The few studies that have examined body composition after a carbohydrate-restricted diet have reported enhanced fat loss and preservation of lean body mass in obese individuals. The role of hormones in mediating this response is unclear. We examined the effects of a 6-week carbohydrate-restricted diet on total and regional body composition and the relationships with fasting hormone concentrations. Twelve healthy, normal-weight men switched from their habitual diet (46% carbohydrate) to a carbohydrate-restricted diet (8% carbohydrate) for 6 weeks and 8 men served as controls, consuming their normal diet. Subjects were encouraged to consume adequate dietary energy to maintain body mass during the intervention. Total and regional body composition and fasting blood samples were assessed at week 0, 3, and 6 of the experimental period. Fat mass was significantly (P < .05) decreased (−3.4 kg) and lean body mass significantly increased (+1.1 kg) at week 6. There was a significant decrease in serum insulin (+34%), and an increase in thyroid hormone (T4 +11%) and the free T3 index (+15%). Approximately 70% of the variability in fat loss on the carbohydrate-restricted diet was accounted for by the decrease in serum insulin concentrations. There were no significant changes in glucagon, total or free testosterone, sex hormone-binding globulin (SHBG), insulin-like growth factor-I (IGF-I), cortisol, or triiodothyronine (T3) uptake, nor were there significant changes in body composition or hormones in the control group. Thus, we conclude that a carbohydrate-restricted diet resulted in a significant reduction in fat mass and a concomitant increase in lean body mass in normal-weight, men which may be partially mediated by the reduction in circulating insulin concentrations. Copyright 2002, Elsevier Science (USA). All rights reserved.

THE POPULARITY of diets with the common theme of restricting intake of carbohydrate while increasing protein and fat has increased in recent years. Surprisingly few scientific studies have examined the physiologic effects of a carbohydrate-restricted diets. Extreme restriction of carbohydrate results in a ketogenic state of ketosis and these diets are commonly referred to as "ketogenic diets." The most common application of ketogenic diets is for weight loss. Several studies have documented that very low carbohydrate diets result in greater weight loss compared to isocaloric diets higher in carbohydrate. Yet few studies have examined the effects on body composition.

To our knowledge, only 3 studies have assessed body composition responses to a carbohydrate-restricted diet. These studies involved hypotensive diets in a small number of obese subjects. Nevertheless, they indicate that a carbohydrate-restricted diet low in energy results in body composition changes that favor loss of fat mass and preservation (perhaps even increase) in lean body mass. The mechanism(s) explaining the greater fat loss and preservation of lean body mass on a carbohydrate-restricted diet remains unclear. The regulation of lipolysis/spoilage and protein synthesis/misfolding is highly influenced by the endocrine system. For example, insulin is a potent inhibitor of lipolysis at physiological concentrations, whereas cortisol, thyroid hormones, and glucagon stimulate lipolysis. Protein balance is influenced by simultaneously.

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MATERIALS AND METHODS

Experimental Design

The study design involved a group of normal-weight men that switched from their habitual diet (48% carbohydrate) to a very low carbohydrate diet (8% carbohydrate) for 6 weeks. Body composition was measured and 2 consecutive 24-hour fasting blood samples were collected at weeks 0, 3, and 6 of the diet intervention. A separate group of normal-weight men was recruited to establish reliability of the dependent variables. Control subjects continued to follow their habitual diet, and performed the same experimental tests as the intervention subjects.

Subjects

Twenty healthy men free of metabolic and endocrine disorders volunteered to participate. Twelve subjects volunterred to switch from their habitual diet to a carbohydrate-restricted diet for 6 weeks (mean ± SD; age, 36.7 ± 10.6 years; body mass, 89.2 ± 8.3 kg; percent fat, 20.5 ± 6.2%) and the remaining 8 subjects served as controls (age, 35.0 ± 13.0 years; body mass, 85.4 ± 12.8 kg; percent fat, 22.2 ± 9.1%). The subjects had not lost or gained weight in the previous year. None used diet.
CARBOHYDRATE RESTRICTION AND BODY COMPOSITION

attending to special diets or regular consumers of nutritive supple-
ments, and habitually consumed between 19% and 41% of energy as fat (as
assessed via a 7-day food diary). All subjects were non-smokers, and
not currently taking any medication known to affect the hormones
measured in this study. Subjects were moderately active performing a
variety of different aerobic and weight-bearing routines, but none were
competitive athletes. In the carbohydrate-restricted diet group, 1 sub-
ject was sedentary, 5 performed regular aerobic exercise (2 x 4
 workouts/week for 15 to 90 minutes) and moderate exercise
(2 x 0.5 workouts/week for 45 to 120 minutes). Subjects were
required to maintain their current level of physical activity during the
study. All subjects were informed of the purpose and possible risks
of the study and provided written informed consent document ap-
proved by the institutional review board.

Diary Intervention

The aim of the intervention diet was to reduce carbohydrate intake to
5% to 10% of energy. The diet was designed so that fat comprised
approximately 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, steak),
poultry (e.g. chicken, turkey), fish, oils, cheese, eggs, various nuts/seeds
and peanut butter, vegetables, salads with low-carbohydrate dressing,
potato 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),
bread, cereals, beans, rice, desserts/swifts, or any other foods con-
taining significant amounts of carbohydrates. A portion of the foods
consumed during the intervention diet (<50% to 40% of total energy)
were provided to subjects during weekly meetings to review compli-
ance with the registered dietitian. These foods included pumpkin seeds,
russet potatoes, low-carbohydrate bars, shakes, and make it/1 eating
Nuttinuts, Inc., Happsau, NY) and potato powders (Super Whey
Fat and Fuel Plexa Line, Twin Laboratories, Happsau, NY). Subjects
were also provided with a daily multivitamin/mineral complex (Daily
One Cap With Iron, Twin Laboratories).

Each subject received individual dietary instruction weekly on how
to consume meals within the specified nutrient goals and to assess
intake during the intervention diet. Weekly food logs containing specific
lists of appropriate foods, recipes, and sample meal plans that were
comparable with their individual preferences and the nutrient profile
goals of the intervention diet. Food monitoring sheets and scales were
provided to all subjects prior to the study to assist in the estimation of
portion sizes of foods and beverages. Subjects keep records every day
of the experiment (7 days during baseline and 42 days during the very
low carbohydrate diet) and the control group kept 7-day records during
weeks 1 and 4. All recorded days were analyzed for nutrient content
(Nutritionist V. Version 2.3, N-Squared Computing, Fitit Dishawer
Division. The Health Corporation, San Bruno, CA). Subjects were also
provided with log sheets to record any physical activity performed
during the experimental period.

Body Composition

Total and regional body composition was assessed using dual-energy
X-ray absorptiometry (DXA) with a total body-scanner (Polariscan,
Lunar Corp., Madison, WI) that uses a constant potential x-ray source of
76 kV and a certain filter that produces dual-energy x-rays of 52 and
62 kV. Soft tissue mass, which comprised of fat mass and lean body
mass, is measured pixel-by-pixel as a beam of photons penetrate the
body. The speed and energy of the dual-energy x-rays is attenuated by
approximately 6 minutes while the scanning arm of the DXA passed
teen their body from head to toe in parallel 10 cm strips. Percent body
fat from the DXA imaging was subsequently calculated as fat mass
divided by the total soft tissue mass plus the estimated bone mineral
content. Regional analyses of the trunk, arm, and leg regions were
automatically calculated according to anatomic landmarks by the
calculator software. All analyses were performed by the same techni-
cian using computer algorithms (software version 2.1.2708). Quality
assurance was assessed by analyzing a phantom spine provided by the
company and daily calibrations were performed prior to all scans using
a calibration block provided by the manufacturer. Interclass correlation
coefficients for DXA procedures were obtained using an independent
body mass, fat mass from repeated scans on a group of men and women
in our laboratory.

Blood Collection and Analyses

Fastin blood samples were obtained on 2 separate days at weeks 0,
3, and 8 after a 12-hour overnight fast and abstinence from alcohol and
smoking for at least 4 hours. Subjects reported to the laboratory
between 7 am and 9 am, rested quietly for 10 minutes in the supine
position, and blood was obtained from an antecubital vein with a
20-gauge needle and Vacutainer (Becton Dickenson, Franklin Lakes,
NJ). Within 15 minutes, whole blood was centrifuged (1200 x for 15
minutes at 4 °C) and the resultant plasma or serum divided into aliquots.
A comprehensive metabolic screening profile was performed that
assessed serum glucose, albumin, total protein, minerals (sodium,
potassium, chloride, calcium, phosphorus, magnesium, iron), renal
function (blood urea nitrogen [BUN], uric acid, creatinine, bilirubin),
and liver function (alkaline phosphatase [ALP], alanine aminotransfer-
ase, aspartate aminotransferase, gamma-glutamyl transferase [GGT],
leakage dehydrogenase). Fasting serum [hydroxybutyrate concen-
trations were enzymatically determined in duplicate using a commercially
available kit (Sigma Diagnostics, St Louis, MO) and spectrophotome-
tric analysis (Spectronic 601, Milton Roy Co, Rochester, NY). Intra-
assay variance was 5%. Serum insulin concentrations were deter-
mined in duplicate using an enzyme-linked immunosorbent assay
(ELISA, Diagnostic Systems Laboratory, Webster, TX) with a sensi-
tivity of 0.107 pM -1 and an intra-assay variance of 8.0%. Thyroid
function tests included determination of triiodothyronine (T3) uptake
using a solid phase [125I]radioimmunoassay (RIA) and total thyroxine
(T4) concentrations using an ELISA kit with a sensitivity of 2.322
nMol/L -1. Intra-assay variance was 2.62%. The free, t3 index was
measured using [125I] T3 uptake (RIA) and a free, t3 index of 0.26. The
protein concentrations were determined in duplicate serum samples
using an enzyme immunosorbent assay (EIA; Diagnostic Systems Labora-
tory) with a sensitivity of 3.76 E13 M -1 and an intra-assay variance of
4.9%. Testosterone concentrations were determined in duplicate serum
samples using an EIA kit with a sensitivity of 0.14 nMol/L -1 and an
intra-assay variance of 2.5%. Insulin-like growth factor-I (IGF-I)
concentrations were determined in duplicate serum samples using an
enzyme immunosorbent assay (EIA; Diagnostic Systems Laboratory)
with a sensitivity of 2.56 E13 M -1 and an intra-assay variance of
14.2%. Glucagon concentrations were determined in duplicate plasma
samples using a double antibody [125I] RIA with a sensitivity of 3.7
pM -1. Absorbance for all ELISA and EIA samples were read on a
multiwell counter (Waltar/1420 Victor, Waltar Oy, Turku, Finland).

Statistical Analyses

Two-facing samples were obtained for each blood variable and the
mean of these 2 values used for statistical analysis. An analysis of
variance (ANOVA) with repeated measures was used to evaluate changes in body composition and hormones over time. Pearson's signifi-
cant F value was achieved, the Fisher's least significant differences
(LSD) test was used to locate the pairwise differences between
weeks. Relationships among the changes in hormones and body com-

positive were examined using Pearson's product-moment correlation coefficients. The level of significance was set at P < 0.05.

RESULTS

Dietary Inoue

Daily intakes of dietary energy and nutrients are presented in Table 1. All dietary nutrients were significantly different during the carbohydrate-restricted diet with the exception of dietary energy and alcohol consumption. Dietary protein, fat, and cholesterol were significantly greater and dietary carbohydrate was significantly lower (8% of total energy) during the carbohydrate-restricted diet. There were no significant changes in dietary nutrient intake in the control group. Data from exercise logs indicated that there were no changes in physical activity patterns of subjects.

Total and Regional Body Composition

Compared to body mass at week 0 (79.2 ± 8.3 kg), there was a small but significant decrease in body mass at weeks 3 (77.5 ± 7.7 kg) and 6 (77.0 ± 7.5 kg). There was no change in body mass in the control group (85.4 ± 12.8 to 85.8 ± 12.0 kg). Total and regional body composition responses are presented in Table 2 and Fig 1. Although the decrease in body mass was small (-2.2 kg), there was a significant decrease in total body percent fat at week 3 that significantly decreased further at week 6. Fat mass was significantly decreased at week 3 (-1.1 kg) and continued to decrease at week 6 (-3.3 kg). Soft tissue lean body mass significantly increased at week 6 (+1.1 kg). This same pattern of change in body composition (decreased fat mass and increased lean body mass) was observed for the arm, leg, and trunk regions as well. There were no significant changes in body mineral content. There were no significant changes in total and regional body composition in the control group.

Metabolic Responses

Serum β-hydroxybutyrate concentrations were significantly increased at week 3 (+427%) and remained significantly elevated at week 6 (+279%) in the carbohydrate-restricted group. All subjects demonstrated β-hydroxybutyrate concentrations above 0.20 mmol·L⁻¹ indicating compliance with the carbohydrate-restricted diet. All changes in the metabolic screening profile were small to moderate and within normal expected values for both the carbohydrate-restricted and control groups. There were significant decreases in serum AP (-10%), carbonic anhydrase (109) and GGT (18%), and significant increases in BUN (+43%), the BUN:creatinine ratio (+43%), and chloride (+3%) after the carbohydrate-restricted diet.

Hormonal Responses

There were no significant changes in any hormones in the control group (Table 3). After the carbohydrate-restricted diet, there was a significant decrease in serum insulin concentrations at week 3 (-19.4%) and week 6 (-34.2%). After 6 weeks of the carbohydrate-restricted diet there was also a significant increase in total T₄ (10.8%) and the free T₄ index (12.5%). There were no significant changes in glucagon, testosterone, sHbG, cortisol, IGF-I, or T₃ uptake. The only hormone significantly correlated with change in body composition was insulin. Using the week 6 data, the percent change in insulin was significantly related to the change in total and regional fat mass (r = -0.709 to -0.819) and percent fat (r = -0.709 to -0.830) (Fig 2).

DISCUSSION

The primary objective of this study was to examine how healthy normal-weight men respond to a carbohydrate-restricted diet and to examine the relationship with potential changes in the correlating hormonal milieu. Subjects consumed
Table 2. Total and Regional Percent Fat, LBM, FM, and BMC Responses to a 6-Week Carbohydrate-Restricted Diet

<table>
<thead>
<tr>
<th>Week</th>
<th>Low Carbohydrate Group (N = 7a)</th>
<th>Control Group (N = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Week 3</td>
<td>Week 5</td>
</tr>
<tr>
<td>Total</td>
<td>20.5 ± 2.2</td>
<td>18.8 ± 5.0*</td>
</tr>
<tr>
<td>LBM (kg)</td>
<td>60.4 ± 5.6</td>
<td>60.2 ± 5.0</td>
</tr>
<tr>
<td>FM (kg)</td>
<td>16.7 ± 5.9</td>
<td>15.0 ± 5.4*</td>
</tr>
<tr>
<td>BMC (kg)</td>
<td>3.4 ± 2.4</td>
<td>3.3 ± 2.4</td>
</tr>
<tr>
<td>Arms</td>
<td>13.5 ± 5.0</td>
<td>12.0 ± 5.3*</td>
</tr>
<tr>
<td>LBM (kg)</td>
<td>8.2 ± 0.8</td>
<td>8.2 ± 0.8</td>
</tr>
<tr>
<td>FM (kg)</td>
<td>1.4 ± 0.7</td>
<td>1.2 ± 0.6*</td>
</tr>
<tr>
<td>BMC (kg)</td>
<td>0.5 ± 0.1</td>
<td>0.5 ± 0.1</td>
</tr>
<tr>
<td>Legs</td>
<td>20.1 ± 5.5</td>
<td>18.7 ± 5.3*</td>
</tr>
<tr>
<td>LBM (kg)</td>
<td>18.9 ± 2.3</td>
<td>18.7 ± 2.2</td>
</tr>
<tr>
<td>FM (kg)</td>
<td>5.4 ± 1.8</td>
<td>4.9 ± 1.8*</td>
</tr>
<tr>
<td>BMC (kg)</td>
<td>1.3 ± 0.2</td>
<td>1.3 ± 0.2</td>
</tr>
<tr>
<td>Trunk</td>
<td>21.9 ± 7.5</td>
<td>21.8 ± 6.7*</td>
</tr>
<tr>
<td>LBM (kg)</td>
<td>28.1 ± 3.0</td>
<td>28.0 ± 2.4</td>
</tr>
<tr>
<td>FM (kg)</td>
<td>9.4 ± 3.6</td>
<td>8.3 ± 3.1*</td>
</tr>
<tr>
<td>BMC (kg)</td>
<td>1.1 ± 0.1</td>
<td>1.1 ± 0.1</td>
</tr>
</tbody>
</table>

*Note: Values are mean ± SD.

Abbreviations: LBM, soft tissue lean body mass; FM, fat mass; BMC, bone mineral content.

*P < 0.05 vs. corresponding week 0 value.

Fig 1. Changes in body composition after a 6-week carbohydrate-restricted diet. BM, body mass; FM, fat mass; LBM, lean body mass; BMC, bone mineral content. *P ≤ 0.05 from week 0 to week 6.

a diet that consisted of 8% carbohydrate (<50 g/d), 61% fat, and 30% protein. Adaptation to this carbohydrate-restricted diet resulted in a significant decrease in percent body fat and increase in lean body mass. Serum insulin was significantly decreased and serum total T4 increased. The decrease in serum insulin resulting from the reduction in carbohydrate was associated with the decrease in serum insulin. The contribution of other circulating metabolites (e.g., ketone bodies) and hormones (e.g., thyroid hormones) in mediating the changes in fat mass and lean body mass on a carbohydrate-restricted diet remain unclear.

Similar to our prior work, a significant decrease in body
Table 3. Hormonal Responses to a 6-Week Carbohydrate-Restricted Diet

<table>
<thead>
<tr>
<th>Week 0</th>
<th>Week 1</th>
<th>Week 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin (μM/ L^-1)</td>
<td>24.7 ± 16.3</td>
<td>19.1 ± 12.7</td>
</tr>
<tr>
<td>Glucagon (μM/ L^-1)</td>
<td>25.9 ± 10.4</td>
<td>25.6 ± 4.9</td>
</tr>
<tr>
<td>Total Insulin (μM/ L^-1)</td>
<td>20.7 ± 8.1</td>
<td>18.8 ± 7.8</td>
</tr>
<tr>
<td>Carbohydrate (μM/ L^-1)</td>
<td>618 ± 324</td>
<td>513 ± 105</td>
</tr>
<tr>
<td>SNS (μM/ L^-1)</td>
<td>452 ± 32.0</td>
<td>345 ± 22.3</td>
</tr>
<tr>
<td>IGF-I (μM/ L^-1)</td>
<td>39.8 ± 8.7</td>
<td>39.6 ± 14.0</td>
</tr>
<tr>
<td>TNF-α (μM/ L^-1)</td>
<td>32.8 ± 3.5</td>
<td>32.8 ± 2.9</td>
</tr>
<tr>
<td>Total TNF-α (μM/ L^-1)</td>
<td>58.2 ± 1.5</td>
<td>64.5 ± 10.1</td>
</tr>
<tr>
<td>Free T4 index</td>
<td>19.2 ± 3.1</td>
<td>21.1 ± 3.5</td>
</tr>
</tbody>
</table>

NOTE: Values are mean ± SD.

Abbreviations: SNS, serum hormone-binding globulin; IGF, insulin-like growth factor; T4, thyroid index; TNF-α, tumor necrosis factor alpha.

Fig 2. Correlation between the percent change in insulin concentrations and the change in total body fat mass (top) and percent fat loss (bottom) in response to a 6-week carbohydrate-restricted diet.
relatively low concentrations of insulin with a half-maximal effect occurring at a concentration of 12 pmol l^-1 and a maximal effect at a concentration of about 250 to 300 pmol l^-1. The significant reduction in insulin levels due to 23.7 to 15.6 pmol l^-1 may have been permissive to mobilisation of body fat on the carbohydrate-restricted diet. Although there cause and effect relationship cannot be established, it is interesting to note that there was a significant correlation between the decrease in insulin concentrations and the decrease in body fat on the carbohydrate-restricted diet (Fig. 2). We found the association with mean values circulating concentrations of hormones, which do not necessarily reflect changes in hormone bioavailability or secretion or acute uptake and signal induction. Thus, other hormones that affect lipid metabolism that were neither measured (eg, growth hormone, epinephrine) nor correlated to the change in fat mass (eg, cortisol, glucagon) may also contribute to the proportionally large decrease in fat mass. The significant increase in lean body mass on the carbohydrate-restricted diet was not explained. Infusions of β-hydroxybutyrate [the major ketone in the circulation] have been shown to reduce proteolysis during starvation. 26 Young et al. compared 3 isocaloric (1.80 kcal/g) isotopes ([15 g] diet differing in carbohydrate content (30, 60, and 104 g) consumed for 9 weeks in obese men. The diet with the lowest amount of carbohydrate (30 g/d) was associated with increased ketones, greater fat loss, and greater nitrogen retention and preservation of lean tissue compared to the diets with more carbohydrate. The overall effect of elevated ketones on nitrogen retention must be considered in the context of other stimuli (eg, growth hormone, testosterone, insulin) and inhibitory (eg, cortisol, catecholamines) hormones that regulate protein balance. We hypothesized that elevated β-hydroxybutyrate concentrations may have played a minor role in preventing catabolism of lean tissue on the carbohydrate-restricted diet but other metabolic hormones were likely involved (eg, growth hormone). Similar to our prior study, 26 we observed a significant decrease in fasting insulin concentrations after the carbohydrate-restricted diet. Decreases in insulin concentrations have been reported in response to 3 to 4 days of a low-carbohydrate diet high in fat. 27 The mechanism for such a response probably resides in the greater reliance on fat oxidation induced by the carbohydrate restriction and subsequent reduced requirement for insulin to assist glucose uptake. The significant increase in total T₃ and the free T₃ index was unexpected. Other studies have reported decreases in T₃ and no change to T₄ in response to reducing carbohydrate. 13,12,17,18 However, carbohydrate-restricted very low calorie diet caused a decline in T₃, but a carbohydrate-rich very low calorie diet. 26 In the present study, the nonsignificant change in T₄ uptake suggests that T₃ and T₄ binding proteins were not affected by carbohydrate restriction. Thus, the significant increase in total T₄ may represent an increase in the biologically active hormone available to cells. This interpretation should be made with caution since we did not directly measure concentrations of free T₄ or T₃, our diet was measured metabolic rate in these subjects. All the subjects adapted well to the restricted-carbohydrate diet and there were no adverse responses in any of the biochemical variables measured. Similar to data from prior carbohydrate-restricted diet studies, 26 there was a significant increase in serum BUN concentrations as the BUN/creatinine ratio, whereas creatinine concentrations remained stable. A disproportionate rise in BUN relative to creatinine is not indicative of renal areas. The increased BUN probably due to the greater amount of dietary protein available for hepatic urea synthesis leading to increased urea formation during the carbohydrate-restricted diet. Krebill et al. observed progressively higher BUN concentrations in healthy men consuming a low-carbohydrate diet that was gradually increased in protein so that the protein to fat ratio was raised in increments from 30%-70% to 70%-30%. In summary, a 6-week carbohydrate-restricted diet resulted in a favorable response in body composition (decreased fat mass and increased lean body mass) in normal-weight men. Our results indicate that endocrine adaptations may partially mediate the accelerated fat loss, in particular the decrease in circulating insulin concentrations. Further study of the metabolic and hormonal adaptations associated with carbohydrate-restricted diets is warranted considering the potential for favorable effects on body composition, especially given the widespread frequency of obesity in the United States. 28

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