

Lack of suppression of circulating free fatty acids and hypercholesterolemia during weight loss on a high-fat, low-carbohydrate diet^{1–3}

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ABSTRACT

Background: Little is known about the comparative effect of weight-loss diets on metabolic profiles during dieting.

Objective: The purpose of this study was to compare the effect of a low-carbohydrate diet (≤ 20 g/d) with a high-carbohydrate diet (55% of total energy intake) on fasting and hourly metabolic variables during active weight loss.

Design: Healthy, obese adults ($n = 32$; 22 women, 10 men) were randomly assigned to receive either a carbohydrate-restricted diet [High Fat; mean \pm SD body mass index (BMI; in kg/m^2): 35.8 ± 2.9] or a calorie-restricted, low-fat diet (High Carb; BMI: 36.7 ± 4.6) for 6 wk. A 24-h in-patient feeding study was performed at baseline and after 6 wk. Glucose, insulin, free fatty acids (FFAs), and triglycerides were measured hourly during meals, at regimented times. Remnant lipoprotein cholesterol was measured every 4 h.

Results: Patients lost a similar amount of weight in both groups ($P = 0.57$). There was an absence of any diet treatment effect between groups on fasting triglycerides or on remnant lipoprotein cholesterol, which was the main outcome. Fasting insulin decreased ($P = 0.03$), and both fasting ($P = 0.040$) and 24-h FFAs ($P < 0.0001$) increased within the High Fat group. Twenty-four-hour insulin decreased ($P < 0.05$ for both groups). Fasting LDL cholesterol decreased in the High Carb group only ($P = 0.003$). In both groups, the differences in fasting and 24-h FFAs at 6 wk were significantly correlated with the change in LDL cholesterol (fasting FFA: $r = 0.41$, $P = 0.02$; 24-h FFA: $r = 0.52$, $P = 0.002$).

Conclusions: Weight loss was similar between diets, but only the high-fat diet increased LDL-cholesterol concentrations. This effect was related to the lack of suppression of both fasting and 24-h FFAs. *Am J Clin Nutr* 2010;91:578–85.

INTRODUCTION

Obesity has become a worldwide epidemic, leading to an increased incidence of cardiovascular disease (CVD) morbidity and mortality (1). It is associated with an increased incidence of coronary artery disease, a likely composite effect of more hypertension, glucose intolerance, inflammation, and dyslipidemia (2). The dyslipidemia of obesity is typically associated with insulin resistance, including higher concentrations of fasting triglycerides and lower concentrations of HDL cholesterol. The added risk of atherosclerosis associated with postprandial lipids presumably relates to the increase in fasting plasma triglycerides,

including VLDL and chylomicron triglycerides, chylomicron remnants, and/or lipolysis products (3).

Although there remain several options for the treatment of obesity, dietary modification remains the mainstay of therapy (4). Within the past 10 y, there has been a resurgence of the use of diets that contain a very low proportion of carbohydrates but are unrestricted in calories, dietary fat, and/or protein intake (low-carbohydrate diets). It is claimed that low-carbohydrate diets have more favorable effects on metabolic abnormalities found in insulin resistance syndromes, including serum triglyceride concentrations, HDL-cholesterol concentrations, and small, dense LDL-cholesterol particles (5). Despite these claims, little is known about the comparative effect of the diets on metabolic profiles during active weight loss.

We therefore sought to compare the effect of a high-fat/low-carbohydrate diet with a high-carbohydrate/low-calorie diet on fasting and hourly glucose, insulin, free fatty acids (FFAs), triglycerides, and remnant lipoprotein cholesterol (RLP cholesterol) during active weight loss over 6 wk of dieting.

SUBJECTS AND METHODS

Study population

This cohort of subjects was part of a multi-institutional National Institutes of Health-funded study that included the University of Colorado Denver Anschutz Medical Campus, Washington University, and the University of Pennsylvania. The larger parent study included adult men and women (age: 18–65 y old) with a body mass index (BMI; in kg/m^2) >30 and <40 . Subjects with evidence of significant organ system dysfunction,

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metabolic diseases, CVD, or diabetes were not eligible to participate. Similarly, subjects who were taking steroids, lipid-lowering agents, medications for weight loss (including herbal therapy), or medications known to affect appetite, food intake, or energy expenditure were excluded. Women who were pregnant or intending to become pregnant within 2 y of initiation of the study, or who were lactating, were excluded. Subjects with severe psychiatric illness or any other serious illness that may have adversely affected dieting as judged by the investigators were also excluded. From patients enrolled in the parent study at the University of Colorado Denver site, 35 obese men and women (BMI: 30–40) were presented with the opportunity to enroll in this substudy. Thirty-two subjects provided their written informed consent. The current substudy was approved by the Colorado Multiple Institutional Review Board. Patients were recruited during 2004–2006.

Study design

The 32 enrolled healthy obese subjects were randomly assigned in an unblinded fashion to receive either a carbohydrate-restricted diet (High Fat group) or a calorie-restricted, low-fat diet (High Carb group) for 6 wk. Subjects were stratified by age, sex, and BMI before being randomly assigned to a group. All subjects underwent a comprehensive behavioral weight-control program used in previous weight-loss studies at the University of Pennsylvania (6). All subjects attended weekly group sessions that varied between the 2 treatment conditions only in the type of diet plan prescribed. Two 24-h feeding studies were performed: at baseline before randomization and 6 wk after the weight-loss intervention began. Although the 2 investigations (parent and substudy) were related, the measurements for each study were entirely separate, except for the weekly body-weight measurements.

Laboratory procedures

The subjects' weights were measured weekly by using a single calibrated scale. Fasting blood samples were obtained from all subjects at weeks 0 and 6 for determination of lipid profiles and RLP cholesterol. Glucose and serum lipid concentrations were obtained after a 12-h overnight fast (Synchro LX20 Clinical Chemistry System; Beckman Coulter, Fullerton, CA).

Total cholesterol and triglycerides were measured enzymatically with a colorimetric endpoint (Roche Diagnostic Systems, Indianapolis, IN), as were HDL-cholesterol concentrations (Diagnostic Chemicals Ltd, Oxford, CT). LDL cholesterol was calculated from plasma total and HDL cholesterol and triglyceride concentrations (7). Serum insulin concentrations were measured by radioimmunoassay (Laboratory Corporation of America, Burlington, NC). RLP cholesterol was measured by using the JIMRO assay of Nakajima et al (8).

Parent study

Subjects in both treatment conditions at all study sites received a comprehensive, state-of-the-art behavioral weight-control program used in previous weight-loss studies (9). All participants attended group sessions (each 90 min) weekly from weeks 1 through 20, every other week from weeks 21 through 40, and every 8 wk from weeks 41 through 104. This totaled 38 small

group sessions. Groups included 10 participants; the same group remained together throughout the 104 wk. Group sessions varied between the 2 treatment conditions (High Fat compared with High Carb) only in the type of diet plan that was prescribed. Groups were led by a behavioral psychologist or a registered dietitian with extensive experience in weight management.

This substudy took place over the first 6 wk of weight loss. During this phase (weeks 1–20 for the larger parent study), subjects were instructed in traditional behavioral methods of weight control such as self-monitoring, stimulus control, slowed eating, and related behaviors. They were given a treatment manual to reinforce the topics covered in the group sessions. Treatment was based on a protocol developed by Thomas Wadden and Gary Foster (10), which has been used in previous studies (11, 12). Subjects' progress was reviewed at these meetings. The only difference between the 2 treatments was the diet that they were prescribed. Each treatment session was audiotaped and forwarded to Foster and his staff, who reviewed the sessions to ensure that the curriculum was being followed accurately by the treatment providers.

Each participant (both interventions) was asked to take a study-supplied multivitamin during the weight-loss intervention. Adherence to dietary advice was monitored by using urine ketone measurements at later time points in the parent study. However, during the first 6 wk, dietary adherence was monitored and encouraged at the weekly sessions.

High-fat/low-carbohydrate-diet treatment

Participants in the high-fat condition were instructed to consume a diet that was low in carbohydrate and thus higher in percentage fat and/or protein (13). The central feature of this approach is carbohydrate restriction with unlimited consumption of fat and protein. Subjects were told that polyunsaturated fats and monounsaturated fats were healthier sources of dietary fatty acids than were saturated fats, but it was clear that the primary goal was to limit carbohydrate by whatever means were required. Participants were provided a treatment manual, which described the rationale for a low-carbohydrate diet as well as numerous suggestions for meal plans. The treatment manual for the high-carbohydrate diet plan was modified to make it parallel to the high-fat (low-carbohydrate) recommendations.

This substudy took place during the first phase ("induction") of the intervention. During this phase, participants were instructed to consume ≈ 20 g carbohydrate/d. They were told to eat until full while remaining within the carbohydrate limit.

High-carbohydrate/low-fat-diet treatment

Participants in the high-carbohydrate-diet treatment were encouraged to consume a diet consistent with the US Department of Agriculture's Food Guide Pyramid. Consistent with the comprehensive, group-based, behavioral weight-loss program developed by Foster et al (9), subjects were not prescribed a diet but instead were encouraged to make small changes in dietary intake that included reducing fat and increasing the consumption of fruit, vegetables, breads, and cereals.

This substudy took place during the first phase ("weight loss") of the treatment program. Participants consumed a self-selected calorie-controlled diet, with the goal of inducing steady weight

loss. Suggested caloric intakes for women initially were set at 1200–1500 kcal/d, with the higher intakes recommended for those with a BMI > 36. Men were instructed to eat 1500–1800 kcal/d, again with the higher intakes recommended for those with a BMI > 36. Subjects were encouraged to consume ≈30% of calories from fat, 15% from protein, and 55% from carbohydrate.

Twenty-four-hour feeding studies

All subjects underwent a baseline 24-h feeding study, during which time they were admitted to the in-patient Clinical Translational Research Center (CTRC) at the University of Colorado Hospital, Anschutz Medical Campus. Before their arrival, they were instructed to strictly adhere to the macronutrient targets according to their randomized assignment. The macronutrient composition of all provided meals consisted of 55% carbohydrates, 30% fat, and 15% protein. For the provided diets during admission, calorie amounts for each participant were estimated by using the Harris-Benedict equation with an adjusted weight and an activity factor of 1.3 (14). During each 24-h feeding study, the subject was admitted to the CTCRC in the morning (0630) after a 12-h fast. Three meals were given at regimented times (0800, 1200, and 1800). The first blood sample was drawn just before breakfast at 0800. Estimated required daily calories were distributed as follows: 25% of total calories at breakfast, 40% at lunchtime, and 35% at dinner. Subjects were required to consume each meal within 20 min and were prohibited from consuming any additional calories during the 24-h period.

The second 24-h study was engineered to be identical to the initial 24-h feeding study, but the meal macronutrient composition reflected the randomized diet assigned to each subject. Calorie amounts for each participant were based on the height and weight measured during the week of the admission. Thus, for patients adhering to the high-fat diet at home, the macronutrient composition of their second CTCRC study meal was <10% carbohydrate with the remainder as fat and protein. For patients adhering to the high-carbohydrate diet at home, the macronutrient composition of their second CTCRC study meal comprised 30% fat, 15% protein, and 55% carbohydrate. This design allowed a comparison with the standardized baseline study results and a robust assessment of the randomized diets. Glucose, insulin, FFA, and triglycerides were measured hourly. RLP-cholesterol concentrations were measured every 4 h. These data were evaluated as incremental area under the curve (AUC), estimated by using the trapezoidal rule. Incremental area subtracts baseline (time = 0) from the total AUC and was used because fasting measures (time = 0) were evaluated separately.

Statistical analysis

Results are expressed as means ± SDs where distributions were approximately normal; median and interquartile ranges are reported for RLP, HDL, and LDL cholesterol, as these were right-skewed. Diet differences at 6 wk were evaluated by analysis of covariance, where the covariate was the baseline value of the measure. Within-diet differences over 6 wk were evaluated using paired *t* tests. The relations between fasting LDL cholesterol and fasting or 24-h FFA were evaluated by using the

Pearson product-moment correlation coefficient. All outcome measures were checked for differences at baseline between diet groups by using a 2-tailed, 2-sample *t* test. RLP, LDL, and HDL cholesterol were log-transformed for analysis to stabilize the variance.

The study was designed to achieve 80% power to detect a 2.6 ± 2.47 mg/dL difference due to diet in average difference from baseline in postprandial RLP cholesterol at 6 wk at the $\alpha = 0.05$ level with 15 subjects in each diet group (15, 16). Statistical analyses were performed in SAS, version 9.1 (SAS Institute, Cary, NC), and statistical significance was defined as a 2-sided *P* value < 0.05. No adjustments were made for multiple endpoints or ad hoc testing.

RESULTS

Characteristics of the study cohort

The baseline characteristics of the study cohort are shown in **Table 1**. Subjects in each diet group were stratified according to age, sex, and weight. There were no significant differences in baseline lipid, glucose, or insulin measures between the diet groups.

Weight loss

Within both diet groups, patients lost weight. At 6 wk, the average weight loss in the High Carb and High Fat groups was -6.0 ± 3.5 kg ($P < 0.0001$) and -6.2 ± 4.8 kg ($P < 0.0001$), respectively. There was no significant difference in weight loss between the 2 diet groups ($P = 0.57$).

Fasting and 24-h feeding studies at baseline

At baseline, there were no differences between diet treatments in any of the fasting or 24-h outcome measures, including concentrations of lipoprotein, FFA, glucose, insulin, and triglyceride (Table 1).

Changes in glucose, insulin, triglycerides, and FFA after 6 wk

The difference between baseline and 6 wk in fasting and concentrations of 24-h serum lipoprotein, FFA, glucose, insulin, and triglyceride is shown in **Table 2**. Average measures by diet group at 6 wk for fasting and 24-h measures are also shown in Table 2. At 6 wk, fasting LDL cholesterol was significantly reduced between baseline and 6 wk within the High Carb group (mean ± SD high-carbohydrate change: -6.7 ± 7.8 mg/dL; $P = 0.003$) but not within the High Fat group. Instead, fasting LDL cholesterol tended to increase over 6 wk within the High Fat group, although not significantly (mean ± SD change: 11.7 ± 28.2 mg/dL; $P = 0.13$). There was no significant change in fasting FFA within the High Carb group at 6 wk, nor were the fasting FFA concentrations different between groups (Table 2). However, there was an increase in fasting FFA within the High Fat group (mean ± SD High Fat change: 159 ± 291 μmol/L; $P = 0.04$) (Table 2). There was no difference in fasting glucose or insulin between diet groups (Table 2). However, as seen in Table 2, there was a significant reduction in fasting insulin concentrations within the High Fat group (mean ± SD change: -4.7 ± 8.0 μU/mL; $P = 0.03$). Fasting triglycerides were significantly



TABLE 1

Baseline characteristics and outcome measures by randomization group¹

	High carbohydrate	High fat	P ²
No. of participants	16	16	—
Age (y)	43.4 ± 8.5 ³	42.8 ± 10.0	0.45
Female [n (%)]	12 (75)	10 (62)	—
Male [n (%)]	4 (25)	6 (38)	0.7
Weight (kg)	103 ± 11	101 ± 13	—
Female	101 ± 8	96 ± 10	—
Male	111 ± 20	111 ± 12	0.68
Fasting measures			
RLP-C (mg/dL) ⁴	2.85 (1.91, 6.31) ⁵	4.31 (2.73, 6.13)	0.80
HDL-C (mg/dL)	46.5 (40.5, 56.5)	42.0 (34.0, 62.5)	—
Female	50.0 (42.0, 58.5)	53.5 (43.0, 74.0)	—
Male	40.5 (36.5, 49.0)	36.5 (31.0, 41.0)	0.88
LDL-C (mg/dL)	102 (93.2, 115.0)	109 (77.4, 126.0)	0.73
FFA (μmol/L)	634 ± 178	631 ± 164	0.96
Glucose (mg/dL)	85.6 ± 8.3	85.5 ± 10.9	0.97
Insulin (μU/mL)	10.7 ± 6.1	11.3 ± 7.3	0.79
Triglycerides (mg/dL)	117 ± 55.4	124 ± 58.1	0.73
AUC			
RLP-C (mg/dL)	116 (50.9, 178)	113 (47.5, 176)	0.93
FFA (μmol/L)	4787 ± 1327	5200 ± 1119	0.35
Glucose (mg/dL)	1428 ± 112	1448 ± 108	0.62
Insulin (μU/mL)	492 ± 304	556 ± 421	0.63
Triglycerides (mg/dL)	2329 ± 1031	2513 ± 1186	0.64

¹ RLP-C, remnant lipoprotein cholesterol; HDL-C, HDL cholesterol; LDL-C, LDL cholesterol; FFA, free fatty acid; AUC, area under the curve. RLP-C, HDL-C, and LDL-C values were approximately log-normally distributed; all statistical tests were performed by using the natural log of these variables.

² P values obtained by using 2-group *t* tests or Fisher's exact test for independent proportions, as appropriate.

³ Mean ± SD (all such values).

⁴ One data point was lost on this measure; *n* = 15 in the high-carbohydrate-diet group.

⁵ Median; interquartile range in parentheses (all such values).

reduced between baseline and 6 wk within both diet groups (mean ± SD high-carbohydrate change: -26.9 ± 41.3 mg/dL; *P* = 0.02; mean ± SD high-fat change: -43.6 ± 59.5 mg/dL; *P* = 0.01) (Table 2).

After 6 wk, 24-h glucose was decreased only within the High Fat group (mean ± SD: -133 ± 82.9 mg/dL, *P* < 0.0001) (Table 2). However, 24-h insulin was decreased within both diet groups (mean ± SD high-carbohydrate change: -141.9 ± 207.6 μU/mL; *P* = 0.02; mean ± SD high-fat change: -338.7 ± 398.1 μU/mL; *P* = 0.004) (Figure 1A, Table 2). At 6 wk, there was no significant difference in 24-h AUC for triglycerides between groups (Table 2). However, during both the baseline study for all subjects and the High Carb group at 6 wk, FFA concentrations were suppressed by meal intake (Figure 1B). However, the High Fat group failed to show any decline in FFA throughout the 24-h period. In fact, there was a 2-fold increase in 24-h FFA within the High Fat group between baseline and 6 wk (mean ± SD high-fat change: 4486 ± 2293 μmol/L; *P* < 0.0001) and a significant difference in 24-h FFA between groups (mean ± SD high-carbohydrate 24-h FFA compared with high-fat 24-h FFA: 4798 ± 1373 compared with 9686 ± 1801 μmol/L, respectively; *P* < 0.0001) (Table 2).

Changes in lipoproteins over 6 wk

Fasting RLP cholesterol decreased within both diet groups (median ± SD high-carbohydrate change: -1.9 ± 3.3 mg/dL; *P* = 0.004; median ± SD high-fat change: -2.7 ± 3.1 mg/dL;

P = 0.04) (Table 2). Fasting HDL cholesterol decreased between baseline and 6 wk only within the High Carb group (mean ± SD high-carbohydrate change: -5.1 ± 7.8 mg/dL; *P* = 0.02.) In addition, fasting LDL cholesterol decreased over 6 wk only within the High Carb group (mean ± SD high-carbohydrate change: -6.7 ± 7.8 mg/dL; *P* = 0.003). A between-diet comparison of the change in fasting LDL cholesterol between baseline and 6 wk was found to be statistically significant (*P* = 0.01) (Table 2).

Twenty-four-hour RLP-cholesterol concentrations decreased between baseline and 6 wk within both diet groups (mean ± SD high-carbohydrate change: -50.2 ± 54.8 mg/dL; *P* = 0.003; mean ± SD high-fat change: -59.7 ± 70.8 mg/dL; *P* = 0.004) (Table 2). Twenty-four-hour HDL- and LDL-cholesterol concentrations were not measured.

Differential increase in FFA concentrations and relation to LDL cholesterol

As stated previously, over the 6-wk study period, there was a significant increase in average fasting and 24-h FFA concentrations only within the High Fat group (Table 2). The differences in both fasting (Figure 2A) and 24-h FFA (Figure 2B) at 6 wk were significantly correlated with the difference in LDL-cholesterol values in both diet groups (fasting: *r* = 0.41, *P* = 0.02; 24 h: *r* = 0.52, *P* = 0.002). This relation appears to have been driven by the greater differences within the High Fat group.



TABLE 2Differences from baseline within and between diet groups (6 wk – baseline)¹

Measure	Difference	<i>p</i> ²	Values	<i>p</i> ³
Weight loss (kg)				
High carbohydrate	−6.0 ± 3.5 ⁴	<0.0001	95.4 ± 11.3	
High fat	−6.2 ± 4.8	<0.0001	97.3 ± 12.9	0.57
Fasting measures				
RLP-C ⁵ (mg/dL)				
High carbohydrate	−1.9 ± 3.3	0.004	2.5 (0.8, 4.6) ⁶	
High fat	−2.7 ± 3.1	0.04	2.0 (1.0, 3.4)	0.42
HDL-C ⁵ (mg/dL)				
High carbohydrate	−5.1 ± 7.8	0.02	43.0 (38.5, 46.5)	
High fat	−2.6 ± 13.1	0.49	41.0 (34.0, 59.5)	0.36
LDL-C ⁵ (mg/dL)				
High carbohydrate	−6.7 ± 7.8	0.003	91.5 (85.6, 113.2)	
High fat	11.7 ± 28.2	0.13	121 (97.7, 130.0)	0.01
FFA (μmol/L)				
High carbohydrate	25.4 ± 269	0.71	660 ± 191	
High fat	159 ± 291	0.04	790 ± 259	0.12
Glucose (mg/dL)				
High carbohydrate	−0.1 ± 6.0	0.94	85.5 ± 6.5	
High fat	0.9 ± 10.5	0.73	86.4 ± 7.6	0.65
Insulin (μU/mL)				
High carbohydrate	−2.8 ± 5.3	0.06	7.9 ± 5.7	
High fat	−4.7 ± 8.0	0.03	6.6 ± 3.6	0.37
Triglycerides (mg/dL)				
High carbohydrate	−26.9 ± 41.3	0.02	90.1 ± 48.7	
High fat	−43.6 ± 59.5	0.01	80.4 ± 29.3	0.35
AUC (24 h)				
RLP-C (mg/dL)				
High carbohydrate	−50.2 ± 54.8	0.003	70.7 (35.3, 97.8)	
High fat	−59.7 ± 70.8	0.004	63.7 (32.2, 103.5)	0.94
FFA (μmol/L)				
High carbohydrate	10.8 ± 1269.6	0.97	4798 ± 1373	
High fat	4486 ± 2293	<0.0001	9686 ± 1801	<0.0001
Glucose (mg/dL)				
High carbohydrate	35.7 ± 79.5	0.09	1464 ± 92.5	
High fat	−133 ± 82.9	<0.0001	1315 ± 58.5	<0.0001
Insulin (μU/mL)				
High carbohydrate	−141.9 ± 207.6	0.02	350 ± 152	
High fat	−338.7 ± 398.1	0.004	217 ± 56.5	0.0002
Triglycerides (mg/dL)				
High carbohydrate	−350 ± 673	0.06	1979 ± 1092	
High fat	−793 ± 872	0.002	1720 ± 622	0.11

¹ RLP-C, remnant lipoprotein cholesterol; HDL-C, HDL cholesterol; LDL-C, LDL cholesterol; FFA, free fatty acid; AUC, area under the curve.² *P* values obtained by using a *t* test of the 6-wk difference.³ *P* values for the diet difference at 6 wk by ANCOVA with regression of the 6-wk measure on the baseline measure and diet.⁴ Mean ± SD (all such values).⁵ RLP-C, HDL-C, and LDL-C values were approximately log-normally distributed; all statistical tests were performed by using the natural log of these variables.⁶ Median; interquartile range in parentheses (all such values).

DISCUSSION

The aim of this study was to compare the metabolic and lipid/lipoprotein effects of a high-fat/low-carbohydrate diet with a high-carbohydrate/low-calorie diet during active weight loss. Of interest, weight loss was similar in both diet groups, allowing for better definition of the role of macronutrient composition in the changes on plasma lipids during active weight loss. There was an absence of any diet treatment effect between groups on fasting triglycerides or RLP cholesterol, the main outcome. Similarly, the 24-h data revealed no differences in AUC for triglycerides or RLP cholesterol after 6 wk. Expected diet treatment effects over

6 wk between the groups were observed for LDL cholesterol and 24-h AUC for FFA, glucose, and insulin. There was also an interesting association between the change in LDL cholesterol and changes in both fasting and 24-h FFA. Within the groups, it was observed that fasting triglycerides were reduced between baseline and 6 wk on both diets. Although low-carbohydrate weight-loss diets have been reported to have more favorable effects on serum triglyceride concentrations (3), these data suggest that the amount of weight reduction at 6 wk is more important than dietary composition in driving changes in fasting and 24-h triglycerides, as well as other postprandial lipid



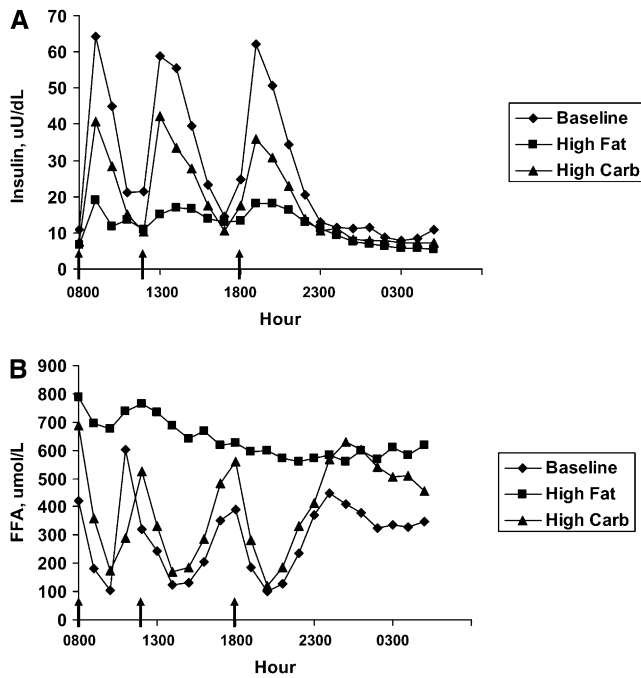


FIGURE 1. A: Decrease in 24-h insulin (baseline – 6 wk) for both the high-fat- and high-carbohydrate-diet groups (High Fat and High Carb). B: Lack of free fatty acid (FFA) suppression with meal intake over 24 h for the High Fat group. In both panels, baseline data show the trend for all participants (High Fat/High Carb groups combined). Data for the 2 separate groups ($n = 16$ per diet condition) were collected 6 wk into the weight-loss period. Arrows indicate meal times.

variables. With more weight loss, a greater reduction in triglycerides would be expected.

Changes in metabolic variables were observed both within and between the diet intervention groups in this study. Careful investigations of the effects of varying diets on diurnal metabolic patterns in normal and hyperlipidemic patient populations have been carried out in recent decades (17–20). Although our data support previous observations, they were collected during active weight loss, making their metabolic context different from other studies. In obese patients, increased rates of adipose tissue intracellular triglyceride lipolysis are expected (21). The failure of the high-fat diet to suppress FFA concentrations in this study may have contributed to relative overproduction of VLDL triglycerides by the liver (22) compared with the High Carb group, in whom decreases would have been expected (23). Subjects in the High Fat, but not the High Carb, group also failed to suppress FFA concentrations over 24 h after 6 wk of intervention, including after meals (Figure 1B). As expected, patterns of 24-h insulin concentrations in the High Fat group lacked a physiologic pattern, remaining low and blunted (Figure 1A), which assumedly contributed to the lack of FFA suppression. It is possible, on the other hand, that the patterns of lower fasting and 24-h FFA in the High Carb group were secondary to higher rates of reesterification of FFA (24). This would be a possibility due to the presence of greater carbohydrate-induced insulin concentrations over 24 h (Table 2).

It was also interesting that fasting LDL-cholesterol concentrations were significantly decreased at 6 wk in the High Carb group (change from baseline: -6.7 ± 7.8 mg/dL; $P = 0.003$), a finding that was not seen in the High Fat group. In fact, sub-

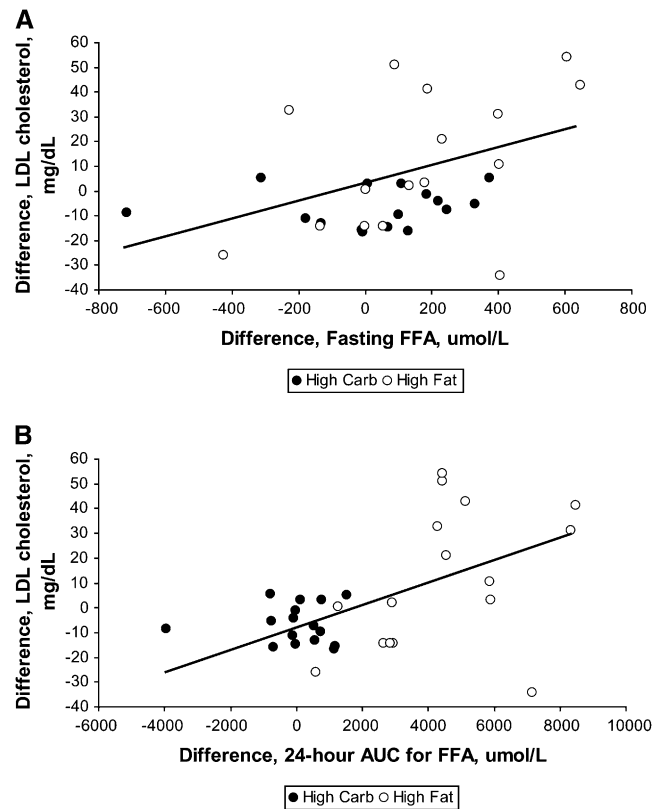


FIGURE 2. A: Relation between the difference in fasting free fatty acid (FFA; baseline – 6 wk) and the difference in LDL cholesterol (baseline – 6 wk; $r = 0.41$, $P = 0.02$; $n = 16$ per diet condition) in high-fat- and high-carbohydrate-diet groups (High Fat and High Carb). B: Relation between the difference in 24-h area under the curve (AUC) for FFA (baseline – 6 wk) and the difference in LDL cholesterol (baseline – 6 wk; $r = 0.52$, $P = 0.002$; $n = 16$ per diet condition).

jects randomly assigned to receive the high-fat diet had a trend toward an increase in LDL-cholesterol concentrations (change from baseline: $+11.7 \pm 28.2$ mg/dL; $P > 0.05$; Table 2). We further found that across participants, the change in both fasting and 24-h LDL-cholesterol concentrations over 6 wk was correlated with the increase in 24-h FFA over 6 wk, a finding particularly influenced by the High Fat group (Figure 2B). Again, in a setting where weight loss was similar between diets, it appears that the effects of macronutrient composition are stronger than the effects of weight loss on LDL cholesterol, a marker of CVD risk. This is in contrast to the triglyceride profile, which appears to be more strongly dictated by the amount of weight loss instead of macronutrient composition.

To our knowledge, this is the first study to evaluate the effect of macronutrient composition on 24-h lipoprotein concentrations during the most active phase of weight reduction (6 wk). Previous studies have investigated between-group differences of the effects of varying diets on fasting plasma lipid concentrations. In these studies, the majority of weight loss occurred during the first 3–6 mo. Stern et al (25) compared the effects of a low-carbohydrate with a high-carbohydrate diet. Whereas weight loss was similar at 1 y in both groups, those in the high-carbohydrate group were still slowly losing weight. Subjects on the low-carbohydrate diet, however, had more favorable effects on fasting triglycerides and HDL-cholesterol concentrations. Brehm et al



(26) found that a very-low-carbohydrate diet was more effective than a low-fat diet in producing short-term weight loss over 6 mo in healthy women, when some weight loss continued in both groups. In this study, there were no deleterious effects on fasting plasma lipids and lipoproteins. A study comparing a low-carbohydrate diet, a Mediterranean diet, and a low-fat diet showed weight losses of 5.5, 4.6, and 3.3 kg, respectively, over 2 y (27). Associated with the greater amount of weight loss after 7–24 mo of weight stability was a relative reduction in the ratio of total cholesterol to HDL cholesterol (20% in the low-carbohydrate group; 12% in the low-fat group). Most recently, the large randomized controlled trial of Sacks et al (28) showed that weight loss was similar among 4 diet groups at 2 y (low-fat/average-protein, low-fat/high-protein, high-fat/average-protein, high-fat/high-protein). Whereas 6 wk data were not reported, across diet groups weight loss reached a plateau by 6 mo, when there were no differences between diet groups in LDL cholesterol or fasting plasma triglycerides.

An interesting finding in this study was the relation between FFA and LDL-cholesterol concentrations (Figure 2, A and B). The High Fat group exhibited both higher fasting FFA and LDL-cholesterol concentrations at 6 wk. The rise in LDL cholesterol is consistent with the composition of the high-fat diet, which contains $\geq 20\%$ of the calories as saturated fat (3 times more than the recommended intake) (29). Cholesteryl ester transfer protein, a protein that transfers cholesteryl esters from HDL to VLDL and LDL, may be responsible for the increased atherogenicity seen with dietary saturated fat. This has been implied in a nonhuman primate model in which positive associations between cholesteryl ester transfer protein activity, VLDL-, and LDL-cholesterol concentrations were found in animals fed a diet high in saturated fat (30). As in the nonhuman primates, these data also revealed a significant relation between the change in LDL-cholesterol and FFA concentrations between baseline and 6 wk. To our knowledge, no other clinical investigations have shown this relation between FFA over 24 h and LDL cholesterol during active weight loss. The relation does have biological plausibility. First, as shown, a diet severely restricted in carbohydrate would substantially reduce glucose and insulin excursions in the fed state, leading to less suppression of adipose tissue triglyceride lipolysis. Convincing data exist that show the ability of dietary saturated fatty acids to increase LDL cholesterol in animals and humans (31–33). The changes in LDL cholesterol might be explained by diet-induced down-regulation of LDL receptor activity through cleavage inhibition of the precursor protein (sterol regulatory element binding protein) (34–36). This chain of events would allow for less hepatic clearance of LDL cholesterol. Overall, this relation is of interest, but its mechanism requires further elucidation, particularly during weight loss.

Potential limitations

This study was small, thus not powered to detect small differences. Moreover, the sample was limited to healthy obese subjects instead of including those with CVD and/or diabetes mellitus. If it is known that a particular diet composition raises LDL cholesterol even in the short-term, however, the safety of such an intervention in persons with known CVD would need to be carefully considered. It is also a limitation that the specific

types of dietary fatty acids consumed (monounsaturated, polyunsaturated, or saturated) are not known. The study design did not insist on dietary records. Thus, despite close and careful surveillance of the participants, macronutrient compliance cannot be assured. Furthermore, the isocaloric diets during the 6-wk inpatient feeding study were not the same composition as at baseline. The second feeding study was engineered to reflect the randomized diet treatment and to maximize comparability between the diets. Finally, in this study, concentrations of LDL cholesterol were estimated by using the Friedewald equation (7) while subjects were in active weight loss. Direct LDL measurements and those from the calculated Friedewald formula are highly correlated (37, 38). A comparison between Friedewald and the LDL apolipoprotein B formula, felt to be a more accurate assessment of LDL cholesterol (39), showed that both were in good agreement with the β -quantification ($r = 0.96$ and 0.97 , respectively) (40). In a study of 27,331 healthy women with triglyceride concentrations ≤ 400 mg/dL, baseline fasting Friedewald LDL cholesterol was compared with fasting and non-fasting direct LDL cholesterol for incident CVD during an 11-y period (41). Whereas the association of LDL cholesterol with CVD by the 2 methods was nearly identical in fasting samples, the direct LDL-cholesterol concentrations were lower and were felt to misclassify many individuals into a lower National Cholesterol Education Program risk category. Thus, whereas validation may be required during weight loss, the Friedewald equation for calculating LDL cholesterol, particularly in subjects with normal fasting triglyceride concentrations, appears to be appropriate at this time.

Conclusions

In conclusion, our data indicate that a high-fat diet, instead of a calorie- and fat-restricted diet, increased LDL-cholesterol concentrations over 6 wk, and that this effect related at least in part to the lack of suppression of both fasting FFA and FFA measured hourly for 24 h. Despite this adverse effect, weight loss was not greater in the High Fat group. Thus, these data suggest that a high-fat diet may have adverse metabolic effects during active weight loss.

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