# A Ketogenic Diet Favorably Affects Serum Biomarkers for Cardiovascular Disease in Normal-Weight Men[1]

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ABSTRACT Very low-carbohydrate (ketogenic) diets are popular yet little is known regarding the effects on serum biomarkers for cardiovascular disease (CVD). This study examined the effects of a 6-wk ketogenic diet on fasting and postprandial serum biomarkers in 20 normal-weight, normolipidemic men. Twelve men switched from their habitual diet (17% protein, 47% carbohydrate and 32% fat) to a ketogenic diet (30% protein, 8% carbohydrate and 61% fat) and eight control subjects consumed their habitual diet for 6 wk. Fasting blood lipids, insulin, LDL particle size, oxidized LDL and postprandial triacylglycerol (TAG) and insulin responses to a fat-rich meal were determined before and after treatment. There were significant decreases in fasting serum TAG (−33%), postprandial lipemia after a fat-rich meal (−29%), and fasting serum insulin concentrations (−34%) after men consumed the ketogenic diet. Fasting serum total and LDL cholesterol and oxidized LDL were unaffected and HDL cholesterol tended to increase with the ketogenic diet (+11.5%; $P = 0.066$). In subjects with a predominance of small LDL particles pattern B, there were significant increases in mean and peak LDL particle diameter and the percentage of LDL-1 after the ketogenic diet. There were no significant changes in blood lipids in the control group. To our knowledge this is the first study to document the effects of a ketogenic diet on fasting and postprandial CVD biomarkers independent of weight loss. The results suggest that a short-term ketogenic diet does not have a deleterious effect on CVD risk profile and may improve the lipid disorders characteristic of atherogenic dyslipidemia. J. Nutr. 132: 1879–1885, 2002.

KEY WORDS: • *triglycerides* • *postprandial lipemia* • *lipoprotein subclasses*

Cardiovascular disease (CVD)[3] is the leading cause of mortality in most industrialized countries including the United States (1). Diet is a major weapon used in the fight against CVD because of its influence on the myriad of CVD risk factors. Current dietary recommendations call for a low-fat (<30% of energy), low saturated fat (<7% total energy), low cholesterol (<300 mg/d) diet (2). However, high-carbohydrate diets are controversial (3,4), because they raise plasma triacylglycerols (TAG) (5) and may adversely affect LDL composition (6,7). There has been an alarming increase in the popularity of diets with the common theme of reducing carbohydrate, prompting concern regarding their safety (8). Despite the popularity of very low-carbohydrate diets, very few scientific studies have evaluated how these diets affect CVD risk factors (9) and no studies have examined the effect on recently identified CVD biomarkers (i.e., LDL particle size, postprandial lipemia, oxidized LDL, etc).

A recent meta-analysis of prospective studies indicated that elevated fasting TAG is an independent risk for CVD (10). The atherogenicity of TAG-rich lipoproteins in the postprandial state may play a greater role than fasting TAG, prompting some authors to suggest that elevated postprandial lipemia is a better predictor of CVD than fasting TAG (11,12). Abnormal postprandial lipemia precipitates production of highly atherogenic small LDL particles and a reduction in HDL cholesterol (12), all of which contribute to the causal role for elevated postprandial lipemia in the pathogenesis and progression of CVD.

Individuals with a predominance of large buoyant LDL cholesterol have been classified as pattern A, whereas those with a predominance of small dense LDL particles are pattern B (10). Individuals exhibiting higher levels of small dense LDL have a greater than 3-fold risk of CVD (13,14). This is most likely a result of the longer half-life and increased susceptibility to oxidative modification (15). The fact that LDL is extremely susceptible to oxidative damage has been known for some time (16), with it now appearing that the oxidation of LDL plays an important role in atherogenesis (17).

The therapeutic value of diet interventions aimed at improving CVD risk should take into account factors other than just fasting total cholesterol, LDL cholesterol, HDL cholesterol, and TAG. In this study, we evaluated the effect of a ketogenic diet on both fasting and postprandial lipoprotein metabolism including measures of postprandial lipemia, LDL size and LDL oxidation. As a first step, we studied a normal-

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[3] Abbreviations used: CVD, cardiovascular disease; TAG, triacylglycerol.


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weight normolipidemic population to minimize the confounding effects of weight loss or metabolic abnormalities on the dependent variables. Based on our previous work showing a reduction in fasting TAG and postprandial lipemia after a ketogenic diet rich in monounsaturated fat and supplemented with (n-3) PUFA (9), we hypothesized that the ketogenic diet used in this study would result in a similar TAG response, which would in turn result in increased HDL cholesterol and an increase in the average size of LDL particles.

## MATERIALS AND METHODS

***Subjects.*** Twenty healthy white men free of metabolic and endocrine disorders volunteered to participate in this investigation. To enhance compliance to the rigorous ketogenic dietary treatment, we initially asked subjects if they would be willing to restrict carbohydrate to <10% of total energy for 6 wk. Twelve subjects volunteered to switch from their habitual diet to a ketogenic diet for 6 wk (mean ± SD; age: 36.7 ± 11.6 y; body mass: 79.2 ± 8.3 kg; fat: 20.5 ± 6.2%) and the remaining eight subjects served as controls (mean ± SD; age: 35.0 ± 13.0 y; body mass: 85.4 ± 12.8 kg; fat: 22.2 ± 9.0%). Subjects in the ketogenic diet group had fasting serum total cholesterol concentrations ranging from 2.87 to 5.82 mmol/L and TAG concentrations from 0.62 to 2.24 mmol/L. There were no significant differences in any physical characteristics or serum lipids concentrations between groups at the start of the study. The subjects had not lost or gained weight in the previous year, were not adhering to special diets or regular consumers of nutritional supplements, and habitually consumed between 19% and 43% of energy as fat (assessed via a 7-d food diary). All subjects were nonsmokers and were not prescribed any medication known to affect serum lipoproteins. All subjects were informed of the purpose and possible risks of this investigation before signing an informed consent document approved by an Institutional Review Board at Ball State University.

***Experimental design.*** The study design involved normal-weight normolipidemic men that either switched from their habitual diet (32% fat) to a ketogenic diet (61% fat) or maintained their habitual diet (control group) for 6 wk. Body weight was assessed and two 12-h fasting blood samples were collected at wk 0, 3 and 6. Postprandial TAG and insulin responses to a fat-rich test meal were measured at wk 0 and 6 only in the ketogenic group.

***Dietary intervention.*** The aim of the intervention diet was to reduce carbohydrate intake to <10% of energy. The diet was designed so that fat comprised ~60% of energy with no restrictions on the type of fat from saturated and unsaturated sources or cholesterol levels. The actual diets consumed were mainly comprised of beef (e.g., hamburger and steak), poultry (e.g., chicken and 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. Foods avoided or consumed infrequently included fruits and fruit juices, most dairy products with the exception of hard cheeses and heavy cream, breads, cereals, beans, rice, desserts/sweets, or any other foods containing substantial amounts of carbohydrate. A portion of the food consumed during the intervention diet (~30–40% of total energy) was provided to subjects during weekly meetings to review compliance with the registered dietitian. These foods included pumpkin seeds, roasted cheese, low-carbohydrate bars, shakes, and bake mix (Atkins Nutritionals, Hauppauge, NY) and protein powders (Super Whey Fuel and Fuel Plex Lite; Twin Laboratories, Hauppauge, NY). Subjects were also provided with a daily multivitamin/mineral complex (Daily One Caps With Iron; Twin Laboratories).

Each subject received individual dietary instruction weekly on how to consume meals within the specified nutrient goals and to assess compliance. Subjects were provided with a packet outlining specific lists of appropriate foods, recipes and sample meal plans that were compatible with their individual preferences and the nutrient profile goals of the ketogenic diet. Food measuring utensils and scales were provided to all subjects before the study to assist in the estimation of portion sizes of foods and beverages. Subjects were encouraged to maintain adequate energy intake to maintain body weight. If body weight changed >1 kg, dietary counseling was provided. The ketogenic group kept records each day of the experiment (7 d during baseline and 42 d during the ketogenic diet) and the control group kept 7-d records during wk 1 and 6. All recorded days were analyzed for nutrient content (Nutritionist V, Version 2.3; N-Squared Computing, First Databank Division, The Hearst Corporation, San Bruno, CA). All intervention foods and supplements were entered into the software database and included in the analysis of nutrient intake. Additionally, dietary compliance was monitored using Ketostix reagent strips (Bayer Corporation, Elkhart, IN), which determine qualitatively the presence of acetoacetic acid in urine. Each subject maintained a record of color changes on the reagent strips performed daily at ~0800.

***Fasting blood collection.*** Fasting blood samples were obtained on two separate days at wk 0, 3 and 6 after a 12-h 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, and blood was obtained from an antecubital vein with a 20-gauge needle and vacutainers.

***Oral fat tolerance test.*** Postprandial TAG and insulin responses to a fat challenge were assessed at wk 0 and 6 using standard procedures in our laboratory (9). 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, which was kept patent with a constant saline drip (60 mL/h). Subjects rested in a seated position for 10 min and two baseline blood samples were obtained separated by 10 min with a 10-mL syringe. The first 3 mL of blood withdrawn was discarded to avoid dilution of the sample and ~10 mL was subsequently withdrawn and processed. The test meal then was consumed under supervision to ensure that the entire meal was ingested within a 15-min period. The test meal was designed to be rich in fat and low in carbohydrate similar to the ketogenic diet and contained 5.44 MJ (11% carbohydrate, 2% protein, 87% fat, 52 g saturated fat, 59 g monounsaturated fat, 12 g polyunsaturated fat, and 266 mg cholesterol). Postprandial blood samples were obtained immediately after the meal and hourly for a total of 8 h to assess the magnitude and time course of postprandial lipemia. Subjects rested quietly in a seated position and consumed exactly one liter of water only during the 8-h postprandial period. All subjects completed the entire meal and no adverse side effects (stomach distress, nausea, etc.) were reported.

***Blood analyses.*** Serum collected for the determination of LDL particle size, oxidized LDL, insulin and β-hydroxybutyrate were immediately stored at −80°C. The remaining serum was analyzed for glucose, total cholesterol, HDL cholesterol, and TAG using automated techniques (Roche Modular; Roche Diagnostics, Indianapolis, IN) with calculated precision values < 3%. Concentrations of LDL and VLDL cholesterol were calculated from total cholesterol, HDL cholesterol, and TAG (18). Fasting serum β-hydroxybutyrate concentrations were enzymatically determined in duplicate using a commercially available kit (#310A; Sigma Diagnostics, St. Louis, MO) and spectrophotometric analysis (Spectronic 601; Milton Roy, Rochester, NY). The intra-assay CV was 0.9%. Fasting serum and postprandial plasma insulin concentrations were determined in duplicate using an ELISA kit with a sensitivity of 1.81 pmol/L (#10-1600; Diagnostic Systems Laboratory, Webster, TX) (19). Intra- and inter-assay CV were 5.5% and 3.2%, respectively. Fasting oxidized LDL were also determined in duplicate using an enzyme-linked immunosorbent assay (American Laboratory Products Company, Windham, NH) with a solid two-site enzyme immunoassay that is based on the direct sandwich technique in which two monoclonal antibodies are directed against separate antigenic determinants on the oxidized apolipoprotein B molecule. Intra-assay CV was 7.9%. Absorbances were read on a multilabel counter (Wallac1420 Victor$^2$; Wallac Oy, Turku, Finland).

***LDL subclasses.*** Separation of LDL subclasses was performed using nongradient polyacrylamide gel electrophoresis (Lipoprint LDL System; Quantimetrix, Redondo Beach, CA) previously described in detail (20,21). High-resolution 3% polyacrylamide gel tubes were used for electrophoresis. Seven bands of LDL were quantitatively evaluated using computer software (NIH imaging software, utilizing the Lipoprint LDL macro; Quantimetrix), which divides the scanned gel image at designated Rf values identified by their relative mobility.

This is based on differences in particle size (smaller particles migrate further) and calculates the area under the curve for each fraction. We report the relative percentage of LDL cholesterol in each band and the mean and peak particle diameter. The peak particle diameter for phenotype A is generally >25.5 nm, in contrast the major peak for phenotype B is usually <25.5 nm (22). The determination of a sample being characterized as either phenotype A or B is based on LDL migration rates and is described in detail in Hoefner et al. (20).

***Insulin sensitivity.*** Because high-fat diets have been associated with insulin resistance, we estimated insulin sensitivity using the homeostasis model analysis using fasting glucose and insulin concentrations (23). Assuming that normal-weight subjects aged <35 y have a insulin resistance of 1, the values for a subject can be assessed from the insulin and glucose concentrations by the formula: insulin resistance (near approximation) = insulin/($22.5e^{-\ln \text{glucose}}$).

***Statistical analyses.*** Means and SD were calculated for all variables using conventional methods. Two fasting samples were obtained for each blood variable and the mean of these two values used for statistical analysis. A two-way repeated-measures ANOVA was used to evaluate changes over time in the ketogenic and control groups. When a significant $F$ value was achieved, Fisher's least significant difference test was used to locate the pair-wise differences between means. The total area (serum concentration × time) under the line connecting the postprandial TAG and insulin responses was calculated using the trapezoidal method. The change in body weight was used as a covariate in all analyses. Relationships between variables were examined using Pearson's product-moment correlation coefficient. A criterion $\alpha$-level of $P \leq 0.05$ was used for all statistical comparisons.

## RESULTS

***Body mass and dietary intake.*** All dietary nutrients were significantly different when men consumed the ketogenic diet compared with their habitual diet with the exception of dietary energy and alcohol consumption (**Table 1**). Dietary protein, fat and cholesterol were significantly greater and dietary carbohydrate was significantly lower (8% of total energy) during the ketogenic diet period. There were no significant changes in dietary nutrient intake in the control group from 0 to 6 wk. There was a small but significant decrease in body mass in the ketogenic group (−2.2 ± 1.7 kg) but not the control group (+0.4 ± 4.1 kg).

***Fasting serum metabolic and insulin responses.*** Compared with baseline in the men who followed the ketogenic diet, serum β-hydroxybutyrate concentrations were significantly increased at wk 3 (+427%) and remained significantly elevated at wk 6 (+250%; **Table 2**). All subjects following the ketogenic diet had β-hydroxybutyrate concentrations > 0.20 mmol/L, indicating compliance with the ketogenic diet. Serum insulin concentrations were significantly reduced at 3 and 6 wk (−34.2%) in the ketogenic group but unchanged in the control group (Table 2). From 0 to 6 wk, the estimation of insulin resistance was not affected in the ketogenic group (0.75 ± 0.62 → 0.52 ± 0.35) or the control group (0.52 ± 0.41 → 0.51 ± 0.41).

***Fasting serum lipid responses.*** There were significant increases in total and LDL cholesterol that returned to values that were not significantly different than wk 0 values at the end of the ketogenic diet period (Table 2). Individual responses in total cholesterol were variable, decreasing in five subjects (range: −2 to −17%) and increasing in seven subjects (range: 1 to 60%). LDL cholesterol decreased in four subjects (range: −4 to −11%) and increased in seven subjects (range: 2 to 70%; **Fig. 1**). Compared with wk 0 in the ketogenic group, there was a significant increase in HDL cholesterol at wk 3 and a trend ($P = 0.066$) at wk 6. Serum HDL cholesterol decreased in three subjects (range: −6 to −20%) and increased in nine subjects (range: 1 to 71%; Fig. 1). There was a significant reduction in VLDL cholesterol at wk 3 and 6 of the ketogenic diet. Serum TAG were significantly reduced at wk 3 (−30.9%) and wk 6 (−33.0%). All but one subject had a decrease in fasting TAG (range: −1 to −50%). There were no significant changes in serum lipids in the control group.

***LDL subclass profiles.*** There was a significant increase in peak particle diameter and the LDL-1 subclass distribution after the ketogenic diet was consumed (**Table 3**). We also analyzed subjects classified as pattern A or pattern B at the start of the study. Before the diet intervention, seven subjects were classified as pattern A and five subjects were pattern B. Three of the five pattern B subjects switched to pattern A after the ketogenic diet; all seven pattern A subjects remained pattern A after the ketogenic diet. At the start of the study,

**TABLE 1**

*Daily intake of dietary energy and nutrients in men who switched from their habitual diet to a ketogenic diet for 6 wk and in a control group who continued to consume their habitual diet for 6 wk*[1]

| | Ketogenic diet (wk 0) | Ketogenic diet (wk 6) | Habitual diet (wk 0) | Habitual diet (wk 6) |
|---|---|---|---|---|
| Energy, *kJ* | 10627 ± 2469 | 9770 ± 1569 | 8489 ± 1962 | 7594 ± 816 |
| Protein, *g* | 113 ± 40[b] | 176 ± 45[a] | 82 ± 16[b] | 70 ± 10[b] |
| Protein, %[2] | 17 ± 4[b] | 30 ± 5[a] | 16 ± 2[b] | 15 ± 1[b] |
| Carbohydrate, *g* | 306 ± 100[a] | 46 ± 10[b] | 287 ± 79[a] | 271 ± 47[a] |
| Carbohydrate, % | 48 ± 10[a] | 8 ± 3[b] | 55 ± 5[a] | 59 ± 7[a] |
| Total fat, *g* | 91 ± 31[b] | 157 ± 27[a] | 65 ± 18[b] | 50 ± 14[b] |
| Total fat, % | 32 ± 8[b] | 61 ± 4[a] | 29 ± 5[b] | 25 ± 8[b] |
| Saturated fat, *g* | 31 ± 12[b] | 56 ± 11[a] | 21 ± 6[b] | 16 ± 6[b] |
| Saturated fat, % | 14 ± 4[b] | 25 ± 2[a] | 14 ± 4[b] | 12 ± 4[b] |
| Monounsaturated fat, *g* | 27 ± 11[b] | 57 ± 12[a] | 14 ± 5[c] | 12 ± 8[c] |
| Monounsaturated fat, % | 12 ± 4[b] | 25 ± 3[a] | 9 ± 2[b] | 9 ± 5[b] |
| Polyunsaturated fat, *g* | 12 ± 6[b] | 24 ± 5[a] | 9 ± 3[b] | 6 ± 3[b] |
| Polyunsaturated fat, % | 6 ± 2[b] | 11 ± 2[a] | 6 ± 1[b] | 4 ± 2[b] |
| Cholesterol, *mg* | 332 ± 126[b] | 741 ± 254[a] | 130 ± 14[c] | 118 ± 7[c] |
| Alcohol, % | 3 ± 3 | 1 ± 2 | 0 ± 0 | 0 ± 1 |

[1] Values are means ± SD. Ketogenic diet group, $n = 12$. Control group, $n = 8$. Means in a row with different superscripts differ ($P \leq 0.05$).
[2] Percentage of total energy intake.

**TABLE 2**

*Fasting blood lipid, metabolic and insulin responses in men who switched from their habitual diet to a ketogenic diet for 6 wk and in a control group who continued to consume their habitual diet for 6 wk*[1]

| | Ketogenic group ($n$ = 12) | | | | Control group ($n$ = 8) | | |
|---|---|---|---|---|---|---|---|
| | Wk 0 | Wk 3 | Wk 6 | Percent Δ[2] | Wk 0 | Wk 6 | Percent Δ |
| TC, *mmol/L* | 4.27 ± 0.8[b] | 4.78 ± 0.9[a] | 4.47 ± 0.81[b] | 4.7% | 4.24 ± 1.0[b] | 4.10 ± 1.2[b] | −3.3% |
| TAG, *mmol/L* | 1.09 ± 0.5[a] | 0.75 ± 0.3[b] | 0.73 ± 0.3[b] | −33.0% | 1.14 ± 0.3[a] | 1.08 ± 0.7[a] | −5.3% |
| HDL-C, *mmol/L* | 1.22 ± 0.2[b] | 1.43 ± 0.3[a] | 1.36 ± 0.4[b] | 11.5% | 1.16 ± 0.2[b] | 1.16 ± 0.5[b] | 0.0% |
| LDL-C, *mmol/L* | 2.87 ± 0.8[b] | 3.22 ± 0.9[a] | 2.99 ± 0.8[b] | 4.2% | 2.89 ± 0.9[b] | 2.74 ± 1.1[b] | −5.2% |
| VLDL-C, *mmol/L* | 0.17 ± 0.1[a] | 0.12 ± 0.0[b] | 0.12 ± 0.0[b] | −29.4% | 0.18 ± 0.0[a] | 0.20 ± 0.1[a] | 11.1% |
| TC/HDL | 3.60 ± 0.9 | 3.45 ± 0.88 | 3.45 ± 0.9 | −4.2% | 3.67 ± 0.7 | 3.59 ± 0.8 | −2.2% |
| Insulin, *pmol/L* | 23.7 ± 16.3[a] | 19.1 ± 12.2[b] | 15.6 ± 8.9[b] | −34.2% | 21.5 ± 6.7[a] | 24.3 ± 9.9[a] | 13.0% |
| Glucose, *mmol/L* | 5.00 ± 0.4 | 4.84 ± 0.4 | 4.99 ± 0.3 | −0.2% | 5.00 ± 0.3 | 5.09 ± 0.3 | 1.8% |
| β-HBA, *mmol/L* | 0.08 ± 0.1[b] | 0.40 ± 0.3[a] | 0.28 ± 0.09[a] | 250.0% | 0.09 ± 0.1[b] | 0.10 ± 0.1[b] | 11.1% |

[1] Values are means ± SD. Data were analyzed with a two-way ANOVA using body weight as a covariate. Ketogenic Diet group, $n$ = 12, Control group, $n$ = 8. Means in a row with different superscripts differ ($P \leq 0.05$). TC, total cholesterol; TAG, triacylglycerol; β-HBA, β-hydroxybutyrate.
[2] Percent change from wk 0 to wk 6.

pattern B subjects had significantly smaller mean and peak LDL particle diameters, a significantly greater percentage of LDL-3 and LDL-4, and a significantly smaller percentage of LDL-1 compared with pattern A subjects. There were no significant changes in the percentage of any LDL subclasses or the mean and peak particle size in pattern A subjects. There was a significant increase in peak LDL particle diameter from 25.28 nm to 26.16 nm after the ketogenic diet in pattern B subjects (**Fig. 2**), and also a significant increase in mean LDL particle diameter. There was a significant increase in the percentage of LDL-1 and a significant decrease in the percentages of LDL-3 and LDL-4 after the ketogenic diet in pattern B subjects. There were no changes from 0 to 6 wk in the concentrations of oxidized LDL in either the ketogenic group (44.38 ± 33.7 U/L → 46.45 ± 15.6 U/L) or control group (36.49 ± 10.9 U/L → 39.56 ± 16.9 U/L).

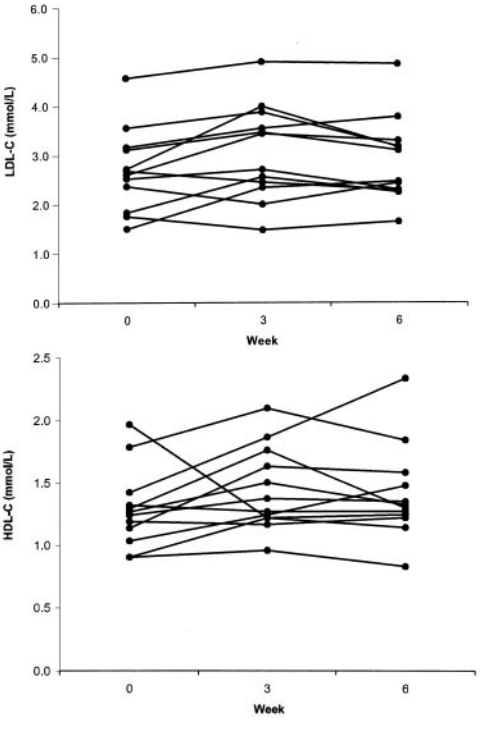


**FIGURE 1** Individual responses of men ($n$ = 12) in LDL-cholesterol (C; upper graph) and HDL-C (lower graph) after consuming a ketogenic diet for 6 wk in normolipidemic, normal-weight men.

***Postprandial TAG and insulin responses.*** Postprandial TAG concentrations peaked 3 h after the meal and started to decline toward fasting values ~5 h after the meal (**Fig. 3**). Compared with wk 0, peak postprandial TAG concentrations were significantly lower (−24%) after the ketogenic diet (2.57 ± 1.4 to 1.96 ± 0.7 mmol/L). The area under the postprandial TAG curve was also significantly lower (−29%) after the ketogenic diet (17.47 ± 9.3 to 12.39 ± 4.2 mmol/L × h). Postprandial insulin concentrations peaked immediately after the meal at wk 0 and 1 h after the meal at wk 6. Compared with wk 0, the area under the postprandial insulin curve was unaffected at wk 6 (339 ± 168 to 283 ± 140 pmol/L × h).

## DISCUSSION

The primary objective of this study was to examine how healthy normolipidemic, normal-weight men respond to a ketogenic diet in terms of fasting and postprandial CVD biomarkers. Ketogenic diets have been criticized on the grounds they jeopardize health (8); however, very few studies have directly evaluated the effects of a ketogenic diet on fasting and postprandial risk factors for CVD. Subjects consumed a diet that consisted of 8% carbohydrate (<50 g/d), 61% fat, and 30% protein. Adaptation to this ketogenic diet resulted in significant reductions in fasting TAG (−33%), postprandial lipemia after a fat-rich meal (−29%), and fasting insulin concentrations (−34%). There were significant increases in LDL particle size, and no change in the oxidative LDL concentrations. There was a significant increase in HDL cholesterol at wk 3 after the ketogenic diet. Collectively, the responses in serum lipids, insulin and lipid subclasses to the ketogenic diet were favorable in terms of overall CVD risk profile.

Only a few studies have examined the effects of a diet with very low amounts of carbohydrate on blood lipids (9,24). Our laboratory recently examined the effects of a ketogenic diet rich in monounsaturated fat and supplemented with (n-3) PUFA on blood lipids in normolipidemic men (9). Fasting TAG, total cholesterol, LDL cholesterol, and HDL cholesterol changed −55%, +2%, +10%, and +10%, respectively (9).

**TABLE 3**

*Serum LDL subclass responses in 12 men who consumed a ketogenic diet who started as either pattern A or pattern B*[1,2]

| | % LDL-1, 27.7 nm | % LDL-2, 26.1 nm | % LDL-3, 24.5 nm | % LDL-4, 23.0 nm | Peak LDL size, nm | Mean LDL size, nm |
|---|---|---|---|---|---|---|
| Ketogenic group ($n$ = 12) | | | | | | |
| Wk 0 | 13.9 ± 6.4[b] | 16.6 ± 5.0 | 6.1 ± 5.2 | 2.0 ± 3.9 | 26.19 ± 10.7[b] | 26.28 ± 7.8 |
| Wk 3 | 18.4 ± 6.8[a] | 19.2 ± 5.5 | 5.5 ± 3.7 | 0.8 ± 1.0 | 26.76 ± 7.8[a] | 26.54 ± 4.3 |
| Wk 6 | 18.2 ± 6.2[a] | 19.4 ± 5.7 | 4.7 ± 3.6 | 0.6 ± 1.3 | 26.71 ± 7.8[b] | 26.57 ± 4.5 |
| Pattern A subjects ($n$ = 7) | | | | | | |
| Wk 0 | 18.2 ± 4.1 | 17.0 ± 4.9 | 2.0 ± 1.6 | 0.1 ± 0.2 | 26.83 ± 5.1 | 26.80 ± 1.6 |
| Wk 3 | 21.8 ± 6.4 | 19.1 ± 5.5 | 4.2 ± 2.7 | 0.4 ± 0.5 | 27.04 ± 7.8 | 26.70 ± 3.0 |
| Wk 6 | 22.2 ± 4.1 | 19.0 ± 7.1 | 2.8 ± 1.8 | 0.1 ± 0.3 | 27.10 ± 7.2 | 26.81 ± 2.5 |
| Pattern B subjects ($n$ = 5) | | | | | | |
| Wk 0 | 7.9 ± 3.1[b] | 16.0 ± 6.1 | 11.7 ± 2.3[b] | 4.7 ± 5.5[a] | 25.28 ± 9.9[b] | 25.54 ± 7.0[b] |
| Wk 3 | 13.7 ± 4.2[a] | 19.2 ± 6.1 | 7.2 ± 4.6[a] | 1.3 ± 1.2[b] | 26.37 ± 6.6[a] | 26.32 ± 5.3[a] |
| Wk 6 | 12.6 ± 3.8[a] | 19.9 ± 3.7 | 7.3 ± 4.0[a] | 1.4 ± 1.9[b] | 26.16 ± 5.1[a] | 26.22 ± 4.6[a] |

[1] Values are means ± SD. Means in a column within a group with different superscripts differ ($P \leq 0.05$).
[2] Individuals with pattern A have a predominance of large LDL particles and those with pattern B have a predominance of smaller LDL particles.

Larosa et al. (24) examined the effects of a hypocaloric ketogenic diet on blood lipids in moderately overweight normolipidemic subjects. Fasting TAG, total cholesterol, LDL cholesterol and HDL cholesterol changed −33%, +6%, +18% and −6%, respectively. Corresponding changes in serum lipids in this study for fasting TAG, total cholesterol, LDL cholesterol, and HDL cholesterol were −33%, +4%, +4%, and +11%, respectively. Confounding variables in these studies include varying degrees of weight loss (−2.2 to −7.7 kg) and slight differences in the type of fat consumed. Nevertheless, these studies collectively indicate that carbohydrate restriction result in significant decreases in serum TAG, small increases in total and LDL cholesterol, and moderate increases in HDL cholesterol in normolipidemic individuals. The small but significant weight loss (−2.2 kg) could have partially explained the HDL and TAG responses in this study. A meta-analysis by Dattilo and Kris-Etherton (25), showed that for every kilogram decrease in body weight during weight loss, HDL-C increases 0.009 and TAG decreases 0.015 mmol/L. Using these estimates, the −2.2 kg weight loss would have been predicted to increase HDL by 0.198 mmol/L and decrease TAG by 0.033 mmol/L, which amounts to only 14% and 9% of the observed changes in these parameters. Thus, dietary composition most likely contributed to the changes in blood lipids in this study.

There was a significant decrease in postprandial lipemia after the fat-rich meal (−29%), which was significant but somewhat lower than the decrease (−50%) we observed in response to a ketogenic diet rich in monounsaturated fat and supplemented with (n-3) polyunsaturated fatty acids (9). In contrast to our results, Miller et al. (26) reported that a low-fat (19% of total energy)/high-carbohydrate (64% of total energy) diet significantly reduced postprandial lipemia compared with a diet higher in fat (41% of total energy) in normolipidemic men. The high-fat diet in the study by Miller et al. (26) still contained significant amounts of carbohydrate (42% of total energy), which likely explains the conflicting results with our postprandial TAG response in men that consumed a very low carbohydrate (8% of total energy) diet.

The significant reduction in fasting TAG was probably due to the combination of a reduced VLDL production rate, which has been shown to increase on a high-carbohydrate diet (27), and an increase in TAG removal because high-fat diets (46–65% of total energy) significantly increase postheparin plasma LPL activity and skeletal muscle LPL activity in humans (28–30). A greater VLDL-TAG pool size would also compete

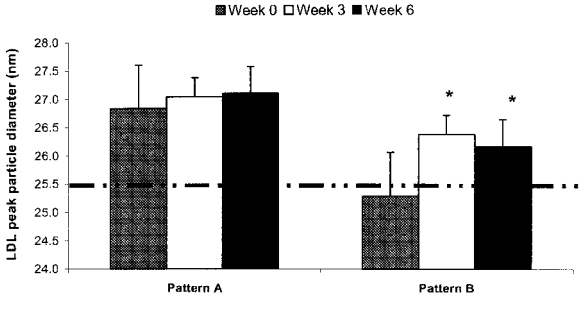


**FIGURE 2** Peak LDL particle size responses to a ketogenic diet in normolipidemic, normal-weight men classified as pattern A ($n$ = 7) or pattern B ($n$ = 5) at the start of the diet. Individuals with pattern A have a predominance of large LDL particles and those with pattern B have a predominance of smaller LDL particles. The peak particle diameter for pattern B is usually <25.5 nm. *$P \leq 0.05$ from corresponding wk 0 value.

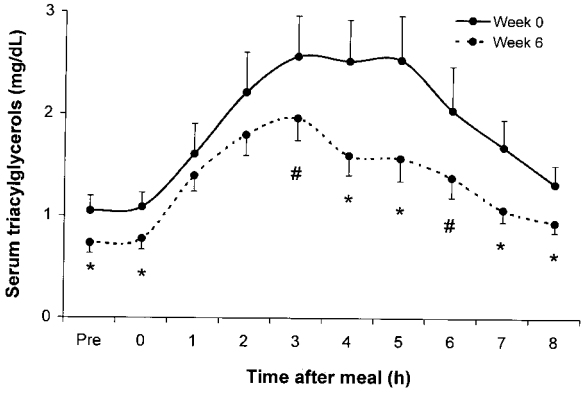


**FIGURE 3** Postprandial serum triacylglycerol (TAG) responses (mean ± SD; $n$ = 12) to a fat-rich meal before (wk 0) and after (wk 6) a ketogenic diet ($n$ = 12) in normolipidemic, normal-weight men. The area under the postprandial TAG curve was significantly ($P \leq 0.05$) lower (−29%) after the ketogenic diet (17.47 ± 9.3 to 12.39 ± 4.2 mmol/L × h). *$P \leq 0.05$ from corresponding wk 0 value. #$P \leq 0.01$ from corresponding wk 0 value.

with TAG from intestinal origin for removal during the postprandial period. Thus, elevated fasting TAG (primarily VLDL-TAG) is associated with enhanced postprandial TAG (primarily chylomicron-TAG) due to competition for removal (31). It follows then that a reduction in fasting TAG should be directly related to a reduction in TAG responses to a fat-rich meal, which was the case in this study ($r = 0.59$; $P < 0.05$). Although the majority of studies have reported significant correlations between changes in fasting and postprandial TAG (9), a recent study demonstrated that a dietary regimen that lowered fasting TAG did not result in a reduction in postprandial TAG (32), emphasizing the importance of measuring postprandial TAG to assess overall CVD risk.

Dietary cholesterol intake increased >100% (332–741 mg/d) when subjects switched to the ketogenic diet, which would be predicted to result in significant increases in total cholesterol and LDL cholesterol, although these were not significantly elevated after 6 wk of the ketogenic diet. There is great variability in the responsiveness of blood cholesterol after increases in dietary cholesterol, which may be due to differences in hormonal factors, obesity, and genetic predisposition (33). There were changes in the distribution of the LDL subfractions that would be considered favorable in terms of CVD. We observed general increases in the mean and peak LDL particle sizes during the ketogenic diet, which were more pronounced in subjects that exhibited a pattern B distribution at the start of the study. Individuals with pattern B exhibit a predominance of small dense lipoproteins and this distribution is associated with increased risk of CVD (13,14) and was recently shown to be the best discriminate factor for the presence of CVD even when adjusting for other risk factors (21). Although the characterization of pattern B is likely to have a genetic origin (22), changes in diet are known to influence the distribution of LDL subclasses. For example, switching to a fat-rich diet (46% vs. 24% of total energy) was shown to increase mean particle diameter and large LDL-1 mass and decrease small dense LDL-III cholesterol (28), while reductions in dietary fat have the opposite effect (6,7). Despite the changes in LDL size, we did not observe any significant changes in oxidized LDL concentrations. Collectively these studies indicate that when dietary fat is reduced, the distribution of LDL moves toward a smaller more dense particle and when dietary fat is increased the distribution of LDL moves toward a larger less dense particle. The reason some individuals are more stable in their LDL subclass distribution in response to changes in diet is unknown but is likely to reflect complex interactions between metabolic and genetic traits that are influenced to varying extents depending on the level of dietary fat (1,7).

We observed a significant decrease in fasting and postprandial insulin responses after the ketogenic diet. Decreases in resting insulin concentrations have been reported in response to 3–4 d of a low-carbohydrate diet high in fat (34–38). The mechanism for such a response probably resides in the greater reliance on fat oxidation induced by dietary carbohydrate restriction (39) and subsequent reduced requirement for insulin to assist in glucose uptake. To our knowledge, the reduced postprandial insulin response to a fat-rich meal observed after a ketogenic diet has not been reported in the literature. According to our estimate of insulin resistance using fasting levels of glucose and insulin, subjects in this study were not insulin resistant and there was no adverse effect of the ketogenic diet on insulin sensitivity. This is in agreement with other studies showing no adverse effects on glucose metabolism or insulin resistance after ketogenic diets using the insulin clamp technique (40,41).

Numerous studies now suggest that high-carbohydrate diets can raise TAG levels, create small, dense LDL particles, and reduce HDL cholesterol (i.e., atherogenic dyslipidemia)—a combination along with insulin resistance, that has been termed syndrome X (42,43). Syndrome X is postulated to be resistance to insulin-mediated glucose disposal by muscle (44), 30% of adult males and 10% to 15% of postmenopausal women have this particular syndrome X profile, which is associated with several-fold increase in heart disease risk. Replacing saturated fat with carbohydrate appears to accentuate insulin concentrations and the atherogenic dyslipidemia associated with syndrome X (44,45). The ketogenic diet in this study resulted in favorable responses in fasting TAG, postprandial lipemia, HDL-C, LDL particle size, and insulin levels in healthy normolipidemic men. Although the duration of the diet was short (6 wk), these data suggest that a ketogenic diet does not have an adverse effect on accepted biochemical risk factors for CVD and improves those associated with syndrome X.

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