bacon, tofu, and soy milks. Nuts included almonds, cashews, hazelnuts, macadamia, pecans, and pistachios. The fat sources were nuts (43.6% of total fat), vegetable oils (24.4%), soy products (18.5%), avocado (7.1%), cereals (2.7%), fruits and vegetables (2.3%), and seitan products (1.4%). Participants were able to purchase at the research center the "no" starch high protein nut bread and three of the seitan (wheat gluten) products used in the study which were not available in Canada. The control, high-carbohydrate lacto-ovo vegetarian diet (58% carbohydrate, 16% protein and 25% fat) emphasized whole wheat cereals and cereal fiber, as well as low-fat or skim milk dairy products and liquid egg substitute to reduce saturated fat and cholesterol intakes. These diets have been published previously [1]. Participants were given a copy of the menu plans that outlined the food items and amounts prescribed during the metabolic phase. These menu plans served as a reference during the ad libitum phase.

Furthermore, participants were given an exchange list of the items prescribed on the menu plan. The goal was to enhance adherence.

Self-taring electronic scales (My Weigh Scales, Vancouver, BC or Tanita Corporation, Arlington Heights, IL) were provided to all participants and they were instructed to weigh all food items while recording the seven-day food diary in the week prior to monthly clinic visits. Adherence to the three principal cholesterol-lowering components [vegetable proteins (soy and gluten), nuts, and viscous fibers] of the low-carbohydrate diet was assessed from the completed monthly seven-day food records. The amount of each component provided during the metabolic phase remained the same as that prescribed during the ad libitum phase.

Neither the dietitians nor participants could be blinded, but equal emphasis was placed on the potential importance for health of both diets. The analytical technicians were blinded to diet allocation, as was the statistician, up to analysis of the primary outcome. Participants were offered no financial compensation for participation in the study.

Analyses

The analytical techniques have been reported previously [1]. Serum was analyzed in the J. Alick Little Lipid Research Laboratory [35]. LDL-C (in mmol/L) was calculated by the method of Friedewald et al. [1], using all data including the two participants who had baseline and during study triglyceride values above 4.5 mmol/L (3 values on low-carbohydrate diet and 2 on high-carbohydrate diet, maximum triglyceride < 6.5 mmol/L) (exclusion of these two individuals did not alter the findings). The methods for analyzing apolipoproteins A1 and B, high sensitivity C-reactive protein (hs-CRP), blood glucose, insulin, HbA1c, and homeostasis model assessment – insulin resistance model (HOMA-IR) have been described previously [1]. Exercise data were

calculated as metabolic equivalents (METs) [41]. The absolute 10-year CHD risk score was calculated using the Framingham risk equation [42].

Diets were assessed for macronutrients, fatty acids, cholesterol and fiber using a computer program based on the USDA database [43] and developed in our laboratory to allow the addition of the macronutrient content of study foods obtained from food labels or directly from food manufacturers. The nutritional profiles of the diets were calculated from the 7-day food records completed once a month throughout the study and mean intakes are presented.

Adherence to the three principal cholesterol-lowering components [vegetable proteins (soy and gluten), nuts, and viscous fibers] of the low-carbohydrate diet was estimated from the 7-day food records. Each component was assessed as contributing 1/3 or 33.3% to the LDL-C reduction. When the amount consumed was equivalent to the amount prescribed a 33.3% compliance would be recorded for that component. The sum of the three components if consumed as prescribed would equal 100% adherence.

Statistical Analyses

Results are expressed as means ± SEM or 95% confidence intervals (CIs). Time zero was used as the baseline and refers to the pre-metabolic study baseline [1]. Treatment differences in physical and biochemical measures were assessed using all available data from the 39 participants and a repeated measures mixed model accounting for time of assessment (SAS 9.2) [44] in the Tables (Table 2 and 3) and the Results. The response variable was change from baseline, with diet and week as fixed effects and subject ID nested in diet. There was no adjustment for baseline. Any participant who started the ad libitum treatment was included in the analysis (N=39). The completer analysis included the 23 participants who completed the study (Figure 1).

Multiple imputation (taking the mean of 5 sets of randomly imputed values) was used to present baseline and treatment values in the Tables (2 and 3) and Figures (2 and 3) by generating data for those who dropped out or had missing values [44].

Results

Compliance with the major dietary components [vegetable proteins (soy and gluten), nuts, and viscous fibers] was 33.6% or one-third of that prescribed during the metabolic phase (Table 2). Saturated fat intakes were similar on both treatments whereas intake of monounsaturated fats, vegetable proteins, and soy protein were significantly higher on the low-carbohydrate diet (Table 2). Available carbohydrate intake was significantly lower on the low-carbohydrate diet (Table 2). The attrition rate was 50% (10/20) on the low-carbohydrate and 32% (6/19) on the high-carbohydrate (Figure 1), this equates to a total attrition rate of 41% (16/39). The number of participants who did not complete the study (including dropouts and withdrawals) did not differ between treatments. Three participants were withdrawn by the study physician due to failure to attain LDL-C targets on the low-carbohydrate diet (mean LDL-C = 5.24mmol/L) and one subject on the high-carbohydrate diet (LDL-C = 7.78mmol/L). Participants on the low-carbohydrate diet tended to have larger reductions in body weight over time (Figure 2). The weight loss from baseline to the end of the 6-month ad libitum treatment was -6.9kg [95% CI, -7.7, -6.1] on the low-carbohydrate and –5.8kg [95% CI, -6.6, -5.1] on the control diet with a significant difference between groups (treatment difference [95% CI]: -1.1kg [-2.1, 0.0]; P=0.047) (Table 3). The final reduction in BMI was also greater on the low-carbohydrate versus high-carbohydrate diet (treatment difference [95% CI]: -0.4kg/m^2 [-0.8, 0.0]; P=0.039) (Table 3). Among the completers, there were numerically larger differences between treatments for both body weight

and BMI (treatment difference [95% CI]: -1.8 kg [-3.0, -0.6]; P=0.004 and -0.7 kg/m^2 [-1.1, -0.2]; P=0.004, respectively).

There was a relative increase in recorded exercise by the high-carbohydrate diet participants, whereas there was no relative change in the low-carbohydrate participants (treatment difference [95% CI]: -9.3 [-16.4, -2.2] METs; P=0.012), but this was not reflected in a greater weight loss (Table 3). There were no treatment differences in percent body fat, waist circumference or satiety (Table 3).

Lipids

At the end of the study, the reduction on the low-carbohydrate versus high-carbohydrate diet was greater for LDL-C (treatment difference [95% CI]: -0.49mmol/L [-0.70, -0.28]; P<0.001, for TC (-0.62mmol/L [-0.86, -0.37]; P<0.001, for TC:HDL-C -0.57 [-0.83, -0.32]; P<0.001, for LDL-C:HDL-C (-0.42 [-0.60, -0.24]; P<0.001, and for triglycerides (-0.34mmol/L [-0.57, -0.11]; P=0.005). No treatment difference was seen in HDL-C (Table 3). A similar pattern was observed in the completers. The treatment difference was numerically larger for LDL-C (-0.60mmol/L [-0.84, -0.36]; P<0.0001), TC (-0.73mmol/L [-1.00, -0.45]; P<0.0001), TC:HDL-C (-0.68 [-0.97, -0.39]; P<0.0001), and LDL-C:HDL-C (-0.53 [-0.73, -0.32]; P<0.0001). Values for LDL-C and the TC:HDL-C ratio were consistently lower in participants on the low-carbohydrate diet throughout the study while HDL-C values were not different from baseline (Figure 3 A-C).

Apolipoproteins

ApoB and the ApoB:A1 ratio were reduced more on the low- versus the high-carbohydrate diet at the end of the study (treatment different [95% CI]: -0.11g/L [-0.16, -0.06]; P<0.001 and -0.05

[-0.09, -0.02]; P=0.003, respectively) (Table 3). No significant difference between the diets was observed for ApoA1 concentrations. The pattern of change in the apolipoproteins in the completers reflected the changes seen in the whole group. Figure 3D and 3F show that the low-carbohydrate diet resulted in lower apoB and ApoB:ApoA1 ratios relative to baseline over the course of the study.

C-Reactive Protein, HbA1c, Blood Glucose, Serum Insulin, Insulin Resistance and Blood Pressure

Both treatments reduced hs-CRP with no difference between treatments (Table 3). HbA1c, fasting blood glucose, insulin, and insulin resistance (calculated using the HOMA model) fell similarly on both treatments during the course of the study (Table 3). Systolic and diastolic blood pressure decreased similarly with no treatment differences (Table 3). The completers also failed to show a difference between treatments.

Calculated CHD Risk

The low-carbohydrate diet significantly reduced the calculated 10-year CHD risk relative to the high-carbohydrate diet (2% [-2, -1]; P<0.001) (Table 3). A reduced CHD risk on the low-carbohydrate diet was also observed in the completers (2% [-3, -1]; P<0.001).

Adverse Events

No serious adverse events or events that involved hospitalisation occurred during the study.

Discussion

The present study demonstrated that consumption of a low-carbohydrate vegan diet resulted in a modestly greater body weight reduction compared to a high-carbohydrate diet (7% versus 6% reductions, respectively) over a six-month ad libitum period. These reductions were similar to those reported for low-carbohydrate "Atkins-like" diets[2 3 6 10]. However by comparison with the high-carbohydrate diet, consumption of the low-carbohydrate diet containing vegetable proteins and oils was also associated with significantly reduced concentrations of LDL-C. This LDL-C reduction has not been reported for other low-carbohydrate diet studies in which a large part of the protein and fat originated from animal sources and in which no significant LDL-C reductions were seen [2-6 8]. The sustained reduction in LDL-C, associated with a small incremental weight loss on the 6-month self-selected diet, is a potentially important attribute of the diet in reducing long-term CHD risk [45 46]. Furthermore, as seen in the present study, a low-carbohydrate diet, in which vegetable fat and protein options were encouraged, demonstrated a larger reduction in the TC:HDL-C ratio than that reported at 6 months in weight loss studies employing either a Mediterranean or a high-carbohydrate diet [10].

The majority of studies undertaken to date have been 6 months to one year in duration [2-6 47] with more recent studies of up to 2 years [2 8]. The high dropout rate in the present 6-month study did not prevent identification of significant LDL-C and body weight differences in the intent-to-treat analysis (using all available data). However, the completer data demonstrated an even larger treatment difference in LDL-C favoring the low-carbohydrate treatment. Those on the low-carbohydrate diet showed overall adherence to the major dietary components [vegetable proteins (soy and gluten), nuts, and viscous fibers] at 33.6% of that provided during the metabolic phase [1]. This adherence is similar to the 43.3% seen with the dietary portfolio in the comparison of the metabolic one month [35] and the ad libitum six month studies [48]. In this

study, the LDL-C reduction on the low-carbohydrate metabolic month was also greater than that on the ad libitum 6 months, although the treatment differences were similar [35].

The effect of low-carbohydrate diets on CHD events has not been assessed in randomized controlled trials. Nevertheless, low-carbohydrate diets high in vegetable proteins and oils have been associated with a 30% reduced CHD risk and an 18% reduced incidence of diabetes in cohort studies [30 31]. The median interquantile difference in these studies between the first and 10th decile for vegetable protein and monounsaturated fat (MUFA) intakes, as a marker of increased vegetable oil consumption, was 1.4% and 9.3% expressed as a percentage of total caloric intake [30]. These figures compare with an 8.2% and a 4.6% relative increase in vegetable protein and oil consumption from baseline on the Eco-Atkins diet compared to the control diet. The increases in MUFA were therefore seen in both studies. Recently a Spanish Mediterranean diet emphasizing increased nut or olive oil consumption, and so increasing monounsaturated fat intake by 2-3%, has been shown to significantly reduce cardiovascular events also by approximately 30% [33]. These data provide consistent support for the view that the Eco-Atkins approach would reduce CHD risk in the long term.

The present diet, while lowering LDL-C by 9%, did not result in any significant depression of HDL-C. Lowering LDL-C while maintaining HDL-C would be expected to reduce CHD risk [45 46]. Similarly, reductions in ApoB and the ApoB:A1 ratio were also observed in the present study. These findings further support the potential CHD benefit that this weight loss diet may have [49-51]. It has also been claimed that apolipoproteins may be stronger predictors of CHD events than conventional lipid variables [52-54].

In contrast to the metabolic study, the reductions in systolic and diastolic blood pressure were not significant between the low- and high-carbohydrate diets. Similarly, hs-CRP was unchanged

between treatments, however, the level was significantly reduced with the low-carbohydrate diet compared to baseline. Studies have shown that hs-CRP tended to be lowest on the diets containing the highest proportion of carbohydrate [5]. Low glycemic index and low glycemic load diets have also been associated with lower hs-CRP concentrations [55 56]. These advantages of the higher carbohydrate diet may have reduced any hs-CRP difference between the two diets in the present study.

Soy-containing foods as well as nuts have cholesterol lowering effects [15 17 18 57 58] and may explain the reduction in LDL-C. Viscous fiber in low starch vegetables and β-glucan in oats and barley may also have contributed to the overall cholesterol lowering effect of the diet [9 14 45]. Furthermore, nuts and high fiber food consumption have been associated with lower body weight [59].

The study weaknesses include the relatively small sample size and the high dropout rate. Nevertheless, it is noteworthy that attrition rates were low in the metabolic study when all food was provided [1]. Food availability and preparation may therefore be important factors. Future studies will need to focus on strategies to increase and maintain adherence, especially to the cholesterol lowering components, which all bear US FDA health claims for cardiovascular disease risk reduction. Furthermore, collaboration with food industry may be helpful in addressing concerns of availability, variety, and ease of preparation. In retrospect, a simplified one page eating plan for breakfast, lunch, and dinner with a number of options and amounts for each meal, as we have used in our dietary portfolio studies, might also be helpful [48]. For those who did complete the study, however, there were benefits in weight loss and LDL-C reduction, an additional 2% advantage in body weight reduction compared to the high-carbohydrate diet and a 13% drop in LDL-C for participants consuming a more plant-based low-carbohydrate diet.

Unfortunately it was not possible to predict who would complete the diet based on pre-study data or changes observed during the metabolic phase.

The study's strength is that the prescribed hypocaloric diet was self-selected, meaning the results are more in line with what can be expected under free-living conditions. The breadth of application of the plant-based low-carbohydrate diet, however, remains to be determined, but it may provide an option for some individuals for whom LDL-C reduction is an equal concern to weight loss. If low-carbohydrate dietary options become more generally available the number of individuals who will benefit is likely to increase.

We conclude that a weight loss diet which reduced carbohydrate in exchange for increased intakes of vegetable sources of protein, such as gluten, soy and nuts, together with vegetable oils offers an opportunity to improve both LDL-C and body weight, both being risk factors for CHD. Further trials are warranted to evaluate low-carbohydrate diets, including more plant-based low-carbohydrate diets, on CHD risk factors and ultimately on CHD.

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Dr. Jenkins, together with those responsible for analysis and interpretation of data, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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Contributions

Conception and design - Jenkins, Wong, Kendall, Faulkner, Paul, Mukherjea, Krul, Singer

Acquisition of data - Jenkins, Wong, Kendall, Esfahani, Ng, Leong

Analysis and interpretation of data – Jenkins, Wong, Kendall, Vidgen

Drafting of the manuscript – Jenkins, Wong

Critical revision of the manuscript for important intellectual content – Jenkins, Wong, Kendall, Esfahani, Ng, Leong, Faulkner, Vidgen, Paul, Mukherjea, Krul, Singer

Statistical analysis - Vidgen

Obtaining funding – Jenkins, Kendall, Wong

Administrative, technical, or material support – Wong, Kendall, Esfahani, Ng, Leong, Faulkner

Supervision – Jenkins, Kendall, Wong, Singer

No additional contributions - Paul, Mukherjea, Krul

Role of the Sponsors

None of the funding organizations or sponsors played any significant role in the design and conduct of the study, in the collection, management, analysis, and interpretation of the data, or in the preparation, or approval of the manuscript. However, the named co-authors from Solae LLC reviewed the manuscript.

Disclosures

Dr. Jenkins has served on the Scientific Advisory Board of Sanitarium Company, Agri-Culture and Agri-Food Canada (AAFC), Canadian Agriculture Policy Institute (CAPI), California Strawberry Commission, Loblaw Supermarket, Herbal Life International, Nutritional Fundamental for Health, Pacific Health Laboratories, Metagenics, Bayer Consumer Care, Orafti, Dean Foods, Kellogg's, Quaker Oats, Procter & Gamble, Coca-Cola, NuVal Griffin Hospital, Abbott, Pulse Canada, Saskatchewan Pulse Growers, and Canola Council of Canada; received honoraria for scientific advice from Sanitarium Company, Orafti, the Almond Board of California, the American Peanut Council, International Tree Nut Council Nutrition Research and Education Foundation and the Peanut Institute, Herbal Life International, Pacific Health Laboratories, Nutritional Fundamental for Health, Barilla, Metagenics, Bayer Consumer Care, Unilever Canada and Netherlands, Solae LLC, Oldways, Kellogg's, Quaker Oats, Procter & Gamble, Coca-Cola, NuVal Griffin Hospital, Abbott, Canola Council of Canada, Dean Foods, California Strawberry Commission, Haine Celestial, Pepsi, and Alpro Foundation; has been on the speakers panel for the Almond Board of California; received research grants from

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Figure Legends

Figure 1: Patient Flow Diagram.

Figure 2: Weight loss during the study on both diets.

Figure 3: Mean (A) LDL-C, (B) HDL-C, (C) TC:HDL-C, (D) apoplipoprotein B (apoB) and (E) apolipoprotein A1 (apoA1), (F) ApoB:ApoA1 ratio between the two treatments during the metabolic and ad libitum phases.

Tables

Supplementary Table 1: Baseline Characteristics for Those Who Started the 6-Month Self-Selected Diets

Supplementary Table 2: Nutritional Profiles on the High and Low Carbohydrate Diets

Supplementary Table 3: Effect of high and low carbohydrate diets on body weight, blood lipids, apolipoproteins and 10-yr CHD risk

Effect of a Six Months of a Vegan Low-Carbohydrate ("Eco-Atkins") Diet Improveson Cardiovascular Risk Factors and Body Weight in Hyperlipidemic Adults: A Randomized Controlled Trial

David JA Jenkins, MD[1-5] Julia MW Wong, PhD[1,3] Cyril WC Kendall, PhD[1,3] Amin Esfahani, MSc[1,3] Vivian WY Ng, RD[1,3] Tracy CK Leong, BASc[1,3] Dorothea A Faulkner, PhD[1,3] Ed Vidgen, BSc[1,3] Gregory Paul, PhD[6] Ratna Mukherjea, PhD[6] Elaine S. Krul, PhD[6] William Singer, MD[1-4]

Departments of [1]Nutritional Sciences, [2]Medicine, Faculty of Medicine, University of Toronto, Toronto, Ontario, Canada; [3]Clinical Nutrition & Risk Factor Modification Center, St. Michael's Hospital, Toronto, Ontario, Canada; [4]Department of Medicine, Division of Endocrinology and Metabolism, [5]Li Ka Shing Knowledge Institute, St. Michael's Hospital, Toronto, Ontario, Canada; [6]Solae LLC, St. Louis, Missouri, USA

JMWW current affiliation is the New Balance Foundation Obesity Prevention Center, Boston Children's Hospital, Boston, MA, USA, and Department of Pediatrics, Harvard Medical School, Boston, MA, USA.

AE current affiliation is New York Medical College, School of Medicine, Valhalla, NY, USA.

Address correspondence and reprint requests to David JA Jenkins, Clinical Nutrition and Risk Factor Modification Center, St. Michael's Hospital, 61 Queen St. East, Toronto, Ontario, CANADA, M5C 2T2. Phone: (416) 978-4752; Fax: (416) 978-5310; EM: cyril.kendall@utoronto.ca

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Running Title: Weight loss in hyperlipidemia on a vegan diet

Trial Registration: #NCT00256516

Keywords: weight loss, vegetable proteins, nuts, soy, vegan diet, hyperlipidemia

Contributions

Conception and design - Jenkins, Wong, Kendall, Faulkner, Paul, Mukherjea, Krul, Singer

Acquisition of data - Jenkins, Wong, Kendall, Esfahani, Ng, Leong

Analysis and interpretation of data – Jenkins, Wong, Kendall, Vidgen

Drafting of the manuscript – Jenkins, Wong

Critical revision of the manuscript for important intellectual content – Jenkins, Wong, Kendall, Esfahani, Ng, Leong, Faulkner, Vidgen, Paul, Mukherjea, Krul, Singer

Statistical analysis - Vidgen

Obtaining funding – Jenkins, Kendall, Wong

Administrative, technical, or material support – Wong, Kendall, Esfahani, Ng, Leong, Faulkner

Supervision – Jenkins, Kendall, Wong, Singer

No additional contributions - Paul, Mukherjea, Krul

Abstract

Objective: Low-carbohydrate diets may be useful for weight loss. Diets high in vegetable proteins and oils may reduce the risk of coronary heart disease (CHD). The main objective was to determine the longer term effect of a diet that was both low-carbohydrate and plant-based on weight loss and LDL-C.

Design, Setting, Participants: A parallel design study of 39 overweight hyperlipidemic men and postmenopausal women conducted at a Canadian university-affiliated hospital nutrition research center from April 2005 to November 2006.

Intervention: Participants were advised to consume either a low-carbohydrate vegan diet or a high-carbohydrate lacto-ovo vegetarian diet for six-months after completing one-month metabolic (all foods provided) versions of these diets. The prescribed macronutrient intakes for the low- and high-carbohydrate diets were: 26% and 58% of energy from carbohydrate, 31% and 16% from protein and 43% and 25% from fat, respectively.

Primary Outcome: Change in body weight.

Results: Twenty-three participants (50% test, 68% control) completed the six-month ad libitum study. The approximate 4kg weight loss on the metabolic study was increased to -6.9kg on low-carbohydrate and -5.8kg on high-carbohydrate six-month ad libitum treatments (treatment difference [95% CI]: -1.1kg [-2.1, 0.0], P=0.047). The relative LDL-C and triglyceride reductions were also greater on the low-carbohydrate treatment (treatment difference [95% CI]: -0.49mmol/L [-0.70, -0.28], P<0.001 and -0.34mmol/L [-0.57, -0.11], P=0.005, respectively), as were the TC:HDL-C and apolipoprotein B:A1 ratios (-0.57 [-0.83, -0.32], P<0.001 and -0.05 [-0.09, -0.02], P=0.003, respectively).

Conclusions: A self-selected low-carbohydrate vegan diet, containing increased protein and fat from gluten and soy products, nuts, and vegetable oils, had lipid lowering advantages over a high-carbohydrate, low-fat weight loss diet, thus improving heart disease risk factors.

Trial Registration: clinicaltrials.gov (http://www.clinicaltrials.gov/), #NCT00256516

Abstract Word Count: 273 (up to 300 allowed)

Article Summary

Article Focus

- Low-carbohydrate diets may be useful for weight loss. Diets high in vegetable proteins and oils may reduce the risk of coronary heart disease (CHD).
- The objective of the randomized clinical trial was to determine the longer term effect of a diet that was both low-carbohydrate and plant-based on weight loss and LDL-C.
- We have already reported the effect of this dietary strategy in producing a difference of 8% in LDL-C reduction between calorie-restricted diets (60% of estimated calorie requirements) when all food was provided. We now report findings after these same participants continued on their respective diets for an additional 6 months, under self-selected conditions, in order to gain insights into the real life effectiveness of this diet.

Key Messages

- By comparison with the high-carbohydrate diet, consumption of the low-carbohydrate diet containing vegetable proteins and oils was also associated with significantly reduced concentrations of LDL-C. This LDL-C reduction has not been reported for other low-carbohydrate diet studies in which a large part of the protein and fat originated from animal sources.
- The present study also demonstrated that consumption of a low-carbohydrate vegan diet resulted in modestly greater body weight reductions compared to a high-carbohydrate diet (7% versus 6% reductions, respectively) over a six-month ad libitum period.

- The sustained reduction in LDL-C, associated with ~~only~~ a small incremental weight loss on the 6-month self-selected diet, is a potentially important attribute of the diet in reducing long-term CHD risk

Strengths and Limitations of this Study

The study weaknesses include the relatively small sample size and the high dropout rate. Nevertheless, ~~high dropout rates have been reported in similar dietary studies and~~ it is noteworthy that attrition rates were low in the metabolic study when all food was provided [1]. Food availability and preparation may therefore be important factors. For those who did complete the study, however, there were benefits in weight loss and LDL-C reduction, an additional 2% advantage in body weight reduction compared to the high-carbohydrate diet and a 13% drop in LDL-C for participants consuming a more plant-based low-carbohydrate diet. The study's strength is that the prescribed hypocaloric diet was self-selected, meaning the results are more in line with what can be expected under free-living conditions. The breadth of application of the plant-based low-carbohydrate diet, however, remains to be determined, but it may provide an option for some individuals for whom LDL-C reduction is an equal concern to weight loss. If low-carbohydrate dietary options become more generally available the number of individuals who will benefit is likely to increase.

Introduction

Many popular weight loss diets emphasize carbohydrate restriction (Atkins, Eddies, South Beach, Zone). Their success is determined by the level of compliance with the prescribed diets [2-7]. However, a high content of animal products, rich in saturated fat and cholesterol, may make conventional low-carbohydrate diets less appropriate for those with hypercholesterolemia [3 8]. Even during active weight loss, these high saturated fat diets do not lower serum LDL-C below baseline [3 8] and there is concern that if such diets continue to be eaten when weight loss has ceased, a more atherogenic blood lipid profile may result [9]. These concerns have prompted exploration of other weight loss strategies, but only modest reductions in LDL-C have been observed [10].

By contrast vegan diets significantly lower LDL-C [11]. Trials of vegan and vegetarian diets also reduce progression of coronary heart disease (CHD) [12] and improve diabetes control [13]. Plant food components such as vegetable proteins, vegetable oils, nuts and viscous fibers, reduce serum lipids in many studies [14-19] and may increase flow mediated vasodilatation [20-23]. Nuts, fiber and vegetarian diets in general, all reduce CHD and diabetes in cohort studies [24-29]. Finally, in cohort studies, low-carbohydrate diets, high in vegetable oils and proteins as opposed to animal products, reduce CHD events and diabetes incidence in women [30 31], while lower red meat intake reduces total, cardiovascular and cancer mortality [32]. Most recently a large randomized controlled trial confirmed the effect of nuts and increased vegetable oil (olive oil) intake in reducing cardiovascular events in the context of a Mediterranean diet [33].

In view of the apparent success of low-carbohydrate diets for weight loss and the demonstration that relatively high-carbohydrate vegetarian and vegan diets, and diets low in animal products, lower CHD risk factors [34-37], we designed a diet that combined both vegan and low-

carbohydrate elements to determine whether such a diet captured both the weight loss and CHD risk reduction advantages. We have already reported the effect of this dietary strategy in producing a difference of 8% in LDL-C reduction between calorie-restricted diets (60% of estimated calorie requirements) when all food was provided [1]. We now report findings after these same participants continued on their respective diets for an additional 6 months, under self-selected conditions, in order to gain insights into the real life effectiveness of this diet. The results of the metabolic (all foods provided) study have been reported previously and had demonstrated a CHD risk factor advantage, but with no greater weight loss than the control diet [1].

Methods

Participants

Forty-seven overweight participants, recruited by newspaper advertisement and hospital clinic notices, undertook the one-month metabolic first phase of the study (Figure 1) that has been previously reported [1]. At the start of the study, participants were given the option to participate in both the metabolic and ad libitum phases or only the metabolic phase. On completion of the metabolic phase, thirty-nine participants (19 control and 20 test participants) continued for an ad libitum six-month study and their data (n=39) were used in the final analysis (Table 1). The study was conducted at a Canadian university-affiliated hospital nutrition research center from April 2005 to November 2006. All participants had high normal to raised LDL-C levels (>3.4mmol/L at diagnosis) and a body mass index > 27 kg/m^2. Details of the eligibility criteria have been previously reported [1]. After recruitment, the 11/39 participants who were taking

lipid lowering medications discontinued their medications at least two weeks prior to starting and for the study duration (Table 1).

Study Protocol

The intervention was a randomized parallel study stratified by sex in which participants were randomized to either low- or high-carbohydrate, calorie-reduced diets. The first month was the previously reported metabolically controlled study [1]. For the following six-months, participants continued on the diet to which they had been assigned as a self-selected (ad libitum) diet. Anthropometric, blood pressure and blood lipid measurements were repeated at monthly intervals. Insulin and HbA1c were measured at baseline and at the start and end of the ad libitum treatment. Percentage body fat was measured at baseline and end of the ad libitum treatment by bioelectrical impedance (Quantum II; RJL Systems, Clinton Township, Michigan). Seven-day diet and exercise histories were recorded in the week prior to each monthly visit. These histories were reviewed and discussed with the dietitian and appropriate dietary counselling was provided to enhance adherence. The overall feeling of satiety for the previous week was assessed at each study visit using a 9-point bipolar semantic scale, where –4 was extremely hungry, 0 was neutral, and +4 was uncomfortably full [1 35]. No exercise advise was given during the study, but alterations in exercise were allowed and recorded.

The Ethics Committees of St. Michael's Hospital and the University of Toronto, and the Therapeutic Products Directorate of Health Canada approved the study. Written informed consent was obtained from the participants. The study's clinical trial registration number was #NCT00256516.

Diets

As with the previous metabolic study, participants were encouraged to eat only 60% of their estimated caloric requirements in order to continue the body weight reduction started on their metabolic phase [38-40]. The prescribed test diet was a low-carbohydrate vegan diet containing 26% of calories from carbohydrate, 31% of calories from vegetable proteins and 43% from fat (primarily vegetable oils). Carbohydrate sources on the low-carbohydrate diet featured viscous fiber-containing foods (such as oats and barley) and low-starch vegetables (emphasizing okra and eggplant) for the relatively limited amount of carbohydrate allowed. The vegetable proteins were prescribed as gluten (54.8% of total protein), soy (23.0%), fruits and vegetables (8.7%), nuts (7.5%), and cereals (6.0%). Gluten was contained in the nut bread and wheat gluten (also called "seitan") products. Soy protein was present in the form of burgers, ~~veggie bacon,~~ deli slices, breakfast links, veggie bacon, tofu, and soy milks. Nuts included almonds, cashews, hazelnuts, macadamia, pecans, and pistachios. The fat sources were nuts (43.6% of total fat), vegetable oils (24.4%), soy products (18.5%), avocado (7.1%), cereals (2.7%), fruits and vegetables (2.3%), and seitan products (1.4%). Participants were able to purchase at the research center the "no" starch high protein nut bread and three of the seitan (wheat gluten) products used in the study which were not available in Canada. The control, high-carbohydrate lacto-ovo vegetarian diet (58% carbohydrate, 16% protein and 25% fat) emphasized whole wheat cereals and cereal fiber, as well as low-fat or skim milk dairy products and liquid egg substitute to reduce saturated fat and cholesterol intakes. These diets have been published previously [1]. Participants were given a copy of the menu plans that outlined the food items and amounts prescribed during the metabolic phase. These menu plans~~is~~ served as a reference during the ad

libitum phase. Furthermore, participants were given an exchange list of the items prescribed on the menu plan. The goal was to enhance adherence.

Self-taring electronic scales (My Weigh Scales, Vancouver, BC or Tanita Corporation, Arlington Heights, IL) were provided to all participants and they were instructed to weigh all food items while recording the seven-day food diary in the week prior to monthly clinic visits. Adherence to the three principal cholesterol-lowering components [vegetable proteins (soy and gluten), nuts, and viscous fibers] of the low-carbohydrate diet was assessed from the completed monthly seven-day food records. The amount of each component provided during the metabolic phase remained the same as that prescribed during the ad libitum phase.

Neither the dietitians nor participants could be blinded, but equal emphasis was placed on the potential importance for health of both diets. The analytical technicians were blinded to diet allocation, as was the statistician, up to analysis of the primary outcome. Participants were offered no financial compensation for participation in the study.

Analyses

The analytical techniques have been reported previously [1]. Serum was analyzed in the J. Alick Little Lipid Research Laboratory [35]. ~~and~~ LDL-C (in mmol/L) was calculated by the method of Friedewald et al. [1], ~~.~~ using all data including the two participants who had baseline and during study triglyceride values above 4.5 mmol/L (3 values on low-carbohydrate diet and 2 on high-carbohydrate diet, maximum triglyceride < 6.5 mmol/L) (exclusion of these two individuals did not alter the findings). The methods for analyzing apolipoproteins A1 and B, high sensitivity C-reactive protein (hs-CRP), blood glucose, insulin, HbA1c, and homeostasis model assessment – insulin resistance model (HOMA-IR) have been described previously [1]. Exercise data were

calculated as metabolic equivalents (METs) [41]. The absolute 10-year CHD risk score was calculated using the Framingham risk equation [42].

Diets were assessed for macronutrients, fatty acids, cholesterol and fiber using a computer program based on the USDA database [43] and developed in our laboratory to allow the addition of the macronutrient content of study foods obtained from food labels or directly from food manufacturers. The nutritional profiles of the diets were calculated from the 7-day food records completed once a month throughout the study and mean intakes are presented.

Adherence ~~with~~to the three principal cholesterol-lowering components [vegetable proteins (soy and gluten), nuts, and viscous fibers] of the low-carbohydrate diet was estimated from the 7-day food records. Each component was assessed as contributing 1/3 or ~~by applying~~ 33.3% to the LDL-C reduction. When the amount consumed was equivalent to the amount prescribed a 33.3% compliance would be recorded for that component. ~~adherence factor to the recorded intake for each of the three main components.~~ The sum of the three components if consumed as prescribed would equal 100% adherence.

Statistical Analyses

Results are expressed as means ± SEM or 95% confidence intervals (CIs). Time zero was used as the baseline and refers to the pre-metabolic study baseline [1]. Treatment differences in physical and biochemical measures were assessed using all available data from the 39 participants and a repeated measures mixed model accounting for time of assessment (SAS 9.2) [44] in the Tables (Table 2 and 3) and the Results. The response variable was change from baseline, with diet and week as fixed effects and subject ID nested in diet. There was no adjustment for baseline. Any

participant who started the ad libitum treatment was included in the analysis (N=39). The completer analysis included the 23 participants who completed the study (Figure 1).

Multiple imputation (taking the mean of 5 sets of randomly imputed values) was used to present baseline and treatment values in the Tables (2 and 3) and Figures (2 and 3) by generating data for those who dropped out or had missing values [44].

Results

Compliance with the major dietary components [vegetable proteins (soy and gluten), nuts, and viscous fibers] was 33.6% or one-third of that prescribed during the metabolic phase (Table 2). Saturated fat intakes were similar on both treatments whereas intake of monounsaturated fats, vegetable proteins, and soy protein were significantly higher on the low-carbohydrate diet (Table 2). Available carbohydrate intake was significantly lower on the low-carbohydrate diet (Table 2). The attrition rate was 50% (10/20) on the low-carbohydrate and 32% (6/19) on the high-carbohydrate (Figure 1), this equates to a total attrition rate of 41% (16/39). The number of participants who did not complete the study (including dropouts and withdrawals) did not differ between treatments. Three participants were withdrawn by the study physician due to failure to attain LDL-C targets on the low-carbohydrate diet (mean LDL-C = 5.24mmol/L) and one subject on the high-carbohydrate diet (LDL-C = 7.78mmol/L). Participants on the low-carbohydrate diet tended to have larger reductions in body weight over time (Figure 2). The weight loss from baseline to the end of the 6-month ad libitum treatment was -6.9kg [95% CI, -7.7, -6.1] on the low-carbohydrate and –5.8kg [95% CI, -6.6, -5.1] on the control diet with a significant difference between groups (treatment difference [95% CI]: -1.1kg [-2.1, 0.0]; P=0.047) (Table 3). The final reduction in BMI was also greater on the low-carbohydrate versus high-carbohydrate diet

(treatment difference [95% CI]: -0.4kg/m^2 [-0.8, 0.0]; P=0.039) (Table 3). Among the completers, there were numerically larger differences between treatments for both body weight and BMI (treatment difference [95% CI]: -1.8 kg [-3.0, -0.6]; P=0.004~~1~~ and -0.7 kg/m^2 [-1.1, -0.2]; P=0.00~~4~~~~39~~, respectively).

There was a relative increase in recorded exercise by the high-carbohydrate diet participants, whereas there was no relative change in the low-carbohydrate participants (treatment difference [95% CI]: -9.3 [-16.4, -2.2] METs; P=0.012), but this was not reflected in a greater weight loss (Table 3). There were no treatment differences in percent body fat, waist circumference or satiety (Table 3).

Lipids

At the end of the study, the reduction on the low-carbohydrate versus high-carbohydrate diet was greater for LDL-C (treatment difference [95% CI]: -0.49mmol/L [-0.70, -0.28]; P<0.001, for TC (-0.62mmol/L [-0.86, -0.37]; P<0.001, for TC:HDL-C -0.57 [-0.83, -0.32]; P<0.001, for LDL-C:HDL-C (-0.42 [-0.60, -0.24]; P<0.001, and for triglycerides (-0.34mmol/L [-0.57, -0.11]; P=0.005). No treatment difference was seen in HDL-C (Table 3). A similar pattern was observed in the completers. The treatment difference was numerically larger for LDL-C (-0.60mmol/L [-0.84, -0.36]; P<0.0001), TC (-0.73mmol/L [-1.00, -0.45]; P<0.0001), TC:HDL-C (-0.68 [-0.97, -0.39]; P<0.0001), and LDL-C:HDL-C (-0.53 [-0.73, -0.32]; P<0.0001). Values for LDL-C and the TC:HDL-C ratio were consistently lower in participants on the low-carbohydrate diet throughout the study while HDL-C values were not different from baseline (Figure 3 A-C).

Apolipoproteins

ApoB and the ApoB:A1 ratio were reduced more on the low- versus the high-carbohydrate diet at the end of the study (treatment different [95% CI]: -0.11g/L [-0.16, -0.06]; P<0.001 and -0.05 [-0.09, -0.02]; P=0.003, respectively) (Table 3). No significant difference between the diets was observed for ApoA1 concentrations. The pattern of change in the apolipoproteins in the completers reflected the changes seen in the whole group. Figure 3D and 3F show that the low-carbohydrate diet resulted in lower apoB and ApoB:ApoA1 ratios relative to baseline over the course of the study.

C-Reactive Protein, HbA1c, Blood Glucose, Serum Insulin, Insulin Resistance and Blood Pressure

Both treatments reduced hs-CRP with no difference between treatments (Table 3). HbA1c, fasting blood glucose, insulin, and insulin resistance (calculated using the HOMA model) fell similarly on both treatments during the course of the study (Table 3). Systolic and diastolic blood pressure decreased similarly with no treatment differences (Table 3). The completers also failed to show a difference between treatments.

Calculated CHD Risk

The low-carbohydrate diet significantly reduced the calculated 10-year CHD risk relative to the high-carbohydrate diet (2% [-2, -1]; P<0.001) (Table 3). A reduced CHD risk on the low-carbohydrate diet was also observed in the completers (2% [-3, -1]; P<0.001).

Adverse Events

No serious adverse events or events that involved hospitalisation occurred during the study.

Discussion

The present study demonstrated that consumption of a low-carbohydrate vegan diet resulted in a modestly greater body weight reduction compared to a high-carbohydrate diet (7% versus 6% reductions, respectively) over a six-month ad libitum period. These reductions were similar to those reported for low-carbohydrate "Atkins-like" diets[2 3 6 10]. However by comparison with the high-carbohydrate diet, consumption of the low-carbohydrate diet containing vegetable proteins and oils was also associated with significantly reduced concentrations of LDL-C. This LDL-C reduction has not been reported for other low-carbohydrate diet studies in which a large part of the protein and fat originated from animal sources and in which no significant LDL-C reductions were seen [2-6 8]. The sustained reduction in LDL-C, associated with ~~only~~ a small incremental weight loss on the 6-month self-selected diet, is a potentially important attribute of the diet in reducing long-term CHD risk [45 46]. Furthermore, as seen in the present study, a low-carbohydrate diet, in which vegetable fat and protein options were encouraged, demonstrated a larger reduction in the TC:HDL-C ratio than that reported at 6 months in weight loss studies employing either a Mediterranean or a high-carbohydrate diet [10].

The majority of studies undertaken to date have been 6 months to one year in duration [2-6 47] with more recent studies of up to 2 years [2 8] ~~and, as with the present study, a number of these studies had a high dropout rate [2 3 5 47]~~. The high dropout rate in the present 6-month study did not prevent identification of significant LDL-C and body weight differences in the intent-to-treat analysis (using all available data). However, the completer data demonstrated an even larger treatment difference in LDL-C favoring the low-carbohydrate treatment. Those on the low-carbohydrate diet showed overall adherence to the major dietary components [vegetable proteins

(soy and gluten), nuts, and viscous fibers] at 33.6% of that provided during the metabolic phase [1]. This adherence is similar to the 43.3% seen with the dietary portfolio in the comparison of the metabolic one month [35] and the ad libitum six month studies [48]. In this study, ~~comparison also just under half~~ the LDL-C reduction on the low-carbohydrate metabolic month was also greater than that on the ad libitum 6 months, although the treatment differences were similar ~~(13-14%) seen on the ad libitum compared to the metabolic study~~ [35].

The effect of low-carbohydrate diets on CHD events has not been assessed in randomized controlled trials. Nevertheless, low-carbohydrate diets high in vegetable proteins and oils have been associated with a 30% reduced CHD risk and an 18% reduced incidence of diabetes in cohort studies [30 31]. The median interquantile difference in these studies between the first and 10th decile for vegetable protein and monounsaturated fat (MUFA) intakes, as a marker of increased vegetable oil consumption, was 1.4% and 9.3% expressed as a percentage of total caloric intake [30]. These figures compare with an 8.2% and a 4.6% relative increase in vegetable protein and oil consumption from baseline on the Eco-Atkins diet compared to the control diet. The increases in MUFA were therefore seen in both studies. Recently a Spanish Mediterranean diet emphasizing increased nut or olive oil consumption, and so increasing monounsaturated fat intake by 2-3%, has been shown to significantly reduce cardiovascular events also by approximately 30% [33]. These data provide consistent support for the view that the Eco-Atkins approach would reduce CHD risk in the long term.

The present diet, while lowering LDL-C by 9%, did not result in any significant depression of HDL-C. Lowering LDL-C while maintaining HDL-C would be expected to reduce CHD risk [45 46]. Similarly, reductions in ApoB and the ApoB:A1 ratio were also observed in the present study. These findings further support the potential CHD benefit that this weight loss diet may

have [49-51]. It has also been claimed that apolipoproteins may be stronger predictors of CHD events than conventional lipid variables [52-54].

In contrast to the metabolic study, the reductions in systolic and diastolic blood pressure were not significant between the low- and high-carbohydrate diets. Similarly, hs-CRP was unchanged between treatments, however, the level was significantly reduced with the low-carbohydrate diet compared to baseline. Studies have shown that hs-CRP tended to be lowest on the diets containing the highest proportion of carbohydrate [5]. Low glycemic index and low glycemic load diets have also been associated with lower hs-CRP concentrations [55 56]. These advantages of the higher carbohydrate diet may have reduced any hs-CRP difference between the two diets in the present study.

Soy-containing foods as well as nuts have cholesterol lowering effects [15 17 18 57 58] and may explain the reduction in LDL-C. Viscous fiber in low starch vegetables and β-glucan in oats and barley may also have contributed to the overall cholesterol lowering effect of the diet [9 14 45]. Furthermore, nuts and high fiber food consumption have been associated with lower body weight [59].

The study weaknesses include the relatively small sample size and the high dropout rate. Nevertheless, ~~high dropout rates have been reported in similar dietary studies and~~ it is noteworthy that attrition rates were low in the metabolic study when all food was provided [1]. Food availability and preparation may therefore be important factors. Future studies will need to focus on strategies to increase and maintain adherence, especially to the cholesterol lowering components, which all bear US FDA health claims for cardiovascular disease risk reduction. Furthermore, collaboration with food industry may be helpful in addressing concerns of availability, variety, and ease of preparation. In retrospect, a simplified one page eating plan for

breakfast, lunch, and dinner with a number of options and amounts for each meal, as we have used in our dietary portfolio studies, might also be helpful [48]. For those who did complete the study, however, there were benefits in weight loss and LDL-C reduction, an additional 2% advantage in body weight reduction compared to the high-carbohydrate diet and a 13% drop in LDL-C for participants consuming a more plant-based low-carbohydrate diet. Unfortunately it was not possible to predict who would complete the diet based on pre-study data or changes observed during the metabolic phase.

The study's strength is that the prescribed hypocaloric diet was self-selected, meaning the results are more in line with what can be expected under free-living conditions. The breadth of application of the plant-based low-carbohydrate diet, however, remains to be determined, but it may provide an option for some individuals for whom LDL-C reduction is an equal concern to weight loss. If low-carbohydrate dietary options become more generally available the number of individuals who will benefit is likely to increase.

We conclude that a weight loss diet which reduced carbohydrate in exchange for increased intakes of vegetable sources of protein, such as gluten, soy and nuts, together with vegetable oils offers an opportunity to improve both LDL-C and body weight, both being risk factors for CHD. Further trials are warranted to evaluate low-carbohydrate diets, including more plant-based low-carbohydrate diets, on CHD risk factors and ultimately on CHD.

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Role of the Sponsors

None of the funding organizations or sponsors played any significant role in the design and conduct of the study, in the collection, management, analysis, and interpretation of the data, or in the preparation, or approval of the manuscript. However, the named co-authors from Solae LLC reviewed the manuscript.

Disclosures

Dr. Jenkins has served on the Scientific Advisory Board of Sanitarium Company, Agri-Culture and Agri-Food Canada (AAFC), Canadian Agriculture Policy Institute (CAPI), California Strawberry Commission, Loblaw Supermarket, Herbal Life International, Nutritional Fundamental for Health, Pacific Health Laboratories, Metagenics, Bayer Consumer Care, Orafti,

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Figure Legends

Figure 1: Patient Flow Diagram.

Figure 2: Weight loss during the study on both diets.

Figure 3: Mean (A) LDL-C, (B) HDL-C, (C) TC:HDL-C, (D) apoplipoprotein B (apoB) and (E) apolipoprotein A1 (apoA1), (F) ApoB:ApoA1 ratio between the two treatments during the metabolic and ad libitum phases.

Table 1: Baseline Characteristics for Those Who Started the 6-Month Self-Selected Diets (n=39)

	High-carbohydrate (n=19)	Low-Carbohydrate (n=20)
Age (y)	55.3 ± 1.8	57.6 ± 1.4
Males/Females	6/13	9/11
Body Weight, kg	85.4 [79.3, 91.6]	83.7 [78.5, 89.0]
Body Mass Index, kg/m^2	31.1 [29.9, 32.4]	31.1 [29.8, 32.4]
Blood Pressure, mm Hg		
Systolic	122 [116, 128]	128 [123, 132]
Diastolic	75 [72, 79]	77 [74, 80]
Cholesterol, mmol/L		
Total	6.75 [6.28, 7.21]	6.76 [6.21, 7.31]
LDL-C	4.40 [3.99, 4.82]	4.53 4.14, 4.93]
HDL-C	1.36 [1.22, 1.50]	1.21 [1.06, 1.36]
Triglycerides, mmol/L	2.16 [1.62, 2.70]	2.23 [1.65, 2.80]
Ratios		
TC:HDL-C	5.17 [4.54, 5.80]	5.81 [5.20, 6.41]
LDL-C: HDL-C	3.35 [2.95, 3.75]	3.89 [3.49, 4.29]
Medications		
Lipid lowering (prior to start of study)	4	7
Blood pressure	3	6
Diabetes	0	0
Thyroid	2	1

Values represent mean ± SEM or 95% confidence intervals (CIs).
No significant differences betweeen treatments at baseline assessed by two sample t-test (two-tailed).

Table 2: Nutritional Profiles on the High and Low Carbohydrate Diets (n=39)

	High Carbohydrate			Low Carbohydrate				
	Week 0[b]	Ad Libitum[b]		Week 0[b]	Ad Libitum[b]		Between-Treatment Difference[c]	P-value[d]
Calories (kcal)	1598 [1421, 1775]	1347 [1140, 1553]		1840 [1550, 2130]	1388 [1234, 1541]		-248 [-391, -106]	0.001
% of Total Calories								
Available Carbohydrate	46.3 [42.2, 50.4]	53.9 [50.2, 57.5]		43.8 [40.2, 47.4]	39.6 [35.7, 43.6]		-10.5 [-13.6, -7.5]	< 0.001
Protein	20.6 [18.7, 22.5]	18.4 [17.4, 19.5]		20.1 [18.0, 22.2]	22.7 [20.1, 25.4]		5.9 [4.3, 7.5]	< 0.001
Vegetable Protein	5.6 [5.0, 6.1]	6.7 [6.1, 7.3]		5.7 [5.3, 6.1]	15.0 [11.7, 18.2]		8.2 [6.5, 9.9]	< 0.001
Soy Protein	0 [0, 0]	0.2 [0.1, 0.2]		0 [0, 0]	4.7 [2.7, 6.8]		3.6 [2.9, 4.4]	< 0.001
Fat	30.8 [27.3, 34.4]	27.5 [24.6, 30.4]		34.4 [31.4, 37.5]	36.0 [31.5, 40.5]		5.2 [2.6, 7.7]	< 0.001
Saturated	10.8 [9.1, 12.6]	7.6 [6.2, 8.9]		11.8 [10.3, 13.3]	7.5 [6.6, 8.4]		-0.4 [-1.4, 0.6]	0.401
Monounsaturated	12.3 [10.7, 13.8]	10.4 [9.3, 11.6]		13.0 [11.9, 14.2]	14.8 [13.1, 16.6]		4.6 [3.1, 6.1]	< 0.001
Polyunsaturated*	5.2 [4.6, 5.8]	6.3 [5.4, 7.2]		6.6 [5.5, 7.8]	8.4 [7.5, 9.4]		0.4 [-0.5, 1.4]	0.350
Alcohol	2.2 [0.3, 4.2]	1.9 [0.7, 3.2]		1.6 [0.0, 3.3]	1.1 [0.1, 2.1]		-0.5 [-1.3, 0.2]	0.160
Dietary Fibre (g/1000 kcal)	10.9 [9.2, 12.5]	18.2 [15.2, 21.1]		12.1 [9.9, 14.4]	21.3 [18.8, 23.8]		1.5 [-0.5, 3.5]	0.127
Dietary Cholesterol (mg/1000 kcal)	149 [129, 169]	87 [61, 113]		157 [136, 177]	117 [44, 189]		11 [-22, 23]	0.954
Adherence with "Eco-Atkins" Components[a]								
Viscous Fiber (out of 33.3%)	---	---		---	14.0 [9.4, 18.6]		---	---
Vegetable Protein (soy and gluten) (out of 33.3%)	---	---		---	14.7 [10.3, 19.1]		---	---
Nuts (out of 33.3%)	---	---		---	6.3 [3.3, 9.3]		---	---
Total Adherence (out of 100%)	---	---		---	33.6 [22.1, 45.2]		---	---

Values represent mean ± 95% confidence intervals (CIs).

[a]Adherence represents the mean percentage intake of the prescribed intake of the 3 cholesterol-lowering components [viscous fiber, vegetable protein (soy and gluten), nuts] by expressing the recorded intake for each component as 33.3%. The sum of the 3 components if consumed as prescribed would equal 100% adherence.

[b]Values represent multiple imputation (taking the mean of 5 sets of randomly imputed values) to generate data for those who dropped out or had missing values.

[c]Between Treatment Difference = Change from baseline between the two diets using all available data.

[d]P-values assessed using all available data and a repeated measures mixed model accounting for time of assessment. The response variable was change from baseline, with diet and week as fixed effects and subject ID nested in diet. There was no adjustment for baseline.

*Significantly different between treatments at baseline assessed by two sample t-test (two tailed), P=0.025.

Table 3: Effect of high and low carbohydrate diets on body weight, blood lipids, apolipoproteins and 10-yr CHD risk (n=39)

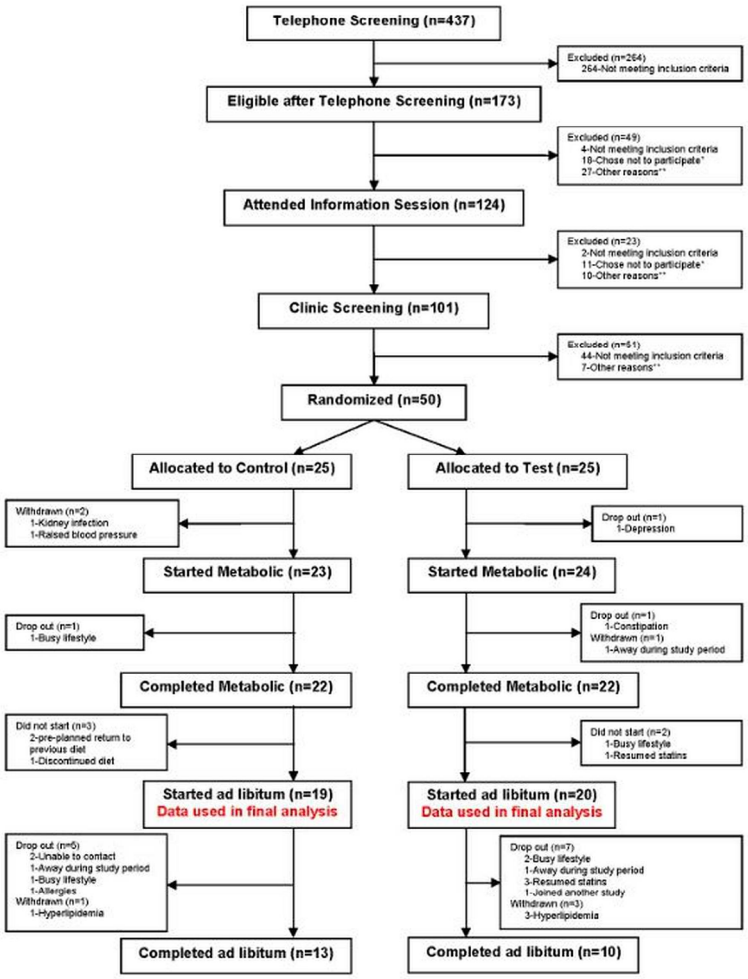
	High Carbohydrate			Low Carbohydrate			
	Week 0[a]	Ad Libitum[a]		Week 0[a]	Ad Libitum[a]	Between Treatment Difference[b]	P-value[c]
Body Weight, kg	85.4 [79.3, 91.6]	80.4 [74.2, 86.6]		83.7 [78.5, 89.0]	76.9 [71.9, 81.9]	-1.1 [-2.1, 0.0]	0.047
BMI	31.1 [29.9, 32.4]	29.2 [27.9, 30.5]		31.1 [29.8, 32.4]	28.7 [27.3, 30.1]	-0.4 [-0.8, 0.0]	0.039
Body Fat, %	38.9 [34.0, 43.8]	35.0 [30.7, 39.2]		35.6 [30.1, 41.1]	31.4 [26.1, 36.6]	-1.7 [-4.0, 0.7]	0.161
Waist Circumference (cm)	102.8 [99.4, 106.2]	97.4 [93.1, 101.6]		99.8 [96.1, 103.5]	93.7 [89.8, 97.7]	0.1 [-1.1, 1.3]	0.861
Fasting Glucose	5.2 [4.9, 5.4]	4.6 [4.5, 4.7]		5.2 [5.0, 5.4]	4.6 [4.4, 4.9]	0.1 [-0.1, 0.2]	0.447
HbA1c (%)	5.2 [5.0, 5.4]	5.2 [5.0, 5.3]		5.3 [5.0, 5.5]	5.2 [5.0, 5.4]	0.0 [-0.2, 0.1]	0.852
Fasting Insulin	50.0 [38.3, 61.7]	36.4 [27.5, 45.4]		47.3 [36.9, 57.6]	33.3 [22.8, 43.9]	-0.6 [-9.1, 8.0]	0.898
HOMA-IR	1.65 [1.17, 2.13]	1.11 [0.81, 1.41]		1.53 [1.19, 1.88]	0.99 [0.68, 1.30]	0.01 [-0.30, 0.33]	0.937
Satiety (-4 to 4)	1.0 [0.7, 1.4]	0.9 [0.7, 1.2]		1.2 [0.8, 1.7]	1.1 [0.8, 1.4]	-0.1 [-0.4, 0.2]	0.440
Exercise, METs	17.4 [12.4, 22.4]	25.8 [21.1, 30.6]		24.0 [12.9, 35.0]	23.9 [15.3, 32.6]	-9.3 [-16.4, -2-2]	0.012
Cholesterol, mmol/L[†]							
Total	6.75 [6.28, 7.21]	6.49 [5.97, 7.02]		6.76 [6.21, 7.31]	6.10 [5.67, 6.53]	-0.62 [-0.86, -0.37]	<0.001
LDL-C	4.40 [3.99, 4.82]	4.40 [3.91, 4.90]		4.53 [4.14, 4.93]	4.06 [3.71, 4.42]	-0.49 [-0.70, -0.28]	<0.001
HDL-C	1.36 [1.22, 1.50]	1.35 [1.22, 1.48]		1.21 [1.06, 1.36]	1.25 [1.10, 1.39]	0.03 [-0.02, 0.07]	0.245
Triglycerides	2.16 [1.62, 2.70]	1.71 [1.35, 2.07]		2.23 [1.65, 2.80]	1.50 [1.22, 1.77]	-0.34 [-0.57, -0.11]	0.005
Ratios							
Tchol:HDL-C	5.17 [4.54, 5.80]	4.92 [4.49, 5.34]		5.81 [5.20, 6.41]	5.13 [4.65, 5.62]	-0.57 [-0.83, -0.32]	<0.001
LDL-C:HDL-C	3.35 [2.95, 3.75]	3.34 [3.00, 3.68]		3.89 [3.49, 4.29]	3.48 [3.06, 3.90]	-0.42 [-0.60, -0.24]	<0.002
Apolipoproteins, g/L[‡]							
Apo A1	1.69 [1.60, 1.78]	1.69 [1.60, 1.77]		1.57 [1.45, 1.69]	1.57 [1.46, 1.67]	-0.02 [-0.06, 0.02]	0.316
Apo B	1.38 [1.26, 1.50]	1.23 [1.13, 1.33]		1.42 [1.30, 1.54]	1.20 [1.10, 1.31]	-0.11 [-0.16, -0.06]	<0.001
Apo B: Apo A1	0.83 [0.74, 0.91]	0.74 [0.68, 0.80]		0.92 [0.84, 0.99]	0.78 [0.70, 0.86]	-0.05 [-0.09, -0.02]	0.003
hs-CRP, mg/dL	2.1 [1.0, 3.3]	1.9 [1.3, 2.4]		3.0 [1.5, 4.5]	2.6 [1.0, 4.1]	-0.4 [-0.9, 0.1]	0.082

Blood Pressure, mmHg								
Systolic	122 [116, 128]	118 [114, 122]		128 [123, 132]	123 [119, 128]		-2 [-5, 2]	0.356
Diastolic	75 [72, 79]	74 [71, 77]		77 [74, 80]	76 [71, 80]		-1 [-3, 1]	0.288
10-yr CHD risk (%)*	8 [6, 9]	7 [6, 9]		12 [9, 14]	9 [7, 11]		-2 [-2, -1]	<0.001

Values represent mean ± 95% confidence intervals (CIs).
†To convert total cholesterol, LDL-C, and HDL-C to mg/dL, divide by 0.0259; to convert triglycerides to mg/dL, divide by 0.0113.
‡To convert apolipoprotein A1 and B to mg/dL, multiply by 100.

[a]Values represent multiple imputation (taking the mean of 5 sets of randomly imputed values) to generate data for those who dropped out or had missing values.
[b]Between Treatment Difference = Change from baseline between the two diets using all available data.
[c]P-values assessed using all available data and a repeated measures mixed model accounting for time of assessment. The response variable was change from baseline, with diet and week as fixed effects and subject ID nested in diet. There was no adjustment for baseline.
*Significantly different between treatments at baseline assessed by two sample t-test (two tailed), P=0.007.

Figure 1



*Chose not to participate (29): busy lifestyle (13), not interested (6), study too demanding (3), currently on another diet (2), no compensation (2), work-related (2), dislike prepackaged foods (1)

**Other reasons (44): unable to contact (19), unable to come to clinic (13), away (5), throat surgery (1), bowel resection (1), high potassium and BP (1), high potassium (1), raised liver function tests (1), not interested (1), medical insurance issue (1)

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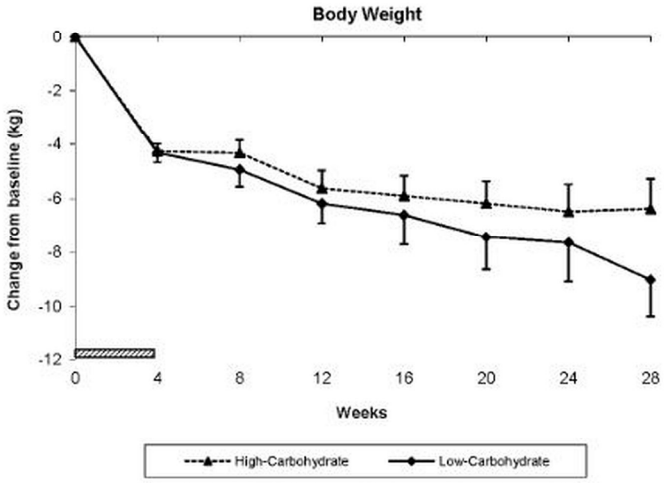

Figure 2: Weight loss during the study on both diets. Values represent mean ± SEM of the change from baseline during the metabolic and ad libitum phases, using multiple imputation (taking the mean of 5 sets of randomly imputed values) to generate data for those who dropped out or had missing values on the ad libitum phase.

The change in weight during the ad libitum phase was significantly reduced (P=0.047) on the low versus the high carbohydrate diet using all available data in the repeated measures mixed model analysis.

Represents the metabolic phase.

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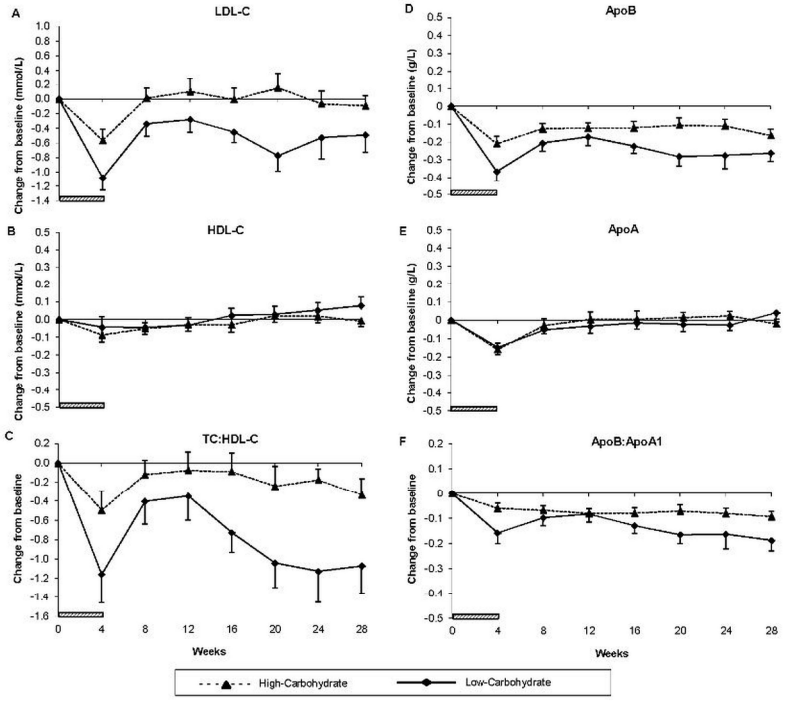

Figure 3:Change in (A) LDL-C, (B) HDL-C, (C) TC:HDL-C, (D) Apolipoprotein B (apoB), (E) Apolipoprotein A1 (apoA1), (F) ApoB:ApoA1 ratio between the two treatments during the metabolic and ad libitum phases. Values represent mean ± SEM of the change from baseline using multiple imputation (taking the mean of 3 sets of randomly imputed values) to generate data for those who dropped out or had missing values for the ad libitum phase.

Significant treatment differences were seen for LDL-C (P<0.001) apo B (P<0.001) and the ratios TC:HDL-C (P<0.001) and apoB:apoA1 (p=0.003) using all available data in the repeated measures mixed model analysis during the ad libitum phase.

Represents the metabolic phase.

277x228mm (300 x 300 DPI)

CONSORT 2010 checklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	1
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	5-6
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	7-8
	2b	Specific objectives or hypotheses	8
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	9
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	NA
Participants	4a	Eligibility criteria for participants	8, also previously published from results of metabolic phase
	4b	Settings and locations where the data were collected	8
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	9-11
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	11-12
	6b	Any changes to trial outcomes after the trial commenced, with reasons	NA
Sample size	7a	How sample size was determined	Continuation with ad libitum phase, metabolic phase published

	7b	When applicable, explanation of any interim analyses and stopping guidelines	NA
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	Continuation with ad libitum phase, randomized metabolic phase published
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	Continuation with ad libitum phase, randomized metabolic phase published
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	Continuation with ad libitum phase, randomized metabolic phase published
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	Continuation with ad libitum phase, randomized metabolic phase published
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those assessing outcomes) and how	11
	11b	If relevant, description of the similarity of interventions	NA

Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	12-13
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	12-13
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	Figure 1, CONSORT Diagram
	13b	For each group, losses and exclusions after randomisation, together with reasons	Figure 1, CONSORT Diagram
Recruitment	14a	Dates defining the periods of recruitment and follow-up	8
	14b	Why the trial ended or was stopped	NA
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	Table 1
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	12
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	13-15, Table 3, Figure 2 & 3
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	Relative effect sizes are given in Results 13-15 and Tables 2 & 3. The absolute differences from each treatment can be derived from Table 2 & 3 and Figures 2 & 3.
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	13-15

Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	15
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	18
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	15-19
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	15-19
Other information			
Registration	23	Registration number and name of trial registry	2
Protocol	24	Where the full trial protocol can be accessed, if available	2
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	2, 20

*We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.