Responses of inflammatory markers to a low-fat, high-carbohydrate diet: effects of energy intake

Sidika E Kasim-Karakas, Alex Tsodikov, Uma Singh, and Ishwaral Jialal

ABSTRACT

Background: Inflammation contributes to atherogenesis. Dietary fats may be proinflammatory.

Objective: The objective was to determine whether energy intake modulates the effects of low-fat, high-carbohydrate intakes on inflammatory markers.

Design: Twenty-two healthy postmenopausal women with a mean (±SD) age of 61 ± 11 y, who were not receiving hormone replacement therapy, were fed eucaloric diets to reduce their fat intake from 35% to 15% of energy. Next, the women consumed a 15%-fat ad libitum diet under free-living conditions. Serum highly sensitive C-reactive protein, interleukin 6, HDL serum amyloid A, and adiponectin concentrations were measured at the end of the eucaloric and ad libitum low-fat, high-carbohydrate intakes.

Results: The eucaloric diet decreased adiponectin from 16.3 ± 2.1 to 14.2 ± 2.0 mg/L (P < 0.05) and increased triacylglycerol from 131 ± 11 to 164 ± 14 mg/dL (P < 0.01). The ad libitum low-fat diet caused 6 kg weight loss and decreased highly sensitive C-reactive protein from 4.3 ± 0.6 to 2.5 ± 0.5 mg/L (P < 0.01), decreased HDL serum amyloid A from 10.3 ± 1.8 to 5.7 ± 1.3 mg/L (P < 0.001), increased adiponectin from 14.2 ± 2.0 to 16.3 ± 1.7 mg/L (P < 0.05), and decreased triacylglycerol from 164 ± 14 to 137 ± 15 mg/dL (P < 0.05).

Conclusion: During the eucaloric phase, the low-fat, high-carbohydrate diet exerted unfavorable effects on the inflammatory markers. In contrast, the ad libitum low-fat, high-carbohydrate intake caused weight loss and affected inflammatory markers favorably. Thus, the energy content of a low-fat, high-carbohydrate diet determines changes in inflammatory markers. Am J Clin Nutr 2006;83:774–9.

KEY WORDS Inflammatory markers, low-fat diet, weight loss, energy intake

INTRODUCTION

Inflammation plays a crucial role in atherogenesis (1). Inflammatory markers are independent predictors of coronary artery disease risk (2–4). High concentrations of C-reactive protein (CRP), serum amyloid A (SAA), and interleukin 6 (IL-6) predict increased risk (3). These markers also correlate with the risk of insulin resistance and the metabolic syndrome (5). In contrast, the adipose tissue cytokine adiponectin correlates with insulin sensitivity and confers protection against the metabolic syndrome and diabetes (6, 7).

Although inflammatory markers are valuable prognostic indicators, it is not yet clear whether the modification of these markers can protect against coronary artery disease (8, 9). Weight and dietary fat intake are among the modifiable lifestyle factors that affect the inflammatory markers: body weight and adiposity correlate directly with serum CRP and IL-6 and inversely with adiponectin (10–13). Weight loss reduces serum CRP and IL-6 (14). Dietary saturated fat, trans fat, and cholesterol intakes also correlate with serum CRP and IL-6, and the restriction of these fats reduces CRP and IL-6 concentrations (15–18). Weight loss may be a more significant regulator than dietary fat because high-fat, very-low-carbohydrate and low-fat diets have been shown to reduce inflammatory markers (19). In fact, a low-fat, high-carbohydrate intake can increase the inflammatory process in the liver (20).

Although dietary fat restriction and weight loss affect the inflammatory markers favorably, it is difficult to distinguish their independent effects because long-term dietary fat restriction usually causes weight loss (21–23). This research aimed to distinguish the effects of dietary fat restriction from those of the energy restriction and weight loss. To accomplish this, the low-fat, high-carbohydrate diet was provided first as a eucaloric metabolic feeding and then the participants followed the diet ad libitum under free-living conditions.

SUBJECTS AND METHODS

Subjects

Twenty-two healthy postmenopausal women with a mean (±SD) age of 61 ± 11 y were recruited after signing the informed consent.  

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able food products. The subjects were not given any goals for free-living conditions using self-selected, commercially available foods. Noncompliance was defined as a difference in energy intake or body weight; the only goal was to restrict dietary fat intake. This was accomplished by providing education and training about the fat contents of food items, types of fats, calculating the amount of energy obtained from fat, principles of low-fat cooking, shopping for low-fat food items, and behavior modification techniques. Each participant also received 3 h of individual counseling with a dietitian to review the general principles of the intervention diet and to address specific questions. During this 8-mo period, the participants attended a potluck participatory dinner once a week. After the dinner, a 30-min session led by a dietitian and attended by the principal investigator was held to provide ongoing reinforcement and support.

**Dietary data**

Seven-day food records were obtained at study entry and once a month during the ad libitum diet. The data were analyzed by using an updated version of NUTRITION DATA SYSTEM 93 (University of Minnesota, Minneapolis, MN). The accuracy of the food records was confirmed by using food-frequency questionnaires.

**Inflammatory markers and biochemical measurements**

Fasting plasma and serum samples were obtained at baseline and at the end of the eucaloric 15%-fat and ad libitum 15%-fat diets (months 4 and 12, respectively). CRP was measured with a highly sensitive (hs) latex-enhanced immunonephelometric assay as reported previously (26). Both interassay and intraassay CVs were <5%. HDL-SAA was measured by enzyme-linked immunoassay, with the use of reagents from the Biosource (Camarillo, CA) in the supernatant fluid of the serum, after precipitation of the other lipoproteins with dextran sulfate and magnesium chloride as described previously (27). The mean CV of 3 batches of pooled serum samples assayed as 10 replicates was 9.2% for HDL-SAA. IL-6 was measured by enzyme-linked immunoassay with the human immunoassay kit (high sensitivity) from R & D Systems (Minneapolis, MN). Adiponectin was measured by radioimmunoassay with a kit from Linco Research (St Charles, MO) with a CV of 4–6.5%, as reported previously (28). Triacylglycerol and cholesterol were measured enzymatically by using kits from Sigma Diagnostics (St Louis, MO) with CVs of 3.6% and 1.9%, respectively. High-density lipoproteins were separated by using dextran sulfate and magnesium chloride precipitation. The CV for HDL cholesterol was 2%. The homeostatic model of assessment (HOMA) of insulin resistance was calculated by using the formula (22.5 × 18)/(fasting plasma insulin × fasting plasma glucose).

**Statistical analysis**

Statistical analyses were performed by using SPSS (version 14; SPSS Inc, Chicago, IL), STATISTICA (version 6.1; StatSoft Inc, Tulsa, OK), and R (version 2.2.0; Internet: http://cran.us.r-project.org/). Changes in energy intake, weight, and inflammatory markers were analyzed by using repeated-measures analysis of variance followed by Tukey’s honestly significant difference post hoc tests. A significance level of 0.05 was used to determine the statistical significance of the observed differences adjusted for multicomparisons. Pearson’s product moment correlation coefficient was used to assess baseline correlations between inflammation markers and anthropometric and metabolic variables as well as the correlation between changes in those variables. The
HDL cholesterol decreased from 66 to 4.3 mg/dL (
high-carbohydrate intake and the eucaloric intake
Comparisons between the long-term ad libitum low-fat,
RESULTS
Two of 22 women had baseline hs-CRP concentrations >10 mg/L. These subjects were considered to have active inflammation and all of their data were excluded from the analysis.
Changes in energy intake and weight
At baseline, self-reported energy intake was 1583 ± 61 kcal/d (Table 1). During the eucaloric phase, energy intake was greater than the self-reported intake (2238 ± 68 kcal/d), despite the small decrease in weight (−1.3 ± 0.4 kg) and body mass index (BMI: in kg/m²; −0.5 ± 0.2) (P < 0.001 for both). During the ad libitum diet, self-reported energy intake decreased significantly (−316 ± 91 kcal/d) and weight loss was greater (−2.5 ± 1.3 kg) (P < 0.05), triacylglycerol decreased from 164 ± 14 to 137 ± 15 mg/dL (P < 0.05).
Combined effects of the low-fat, high-carbohydrate intake and weight loss
When the data from the end of the study were compared with the baseline data, the following significant changes were observed (Table 1): HDL-SAA decreased from 8.7 ± 1.3 mg/dL (P < 0.05), glucose decreased from 101 ± 4 to 86 ± 2 mg/dL (P < 0.05), and HDL cholesterol decreased from 66 ± 5 to 54 ± 3 mg/dL (P < 0.001).
Relations between the inflammatory markers and the metabolic variables
Correlations between the baseline variables
At baseline, hs-CRP and adiponectin concentrations correlated with several of the anthropometric and metabolic variables (Table 2). hs-CRP correlated with weight (r = 0.748, P < 0.001), HDL cholesterol (r = 0.436), and IL-6 (r = 0.508).

TABLE 1
Changes from baseline (t0) in energy intake, weight, and inflammatory markers during eucaloric (eu) 15%–fat (t1) and ad libitum (ad lib) 15%–fat (t2) diets

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Energy (kcal)</th>
<th>Weight (kg)</th>
<th>BMI (kg/m²)</th>
<th>hs-CRP (mg/L)</th>
<th>HDL-SAA (mg/L)</th>
<th>IL-6 (mg/L)</th>
<th>Adiponectin (mg/L)</th>
<th>Glucose (mg/dL)</th>
<th>Insulin (μU/mL)</th>
<th>HOMA</th>
<th>hs-CRP (mg/L)</th>
<th>Adiponectin (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eu 15% fat</td>
<td></td>
<td>1583 ± 61</td>
<td>74.9 ± 3.5</td>
<td>29.1 ± 1.4</td>
<td>3.2 ± 0.6</td>
<td>8.7 ± 1.3</td>
<td>0.9 ± 0.2</td>
<td>16.3 ± 2.1</td>
<td>101 ± 4</td>
<td>15.0 ± 2.8</td>
<td>3.6 ± 0.6</td>
<td>131 ± 11</td>
<td>226 ± 8</td>
</tr>
<tr>
<td>Ad lib 15% fat</td>
<td></td>
<td>2238 ± 68</td>
<td>73.6 ± 3.4</td>
<td>28.6 ± 1.4</td>
<td>4.3 ± 0.6</td>
<td>10.3 ± 1.8</td>
<td>1.2 ± 0.3</td>
<td>14.2 ± 2.0</td>
<td>101 ± 6</td>
<td>12.8 ± 1.6</td>
<td>3.3 ± 0.5</td>
<td>164 ± 14</td>
<td>203 ± 9</td>
</tr>
<tr>
<td>Overall P2</td>
<td></td>
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<td></td>
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<td>t1,t0</td>
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<td>t2,t1</td>
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<tr>
<td>t2,t0</td>
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</tbody>
</table>
| Values determined by ANOVA and Tukey’s honestly significantly different post hoc test: *P < 0.01, †P < 0.001, ‡P < 0.05.

1 All values are x ± SEM, n = 20 for all values except insulin and homeostatic model of assessment of insulin resistance (HOMA), for which n = 11.
2 Values determined with an ANOVA F test for the change over time.
3,4 Values determined by ANOVA and Tukey’s honestly significantly different post hoc test: *P < 0.01, †P < 0.001, ‡P < 0.05.
4 All values are

Effects of the eucaloric low-fat, high-carbohydrate intake
When the data from baseline and the end of eucaloric feeding phase were compared, it was evident that the eucaloric low-fat diet had several adverse effects (Table 1). Adiponectin decreased from 16.3 ± 2.1 to 14.2 ± 2.0 mg/dL (P < 0.05), triacylglycerol increased from 131 ± 11 to 164 ± 14 mg/dL (P < 0.001), and HDL cholesterol decreased from 66 ± 5 to 54 ± 3 mg/dL (P < 0.001).

Comparisons between the long-term ad libitum low-fat, high-carbohydrate intake and the eucaloric intake
Compared with the eucaloric low-fat diet, the ad libitum low-fat diet caused several favorable changes (Table 1). hs-CRP decreased from 4.3 ± 0.6 to 2.5 ± 0.5 mg/dL (P < 0.01), HDL-SAA decreased from 10.3 ± 1.8 to 5.7 ± 1.3 mg/dL (P < 0.001), adiponectin increased from 14.2 ± 2.0 to 16.3 ± 1.7 mg/dL (P < 0.05), glucose decreased from 101 ± 6 to 86 ± 2 mg/dL (P < 0.05), and triacylglycerol decreased from 164 ± 14 to 137 ± 15 mg/dL (P < 0.05).
TABLE 3
Significant Pearson’s product moment correlations between the changes (Δ) in inflammatory markers and changes in anthropometric and metabolic variables during eucaloric 15%–fat (t₁–t₂) and ad libitum 15%–fat (t₁–t₂) diets and the entire study (t₁–t₂)

<table>
<thead>
<tr>
<th></th>
<th>hs-CRP</th>
<th>HDL-SAA</th>
<th>IL-6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Δt₁-t₂</td>
<td>Δt₁-t₂</td>
<td>Δt₁-t₂</td>
<td>Δt₁-t₂</td>
</tr>
<tr>
<td>HDL-SAA</td>
<td>0.570*</td>
<td>0.726**</td>
<td></td>
</tr>
<tr>
<td>IL-6</td>
<td>0.669*</td>
<td>0.710*</td>
<td>0.739*</td>
</tr>
<tr>
<td>Insulin</td>
<td>0.533*</td>
<td>0.484*</td>
<td>0.591*</td>
</tr>
<tr>
<td>HOMA</td>
<td>0.484*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cholesterol</td>
<td>-0.541*</td>
<td>-0.695*</td>
<td>-0.716*</td>
</tr>
</tbody>
</table>

* Significance for two-tailed test: P ≤ 0.01.
** Significance for two-tailed test: P ≤ 0.001.
* P ≤ 0.05.

Correlations between changes during the intervention

Changes in hs-CRP correlated with the changes in IL-6 (r = 0.669 during the eucaloric phase and r = 0.710 during the ad libitum phase; P < 0.001) and with the changes in HDL-SAA (r = 0.570 during the eucaloric phase and r = 0.726 during the ad libitum phase; P < 0.001) (Table 3).

Changes in HDL-SAA correlated with changes in IL-6 (r = 0.739 during the eucaloric phase and r = 0.592 during the ad libitum phase; P < 0.001) and with the changes in insulin resistance variables, such as insulin (r = 0.553 during the eucaloric phase; P < 0.05) and HOMA (r = 0.484 during the eucaloric phase and r = 0.591 at the end of the study; P < 0.05). Changes in HDL-SAA correlated inversely with the changes in total cholesterol (r = -0.541 during the eucaloric phase and r = -0.695 during the ad libitum phase; P < 0.05). Similar inverse correlations existed between IL-6 and total cholesterol [r = -0.716 (P < 0.001) during the eucaloric phase and r = -0.470 (P < 0.05) during the ad libitum phase].

DISCUSSION

This study provided the novel finding that the effects of a low-fat, high-carbohydrate intake on inflammatory risk markers depended on the level of energy intake and weight loss. The changes observed during the eucaloric phase were consistent with an unfavorable coronary artery disease risk profile, whereas the changes during the ad libitum phase were consistent with a favorable risk profile. The differences between baseline and the end of the study were smaller than the differences between the eucaloric and ad libitum phases, which suggests that the low-fat, high-carbohydrate intake partially negated the favorable effects of weight loss.

In a similarly designed study, Santos et al (29) reported that delayed hypersensitivity, as determined by skin testing for common antigens, tended to increase during eucaloric fat restriction. However, this increase became significant only after weight loss.

During the eucaloric phase, the metabolic feeding provided ñ=550 kcal more energy than did the self-reported baseline intake. Despite that the participants lost ñ=1.5 kg in 4 mo, accounting for a 94-kcal energy deficit/d. Thus, the participants had underreported daily energy intake by ñ=450 kcal/d. This finding is consistent with the underreporting that occurred in several other reports (30).

During the eucaloric low-fat, high-carbohydrate phase, despite the small amount of weight loss, several unfavorable changes occurred. Adiponectin decreased and hs-CRP, HDL-SAA, and IL-6 concentrations trended upward. Simultaneously, plasma triacylglycerol increased, HDL cholesterol decreased, and total cholesterol decreased. These findings were consistent with the findings of Liu et al (31), who reported that hs-CRP concentrations correlated directly with the glycemic load of the diet in 244 healthy women who participated in the Women’s Health Study (32). In this cross-sectional study, hs-CRP concentrations in the subjects who consumed the highest dietary glycemic load were twice those of subjects who consumed the lowest dietary glycemic load.

Several mechanistic studies indicate a relation between glucose and carbohydrate intakes and increased inflammation. Mohanty et al (33) were the first to report that glucose ingestion stimulated NADPH oxidase in polymorphonuclear leukocytes and mononuclear cells and increased the generation of reactive
oxygen species (ROS). Simultaneously, α-tocopherol concentrations decreased. Although oral lipid and protein loading (34) and a mixed meal (35) also increased ROS generation; the greatest increase occurred in response to glucose. After a mixed meal, increased generation of ROS was associated with increased nuclear binding of nuclear transcription factor κB in polymorphonuclear leukocytes and mononuclear cells and increased plasma CRP (35). In further support, Esposito et al (36) showed that intravenous glucose administration increased concentrations of the inflammatory markers IL-6, IL-18, and tumor necrosis factor α; this could be prevented by simultaneous infusion of the antioxidant glutathione. We observed direct correlations between the changes in IL-6 and hs-CRP during both the eucaloric and ad libitum phases of our study. The mechanism underlying this relation may have been the changes in oxidative stress, i.e., increases during the eucaloric phase and decreases during the ad libitum phase of the low-fat diet. An additional mechanism may explain the correlations between IL-6 and hs-CRP: dietary carbohydrates stimulate triacylglycerol production in the liver and can cause hepatic steatosis (20, 37). In patients with nonalcoholic fatty liver disease, carbohydrate intake correlates with the histologic evidence of inflammation (20), which then may lead to increased serum concentrations of the inflammatory markers.

Another unexpected finding was the decrease in serum adiponectin during the eucaloric phase. Pischon et al (38) recently reported that in the 532 male participants of the Health Professionals Follow-Up Study, serum adiponectin concentrations correlated inversely with the glycemic load and positively with the total fat content of the diet. However, another smaller cross-sectional study did not observe any relation between dietary macronutrient content and adiponectin concentrations in young male and female university students in Greece (39). Adiponectin is synthesized and secreted by the adipose tissue, and the link between the changes in serum adiponectin and dietary fat and carbohydrate, in the absence of weight change, is not evident.

The ad libitum low-fat, high-carbohydrate intake caused significant weight loss. Serum hs-CRP, SAA, IL-6, and triacylglycerol concentrations decreased and adiponectin concentrations increased. Ampale et al showed that weight loss decreases hs-CRP, IL-6, and HDL–SAA concentrations and increases adiponectin concentrations (14, 19, 31, 40–43). This relation between weight loss and changes in inflammatory markers may also be related to changes in oxidative stress. Dandona et al showed that a 2-d fast (44) as well as a 4-wk hypocaloric diet (45) reduced ROS generation by polymorphonuclear leukocytes and mononuclear cells. Studies investigating the effects of the dietary protein–carbohydrate ratio (46), the fat compared with the carbohydrate cells. Studies investigating the effects of the dietary protein–carbohydrate ratio (46), the fat compared with the carbohydrate

In conclusion, this study showed that, in the absence of energy restriction, a low-fat, high-carbohydrate intake can have unfavorable effects on inflammatory risk markers. In contrast, when accompanied by a decreased energy intake and weight loss, a low-fat, high-carbohydrate intake exerts favorable effects on inflammatory risk markers. All of the studies concluding that macronutrient composition was unimportant were conducted during weight loss. On the other hand, cross-sectional population studies showed that diets with a high glycemic load can adversely affect inflammatory markers. Further research is needed to examine the effects of dietary composition in weight-stable populations.

SEK-K designed the study, supervised the dietary intervention and data collection, wrote the manuscript, and organized the collaborative efforts. AT analyzed the data and helped prepare the manuscript. US carried out the laboratory assays for most of the inflammatory markers and helped prepare the manuscript. IJ supervised the laboratory assays for the inflammatory markers and provided significant advice during manuscript preparation. None of the authors had a conflict of interest.

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