Effects of moderate variations in macronutrient composition on weight loss and reduction in cardiovascular disease risk in obese, insulin-resistant adults

Tracey McLaughlin, Susan Carter, Cindy Lamendola, Fahim Abbasi, Gail Yee, Patricia Schaaf, Marina Basina, and Gerald Reaven

ABSTRACT
Background: Obese, insulin-resistant persons are at risk of cardiovascular disease. How best to achieve both weight loss and clinical benefit in these persons is controversial, and recent reports questioned the superiority of low-fat diets.

Objective: We aimed to ascertain the effects of moderate variations in the carbohydrate and fat content of calorie-restricted diets on weight loss and cardiovascular disease risk in obese, insulin-resistant persons.

Design: Fifty-seven randomly assigned, insulin-resistant, obese persons completed a 16-wk calorie-restricted diet with 15% of energy as carbohydrate and fat, respectively. Baseline and postweight-loss insulin resistance; daylong glucose, insulin, and triacylglycerol concentrations; fasting lipid and lipoprotein concentrations; and markers of endothelial function were quantified.

Results: Weight loss with 60% or 40% of energy as carbohydrate (5.7 ± 0.7 or 6.9 ± 0.7 kg, respectively) did not differ significantly, and improvement in insulin sensitivity correlated with the amount of weight lost (r = 0.50, P < 0.001). Subjects following the diet with 40% of energy as carbohydrate had greater reductions in daylong insulin and triacylglycerol (P < 0.05) and fasting triacylglycerol (0.53 mmol/L; P = 0.04) concentrations, greater increases in HDL-cholesterol concentrations (0.12 mmol/L; P < 0.02) and LDL particle size (1.82 s; P < 0.05), and a greater decrease in plasma E-selectin (5.6 ng/L; P = 0.02) than did subjects following the diet with 60% of energy as carbohydrate.

Conclusions: In obese, insulin-resistant persons, a calorie-restricted diet, moderately lower in carbohydrate and higher in unsaturated fat, is as efficacious as the traditional low-fat diet in producing weight loss and may be more beneficial in reducing markers for cardiovascular disease risk. Am J Clin Nutr 2006;84:813–21.

KEY WORDS Obesity, weight loss, low-carbohydrate diet, macronutrient, insulin resistance, hyperinsulinemia, hypocaloric diet

INTRODUCTION

The prevalence of obesity is increasing rapidly, and obesity is projected to soon become the leading cause of death in the United States (1). The mainstay of treatment for obesity is dietary-induced weight loss, and the American Diabetes Association (ADA), American Heart Association (AHA), and National Institutes of Health, Lung, and Blood Institute (NHLBI) recommend that weight loss is best accomplished by following a low-calorie diet with ≤30% of energy from fat (2–4).

The clinical utility of the low-fat, high-carbohydrate diets recommended by the ADA, AHA, and the NHLBI has been challenged by reports that weight loss is more effectively accomplished by consumption of high-fat, very-low-carbohydrate diets, which have the added benefit of a greater decrease in plasma triacylglycerol and a greater increase in HDL-cholesterol concentrations (5–9). However, evidence shows that short-term benefits of high-fat, very-low-carbohydrate diets are not sustained (10) and that, after the diet was followed for 12 mo, the amount of weight lost by subjects following diets that were either very low in fat and high in carbohydrate or very high in fat and low in carbohydrate did not differ significantly (5, 10, 11). Indeed, irrespective of the diet being consumed, the only variable predicting the amount of weight loss was the self-reported history of dietary compliance (11).

Given the above findings, it appears that the most efficient way to achieve sustained weight loss is to provide to patients a dietary program that they can continue to follow. Although the details are likely to vary from patient to patient, it would be expected that the closer the program is to a patient’s usual dietary habits, the more successful it would be. Consistent with this notion is evidence that the more extreme the diet, the lower the adherence rate over time (12).

Thus, it seems useful to evaluate the effect of more moderate variations in macronutrient content on the ability to lose weight and on the associated changes in cardiovascular disease (CVD) risk factors. It is worth emphasizing that published studies of the effects of differences in macronutrient content on weight loss and related CVD risk factors did not take into consideration the importance of the differences in insulin resistance that exist in obese persons. Because not all obese persons are insulin resistant (13, 14), and because CVD risk is greatest in the subset of obese

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persons who have insulin resistance, it is particularly important
to consider the potential effect of differences in macronutrient
composition on weight loss in this population (14, 15). More-
over, the improvement in insulin sensitivity and the decrease in
CVD risk that occur in association with weight loss occur pri-
marily in those obese persons who are also insulin resistant (13,
15). Thus, it is important both to compare the effects of calorie-
restricted diets with more moderate differences in macronutrient
composition and to perform these comparisons in insulin-
resistant subjects—the persons who will benefit the most from
weight loss. We have addressed both issues by enrolling only
subjects who were obese and insulin resistant and by comparing
their responses to 2 calorie-restricted diets that differed in rela-
tive fat and carbohydrate content by only 20% of total calories.
In addition to assessing the effects of the 2 diets on weight loss, we
evaluated their effect on several CVD risk factors, including
measures of insulin sensitivity; plasma glucose, insulin, and lipopro-
tein concentrations; and markers of endothelial dysfunction.

SUBJECTS AND METHODS

Subjects

Subjects in the greater San Francisco Bay area were recruited
by newspaper advertisements. To qualify, volunteers were re-
quired to have a body mass index (in kg/m²) between 29 and 36,
no recent (≤3 mo) history of weight loss, and no history of major organ disease. At a screening visit to the Stanford University Medical Center General Clinical Re-
search Center (GCRC), a medical history was taken, weight and
height were measured, and hematocrit, creatinine, alanine amino-
transferase, and plasma glucose concentrations were measured be-
fore and after a 75-g oral glucose challenge. Volunteers with
clinical or laboratory evidence of anemia, kidney, liver disease,
or diabetes mellitus were excluded from further participation.

Subjects gave written informed consent. The protocol was
approved by the Stanford University Human Subjects Commit-
tee and conducted according to the regulations of the Health
Insurance Portability and Accountability Act of 1996.

Quantification of insulin-mediated glucose disposal

Volunteers meeting the general eligibility criteria were further
evaluated by quantification of insulin-mediated glucose uptake by
using a modification (16) of the insulin suppression test as originally
described and validated (17, 18). Briefly, after an overnight fast,
subjects were given an infusion of octreotide (0.27 μg · m²/min),
insulin (25 mU · min⁻¹ · m²), and glucose (240 mg · min⁻¹ · m²) for 180
min. Blood was drawn 4 times at 10-min intervals from 150 to 180
min of the infusion to measure plasma glucose (Trinder Assay;
Diagnostic Chemical Limited, Oxford, CT; CV 5.5%) and insu-
lin (ACTIVE Insulin Enzyme-Linked Immunosassay; Diagnostic
Systems Laboratories, Webster, TX; CV 5.2%), and the mean of
these 4 values was used as the steady-state plasma insulin and
plasma glucose (SSPG) concentrations for each subject. Because steady-
state plasma insulin concentrations are similar in all subjects
during the insulin suppression test, the SSPG concentration pro-
vides a direct measure of the ability of insulin to mediate the disposal
of an infused glucose load; the higher the SSPG concentration, the
more insulin-resistant the person. On the basis of a prior study of
the distribution of SSPG concentrations in 490 healthy nondiabetic
adults (19), we classified volunteers as insulin-resistant if their
SSPG concentration was in the top tertile of the SSPG distribu-
tion as previously described. The use of the top tertile for the
purpose of classification is based on prospective studies showing
that it is these persons who are sufficiently insulin resistant to
experience adverse clinical consequences (20, 21).

Measurement of cardiovascular disease risk markers

Subjects meeting the criteria for insulin resistance who were
willing to enter the weight-loss phase of the study were readmit-
ted to the GCRC for measurement of their daylong plasma glu-
cose, insulin, and triacylglycerol concentrations in response to
breakfast and lunch. For this purpose, they were given test meals
containing 15% of energy as protein, 42% of energy as carbo-
hydrate, and 43% of energy as fat at 0900 (20% of daily caloric
intake) and 1200 (40% of daily caloric intake), and blood was
drawn 9 times at hourly intervals, during which test meals were
administered at 0 h (after the 0800 blood draw) and at 4 h (after
the 1200 blood draw). For statistical analyses, daylong insulin,
glucose, and triacylglycerol concentrations were calculated via
the trapezoidal method as the area under the curve (AUC) of the
values for the 9 time points.

Additional blood was drawn before breakfast on the same day
as the meal profile described above, for measurement of lipid and
lipoprotein subclass concentrations by using the Vertical Auto
Profile II method (22, 23). In addition to measuring triacylglyc-
erol and LDL- and HDL-cholesterol concentrations, this method
estimates the concentration of narrow-density LDL cholesterol
to provide a “true” measure of LDL cholesterol that does not
include intermediate-density lipoprotein or lipoprotein(a).
LDL-particle phenotype was based on the measurement of the
LDL-cholesterol peak in the density gradient on a relative
scale of 1 to 200 s, with 0 s representing the most dense and
200 s representing the least dense lipoprotein peak maximum.
Definition of the LDL subclass pattern was based on LDL peak
maximum time: pattern A was defined as an LDL peak of >118
s, pattern B as an LDL peak of <115 s, and the intermediate
pattern as an LDL peak of >115 s and ≤118 s. Apolipoprotein B
(apoB) concentrations were measured as described previously
(24), as were concentrations of soluble intracellular adhesion
molecule 1 (ICAM-1), vascular cellular adhesion molecule 1,
and E-selectin (25) by using an enzyme-linked immunosorbert
assay (R&B, San Bruno, CA); the CVs were 6.1%, 7.7%, and
5.7%, respectively.

Dietary intervention

After completion of baseline measurements, subjects were
randomly assigned to a 16-wk period of caloric restriction, dur-
ing which time they were advised to consume 1 of 2 diets, each
of which contained 15% of total calories as protein, 7% as satu-
rated fat, 200 mg cholesterol/d, and 20 g fiber/d. However, the 2
diets varied in carbohydrate (40% versus 60% of energy), mono-
unsaturated and polyunsaturated fat (38% versus 18% of energy),
and total fat (45% versus 25% of energy). Daily caloric
requirements were calculated by using the Harris-Benedict equa-
tion (26) and an activity factor, and meal plans based on a
750-kcal/d deficit from estimated caloric requirements were
given to each subject. In addition, subjects had an initial 1–2 h of
nutrition education by research dietitians who used the Exchange
Lists for Meal Planning for 2003 of the ADA and the American
Diabetes Association to implement each subject’s individual
macronutrient patterns. Subjects were specifically instructed to restrict exchange-list choices to low-saturated-fat items (eg, selecting only from nonfat or lowfat dairy, monounsaturated and polyunsaturated fat, and very lean to lean protein food lists) and to tally daily servings throughout each day to ensure consumption of prescribed servings from each food group. Subjects prepared their own food and kept detailed food records to enhance compliance and to enable monitoring. Each week, subjects returned to the GCRC for a weigh check and a 15–20-min visit with the study dietitian to review their food records. Compliance with the assigned diet was assessed at weekly follow-up visits by evaluation of each food record for accuracy of macronutrient estimation and compliance with prescribed total calorie and macronutrient composition; this procedure used both Exchange Lists for Meal Planning and FOOD PROCESSOR software (version 8.0; ESHA, Portland, OR). Questionnaires were administered monthly for assessment of satiety and ease of adherence; both were rated on a scale of 1 to 10, in which 1 represented least satiety or ease of adherence and 10 represented complete satiety or ease of adherence.

After the 16 wk of calorie restriction, a 2-wk period of weight maintenance was initiated, during which time participants were instructed about a eucaloric diet that was based on their weight at the end of the intervention but with a macronutrient content similar to that of the hypocaloric diet. At the end of the 14-d period of weight maintenance (ie, week 18), all of the baseline measurements were repeated. However, in this instance, the day-long measurements of plasma glucose and insulin were made in response to the experimental diets, ie, the diet with 40% of energy as carbohydrate (the 40% carbohydrate diet) and that with 60% of energy as carbohydrate (the 60% carbohydrate diet).

Statistical analysis

This study was designed with 90% power, with 2-sided \( \alpha = 0.05 \) (type I error), to detect a 5% difference in weight loss between the 2 groups. On the basis of the SDs observed in the current study, the number of subjects needed to treat to detect this difference was 14/group. Nonnormally distributed variables (ie, triacylglycerol) were log transformed for statistical tests. Statistical analyses used unpaired Student’s \( t \) tests or chi-square tests for comparison of the 2 groups at baseline. Within-group changes were calculated by using paired Student’s \( t \) tests for all subjects combined and for dietary subgroups if the main effect of diet was statistically significant. The between-group differences in magnitude of change for all primary endpoints were compared by using analysis of covariance (ANCOVA), with diet as the main factor and the baseline concentration of the variable as a covariate. Results are presented as the mean (95% CI) absolute difference between groups and the adjusted \( P \) value for between-group comparisons. Evaluation of the significance of within-group changes was done separately by dietary subgroups if between-group comparisons were significant or for both dietary groups combined if the between-group comparisons were not significant. Evaluation of the potential for interaction between weight loss and diet with regard to improvement in insulin sensitivity was done by using ANCOVA after adjustment for baseline SSPG and weight, percentage decline in weight, diet, and diet \( \times \) weight-loss interaction. In ANCOVA models, Tukey’s adjustment for multiple comparisons was employed. Data are presented as mean \( \pm \) SD. We considered \( P < 0.05 \) (type I error), to detect a 5% difference in weight loss and diet criteria, 66 (60%) were insulin resistant as defined by their SSPG concentration at baseline, and all but 1 volunteered to be randomly assigned to 1 of the 2 experimental diets, which yielded an initial cohort of 65 subjects. Four persons in each experimental group (12% of the total group) dropped out before completing the weight-loss period. The baseline characteristics of the 57 persons completing the study—30 assigned to the 60% carbohydrate diet and 27 assigned to the 40% carbohydrate diet—are shown in Table 1. It is apparent from that table that the baseline clinical variables of the 2 groups did not differ significantly.

Estimates from food diaries of the macronutrient content consumed are given in Table 2, and it appears that the differences between the 2 groups in fat and carbohydrate intakes may have

<table>
<thead>
<tr>
<th>Variable</th>
<th>60% Carbohydrate diet ((n = 30))</th>
<th>40% Carbohydrate diet ((n = 27))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>(53 \pm 10)</td>
<td>(48 \pm 11)</td>
</tr>
<tr>
<td>M/F (%)</td>
<td>39/61</td>
<td>46/54</td>
</tr>
<tr>
<td>Ethnicity (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>80</td>
<td>88</td>
</tr>
<tr>
<td>Asian</td>
<td>10</td>
<td>4</td>
</tr>
<tr>
<td>Hispanic</td>
<td>10</td>
<td>8</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>94.3 \pm 14.0</td>
<td>95.0 \pm 10.9</td>
</tr>
<tr>
<td>BMI (kg/m(^2))</td>
<td>33.0 \pm 2.3</td>
<td>32.3 \pm 1.8</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>5.6 \pm 0.6</td>
<td>5.7 \pm 0.4</td>
</tr>
</tbody>
</table>

\(^1\) Between-group comparisons were not statistically significant, \(P > 0.05\) (unpaired Student’s \(t\) test for continuous data and chi-square test for categorical data).

\(^2\) \(\bar{x} \pm SD\) (all such values).

### RESULTS

Of the 106 obese persons meeting the general eligibility criteria, 66 (60%) were insulin resistant as defined by their SSPG concentration at baseline, and all but 1 volunteered to be randomly assigned to 1 of the 2 experimental diets, which yielded an initial cohort of 65 subjects. Four persons in each experimental group (12% of the total group) dropped out before completing the weight-loss period. The baseline characteristics of the 57 persons completing the study—30 assigned to the 60% carbohydrate diet and 27 assigned to the 40% carbohydrate diet—are shown in Table 1. It is apparent from that table that the baseline clinical variables of the 2 groups did not differ significantly.

Estimates from food diaries of the macronutrient content consumed are given in Table 2, and it appears that the differences between the 2 groups in fat and carbohydrate intakes may have

### TABLE 2

<table>
<thead>
<tr>
<th>Macronutrient</th>
<th>60% Carbohydrate diet ((n = 30))</th>
<th>40% Carbohydrate diet ((n = 27))</th>
<th>(P^1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrate</td>
<td>(57 \pm 4)^2</td>
<td>(41 \pm 4)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Protein</td>
<td>(18 \pm 2)</td>
<td>(18 \pm 2)</td>
<td>0.70</td>
</tr>
<tr>
<td>Total fat</td>
<td>(25 \pm 4)</td>
<td>(41 \pm 5)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Saturated fat</td>
<td>(9 \pm 2)</td>
<td>(8 \pm 2)</td>
<td>0.14</td>
</tr>
<tr>
<td>Polyunsaturated or monounsaturated fat</td>
<td>(16 \pm 3)</td>
<td>(33 \pm 4)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

^1 Unpaired Student’s \(t\) test.

^2 \(\bar{x} \pm SD\) (all such values).
been slightly less than intended. Protein intake was 18% in both groups, according to these estimates.

The results in Table 3 indicate that patients lost weight in response to both the 60% carbohydrate (5.7 ± 0.7 kg) and 40% carbohydrate (6.9 ± 0.7 kg) dietary interventions, representing a mean decrease of 5.7% and 6.9% of initial body weight, respectively (P < 0.0001 for weight change in the 2 groups combined). However, the difference in the amount of weight loss was not significant when the 2 diet programs were directly compared. In this context, self-reported satiety (7.7 ± 1) and ease of adherence (7.6 ± 1) also did not differ significantly in the 2 dietary groups, and neither measure correlated with the amount of weight lost.

Similarly, although SSPG concentration declined significantly overall (P < 0.0001 for the 2 groups combined), the difference in the improvement in insulin sensitivity when the 2 diets were compared was not significant. Furthermore, there was no significant interaction between percentage weight loss and diet (the 60% carbohydrate compared with the 40% carbohydrate diet) with respect to decrease in SSPG in the group as a whole. On the other hand, irrespective of diet, the results in Figure 1 show that the more weight that was lost, the greater the magnitude of the decrease in SSPG concentration (ρ = 0.50, P < 0.001 for all subjects combined).

Both systolic and diastolic blood pressures were also significantly lower (P < 0.0001 and P < 0.02, respectively, for the 2 groups combined) after consumption of either of the test diets. However, the magnitude of the decreases in blood pressure responses did not vary as a function of the diet consumed: when the 2 diet groups were directly compared, the differences in systolic and diastolic blood pressures were not significant (P = 0.30 and 0.70, respectively).

Comparison of the changes in daylong insulin, glucose, and triacylglycerol concentrations in response to the 40% and 60% carbohydrate diets, quantified as area under the curve during the 8-h meal profile, is also shown in Table 3. These comparisons, adjusted via ANCOVA for baseline (ie, AUC) values, showed no significant difference in daylong glucose, whereas the decline in daylong insulin was significantly greater in the group following the 40% carbohydrate diet than in the group following the 60% carbohydrate diet, but this difference was not significant when the 2 groups were directly compared. The changes in insulin sensitivity varied by moderate differences (15–20%) in the relative proportion of dietary fat and carbohydrate; this finding is consistent with the results of 2 previously published studies (27, 28). However, neither of those studies limited their subjects to obese, insulin-resistant persons to lose weight was not affected by moderate differences (15–20%) in the relative proportion of carbohydrate and fat in calorie-restricted diets; this finding is consistent with the results of 2 previously published studies (27, 28). However, neither of those studies limited their subjects to obese persons who were also insulin resistant, and, in one of the studies (27), all of the food was prepared in a metabolic kitchen. Consequently, we believe our study is the first to show that providing dietary advice to obese, insulin-resistant persons leads to significant weight loss when they follow calorie-restricted diets with moderate reductions in dietary carbohydrate, and that the amount of weight loss is similar when the relative amounts of dietary fat and carbohydrate vary by 20%.

In addition, insulin sensitivity significantly improved in association with weight loss in both diet groups, and, the more the weight lost, the greater the improvement in insulin sensitivity.
**TABLE 3**  
Changes in weight and cardiovascular disease risk factors in response to a hypocaloric diet containing 60% and 25% or 40% and 45% of energy as carbohydrate and fat, respectively

<table>
<thead>
<tr>
<th>Variable</th>
<th>60% Carbohydrate diet (n = 30)</th>
<th>40% Carbohydrate diet (n = 27)</th>
<th>Between-group comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before diet</td>
<td>After diet</td>
<td>Before diet</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>94.3 ± 14(^4)</td>
<td>88.6 ± 14</td>
<td>95.0 ± 10.9</td>
</tr>
<tr>
<td>SSPG (mmol/L)</td>
<td>12.9 ± 1.8</td>
<td>10.8 ± 2.4</td>
<td>13.7 ± 2.6</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>126 ± 2</td>
<td>121 ± 12</td>
<td>124 ± 12</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)</td>
<td>74 ± 9</td>
<td>72 ± 9</td>
<td>76 ± 7</td>
</tr>
<tr>
<td>Daylong triacylglycerol AUC (mg/dL - 8 h)</td>
<td>605 ± 239</td>
<td>478 ± 54</td>
<td>469 ± 5.5</td>
</tr>
<tr>
<td>Daylong insulin AUC (μU/ml - 8 h)</td>
<td>866 ± 99</td>
<td>880 ± 95</td>
<td>860 ± 97</td>
</tr>
<tr>
<td>Daylong triglyceride AUC (mmol/L - 8 h)</td>
<td>3157 ± 2935</td>
<td>2720 ± 2569(^6)</td>
<td>3286 ± 1700</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>5.2 ± 1.0</td>
<td>4.9 ± 1.0</td>
<td>4.7 ± 0.9</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/L)</td>
<td>3.6 ± 1.0</td>
<td>3.4 ± 1.0</td>
<td>3.0 ± 0.7</td>
</tr>
<tr>
<td>Narrow-density LDL cholesterol (mmol/L)</td>
<td>2.8 ± 0.9</td>
<td>2.7 ± 0.8</td>
<td>2.4 ± 0.6</td>
</tr>
<tr>
<td>Non-HDL cholesterol (mmol/L)</td>
<td>4.1 ± 1.0</td>
<td>3.9 ± 1.0</td>
<td>3.7 ± 0.9</td>
</tr>
<tr>
<td>Apolipoprotein B (mmol/L)</td>
<td>159 ± 39</td>
<td>151 ± 39</td>
<td>143 ± 35</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/L)</td>
<td>41 ± 8</td>
<td>40 ± 9</td>
<td>38 ± 9</td>
</tr>
<tr>
<td>Total:HDL cholesterol</td>
<td>5.1 ± 0.2</td>
<td>5.0 ± 0.2</td>
<td>5.0 ± 0.2</td>
</tr>
<tr>
<td>LDL size (s)</td>
<td>1.16 ± 0.6</td>
<td>1.16 ± 0.5</td>
<td>1.14 ± 0.6</td>
</tr>
<tr>
<td>ICAM-1 (ng/mL)</td>
<td>650 ± 34</td>
<td>596 ± 26</td>
<td>652 ± 40</td>
</tr>
<tr>
<td>VCAM-1 (ng/mL)</td>
<td>856 ± 33</td>
<td>857 ± 28</td>
<td>895 ± 40</td>
</tr>
<tr>
<td>E-selectin (ng/mL)</td>
<td>114 ± 10</td>
<td>92 ± 8</td>
<td>94 ± 10</td>
</tr>
</tbody>
</table>

\(^1\) Baseline values (before diet) did not differ significantly between groups, \(P > 0.05\) (unpaired Student’s \(t\) test). AUC, area under the curve (measured by the trapezoidal method); SSPG, steady-state plasma glucose; ICAM-1, intracellular adhesion molecule 1; VCAM-1, vascular cellular adhesion molecule 1.

\(^2\) ANOVA.

\(^3\) Analysis of covariance for between-group differences in variable change adjusted for baseline value of the variable.

\(^4\) SD (all such values).

\(^5\) Value of variable after diet was significantly different from baseline (\(P < 0.05\), paired Student’s \(t\) test) for both groups combined, but between-group comparison was not statistically significant.

\(^6\) Significantly different from baseline (\(P < 0.05\), paired Student’s \(t\) test) when the between-group comparison was statistically significant.
(r = 0.50, P < 0.001). Furthermore, the diet × weight-loss interaction for insulin sensitivity was not statistically significant, despite a trend toward greater improvement in SSPG in subjects following the 40% carbohydrate diet. This finding is consistent with previous studies of variations in macronutrients in eucaloric diets (29, 30) and suggests that macronutrient composition does not play a major role in determining changes in insulin sensitivity during either weight loss or weight maintenance.

The third CVD risk factor that improved in response to the reduced calorie intake was blood pressure, which was lower in both diet groups after weight loss. The difference in the observed decrease as a function of variation in macronutrient content was not significant.

In contrast, several CVD risk factors—daylong insulin concentrations, lipid and lipoprotein concentrations, and cellular adhesion molecules—improved to a significantly greater degree in the subjects following the 40% carbohydrate diet than in the subjects following the 60% carbohydrate diet. From a mechanistic point of view, the most central finding was that daylong plasma insulin concentrations were significantly lower in subjects following the 40% carbohydrate diet than in those following the 60% carbohydrate diet. This finding is both consistent with considerable prior data and not surprising: the more carbohydrate ingested by insulin-resistant persons, the more insulin they must secrete to maintain glucose tolerance (31–33).

Not only does evidence exist that insulin resistance and compensatory hyperinsulinemia are CVD risk factors (20, 21, 34, 35), but these changes have also been shown to play a pathogenic role in the second area in which the 40% carbohydrate diet was associated with greater clinical benefit: changes in lipid and lipoprotein metabolism (31–33). Hyperinsulinemia in the presence of insulin resistance is associated with hypertriglyceridemia, a low HDL-cholesterol concentration, an increase in small, dense LDL particles, and enhanced postprandial lipemia (31–33), which is presumably secondary to an increase in hepatic VLDL triacylglycerol synthesis and secretion (36, 37). The observation that all of these CVD risk factors improved to a significantly greater degree in subjects following the 40% carbohydrate diet than in those following the 60% carbohydrate diet is a predictable consequence of the fact that daylong insulin concentrations were lower in the insulin-resistant subjects assigned to the former diet. That the decrease in postprandial triacylglycerol concentrations was of significantly greater magnitude in subjects following the 40% diet than in those following the 60% carbohydrate diet may seem paradoxical in view of the former group’s relatively greater fat intake, but published data show that, in eucaloric diets, the postprandial accumulation of plasma triacylglycerol and triacylglycerol-rich remnant lipoproteins is enhanced in response to a higher carbohydrate intake (32, 33), and this is in line with the findings in the current study.

High triacylglycerol and low HDL-cholesterol concentrations; small, dense LDL particles; and an increase in postprandial lipemia are part of the atherogenic lipoprotein profile that is characteristic of insulin-resistant persons, and they lead to greater CVD risk (41). An improvement in these variables should decrease CVD risk in those assigned to the 40% carbohydrate diet, which is reminiscent of the beneficial changes associated with gemfibrozil treatment in the Veterans Affairs HDL Intervention Trial (42). However, in the context of a decrease in CVD risk in the subjects following the 40% carbohydrate diet, it is necessary to address the fact the LDL-cholesterol and narrow-density LDL-cholesterol concentrations increased significantly in persons assigned to this diet. This change was most likely secondary to the decrease in plasma triacylglycerol concentrations that resulted in a shift toward larger, more buoyant LDL particles, and that interpretation is consistent with the fact that apoB concentration did not change—ie, the number of LDL particles did not increase. Furthermore, total:HDL, a marker of CVD risk, did not increase in the subjects following the 40% carbohydrate diet. Consequently, we would argue that these findings do not negate the improved lipid profile seen in subjects who lost weight on the 40% carbohydrate diet.

Finally, evidence exists that the plasma concentrations of several cellular adhesion molecules produced by the endothelium are greater in persons with diseases associated with insulin resistance and increased CVD risk, and the more insulin resistant an apparently healthy person, the higher his or her plasma concentrations of E-selectin and ICAM-1 (43). The decreases observed in plasma concentrations of E-selectin and ICAM-1 support the view that endothelial function improves after moderate weight loss, which is another beneficial effect on CVD risk seen when obese, insulin-resistant persons lose weight. Although the observed changes in adhesion molecule concentrations were seen in both diet groups, the reduction in E-selectin was significantly (P = 0.02) greater in the subjects following the 40% carbohydrate diet than in those following the 60% carbohydrate diet, and ICAM-1 showed a similar, albeit nonsignificant (P = 0.07) trend.

In summary, obese, insulin-resistant persons lose comparable amounts of weight when following calorie-restricted diets that vary moderately in carbohydrate and fat content, and the amount of weight lost is the most important determinant of the improvement in insulin sensitivity. The improvement in insulin sensitivity and the decrease in blood pressure did not vary significantly as a function of the diet ingested. However, beneficial changes associated with weight loss in terms of lipoprotein metabolism, postprandial insulin and triacylglycerol concentrations, and markers of endothelial function improved to a greater degree in

![FIGURE 1. Relation between weight loss and change in insulin sensitivity as measured by steady-state plasma glucose concentrations (SSPG; n = 57) in subjects following the diet with 60% of energy as carbohydrate (60% CHO) and subjects following the diet with 40% of energy as carbohydrate (40% CHO).](https://academic.oup.com/ajcn/article-abstract/84/4/813/4633002)
subjects following the 40% carbohydrate diet than in those following a 60% carbohydrate diet. However, the period of weight loss was relatively short, and the apparent benefit of the 40% carbohydrate diet should be confirmed in a longer study. Furthermore, because the protein content was the same on both diets, we could not evaluate the putative benefit of diets that are relatively high in both fat and protein. On the other hand, in published weight-loss studies using various dietary approaches that are described as “high in protein” (5, 10), protein intake is reported to be in the range of 17% to 22% of total daily calories, which is not significantly different from the estimated protein intake in the current study. Finally, because we provided only dietary recommendations, and not actual meals, a lack of precise dietary control may have obscured metabolic effects related to differences in dietary macronutrient content. However, the data in Table 2 suggest that the patients consumed diets with respective macronutrient contents close to the differences that we planned to evaluate, and, by providing guidelines rather than meals, we

FIGURE 2. Weight loss–associated changes in daylong glucose, insulin, and triacylglycerol concentrations during standardized meal profile testing. Measurements were made before and after weight loss in both diet groups—ie, subjects following a diet with 60% of energy as carbohydrate and 25% of energy as fat (n = 30; panels on the left) and subjects following a diet with 40% of energy as carbohydrate and 45% of energy as fat (n = 27; panels on the right). Standardized meals were given at 0 h and 4 h (arrows). Statistical significance was assessed by using analysis of covariance with area under the curve (AUC) for each dependent variable as a function of intervention status (before compared with after the dietary intervention and adjusted for the baseline concentration of the dependent variable). The diet × status interaction for insulin and triacylglycerol AUCs was significant (P = 0.005 and 0.0004, respectively). P values shown in the figure are from paired t tests comparing the AUCs before and after the dietary intervention (weeks 0 and 18, respectively).
closely approximated real-life conditions. Despite these limitations, our results suggest that a calorie-restricted diet that is moderately greater in unsaturated fat and lower in carbohydrate resulted in amounts of weight loss comparable to those seen with the diet currently recommended by the ADA, AHA, and NHLBI and led to perhaps greater reductions in CVD risk than did the recommended diet. Finally, because the 40% carbohydrate diet does not require major changes in the average US diet, and because compliance, rather than macronutrient content, appears to be the major contributor to effective weight loss (10), a calorie-restricted diet containing 40% carbohydrate may represent an attractive alternative to either a high-fat, very-low-carbohydrate diet or the low-fat, 60% carbohydrate diet currently recommended by national organizations.

TM and GR were responsible for the overall study design; TM was responsible for data collection and analysis; TM and GR were responsible for writing the manuscript; SC and PS provided dietary advice to the subjects; CL was responsible for administrative and human subjects issues; CL, FA, and MB were responsible for patient recruitment and contributed to data collection; and GY performed the laboratory analyses. None of the authors had a personal or financial conflict of interest.

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