An Isoenergetic Very Low Carbohydrate Diet Improves Serum HDL Cholesterol and Triacylglycerol Concentrations, the Total Cholesterol to HDL Cholesterol Ratio and Postprandial Lipemic Responses Compared with a Low Fat Diet in Normal Weight, Normolipidemic Women[1,2]

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ABSTRACT Very low carbohydrate diets are popular, yet little is known about their effects on blood lipids and other cardiovascular disease risk factors. We reported previously that a very low carbohydrate diet favorably affected fasting and postprandial triacylglycerols, LDL subclasses and HDL cholesterol (HDL-C) in men but the effects in women are unclear. We compared the effects of a very low carbohydrate and a low fat diet on fasting lipids, postprandial lipemia and markers of inflammation in women. We conducted a balanced, randomized, two-period, crossover study in 10 healthy normolipidemic women who consumed both a low fat (<30% fat) and a very low carbohydrate (<10% carbohydrate) diet for 4 wk each. Two blood draws were performed on separate days at 0, 2 and 4 wk and an oral fat tolerance test was performed at baseline and after each diet period. Compared with the low fat diet, the very low carbohydrate diet increased ($P \leq 0.05$) fasting serum total cholesterol (16%), LDL cholesterol (LDL-C) (15%) and HDL-C (33%) and decreased serum triacylglycerols (−30%), the total cholesterol to HDL ratio (−13%) and the area under the 8-h postprandial triacylglycerol curve (−31%). There were no significant changes in LDL size or markers of inflammation (C-reactive protein, interleukin-6, tumor necrosis factor-α) after the very low carbohydrate diet. In normal weight, normolipidemic women, a short-term very low carbohydrate diet modestly increased LDL-C, yet there were favorable effects on cardiovascular disease risk status by virtue of a relatively larger increase in HDL-C and a decrease in fasting and postprandial triaclyglycerols. J. Nutr. 133: 2756–2761, 2003.

KEY WORDS: • *postprandial lipemia* • *LDL subclasses* • *inflammation* • *ketogenic diets* • *women*

A large number of people have adopted a dietary strategy aimed at limiting carbohydrate intake. In many instances, carbohydrates are restricted to <10% of total energy. Because these diets increase the production of ketones, very low carbohydrate diets are commonly referred to as ketogenic diets. Despite their popularity among the general population, very low carbohydrate diets have been widely criticized by professional organizations because of a lack of scientific information demonstrating safety and efficacy (1,2). A recent USDA review called for further research into the safety and efficacy of low carbohydrate diets (3).

We reported previously that an 8-wk very low carbohydrate diet rich in monounsaturated fat (MUFA)[4] and supplemented with (n-3) fatty acids increased HDL cholesterol (HDL-C) and decreased fasting triacylglycerols and postprandial lipemia in normolipidemic, normal-weight men (4). We subsequently reported similar responses in lipoproteins after a 6-wk very low carbohydrate diet that was not rich in MUFA nor supplemented with n-3 fatty acids in normolipidemic, normal-weight men (5). In addition, the very low carbohydrate diet significantly increased the size of LDL cholesterol (LDL-C) particles in men who had a predominance of small atherogenic LDL particles at the start of the study. The total cholesterol/HDL-C ratio was reduced but not significantly in both these studies. Thus our earlier research in men indicates that short-term very low carbohydrate diets improve some aspects of lipoprotein metabolism (i.e., decreased fasting and postprandial triacyl-

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[4] Abbreviations used: CRP, C-reactive protein; CVD, cardiovascular disease; HDL-C, HDL cholesterol; hs-CRP, high sensitivity; IDL, intermediate density lipoprotein; IL-6, interleukin-6; LDL-C, LDL cholesterol; LPL, lipoprotein lipase; MUFA, monounsaturated fatty acids; TNF-α, tumor necrosis factor α.

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glycerols, increased HDL-C and increased LDL size) independent of weight loss. Whether similar effects occur in women remains unclear.

In recent years, it has become apparent that low grade vascular inflammation plays a key role in all stages of the pathogenesis of atherosclerosis (6,7). Several blood markers indicative of endothelial dysfunction and vascular inflammation have recently been found to be associated with future cardiovascular risk including proinflammatory cytokines, such as interleukin-6 (IL-6) and tumor necrosis-α (TNF-α), and the acute phase reactant C-reactive protein (CRP) (8,9). More than a dozen population-based studies have shown that CRP predicts future cardiovascular events [reviewed in (7)]. Few data exist on the effects of different diets on CRP. Data from a recent study showed a greater reduction in CRP in women after 3 mo of consuming a low energy [5021 kJ (1200 kcal/d)] very low carbohydrate diet than after consuming an energy-matched low fat diet (10). Whether a very low have-carbohydrate diet would have similar advantages over a low fat diet under conditions of weight maintenance is unknown.

Although it is difficult to estimate the number of people who have adopted a low carbohydrate diet, books advocating carbohydrate restriction have collectively sold millions of copies in recent years. Despite this apparent public interest, few studies have examined the effects of very low carbohydrate diets on blood lipids and other biomarkers of cardiovascular disease, making this an important public health issue. The primary purpose of this study was to examine the effects of an isoenergetic very low carbohydrate diet on fasting lipids, postprandial lipemia and markers of inflammation in normal-weight, normolipidemic women.

SUBJECTS AND METHODS

Subjects. Healthy normal weight (BMI < 25 kg/m^2) normolipidemic women ($n = 10$) volunteered to participate in this investigation; 9 were Caucasian and 1 was Asian American. Their physical characteristics were (mean $\pm$ SD); age, 26.3 $\pm$ 6.1 y; body mass, 59.8 $\pm$ 4.6 kg; BMI, 22.0 $\pm$ 1.8, kg/m^2; body fat, 26.8 $\pm$ 3.0%. The subjects had not lost or gained weight in the previous year. They were not following special diets or regularly consuming nutritional supplements, and they habitually consumed between 22 and 31% of energy as fat (assessed via a 7-d food diary). All subjects were nonsmokers, not prescribed any medication known to affect serum lipoproteins and eumenorrheic, defined as 12 menstrual periods at regular intervals per year. All blood samples were obtained during d 2–4 of the follicular phase to control for possible effects of menstrual phase on lipoproteins, even though the variation is small (11). The study was conducted in accordance with the guidelines of the Institutional Review Board at the University of Connecticut.

Study design. A balanced, randomized, two-period, crossover study design was used. Free-living subjects consumed two experimental diets, i.e., a low fat diet and a very low carbohydrate diet. Subjects consumed each diet for 4 wk followed by a 4-wk break before crossing over to the other diet. Subjects consumed their habitual diet during the 4-wk break between feeding periods. Two blood draws were performed on separate days at 0, 2 and 4 wk and an oral fat tolerance test was performed at baseline and after each feeding period. The length of the diet periods was sufficient to achieve stabilization of blood lipids based on our prior work (4,5).

Experimental diets. Both experimental diets were designed to be isoenergetic. Energy levels were assigned to the nearest 837 kJ (200 kcal)-increment based on resting energy expenditure obtained using indirect calorimetry at the start of the study and appropriate activity factors. Standard diabetic exchange lists were used to ensure a constant energy and macronutrient balance of protein (~20% energy), fat (~25% energy) and carbohydrate (~55% of energy) during the low fat diet period. The low fat diet was also designed to contain $<$10% saturated fat and $<$300 mg cholesterol. Foods encouraged during the low fat diet included whole grains (breads, cereals and pastas), fruit/fruit juices, vegetables, vegetable oils, and low fat dairy and meat products. We developed customized diabetic exchange lists for the very low carbohydrate diet period in order to ensure a constant energy and balance of protein (~30% energy), fat (~60% energy) and carbohydrate (~10% of energy) throughout the day. There were no restrictions on the type of fat from saturated and unsaturated sources or cholesterol levels. For the very low carbohydrate diet, commonly consumed foods included beef (e.g., hamburger, steak), poultry (e.g., chicken, turkey), fish, oils, various nuts/seeds and peanut butter, moderate amounts of vegetables, salads with low carbohydrate dressing, moderate amounts of cheese, eggs, protein powder, and water or low carbohydrate diet drinks. Low carbohydrate bars and shakes (Atkins Nutritionals, Hauppauge, NY) were provided to subjects during the very low carbohydrate diet period. A daily multivitamin/mineral complex (Top Care; Topco Assoc., Skokie, IL) that provided micronutrients at levels $\leq$100% of the recommended dietary allowance was given to subjects during both experimental periods.

All subjects received extensive initial instruction and follow-up by registered dietitians on how to translate foods/meals into diabetic exchanges. Subjects were also provided with a packet outlining specific lists of appropriate foods, recipes and sample meal plans that were compatible with their individual preferences for both experimental periods. Subjects received follow-up counseling on a weekly basis during which time compliance was assessed and further dietetic education provided. Body mass was monitored weekly and the energy level adjusted if body mass fluctuated $>$1 kg from the previous visit.

Subjects received thorough instructions for completing detailed weighed food records during wk 1, 2 and 4 of each experimental period (21 d total). Food measuring utensils and scales were provided to subjects to ensure accurate reporting of the amounts of foods and beverages consumed. Food diaries were analyzed for energy and macro/micronutrient content (NUTRITIONIST PRO, Version 1.3; First Databank, The Hearst Corporation, San Bruno, CA). When analyzing foods using standard nutrient databases, the sum of saturated, monounsaturated and polyunsaturated fat is less than the total fat grams presumably because total fat includes glycerol, *trans* fatty acids and other minor lipid components. To ensure that carbohydrates were restricted throughout the very low carbohydrate diet period, subjects tested their urine daily using reagent strips (Bayer, Elkhart, IN). The test is specific for acetoacetic acid, which produces a relative color change when it reacts with nitroprusside.

Blood collection. Blood samples were obtained on two separate days before, at the midpoint and after each 4-wk period. Samples were obtained after an overnight fast and abstinence from alcohol and strenuous exercise for 24 h. Subjects reported to the laboratory between 0700 and 0900 h, rested quietly for 10 min in the supine position; the blood sample, obtained from an antecubital vein, was then separated by centrifugation at 1500 $\times$ *g* for 15 min at 4°C and stored at $-$80°C for subsequent analysis.

Oral fat tolerance test. An oral fat tolerance test was performed after each experimental period using standard procedures in our laboratory (4,5). Subjects arrived at the laboratory after a 12-h overnight fast and abstinence from alcohol and strenuous exercise for 24 h. A flexible catheter was inserted into a forearm vein and after a 10-min stabilization period, two blood samples (10 min apart) were collected for determination of triacylglycerols. The test meal (150 mL heavy whipping cream, sugar-free pudding, 5 mL canola oil, 28.5 g macadamia nuts) was then consumed. This meal provided 3.55 MJ, 13% carbohydrate, 3% protein, 84% fat, 38 g saturated fat, 33 g monounsaturated fat, 4 g polyunsaturated fat and 207 mg cholesterol. Postprandial blood samples were obtained immediately after the meal and hourly for a total of 8 h. Subjects rested quietly in a seated position and consumed exactly 1 L of water only during the 8 h postprandial period.

Serum lipids. Serum (~3 mL) was sent to a certified medical laboratory (Quest Diagnostics, Wallingford, CT) for determination of total cholesterol, HDL-C and triacylglycerol concentrations using automated enzymatic procedures (Olympus America, Melville, NY).

The Friedwald formula was used to calculate LDL-C (12): [LDL-C = total cholesterol – (HDL-C + triacylglycerol/5)], which was then converted to mmol/L by dividing by 38.7.

LDL subclasses. LDL particle size was determined using nongradient polyacrylamide gel electrophoresis (Lipoprint LDL System; Quantimetrix, Redondo Beach, CA). The method was described in detail in a recent publication by our laboratory (5) and others (13) and validated against nondenaturing gradient gel electrophoresis and NMR spectroscopy (14). Seven bands of LDL, three bands of intermediate density lipoprotein (IDL) and VLDL were quantitatively evaluated using computer software (NIH imaging software, utilizing the Lipoprint LDL macro). The percentage of LDL, IDL and VLDL in each band and mean and peak LDL particle diameter are reported.

High sensitivity C-reactive protein (hs-CRP) and proinflammatory cytokines. Plasma for the determination of the acute-phase reactant hs-CRP was mixed with a diluent to provide optimum pH and ionic strength for the formation of antigen-antibody complexes. The mixture was then added to a suspension of latex-beads coated with specific antibody to human CRP and the degree of light-scattering determined on a Dade-Behring Model 2400 nephelometer (Marburg, Germany). The detection limit of the method is 0.15 mg/L, and the method is linear up to concentrations of 200 mg/L. Proinflammatory cytokines IL-6 and TNF-α were determined in duplicate using a high sensitivity ELISA (HS600 and HSTA50, R&D Systems, Minneapolis, MN) with a VersaMax tunable microplate reader with SoftMax Pro data reduction software (Molecular Devices, Sunnyvale, CA). The sensitivity of the IL-6 assay was 0.094 ng/L and the intra-assay CV was 8.55%. The sensitivity of the TNF-α assay was 0.18 ng/L and the intra-assay CV was 10.97%.

Statistical analysis. All statistical analyses were done with Statistica software, version 5.5 (StatSoft, Tulsa, OK). Means for serum lipids and lipoproteins were calculated from both fasting samples obtained at each time point and used for statistical analysis. A two-way ANOVA with repeated measures was used to evaluate changes over time (pre-, mid-, post-) and diet condition (low fat and very low carbohydrate) for all blood concentrations. Triacylglycerol total area under the curve (AUC) was calculated from individual values obtained during the oral fat tolerance test using the trapezoidal method and analyzed using a one-way, repeated-measures ANOVA. Significant main effects or interactions were further analyzed using a Tukey's post-hoc test. Changes during each of the two diet periods were compared using dependent *t* tests. Relationships between variables were examined using Pearson's product-moment correlation coefficient. The α-level for significance was set at 0.05.

RESULTS

All dietary macronutrients differed significantly when women consumed the very low carbohydrate diet compared with the low fat diet with the exception of dietary energy (**Table 1**). We achieved our goals for each diet with 19% of total energy coming from fat for the low fat diet and 10% of total energy coming from carbohydrate for the very low carbohydrate diet. All subjects were in ketosis throughout consumption of the very low carbohydrate diet as indicated by color changes on the urinary reagent strips (data not shown). There were small, significant decreases in body mass during both treatments, but the changes did not differ between the very low carbohydrate (−1.2 ± 0.8 kg) and the low fat (−0.8 ± 1.0 kg) diet periods.

Serum lipids. There were significant diet by time interaction effects for all serum lipids and the total cholesterol/HDL-C ratio (**Table 2**). Total cholesterol was significantly increased after 2 wk (+17%) and remained significantly elevated at wk 4 (+16%) of the very low carbohydrate diet. Total cholesterol was significantly reduced at wk 2 (−6%) but did not differ at wk 4 of the low fat diet. Serum LDL-C followed the same pattern as total cholesterol and was significantly increased after 2 wk (+16%) and remained significantly elevated at 4 wk (+15%) of the very low carbohydrate diet.

TABLE 1

Daily intakes by normal weight women of dietary energy and nutrients during very low carbohydrate and low fat diet periods[1,2]

Nutrient	Very low carbohydrate	Low fat
Energy, *MJ*	7.5 ± 1.1	6.6 ± 1.2
Protein, *g*	128 ± 19#	68 ± 10
Protein, *% of energy*	29 ± 2#	17 ± 4
Carbohydrate, *g*	43 ± 8#	249 ± 59
Carbohydrate, *% of energy*	10 ± 2#	62 ± 6
Sugar, *g*	12 ± 3#	97 ± 28
Total fat, *g*	118 ± 21#	34 ± 12
Total fat, *% of energy*	60 ± 3#	19 ± 5
Saturated fat, *g*	41 ± 9#	10 ± 4
Monounsaturated fat, *g*	35 ± 7#	9 ± 4
Polyunsaturated fat, *g*	20 ± 5#	6 ± 3
Alcohol, *% of energy*	1 ± 2#	2 ± 2
Cholesterol, *mg*	650 ± 206#	123 ± 72
Dietary Fiber, *g*	12 ± 5#	20 ± 7

[1] Values are means ± SD; $n = 10$. # Different from low fat at that time $P \leq 0.05$.

[2] The analysis was performed on 21 d of diet records during each diet.

LDL-C was significantly reduced at wk 2 (−14%) but did not differ at wk 4 of the low fat diet. Serum HDL-C was significantly increased at wk 2 (+31%) and remained significantly elevated at wk 4 (+33%) of the very low carbohydrate diet. HDL-C did not differ after the low fat diet. Serum triacylglycerols were significantly decreased after 4 wk (−33%) of the very low carbohydrate diet. Serum triacylglycerols were significantly increased at wk 2 (+44%) but did not differ at wk 4 of the low fat diet. Although total cholesterol was moderately increased after the very low carbohydrate diet, the proportionally larger increase in HDL-C significantly decreased the total cholesterol/HDL-C ratio after 4 wk (−13%).

LDL subclasses. There were no changes in the relative percentage or concentration of LDL subclasses after consumption of either diet (**Table 3**). There was a significant decrease in the percentage of VLDL and IDL-B after the very low carbohydrate diet. There was a significant association between peak LDL size at the start of the study and the change in LDL peak size with the very low carbohydrate ($r = 0.76$) and low fat ($r = 0.71$) diets (**Fig. 1**). Three of the 10 women started the very low carbohydrate diet with a predominance of small LDL particles (i.e., pattern B) and they each demonstrated moderate to large increases in peak LDL size (i.e., 26.9 to 27.6, 26.6 to 27.3 and 26.4 to 27.6 nm).

Oral fat tolerance test. Triacylglycerols values generally peaked ~2 h after the meal and gradually returned to baseline after 7–8 h (**Fig. 2**). There were significant time and diet condition × time interaction effects. Compared with the total postprandial triacylglycerol AUC at baseline (9.4 ± 3.1 mmol/L × 8 h), there was a significantly lower response after consumption of the very low carbohydrate diet (7.9 ± 2.7 mmol/L × 8 h) but not the low fat diet (10.3 ± 3.3 mmol/L × 8 h).

High sensitivity C-reactive protein (hs-CRP) and proinflammatory cytokines. There were no significant effects of either diet on hs-CRP (very low carbohydrate, 0.98 ± 0.23 to 0.85 ± 0.24 mg/L; low fat, 0.87 ± 0.33 to 0.83 ± 0.34 mg/L), IL-6 (very low carbohydrate, 1.91 ± 1.14 to 1.03 ± 0.43 ng/L; low fat, 2.40 ± 1.50 to 0.96 ± 0.41 ng/L), or TNF-α (very low

TABLE 2

Serum lipids in normal weight women consuming very low carbohydrate and low fat diets[1]

	Very low carbohydrate			Low fat			
	wk 0	wk 2	wk 4	wk 0	wk 2	wk 4	*P*-value[2]
Total cholesterol, *mmol/L*	4.61 ± 0.88	5.39 ± 0.52*#	5.34 ± 0.89*#	4.78 ± 0.91	4.46 ± 0.70*	4.53 ± 0.88	<0.0001
LDL-C,[3] *mmol/L*	2.94 ± 0.66	3.41 ± 0.37*#	3.37 ± 0.62*#	3.11 ± 0.72	2.70 ± 0.52*	2.96 ± 0.67$	0.0001
HDL-C, *mmol/L*	1.28 ± 0.29	1.67 ± 0.33*#	1.69 ± 0.38*#	1.30 ± 0.28	1.24 ± 0.29	1.20 ± 0.24	<0.0001
Triacylglycerol, *mmol/L*	0.86 ± 0.32	0.69 ± 0.17#	0.60 ± 0.14*#	0.79 ± 0.60	1.14 ± 0.59*	0.82 ± 0.28	0.0290
Total cholesterol/HDL-C	3.72 ± 0.76	3.33 ± 0.56	3.25 ± 0.54*	3.77 ± 0.67	3.73 ± 0.77	3.86 ± 0.70	0.0437
LDL-C/HDL-C	2.39 ± 0.63	2.13 ± 0.52	2.08 ± 0.50	2.48 ± 0.64	2.26 ± 0.54	2.53 ± 0.60	0.0897

[1] Values are mean ± SD, $n = 10$. * Different from wk 0, $P \leq 0.05$; $ different from wk 2, $P \leq 0.05$; # different from low fat at that time, $P \leq 0.05$.
[2] *P*-value for interaction between diet and time.
[3] LDL-C, LDL cholesterol; HDL-C, HDL cholesterol.

carbohydrate, 7.50 ± 0.74 to 5.99 ± 0.38 ng/L; low fat, 7.90 ± 0.97 to 7.29 ± 0.62 ng/L).

DISCUSSION

Very low carbohydrate diets have been criticized because of concerns related to adverse effects on cardiovascular disease (CVD), yet few studies have directly evaluated the effects on circulating risk factors for CVD to support this view (15). The results of this study show that a very low carbohydrate diet favorably affects fasting and postprandial triacylglycerols, HDL-C and the total cholesterol/HDL-C ratio in normal-weight normolipidemic women who were in energy balance and who did not alter their level of physical activity. The results are consistent with responses in men under similar conditions (4,5). There were no significant effects of the very low carbohydrate diet on LDL subclasses and markers of inflammation.

The pattern of increased total cholesterol, LDL-C and HDL-C after a very low carbohydrate diet in normal-weight women is consistent with our prior work in normal-weight men (4,5). However, there were differences in the relative magnitude of the increases. After a 6-wk very low carbohydrate diet period in normal-weight men, total cholesterol, LDL-C and HDL-C increased 5, 4 and 12%, respectively (5). Corresponding changes in this study were 16, 15 and 33%. In both normal-weight men and women, a very low carbohydrate diet causes a disproportional increase in HDL-C such that the total cholesterol/HDL-C is reduced, particularly in normal-weight women. A low HDL-C concentration is strongly and

TABLE 3

Low density lipoprotein subclass concentrations in normal-weight women consuming very low carbohydrate and low fat diets[1]

	Very low carbohydrate		Low fat	
Lipoprotein fraction	wk 0	wk 4	wk 0	wk 4
	%			
VLDL[2]	11.4 ± 2.3	8.0 ± 2.2*#	12.8 ± 4.1	11.3 ± 3.2
IDL-C	10.3 ± 3.7	7.9 ± 1.5	10.2 ± 2.3	10.1 ± 1.7
IDL-B	5.2 ± 1.4	4.7 ± 1.6*#	5.3 ± 0.9	5.8 ± 1.2
IDL-A	7.6 ± 3.5	7.5 ± 3.4	5.8 ± 2.9	7.3 ± 2.8
LDL-1	20.8 ± 6.6	22.3 ± 5.4	18.8 ± 4.6	20.6 ± 4.3
LDL-2	10.6 ± 6.2	12.3 ± 5.5	12.8 ± 6.9	13.7 ± 6.8
LDL-3	1.3 ± 1.9	2.0 ± 2.4	2.2 ± 2.0	2.1 ± 2.8
LDL-4	0.2 ± 0.4	0.3 ± 0.6	0.3 ± 0.6	0.2 ± 0.7
LDL-5	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.2	0.0 ± 0.0
Peak LDL size, *nm*	27.5 ± 0.7	27.6 ± 0.5	27.3 ± 0.7	27.2 ± 6.8
Mean LDL size, *nm*	27.1 ± 0.4	27.0 ± 0.4	26.9 ± 0.4	27.0 ± 0.4

[1] Values are mean ± SD, $n = 10$. * Different from wk 0, $P \leq 0.05$; # different from low fat at that time, $P \leq 0.05$.
[2] $P < 0.05$ for the interaction between diet and time.

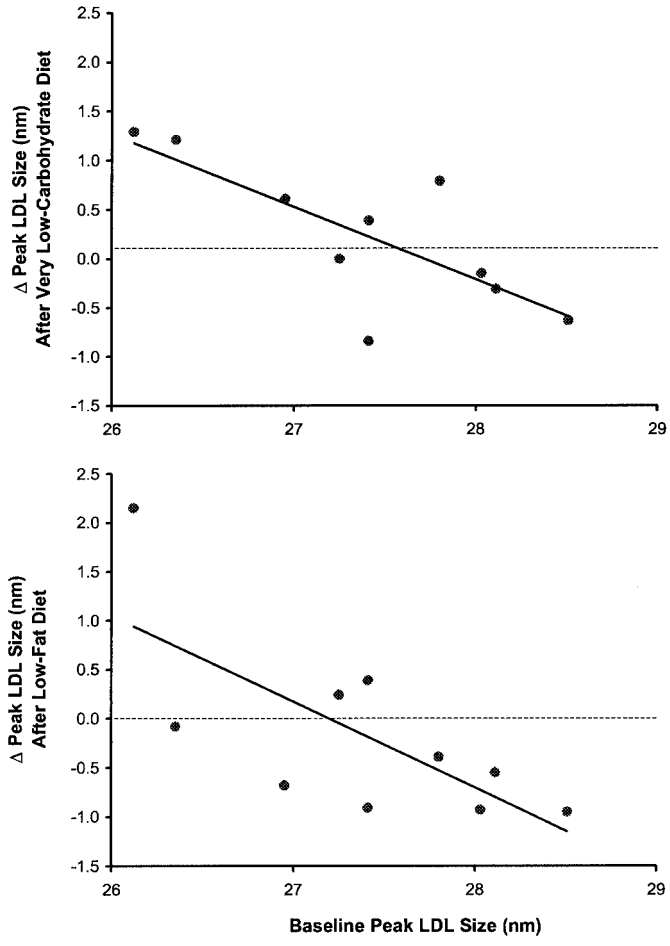

FIGURE 1 Relationship between baseline peak LDL size and the change in peak LDL after 4 wk of consuming very low carbohydrate ($r^2 = 0.58$; $P < 0.05$) and low fat ($r^2 = 0.50$; $P < 0.05$) diets in normal-weight women ($n = 10$).

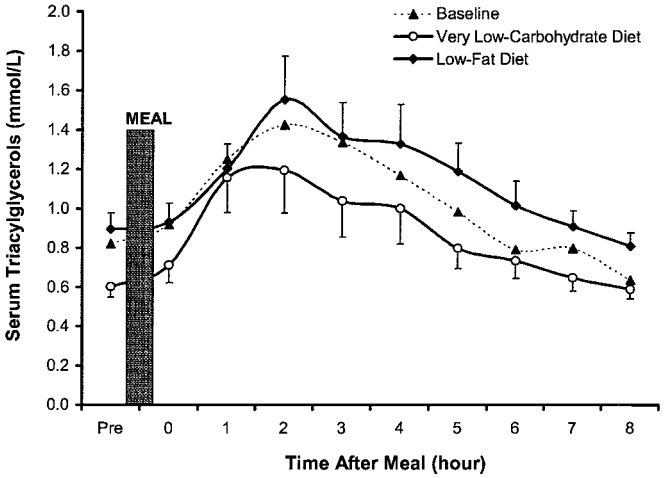

FIGURE 2 Serum triacylglycerol concentrations after ingestion of a high fat meal at baseline and after 4 wk of consuming very low carbohydrate and low fat diets in normal-weight women. Values are means ± SEM, $n = 10$. There were significant time ($P < 0.0001$) and diet × time ($P = 0.039$) effects.

inversely associated with risk for coronary heart disease and a high HDL-C is associated with reduced risk (16). In the Adult Treatment Panel III (17), a high HDL-C concentration >1.55 mmol/L (≥60 mg/dL) is considered a negative risk factor, and its presence evokes removal of one risk factor from the total count used for setting treatment goals for LDL-C. Before consumption of the very low carbohydrate diet, 20% of women met the criterion of high HDL-C (≥60 mg/dL); after the very low carbohydrate diet, 80% met this criterion.

The large increase in HDL-C could have been due to increased production by hepatocytes and the intestinal mucosa and/or increased lipoprotein lipase (LPL)-mediated catabolism of triacylglycerol-rich lipoproteins, which results in disassociation of surface components (i.e., unesterified cholesterol, apoprotein and phospholipid) that are acquired by HDL-C. In mice overexpressing the gene encoding LPL, there is a correlation between increasing postheparin LPL activity and increasing HDL-C, which becomes stronger after consumption of a high fat diet (18). In humans, moderate-to-high fat diets (46–65% of total energy) significantly increase postheparin plasma LPL activity and skeletal muscle LPL activity (19–21), and LPL activity is positively correlated with HDL-C (22–25), especially in response to high fat diets (19). Although speculative, increased tissue expression and activity of LPL may partially explain the mechanism by which very low carbohydrate diets increase HDL-C.

The significant reduction in postprandial lipemia is also consistent with the notion that increased LPL activity may contribute to the lipoprotein changes induced by a very low carbohydrate diet. Although the women in this study had significantly lower fasting and postprandial TAG levels than the men in our previous study (5), the relative reduction in postprandial lipemia was similar (women −31% and men −29%). The very low carbohydrate diet also significantly reduced fasting triacylglycerol concentrations (−30%), suggesting that a decrease in VLDL production rate contributed as well to the reduced postprandial lipemia because a greater VLDL-triacylglycerol pool size competes with triacylglycerols from intestinal origin for removal during the postprandial period. Because fasting and postprandial triacylglycerols are independent risk factors for CVD (26,27), the significant reductions are indicative of decreased CVD risk.

In contrast to our findings in men, there were no changes in the relative percentage or concentration of LDL subclasses after consumption of the very low carbohydrate diet, which may have been because the women had larger particles than the men in our earlier study (5). Previous studies have shown that women have significantly larger, more buoyant LDL particles than men (28–30), even after adjustment for sex-specific differences in triacylglycerols and visceral adipose tissue (31). In men, increasing dietary fat results in larger particles (18) and decreasing fat intake results in smaller particles (32,33). In this study, the response of LDL size to a very low carbohydrate diet was dependent on starting levels (i.e., women with lower peak LDL diameters demonstrated larger increases in response to a low carbohydrate diet). This was true in our earlier study of normal-weight men as well (5). Thus, in men and women with larger LDL particles, a very low carbohydrate diet has little or no effect on LDL subclass distribution, whereas in subjects with a predominance of smaller particles, there appears to be a movement toward larger particles.

Atherosclerosis is no longer considered a disease characterized merely by accumulation of lipid in the arterial wall. There is now substantial evidence revealing the important role of inflammation at all stages of atherosclerosis (6,7). We measured hs-CRP as a marker of systemic inflammation and the proinflammatory cytokines IL-6 and TNF-α because they activate CRP production in the liver. Our results indicate that short-term manipulation of the macronutrient ratio does not affect any of these markers in normal-weight, normolipidemic women. Weight loss has been shown to reduce hs-CRP levels in overweight women (34,35). Thus, a hypoenergetic very low carbohydrate or low fat diet, if used for weight loss, may lower hs-CRP as was shown in a recent study (10).

The results of this study should not be used to make recommendations to the public. This study involved a relatively small and homogenous sample of normal-weight, normolipidemic women. However, the differences in lipid responses to the diets were significant, the results are consistent with our previous work in men and the results agree with data reported by other laboratories, thus making a type I error unlikely (i.e., incorrectly rejecting the null hypothesis). The results indicate that more research should be conducted on larger, more diverse populations for longer periods of time.

In summary, a short-term isoenergetic very low carbohydrate diet significantly decreased fasting and postprandial triacylglycerols, increased HDL-C, decreased the total cholesterol/HDL-C ratio and did not affect markers of inflammation. Our results indicate that in the short term, there is not an adverse response in terms of accepted biomarkers of cardiovascular disease risk in healthy normolipidemic women, even in the absence of large reductions in body mass.

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LITERATURE CITED

1. Blackburn, G. L., Phillips, J. C. & Morreale, S. (2001) Physician's guide to popular low-carbohydrate weight-loss diets. Clev. Clin. J. Med. 68: 761–774.
2. St Jeor, S. T., Howard, B. V., Prewitt, T. E., Bovee, V., Bazzarre, T. & Eckel, R. H. (2000) Nutrition Committee of the Council on Nutrition, Physical Activity, and Metabolism of the American Heart Association. Dietary protein and weight reduction: a statement for healthcare professionals from the Nutrition Committee of the Council on Nutrition, Physical Activity, and Metabolism of the American Heart Association. Circulation 104: 1869–1874.

3. Freedman, M. R., King, J. & Kennedy, E. (2001) Popular diets: a scientific review. Obes. Res. 9 (suppl. 1): 1s–40s.

4. Volek, J. S., Gómez, A. L. & Kraemer, W. J. (2000) Fasting and postprandial lipoprotein responses to a low-carbohydrate diet supplemented with n-3 fatty acids. J. Am. Coll. Nutr. 19: 383–391.

5. Sharman, M. J., Kraemer, W. J., Love, D. M., Avery, N. G., Gómez, A. L., Scheett, T. P. & Volek, J. S. (2002) A ketogenic diet favorably affects serum biomarkers for cardiovascular disease in normal-weight men. J. Nutr. 132: 1879–1885.

6. Ross, R. (1999) Atherosclerosis: an inflammatory disease. N. Engl. J. Med. 340: 115–26.

7. Libby, P., Ridker, P. M. & Maseri, A. (2002) Inflammation and atherosclerosis. Circulation 105: 1135–1143.

8. Blake, G. J. & Ridker, P. M. (2002) Inflammatory bio-markers and cardiovascular risk prediction. J. Intern. Med. 252: 283–294.

9. Blake, G. J. & Ridker, P. M. (2001) Novel clinical markers of vascular wall inflammation. Circ. Res. 89: 763–771.

10. O'Brien, K. D., Brehm, B. J., Seely, R. J., Wener, M., Daniels, S. & D'Alessio, D. A. (2002) Greater reduction in inflammatory markers with a low carbohydrate diet than with a calorically matched, low fat diet. Presented at American Heart Association's Scientific Sessions, November 19, Chicago, IL (abs. #117597).

11. Reed, R. G., Kris-Etherton, P., Stewart, P. W. & Pearson, T. A. (2000) Variation of lipids and lipoprotein in premenopausal women compared with men and postmenopausal women. DELTA (Dietary Effects on Lipoprotein and Thrombogenic Activity) Investigators. Metabolism 49: 1101–1105.

12. Friedewald, W. T., Levy, R. I. & Fredrickson, D. S. (1972) Estimation of the concentration of low-density lipoprotein cholesterol in plasma without use of the preparative ultracentrifuge. Clin. Chem. 18: 499–502.

13. Rajman, I., Kendall, M. J., Cramb, R., Holder, R. L., Salih, M. & Gammage, M. D. (1996) Investigation of low density lipoprotein subfractions as a coronary risk factor in normotriglyceridaemic men. Atherosclerosis 125: 231–242.

14. Hoefner, D. M., Hodel, S. D., O'Brien, J. F., Branum, E. L., Sun, D, Meissner, I. & McConnell, J. P. (2001) Development of a rapid, quantitative method for LDL subfractions with the use of the Quantimetrix Lipoprint LDL System. Clin. Chem. 47: 266–274.

15. Volek, J. S. & Westman, E. C. (2002) Low carbohydrate weight-loss diets revisited. Clev. Clin. J. Med. 69: 849–862.

16. Wilson, P. W., D'Agostino, R. B., Levy, D., Belanger, A. M., Silbershatz, H. & Kannel, W. B. (1980) Prediction of coronary heart disease in the Framingham Offspring Study: role of lipoprotein cholesterol. Am. J. Cardiol. 46: 649–654.

17. National Cholesterol Education Program Expert Panel (2001) Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). NIH publication no. 01–3670. National Heart, Lung and Blood Institute and National Institutes of Health, Bethesda, MD.

18. Clee, S. M., Zhang, H., Bissada, N., Miao, L., Ehrenborg, E., Benlian, P., Shen, G. X., Angel, A., LeBoeuf, R. C. & Hayden, M. R. (1997) Relationship between lipoprotein lipase and high density lipoprotein cholesterol in mice: modulation by cholesteryl ester transfer protein and dietary status. J. Lipid Res. 38: 2079–2089.

19. Campos, H., Dreon, D. M. & Krauss, R. M. (1995) Associations of hepatic and lipoprotein lipase activities with changes in dietary composition and low density lipoprotein subclasses. J. Lipid Res. 36: 462–472.

20. Jackson, R. L., Yates, M. T., McNerney, C. A. & Kashyap, M. L. (1990) Relationship between post-heparin plasma lipases, triglycerides and high density lipoproteins in normal subjects. Horm. Metab. Res. 22: 289–294.

21. Kiens, B., Essen-Gustavsson, B., Gad, P. & Lithell, H. (1987) Lipoprotein lipase activity and intramuscular triglyceride stores after long-term high-fat and high-carbohydrate diets in physically trained men. Clin. Physiol. 7: 1–9.

22. Applebaum-Bowden, D., Haffner, S. M. & Wahl, P. W. (1985) Post-heparin plasma triglyceride lipases: relationships with very low density lipoprotein triglyceride and high density lipoprotein2 cholesterol. Arteriosclerosis 5: 273–282.

23. Mowri, H. O., Patsch, J. R., Ritsch, A., Foger, B., Brown, S. & Patsch, W. (1994) High density lipoproteins with differing apolipoproteins: relationships to postprandial lipemia, cholesteryl ester transfer protein, and activities of lipoprotein lipase, hepatic lipase, and lecithen:cholesterol acyltransferase. J. Lipid Res. 35: 291–300.

24. Nikkila, E. A., Taskinen, M. R., Rehunen, S. & Harkonen, M. (1978) Lipoprotein lipase activity in adipose tissue and skeletal muscle of runners: relation to serum lipoproteins. Metabolism 27: 1661–1670.

25. Patsch, J. R., Prasad, S., Gotto, A. M. Jr. & Patsch, W. (1987) High density lipoprotein2: relationship of the plasma levels of this protein species to its composition, to the magnitude of postprandial lipemia, and to the activities of lipoprotein lipase and hepatic lipase. J. Clin. Investig. 80: 341–347.

26. Austin, M. A., Hokanson, J. E. & Edwards, K. L. (1998) Hypertriglyceridemia as a cardiovascular risk factor. Am. J. Cardiol. 81: 7B–12B.

27. Roche, H. M. & Gibney, M. J. (2000) The impact of postprandial lipemia in accelerating atherothrombosis. J. Cardiovasc. Risk 7: 317–324.

28. Nikkila, M., Pitkajarvi, T., Koivula, T., Solakivi, T., Lehtimaki, T., Laippala, P., Jokela, H., Lehtomaki, E., Seppa, K. & Sillanaukee, P. (1996) Women have a larger and less atherogenic low density lipoprotein particle size than men. Atherosclerosis 37: 1886–1896.

29. Li, Z., McNamara, J. R., Fruchart, J. C., Luc, G., Bard, J. M., Ordovas, J. M., Wilson, P. W. & Schaefer, E. J. (1996) Effects of gender and menopausal status on plasma lipoprotein subspecies and particle sizes. J. Lipid Res. 119: 181–190.

30. Carr, M. C., Hokanson, J. E., Zambon, A., Deeb, S. S., Barrett, P. H., Purnell, J. Q. & Brunzell, J. D. (2001) The contribution of intraabdominal fat to gender differences in hepatic lipase activity and low/high density lipoprotein heterogeneity. J. Clin. Endorinol. Metab. 86: 2831–2837.

31. Lemieux, I., Pascot, A., Lamarche, B., Prud'homme, D., Nadeau, A., Bergeron, J. & Despres, J. P. (2002) Is the gender difference in LDL size explained by the metabolic complications of visceral obesity? Eur. J. Clin. Investig. 32: 909–917.

32. Dreon, D. M., Fernstrom, H. A., Miller, B. & Krauss, R. M. (1994) Low-density lipoprotein subclass patterns and lipoprotein response to a reduced-fat diet in men. FASEB J. 8: 121–126.

33. Dreon, D. M., Fernstrom, H. A., Williams, P. T. & Krauss, R. M. (1999) A very low-fat diet is not associated with improved lipoprotein profiles in men with a predominance of large, low-density lipoproteins. Am. J. Clin. Nutr. 69: 411–418.

34. Heilbronn, L. K., Noakes, M. & Clifton, P. M. (2001) Energy restriction and weight loss on very-low-fat diets reduce C-reactive protein concentrations in obese, healthy women. Arterioscler. Thromb. Vasc. Biol. 21: 968–970.

35. Tchernof, A., Nolan, A., Sites, C. K., Ades, P. A. & Poehlman, E. T. (2002) Weight loss reduces C-reactive protein levels in obese postmenopausal women. Circulation 105: 564–569.