Effects of weight loss from a very-low-carbohydrate diet on endothelial function and markers of cardiovascular disease risk in subjects with abdominal obesity[1–3]

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ABSTRACT

Background: The effects of a very-low-carbohydrate, high-saturated-fat weight-loss diet (LC) on brachial artery flow-mediated dilatation (FMD) and markers of endothelial function are unknown.

Objective: The effect of an LC on markers of endothelial function and cardiovascular disease (CVD) risk was compared with that of an isocaloric high-carbohydrate, low-saturated-fat diet (HC).

Design: FMD and markers of endothelial function ($n = 70$) and CVD risk were measured before and after 8 wk of weight loss. Ninety-nine subjects aged 50.0 ± 8.3 y with a body mass index (in kg/m^2) of 33.7 ± 4.1 completed the study.

Results: Mean (±SD) FMD did not change significantly ($P = 0.55$) with either diet. Pulse wave velocity improved with both diets ($P < 0.01$). Endothelial markers, E- and P selectin, intracellular and cellular-adhesion molecule-1, tissue-type plasminogen activator, and plasminogen activator inhibitor-1 decreased ($P < 0.001$), with no diet effect. Adiponectin did not change significantly. More weight ($P = 0.05$ for diet × time interaction) and more abdominal fat mass ($P = 0.05$ for diet × time interaction) were lost with the LC than with the HC. LDL cholesterol decreased more with the HC than with the LC ($P < 0.05$, time × diet), and C-reactive protein decreased more with the HC than with the LC ($P < 0.05$ for diet × time interaction). Homocysteine increased more with the LC ($P < 0.01$ for diet × time interaction). Folate decreased with the LC and increased with the HC ($P < 0.05$, time; $P < 0.001$ for diet × time interaction).

Conclusion: An LC does not impair FMD. We observed beneficial effects of both diets on most of the CVD risk factors measured. This trial was registered with the Australian Clinical Trials Registry as ACTR N0 12606000203550. *Am J Clin Nutr* 2008;87:567–76.

KEY WORDS Energy-restricted diet, flow-mediated dilatation, body composition, inflammatory markers, endothelial function

INTRODUCTION

Low-carbohydrate diets, such as the Atkins diet, are typically high in saturated fat and are popular weight-loss strategies. However, to date, their efficacy has largely been evaluated with regard to weight, body composition, and lipids (1–4). Obesity is associated with impaired endothelial function as assessed by flow-mediated dilatation (FMD), which precedes the appearance of clinical cardiovascular disease (CVD) and is possibly involved in its pathogenesis (5, 6). FMD is a predictor of future cardiovascular events, independent of other known risk factors for CVD (7). However, the effect of weight loss per se on FMD is contentious; some studies have reported improvements (8, 9) and others either no effect or that reductions in LDL-cholesterol or fasting glucose concentrations were associated with improvements in FMD (10–14).

The effect of dietary composition during weight loss on FMD has not been well explored. We previously observed that weight loss with a low-carbohydrate (33% of energy), low-saturated-fat (7% of energy) diet did not improve FMD (14). We also showed, in a weight-stable study, that FMD was impaired more with a high-saturated-fat diet (19% of energy) than with a low-fat (18% of energy), high-monounsaturated-fat (MUFA) (19% of energy) or high-polyunsaturated-fat (PUFA) (15% of energy) isocaloric diet (15). Other researchers have observed that FMD improved more with a Mediterranean (high-MUFA) diet than with a weight-stable high-saturated-fat diet (16). A very-low-carbohydrate, high-saturated-fat diet has also been shown to increase homocysteine, and high homocysteine concentrations are thought to impair endothelial function (17, 18).

Molecules produced by the endothelium, primarily intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and E- and P selectin are thought to be involved in the pathogenesis of atherosclerosis and are increased in obesity (19–21). High concentrations are also associated with endothelial dysfunction (22). Weight loss has been shown to reduce these molecules, but the effect of a high saturated fat intake, which in weight stability is associated with an increase in P selectin, is not known (15).

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Adiponectin, an insulin-sensitizing adipokine, is reduced in obesity and has been shown to predict endothelial dysfunction (23–25). It has been shown to increase after substantial but not moderate weight loss (25–29). A reduction in adiponectin was observed with a eucaloric low-fat diet, which returned to baseline after weight loss and suggests that dietary fat also affects adiponectin (30).

The aim of the study was to examine the effect of a very-low-carbohydrate, high-saturated-fat weight-loss diet compared with that of an isocaloric conventional high-carbohydrate, low-saturated-fat diet on FMD, concentrations of endothelial derived factors, adiponectin, and cardiometabolic risk factors after weight loss. Our hypothesis was that a very-low-carbohydrate, high-saturated-fat weight-loss diet would impair endothelial function, despite significant weight loss.

SUBJECTS AND METHODS

Subjects

Overweight and obese men and women with abdominal obesity and at least one other additional risk factor for the metabolic syndrome according to the criteria of the International Diabetes Federation were recruited by public advertisement to participate in an 8-wk outpatient weight-loss trial (31). Subjects were excluded if they had a history of liver, cardiovascular, peripheral vascular, respiratory, or gastrointestinal disease; diabetes; or malignancy. One hundred seven subjects aged 24–64 y with a body mass index (BMI; in kg/m^2) of 27–44 and abdominal obesity [waist circumference of 111.0 ± 10.0 ($\bar{x}$ ± SD) cm in men and of 101.3 ± 9.0 cm in women] were enrolled in the study. Thirty-four volunteers were taking antihypertensive medication, and 23 were taking lipid-lowering medication. Three volunteers were excluded from the lipid analysis, and 8 volunteers were excluded from the blood pressure analysis because of changes to their medication.

The study protocol was approved by the Human Ethics Committee of the Commonwealth Scientific Industrial Research Organization (CSIRO) and the University of South Australia, and the subjects provided written informed consent.

Study design

In a parallel design, participants were matched for age, sex, and BMI and were randomly assigned to either an energy-restricted very-low-carbohydrate, high-saturated-fat diet (LC) or an isocaloric conventional high-carbohydrate, low-saturated-fat diet (HC) for 8 wk. At baseline (week 0) and after weight loss (week 8) participants attended the clinical research unit at CSIRO after an overnight fast on 2 consecutive days. At the first clinic visit, participants had height, weight, and blood pressure measured before a venous blood sample was collected for the measurement of lipids, glucose, insulin, folate, homocysteine, and C-reactive protein (CRP). On day 2, all subjects had a second blood sample collected for the measurement of lipids, plasma ketone bodies, and the augmentation index (AI). In a subsample of subjects (n = 70 LC 37 and 33 HC), FMD and pulse wave velocity (PWV) were also measured, and an additional blood sample was collected for the measurement of adhesion molecules, plasminogen activator inhibitor 1 (PAI-1), tissue-type plasminogen activator (tPA), and adiponectin. Subjects also collected a 24-h urine sample at baseline and week 8. Throughout the intervention, participants attended the clinic fortnightly for a weight check and a consultation with a dietitian. Apart from the dietary intervention, subjects were asked to maintain their usual lifestyle throughout the study.

Dietary intervention

The planned macronutrient profiles of the dietary interventions were as follows: 35% of energy as protein, 61% as fat, 20% as saturated fat, and 4% as carbohydrate for the LC and 24% energy as protein, 30% as fat, <8% as saturated fat, and 46% as carbohydrate for the HC. The diets were designed to be isocaloric with a moderate energy restriction of ≈30% (≈6000 kJ for women and 7000 kJ for men) for 8 wk. Key foods for each diet were supplied every 2 wk for the 8 wk to aid compliance. The diet plan was structured to include specific daily quantities of foods to ensure the correct macronutrient and energy requirements (**Table 1**). These foods were listed in a food record that the participants completed daily. Detailed dietary advice, meal planning, and recipe information were provided at baseline and every 2 wk by a qualified dietitian. Scales for weighing food were provided. Three consecutive days (1 weekend and 2 weekdays) from the semiquantitative food record of each 2-wk period were analyzed (12 days in total), while the volunteer was present to ensure accuracy, with a computerized database of Australian foods (FOODWORKS Professional Edition, version 4; Xyris Software 1998, Highgate Hill, Australia).

Body height, weight, and body composition

Body height was measured to the nearest 0.1 cm with a stadiometer (SECA, Hamburg, Germany) while the participants were barefoot. Body weight was measured to the nearest 0.05 kg with calibrated electronic digital scales (AMZ 14; Mercury, Tokyo,

TABLE 1
Food profile of the treatment diets[1]

LC (6000 kJ)	HC (6000 kJ)
125 mL full-fat milk	40 g high-fiber cereal
70 g full-fat cheddar cheese	2 slices whole-grain bread (80 g)
100 g (cooked weight) ham, tuna, beef, chicken, turkey	300 mL skim milk
300 g (raw protein food) beef, chicken, fish	20 g reduced-fat cheese (twice per week)
1 medium (50–55 g) egg	300 g fruit
≥2.5 cups low-carbohydrate vegetables	150 g raw meat, beef, chicken, pork, lamb (5 times/wk)
25 g (5 tsp) oil or butter	150 g fish (1 time/wk)
40 g raw, unsalted mixed nuts	≥2.5 cups vegetables
2 standard alcoholic drinks/wk (optional)	1 medium potato (3 times/wk)
	100 g (dry wt) pasta or rice (4 times/wk)
	100 g bean lentils (2 times/wk)
	200 g diet yogurt (3 times/wk)
	20 g unsalted mixed nuts
	50 g tinned fish (3 times/wk)
	2 tsp polyunsaturated margarine
	3 tsp vegetable oil, eg, olive or canola oil
	2 standard alcoholic drinks/wk (optional)

[1] LC, low-carbohydrate, high-saturated-fat diet; HC, high-carbohydrate, low-saturated-fat diet. 1 cup = 237 mL; 1 tsp = ≈5 mL.

Japan) while the participants were wearing light clothing and no footwear. Body composition was assessed by using dual-energy X-ray absorptiometry (DXA) (Lunar Prodigy; General Electric, Madison, WI). The SE of the measurement for assessments of body composition by DXA were 0.87% for percentage body fat, 0.53 kg (1.6%) for fat-mass, 1.05 kg (2.3%) for lean mass, and 0.02 g/cm^2 (1.3%) for bone mineral density, measured on consecutive days in 11 overweight or obese subjects not involved in this study.

Abdominal fat content was estimated from regional analysis of the DXA scan by drawing a quadrilateral box with the base of the box touching the top of the iliac crest, the lateral borders extending to the edge of the abdominal soft tissue, and the upper margin touching the most inferior aspect of the ribs.

Flow-mediated dilatation

Endothelium-dependent FMD of the right brachial artery was assessed as previously described (32). B-mode ultrasound with a 7.5-MHz linear array transducer (Accuson Aspen Duplex, Mountain View, CA) was used to image the brachial artery in the distal third of the upper arm. A sphygmomanometer cuff was placed around the forearm 2 cm distal to the olecranon process and inflated to 200 mm Hg for 5 min to provide forearm ischemia. Images were recorded 30 s before cuff deflation and every 30 s for 3 min after deflation. After a 10-min rest phase, endothelium-independent dilatation was assessed after the administration of 300 μg sublingual glyceryl-trinitrate. Images were recorded 30 s before administration of glyceryl-trinitrate and every minute for 10 min after admission. All FMD assessments were performed by the same operator, and the intraobserver CV for FMD in this operator's hands was 10.6% on the basis of data from randomly selected healthy individuals ($n = 10$) who were scanned on 2 separate occasions after an overnight fast before the study began. This CV is similar to that reported in other laboratories in similar populations (33, 34).

Pulse wave velocity

Aortic PWV was measured via Doppler recordings in the carotid and femoral arteries (Accuson Aspen Duplex). Approximately 10 consecutive beats were recorded to cover a complete respiratory cycle. A simultaneous electrocardiogram recording was used to calculate the interval between the R-wave and the upstroke of each sound wave. The difference between the average intervals for each artery was calculated. PWV was then determined by dividing the measured surface distance by this difference.

Measurement of the Augmentation Index

Vascular measurements were performed as previously described (35) by using the SphygmoCor blood pressure analysis system (AtCor Medical, Sydney, Australia). All AI measurements were performed by the same operator, and the intraobserver CV for AI in this operator's hands was 16.8% on the basis of data for healthy individuals ($n = 6$) who were tested on 3 separate occasions.

Resting (seated) blood pressure and laboratory analysis

Resting blood pressure (mean of 3 measurements) was measured by automated oscillometry (845XT/XT-IEC; Dinamap, Tampa, FL) while the subjects were seated. Fasting blood samples were collected from a forearm vein into tubes with no additive for lipid, apolipoprotein B (apo B), insulin, CRP, homocysteine, folate, adiponectin, and adhesion molecule measurements; into tubes containing sodium fluoride/EDTA for glucose and ketone body measurements; and into CTAD tubes [BD Vacutainer glass plasma tubes (catalog no. 367946-, 13 × 75 mm, 2.7 mL) and Blue BD Hemogard closures (containing 300 μL of 0.11 mol sodium citrate/L with theophylline, adenosine, and dipyridamole); Becton Dickinson, Franklin Lakes, NJ] for the measurement of PAI-1 and tPA. Plasma or serum was isolated by centrifugation at 2500 rpm at 4 °C for 10 min at 5 °C (Beckman GS-6R centrifuge; Beckman, Irvine, CA) and stored at −80 °C until analyzed.

Biochemical assays were performed in a single assay at the completion of the study. Serum lipids, CRP, apo B, and glucose and urinary urea concentrations were measured in one run on a BM/Hitachi 902 Automatic Analyzer with the use of standard Roche enzymatic kits for lipids, glucose, CRP, and urea and a Roche immunoturbidimetric assay kit for apo B (Roche Diagnostics Co, Indianapolis, IN). Subjects with serum CRP > 10 mg/L at either time point were excluded from the analysis. A modified Friedwald equation [(total cholesterol − HDL cholesterol) – (triacylglycerol × 0.45)] was used to calculate LDL cholesterol (36). Insulin was measured with a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Mercodia Insulin ELISA, catalog no. 10-1113-10; Mercodia AB, Uppsala, Sweden). Plasma ketone concentrations were analyzed in duplicate on a Roche Hitachi auto-analyzer with the use of a RANBUT D-3-Hydroxybutyrate kit (RANDOX Laboratories Ltd, Antrim, United Kingdom).

E selectin, P selectin, and I-CAM were measured in serum by using the Fluorokine multianalyte profiling human adhesion molecule panel (R&D Systems, Minneapolis, MN), according to manufacturer's instructions. Adiponectin and PAI-1 were analyzed by using the human Fluorokine multianalyte profiling human obesity panel (R&D Systems), according to the manufacturer's instructions. Multianalyte profiling was performed on the Luminex-200 system, and fluorescence data were analyzed by using the Liquichip analyzer (version 1.0.5; Qiagen, Melbourne, Australia). VCAM-1 was measured in serum by ELISA (R&D Systems). Plasma concentrations of tPA were also analyzed by using an ELISA (HYPHEN; BioMed, Neuville-sur-Oise, France); the mean intra- and interassay CVs were 6% and 4%, respectively. Plasma vitamin B-12, folate, and homocysteine were measured by the Institute of Medical and Veterinary Sciences (Adelaide, Australia)—a certified commercial laboratory.

Urinalysis

A 24-h urine sample was collected at baseline and week 8 for sodium excretion and urea/creatinine ratio which was used to assess dietary compliance. The urine volume was recorded and aliquots were frozen until analysis. Urea was measured as noted above and sodium and creatinine were measured at the Institute of Medical and Veterinary Sciences.

Statistical analysis

Statistical analyses were performed with the use of SPSS 14.0 for WINDOWS (SPSS Inc, Chicago, IL). Data were tested for

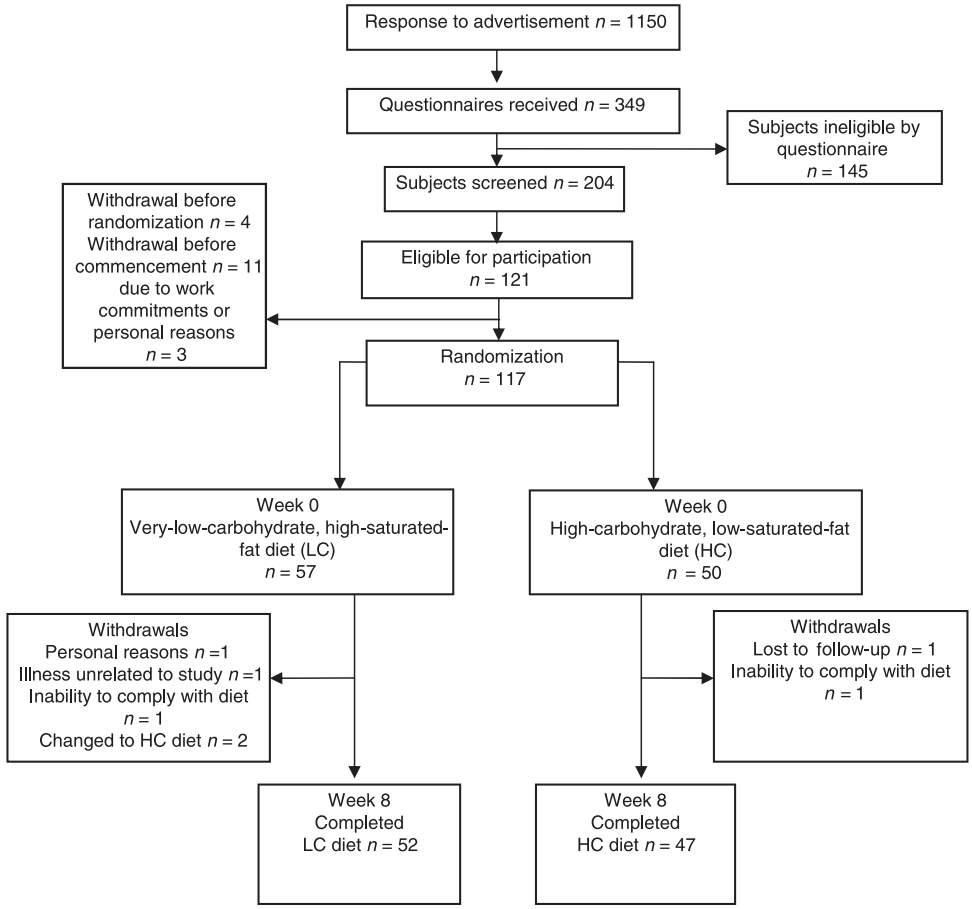

FIGURE 1. Details of subject recruitment and withdrawal are illustrated.

normality, and all variables were normally distributed. One-factor analysis of variance was used to compare baseline characteristics and dietary data. The effect of the dietary intervention was assessed by using repeated-measures analysis of variance, with time as the within-subject factor and diet (LC compared with HC) and sex as the between-subjects factors and change in weight as a covariate where appropriate. Correlational and regression analysis was used to determine relations of changes between variables. Univariate analysis of covariance was used to determine differences between diets after weight loss, with baseline values as a covariate. Statistical significance was set at $P \leq 0.05$. All data are presented as means ± SDs.

RESULTS

Participants

Subject recruitment and withdrawal are presented in (**Figure 1**). Two subjects changed diet allocation from LC to HC after 2 and 4 wk, respectively, and were excluded from the data analysis. The final analysis and the data reported are for 99 participants (n = 52 LC and 47 HC). No significant differences in baseline variables were observed between the groups, except for diastolic blood pressure, which was higher in the HC group (**Table 2**).

Dietary analysis and compliance

Adherence to the dietary interventions was established by concentrations of ketone bodies, the ratio of urinary urea to creatinine, and the dietary analysis data. There was no difference in plasma concentrations of ketone bodies between the groups at baseline (LC: 0.07 ± 0.01 mmol/L; HC: 0.06 ± 0.01 mmol/L; P = 0.40). A significant time × diet effect was evident for plasma ketone bodies ($P < 0.001$), such that concentrations had increased more in the LC group (0.41 ± 0.04 mmol/L) than in the HC group (0.08 ± 0.02 mmol/L) by week 2 and remained higher throughout the intervention. The ratio of urinary urea to creatinine was not different between groups at baseline (LC: 32.6 ±

TABLE 2
Baseline characteristics[1]

	LC (n = 52)	HC (n = 47)
Age (y)	50.5 ± 8.1	49.4 ± 8.2
BMI (kg/m^2)	33.5 ± 4.1	33.9 ± 4.1
Systolic blood pressure (mm Hg)	133 ± 15	136 ± 12
Diastolic blood pressure (mm Hg)	73 ± 12	78 ± 12[2]
Glucose (mmol/L)	5.7 ± 0.6	5.6 ± 0.5
Insulin (mIU/L)	9.6 ± 4.8	11.3 ± 6.0
Total cholesterol (mmol/L)	5.4 ± 1.1	5.3 ± 0.8
HDL cholesterol (mmol/L)	1.4 ± 0.3	1.3 ± 0.4
Triacylglycerol (mmol/L)	1.6 ± 0.7	1.8 ± 1.0
LDL cholesterol (mmol/L)	3.3 ± 1.0	3.2 ± 0.8

[1] All values are $\bar{x}$ ± SD. LC, low-carbohydrate, high-saturated-fat diet; HC, high-carbohydrate, low-saturated-fat diet.
[2] Significantly different from LC, $P < 0.05$ (one-factor ANOVA).

TABLE 3
Dietary intake from 12 d of food records[1]

	LC ($n = 52$)	HC ($n = 47$)	P^2
Energy (kJ)	6608 ± 664	6590 ± 717	NS
Protein (g)	133 ± 10	87 ± 9	<0.001
Fat (g)	103 ± 13	47 ± 7	<0.001
Carbohydrate (g)	20 ± 4	172 ± 26	<0.001
Alcohol (g)	3 ± 4	3 ± 4	NS
Protein (% of energy)	35.0 ± 2.0	24.1 ± 1.6	<0.001
Fat (% of energy)	58.5 ± 2.6	27.8 ± 3.4	<0.001
Saturated fat (% of total energy)	21 ± 2	6 ± 1	<0.001
PUFA (% of energy)	8 ± 1	7 ± 1	<0.001
MUFA (% of energy)	25 ± 2	12 ± 2	<0.001
Carbohydrate (% of energy)	5.1 ± 0.9	46.7 ± 3.4	<0.001
Alcohol (% of energy)	1.4 ± 1.8	1.5 ± 1.6	NS
Cholesterol (mg)	596 ± 89	140 ± 28	<0.001
Saturated fat (g)	37 ± 5	10 ± 2	<0.001
PUFA (g)	14 ± 3	12.0 ± 2	<0.001
n−3 VLC fatty acids	0.53 ± 0.39	0.13 ± 0.07	=0.05
n−6 Linoleic acid	10.61 ± 1.84	8.08 ± 1.35	=0.06
n−3 α-Linolenic acid	0.52 ± 0.06	0.37 ± 0.09	<0.05
MUFA (g)	45 ± 6	21 ± 4	<0.001
Fiber (g)	13 ± 2	32 ± 5	<0.001
Vitamin C (mg)	140 ± 41	178 ± 61	<0.001
Folate (μg)	318 ± 47	348 ± 60	<0.01
Calcium (mg)	908 ± 129	813 ± 84	<0.001

[1] All values are $\bar{x}$ ± SD. LC, low-carbohydrate, high-saturated-fat diet; HC, high-carbohydrate, low-saturated-fat diet; VLC, very long chain; PUFA, polyunsaturated fatty acid; MUFA, monounsaturated fatty acid.
[2] One-factor ANOVA.

10.5; HC: 29.2 ± 6.4; $P = 0.12$) but was significantly different between diets at week 8 (LC: 38.6 ± 8.6; HC: 27.9 ± 6.1; $P = 0.001$ for diet × time interaction). Analyses of the dietary records were consistent with the planned dietary interventions (**Table 3**).

Weight

Weight loss occurred in both groups ($P < 0.001$) and was significantly greater in the LC group (7.9 ± 2.0%) than in the HC group (6.5 ± 2.8%) ($P < 0.01$ for diet × time interaction). Absolute weights before and after weight loss are presented in **Table 4**. BMI also decreased, with a differential effect of diet such that the reduction was greater in the LC group ($P < 0.001$ for time effect, $P < 0.05$ for diet × time interaction; Table 4).

Measures of vascular and endothelial function, adiponectin CRP, homocysteine, folate, and vitamin B-12

FMD did not change with either diet after weight loss (**Table 5**). Change in weight versus change in FMD is presented in **Figure 2**. FMD was negatively correlated with 24-h sodium excretion after weight loss ($r = -0.28$, $P < 0.05$) and negatively correlated with age before ($r = -0.3$, $P < 0.05$) and after weight loss ($r = -0.48$, $P < 0.01$). FID increased after weight loss ($P = 0.05$).

AI did not change with weight loss (Table 5) and was also negatively correlated with 24-h sodium excretion after weight loss ($r = -0.27$, $P < 0.01$). PWV improved after weight loss and no difference was observed between diets ($P < 0.001$ for time effect); this was predominantly related to the change in systolic blood pressure ($P = 0.1$ after adjustment for change in systolic blood pressure; Table 5).

Both systolic blood pressure and diastolic blood pressure decreased from baseline after weight loss ($P < 0.001$), and no difference was observed between diets (Table 5). E selectin, P selectin, ICAM-1, PAI-1, and tPA all decreased with weight loss ($P \leq 0.001$), but VCAM-1 rose with time ($P < 0.001$ for time effect; no diet effect) (Table 5). The changes in E selectin ($r = 0.479$ $P < 0.01$) and ICAM-1 ($r = 0.444$, $P < 0.01$) were strongly related to weight loss. PAI-1 was correlated with abdominal fat mass ($r = 0.4$, $P < 0.01$ before and after weight loss).

Adiponectin did not change with weight loss (Table 5). CRP decreased after weight loss, but to a lesser extent with the LC diet ($P < 0.001$ for time effect, $P < 0.05$ for diet × time interaction); adjustment for weight loss did not change this effect (HC: −0.27 ± 1.5; LC: 1.1 ± 0.63 10 mg/L; $P < 0.05$ for diet × time interaction) (Table 5). There was no correlation between change in LDL and change in CRP.

Homocysteine increased after weight loss ($P < 0.001$ for time effect); the differential effect of diet was such that there was a greater increase in the LC group ($P < 0.01$ for diet × time interaction) (Table 5).

Folate decreased with the LC and increased with the HC ($P < 0.05$ for time effect, $P < 0.001$ for diet × time interaction) (Table 5), whereas vitamin B-12 increased with both diets ($P < 0.05$). There were no correlations between FMD and homocysteine, folate, or vitamin B-12.

TABLE 4
Anthropometric variables at baseline compared with week 8[1]

	LC ($n = 52$)			HC ($n = 47$)		
	Week 0	Week 8	Change	Week 0	Week 8	Change
Weight (kg)[2,3]	94 ± 15.3	87.0 ± 13.9	−7.5 ± 2.6	97.0 ± 14.4	90.7 ± 13.8	−6.2 ± 2.9
Fat mass (kg)[3]	39.5 ± 10.8	33.9 ± 10.2	−5.3 ± 2.5	39.6 ± 8.1	34.2 ± 9.7	−4.9 ± 3.6
Fat (%)[3]	43.8 ± 8.0	41.1 ± 8.8	−2.6 ± 2.6	43.1 ± 8.8	41.0 ± 7.7	−2.4 ± 2.5
Fat-free mass (kg)[3]	50.6 ± 11.3	48.3 ± 10.2	−2.1 ± 2.6	52.8 ± 11.7	50.8 ± 11.3	−1.5 ± 2.0
BMI (kg/m^2)[3]	33.6 ± 4.1	30.9 ± 3.8	−2.7 ± 0.8	33.8 ± 4.2	31.6 ± 4.0	−2.2 ± 1.0
Abdominal fat (kg)[2]	3.2 ± 0.8	2.6 ± 0.8	−0.6 ± 0.4	3.2 ± 0.8	2.8 ± 0.8	−0.4 ± 0.3

[1] All values are $\bar{x}$ ± SD. Body-composition data from dual-energy X-ray scans were available for 95 volunteers (50 LC and 45 HC). LC, low-carbohydrate, high-saturated-fat diet; HC, high-carbohydrate, low-saturated-fat diet. Data were analyzed by using repeated-measures ANOVA.
[2] $P < 0.05$ for diet × time interaction for the change such that the LC group had greater reductions in weight, BMI, and abdominal fat.
[3] $P < 0.001$ for main effect of time.

TABLE 5
Measures of vascular and endothelial function, adiponectin, C-reactive protein (CRP), homocysteine, folate, and vitamin B-12[1]

	LC		HC	
	Week 0	Week 8	Week 0	Week 8
FMD (%)	5.4 ± 3.4	5.6 ± 3.6	6.0 ± 3.8	6.3 ± 4.4
FID (%)[2]	20.1 ± 5.1	20.7 ± 6.4	18.9 ± 7.2	21.0 ± 6.2
PWV (m/s)[3]	10.7 ± 3.0	9.9 ± 2.4	11.1 ± 2.9	9.5 ± 1.5
AI (%)	29.4 ± 9.6	28.9 ± 10.2	28.1 ± 12.8	27.6 ± 9.4
SBP (mm Hg)[3]	133 ± 14	122 ± 12	136 ± 12	123 ± 10
DBP (mm Hg)[3]	74 ± 12	67 ± 13	77 ± 11	70 ± 9[4]
E selectin (ng/mL)[3]	47.0 ± 19.7	31.3 ± 11.8	46.6 ± 23.7	32.6 ± 12.0
P selectin (ng/mL)[3]	94.5 ± 36.9	90.4 ± 32.5	91.4 ± 33.9	82.9 ± 31.9
ICAM-1 (ng/mL)[3]	455 ± 86	375 ± 72	467 ± 122	410 ± 96
VCAM-1 (ng/mL)[3]	685 ± 112	714 ± 136	685 ± 160	714 ± 140
PAI-1 (ng/mL)[3]	8.9 ± 7.1	4.9 ± 3.4	9.9 ± 7.1	6.0 ± 3.9
tPA (ng/mL)[3]	3.0 ± 2.3	2.1 ± 2.4	2.6 ± 2.0	2.0 ± 1.7
Adiponectin (μg/mL)	6.0 ± 2.3	6.3 ± 2.0	5.3 ± 2.2	5.7 ± 2.3
CRP (mg/L)[3,5]	3.2 ± 1.8	2.9 ± 2.0	3.9 ± 2.8	2.8 ± 2.1
Hcy (μmol/L)[3,5]	6.1 ± 1.5	7.1 ± 1.7	6.8 ± 1.6	7.3 ± 1.6
Folate (nmol/L)[2,5]	23.7 ± 9.1	22.7 ± 8.6	20.0 ± 9.0	24.6 ± 8.0
Vitamin B-12 (pmol/L)[2]	307 ± 121	348 ± 152	303 ± 201	312 ± 198

[1] All values are $\bar{x}$ ± SD. LC, low-carbohydrate, high-saturated-fat diet; HC, high-carbohydrate, low-saturated-fat diet; FMD, flow-mediated dilatation; FID, flow-independent dilatation; PWV, pulse wave dilatation; AI, Augmentation Index; Hcy, homocysteine; SBP, systolic blood pressure; DBP, diastolic blood pressure; ICAM-1, intercellular adhesion molecule-1; VCAM-1, vascular cell adhesion molecule-1; PAI-1, plasminogen activator inhibitor 1; tPA, tissue-type plasminogen activator; Hcy, homocysteine. $n = 36$ (LC) and $n = 30$ (HC) for FMD, FID, and PWV. $n = 51$ (LC) and $n = 45$ (HC) for AI. $n = 51$ (LC) and $n = 47$ (HC) for SBP and DBP. $n = 30$ (LC) and $n = 29$ (HC) for E- and P selectin, PAI-1, tPA, and adiponectin. Data were analyzed by using repeated-measures ANOVA.

[2] $P = 0.05$.

[3] $P < 0.001$ for main effect of time.

[4] Significantly different from week 0, $P < 0.05$.

[5] $P < 0.05$ for diet × time interaction such that CRP decreased less with the LC than with the HC before and after adjustment for weight loss (CRP values >10 mgL were excluded from the analysis) and Hcy increased more with the LC than with the HC and folate decreased more with the LC and increased more with the HC.

Cardiometabolic risk factors

Body composition

Fat mass, percentage body fat, and fat-free mass all significantly decreased from baseline (all $P < 0.001$ for time effect; no diet effect) (Table 4). Abdominal fat mass also decreased; the differential effect of diet was such that the reduction was greater in the LC group (Table 4; $P < 0.001$ for time effect, $P = 0.05$ for diet × time interaction).

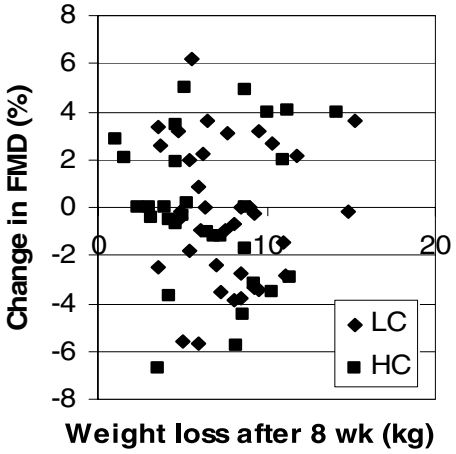

FIGURE 2. Change in weight relative to the change in flow-mediated dilatation (FMD) for subjects in the energy-restricted very-low-carbohydrate, high-saturated-fat diet (LC; $n = 70$) group or the isocaloric conventional high-carbohydrate, low-saturated-fat diet (HC; $n = 33$) group.

Glucose, insulin, total cholesterol, HDL cholesterol, LDL cholesterol, triacylglycerol, and apo B lipoprotein

Glucose and insulin decreased with weight loss ($P < 0.001$ for time), with no effect of diet (**Table 6**). Apo B also decreased after weight loss ($P < 0.001$ for time effect).Total cholesterol was lower after weight loss ($P < 0.001$) and decreased more with the HC ($P < 0.05$ for diet × time interaction) (Table 6). After adjustment for weight loss ($P = 0.003$), the diet × time interaction was strengthened ($P = 0.008$). The change seen was related to the use of lipid medication ($P = 0.015$ for time × medication interaction). The diet effect was seen only in those not taking lipid medications ($n = 75$). LDL cholesterol decreased overall ($P < 0.001$), and the reduction was greater with the HC (−0.36 ± 0.71 mmol/L) than with the LC (−0.15 ± 0.56 mmol/L) ($P < 0.05$ for diet × time interaction; Table 5). After adjustment for weight loss ($P = 0.004$), the diet × time interaction was strengthened ($P = 0.007$). Medication influenced the response in a manner similar to that of total cholesterol ($P = 0.037$ for time × medication interaction).

HDL cholesterol did not change with the HC, but increased with the LC ($P < 0.001$ for diet × time interaction) (Table 5).

TABLE 6
Glucose, insulin, total cholesterol, HDL and LDL cholesterol, triacylglycerol, apolipoprotein B, homocysteine, folate, and vitamin B-12[1]

	LC (n = 52)		HC (n = 47)	
	Week 0	Week 8	Week 0	Week 8
Glucose (mmol/L)[2]	5.7 ± 0.6	5.5 ± 0.5	5.6 ± 0.5	5.4 ± 0.5
Insulin (mIU/L)[2]	9.6 ± 4.8	6.9 ± 5.0	11.3 ± 6.0	7.8 ± 3.1
Total cholesterol (mmol/L)[2,3]	5.4 ± 1.1	5.1 ± 1.2	5.3 ± 0.8	4.8 ± 0.7
HDL cholesterol (mmol/L)[3]	1.4 ± 0.3	1.5 ± 0.3	1.3 ± 0.4	1.3 ± 0.3
LDL cholesterol (mmol/L)[2,3]	3.2 ± 1.0	3.1 ± 1.1	3.2 ± 0.8	2.9 ± 0.6
Triacylglycerol (mmol/L)[2,3]	1.6 ± 0.7	1.1 ± 0.4	1.8 ± 1.0	1.5 ± 0.9
Apolipoprotein B (g/L)[2]	0.97 ± 0.26	0.90 ± 0.28	0.99 ± 0.19	0.89 ± 0.20

[1] All values are $\bar{x}$ ± SD. Subjects who changed lipid medication during the study (n = 3) were excluded from the lipid analysis. LC, low-carbohydrate, high-saturated-fat diet; HC, high-carbohydrate, low-saturated-fat diet. Data were analyzed by using repeated-measures ANOVA.

[2] $P < 0.001$ for main effect of time.

[3] Diet × time interaction before and after adjustment for weight loss such that HDL cholesterol increased with the LC and did not change with the HC, total and LDL cholesterol decreased less with the LC than with the HC, and triacylglycerol decreased more with the LC than with the HC.

Triacylglycerol decreased overall ($P < 0.001$), to a greater extent with the LC diet ($P < 0.05$ for diet × time interaction); this interaction remained significant after adjustment for change in weight ($P < 0.05$).

Urinalysis

Twenty-four–hour urinary sodium excretion decreased at the end of the study equally in both diets (HC: from 193 ± 100 to 135 ± 88 mmol/24 h; LC: 168 ± 63 to 116 ± 52 mmol/24 h; $P <$ 0.001 for time effect; no diet effect).

DISCUSSION

The main finding of this study was that weight loss with an LC did not impair FMD or have any adverse effects on other measures of endothelial function. A number of markers of endothelial function (eg, E- and P selectin, ICAM-1, tPA, and PAI-I) were improved as a result of weight loss with the LC. To our knowledge this has not been reported previously.

We previously found that FMD was reduced by 50% after consumption of a high-saturated-fat diet (20% of energy) in a weight-stable setting (15). It appears that energy restriction and weight loss may negate this adverse effect of saturated fat. In a previous study, LDL cholesterol and apo B increased during the high-saturated-fat, diet but we did not find a relation between changes in LDL cholesterol or apo B and the deterioration in FMD. In the present study, total and LDL cholesterol decreased by ≈8% overall after weight loss, but to a lesser degree with the LC; again, there was no relation with FMD. Other dietary factors, such as walnut and fatty fish intakes, also have a beneficial effect on FMD (37–40). The LC in the present study included both fatty fish and nuts, and we acknowledge that these factors may have had a protective effect on FMD.

The lack of change in FMD in the present study, despite significant weight loss, confirms our previous findings (12, 14). In contrast, Hamdy et al (8) and Williams et al (8, 9) observed improvements in FMD after weight loss (8, 9). Participants in the study by Hamdy et al also undertook an exercise program, which may have had an independent effect on FMD. Bergholm et al (10) observed improvements in endothelial function after weight loss with orlistat use, which correlated with reductions in LDL cholesterol. In contrast, Brook et al (11) did not observe improvement, despite a reduction in LDL cholesterol of 7% after weight loss. Raitakari et al (13) reported improvements in FMD after weight loss, which correlated with reductions in fasting glucose concentrations of 8% but not with reductions in LDL cholesterol or weight loss. There is little evidence to support the relation between FMD and long-term CVD risk. Although FMD was found to be a predictor of future cardiovascular events in one study, it adds only 1% to the prognostic accuracy of traditional CVD risk scores or factors in older adults (7). It is of interest that there was a negative correlation between sodium excretion and FMD after weight loss. There is a well-known relation between salt intake and blood pressure (41), but the correlation between FMD and sodium has not been reported previously. Similarly, AI, also a measure of vascular health, was negatively correlated with sodium excretion. We believe that these findings warrant prospective examination.

Aortic stiffness, of which PWV is an indirect measure, has been shown to be an independent predictor of mortality (42). We found an improvement in PWV after weight loss, with no adverse effect of the high-saturated-fat dietary pattern, which confirmed our previous findings and those of others (12, 43). This finding, in addition to the reduction in blood pressure seen in this and other studies (44), adds to the evidence of the beneficial effects of weight loss on vascular function.

ICAM-1, VCAM-1, E- and P selectin, PAI-1, and tPA have been shown to decrease after moderate weight loss in several studies (12, 45–48), and we observed reductions in these molecules, except for VCAM-1, after weight loss with no adverse effects of a high-saturated-fat diet. We found a small (5%) but significant increase in VCAM-1, which we were unable to explain. However, we believe that this small increase is unlikely to be biologically important.

We found no effect of weight loss on adiponectin. In a previous study we found an improvement in adiponectin after 12 mo but not after 3 mo, which suggests that there is a delay before adiponectin rises (14). Adiponectin concentrations increased in 2 studies (14, 26), but did not change in 5 other studies despite weight losses of 7–8 kg (27–29, 48, 49). The role of adiponectin in vascular risk is controversial (50).

CRP decreased overall, but the reduction with the LC was less than that with the HC, which suggests that a high-saturated-fat diet may influence CRP production. We saw no change in CRP in our earlier study, in which FMD was impaired by a high-saturated-fat diet, and observed reductions in CRP in a previous study of a low-carbohydrate diet during weight loss (15, 17). Weight loss usually reduces CRP but we have not observed an effect of dietary macronutrient composition; this warrants further investigation (51, 52).

We reported previously that homocysteine rose with a very-low-carbohydrate diet, but not with more conventional weight-loss diets; this finding was confirmed in the present study, in which there was a small but statistically significant increase in homocysteine with the LC compared with the HC (17, 53). Elevated plasma homocysteine concentrations are an independent risk factor for all-cause and CVD mortality (54). Concentrations of homocysteine in the present study were half those reported in the Framingham studies, and it is not known whether such small increases in this low range have any detrimental effects on CVD risk. We observed that folate decreased with the LC and increased with the HC. Supplements of folate have been shown to improve FMD (55), but the changes in folate in the present study were not related to FMD. A recent meta-analysis, which evaluated the effects of folic acid supplementation in 16 958 participants, concluded that folic acid does not reduce the risk of CVD. In another study, a multivitamin supplement containing both vitamin B-12 and folate also improved vascular endothelial function in patients with CVD (56). However in the present study while vitamin B-12 rose in both groups there was no effect on FMD.

Greater weight loss with a low-carbohydrate diet than with a conventional low-fat diet has been reported previously (2–4, 57). Subjects in these studies reported similar energy intakes despite differences in weight loss, which suggests that the conventional diet group underreported their intake (3, 4, 57). We also observed similar energy intakes in both groups. We observed greater abdominal fat loss with the LC, but this was not reflected in a superior metabolic risk profile. Hyperglycemia is associated with endothelial dysfunction in cross-sectional studies (58, 59). Rodriguez et al (58) found impaired fasting glucose in 16% of healthy subjects and that FMD was significantly lower in these subjects than in subjects with normal glucose tolerance. Although interventions that lower glucose have been shown to have a beneficial effect on FMD (60, 61), we found no effect of the decrease in glucose concentrations with weight loss on FMD. Obesity and insulin resistance impair FMD, but, despite reductions in insulin with weight loss, we observed no change in FMD. Apo B is an independent predictor of FMD (62), and, although LDL cholesterol decreased more with the HC, because of the decrease in triacylglycerol with the LC, the changes in apo B were not different, which may have accounted for the lack of differences in FMD between diets. A recent systematic review reported that for every 10 kg of weight lost, a decrease of 0.23 mmol/L in total cholesterol (≈5%) can be expected, with similar effects on triacylglycerol and LDL cholesterol (63). According to Mensink et al (64), an increase of 0.032 mmol/L in LDL cholesterol can be expected for every 1% increase in energy from saturated fat in weight stability. We could have expected an increase in LDL of ≈0.2 mmol/L, because saturated fat increased by 7% of energy. Poobalan et al (63) suggests that a reduction of 0.173 mmol/L could be expected from a weight loss of 7.5 kg. The net effect should have been an increase of 0.051 mmol/L in LDL cholesterol, whereas there was a decrease of 0.15 mmol/L in the LC group. A reduction in carbohydrate in the diet with a concomitant increase in saturated fat has been shown to attenuate the expected increase in LDL cholesterol, which may help explain the reduction in LDL cholesterol seen in the present study (65).

In conclusion, short-term weight loss with the LC did not impair FMD. We observed beneficial effects on most of the traditional and new CVD disease risk factors measured with both dietary patterns. The overall risk of CVD does not seem to be different for these 2 types of diet. However, this was a short-term study; the long-term effects of this dietary pattern on vascular function and CVD require investigation.

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The authors' responsibilities were as follows—JBK: contributed to the experimental design, performed the AI measurements and the laboratory analyses relating to endothelial function, drafted the manuscript, and performed the statistical analyses; GDB: had overall responsibility for the study design, coordinated the trial, and contributed to the interpretation of the data and the writing of the manuscript; MN: contributed to the experimental design, data interpretation, and writing of the manuscript; MN and JBK: responsible for the design of the dietary protocols; DPB: oversaw the measurement of the adhesion molecules, tPA, PAI-1, and adiponectin and contributed to the writing of the manuscript; JDB: contributed to the experimental design and writing of the manuscript; and PMC: contributed to the experimental design, statistical analysis, data interpretation, and writing of the manuscript. All authors agreed on the final version of the manuscript. None of the authors had a conflict of interest.

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