Short-term weight loss and hepatic triglyceride reduction: evidence of a metabolic advantage with dietary carbohydrate restriction\textsuperscript{1-3}

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ABSTRACT

Background: Individuals with nonalcoholic fatty liver disease (NAFLD) have excess intrahepatic triglycerides. This is due, in part, to increased hepatic synthesis of fat from carbohydrates via lipogenesis. Although weight loss is currently recommended to treat NAFLD, little attention has been given to dietary carbohydrate restriction.

Objective: The aim of this study was to determine the effectiveness of 2 wk of dietary carbohydrate and calorie restriction at reducing hepatic triglycerides in subjects with NAFLD.

Design: Eighteen NAFLD subjects (n = 5 men and 13 women) with a mean (±SD) age of 45 ± 12 y and a body mass index (in kg/m\textsuperscript{2}) of 35 ± 7 consumed a carbohydrate-restricted (<20 g/d) or calorie-restricted (1200–1500 kcal/d) diet for 2 wk. Hepatic triglycerides were measured before and after intervention by magnetic resonance spectroscopy.

Results: Mean (±SD) weight loss was similar between the groups (−4.0 ± 1.5 kg in the calorie-restricted group and −4.6 ± 1.5 kg in the carbohydrate-restricted group; P = 0.363). Liver triglycerides decreased significantly with weight loss (P < 0.001) but decreased significantly more (P = 0.008) in carbohydrate-restricted subjects (−55 ± 14%) than in calorie-restricted subjects (−28 ± 23%). Dietary fat (r = 0.643, P = 0.004), carbohydrate (r = −0.606, P = 0.008), posttreatment plasma ketones (r = 0.755, P = 0.006), and respiratory quotient (r = −0.797, P < 0.001) were related to a reduction in liver triglycerides. Plasma aspartate, but not alanine, aminotransferase decreased significantly with weight loss (P < 0.001).

Conclusions: Two weeks of dietary intervention (−4.3% weight loss) reduced hepatic triglycerides by ∼42% in subjects with NAFLD; however, reductions were significantly greater with dietary carbohydrate restriction than with calorie restriction. This may have been due, in part, to enhanced hepatic and whole-body oxidation. This trial was registered at clinicaltrials.gov as NCT01262326.


INTRODUCTION

A major sequela of insulin resistance and obesity is non-alcoholic fatty liver disease (NAFLD), a spectrum of pathology ranging from simple triglyceride accumulation in hepatocytes to hepatic steatosis with inflammation (steatohepatitis), fibrosis, and cirrhosis (1). An astounding one-third of adults in a major urban center are affected by NAFLD and, if this prevalence is reflective of the nation as a whole, >71 million individuals have excess liver fat in the United States (2).

Data from rodent models suggest that a complex interplay between processes of lipid accrual (lipogenesis, lipolysis, and diet) and disposal (lipoprotein secretion and fatty acid oxidation) contribute to the deposition of triglycerides in liver. Importantly, hepatic de novo lipogenesis, a process that converts dietary carbohydrate into fat in the postprandial state, was recently documented to be constitutively active in humans with NAFLD (3, 4). Increased fatty acid synthesis generates malonyl-CoA, an intermediate that has been shown to inhibit carnitine palmitoyltransferase-1, the protein responsible for the transport of long-chain fatty acids into mitochondria where they undergo β-oxidation (5–7). These data led us to hypothesize that dietary carbohydrate restriction would be effective at reducing hepatic triglyceride content in NAFLD by limiting the accrual of hepatic lipids via lipogenesis and simultaneously enhancing their disposal via mitochondrial β-oxidation. Indeed, we previously applied this dietary approach in a single case study and showed a marked decline in hepatic triglyceride content after 5 wk, despite the requisite increase in dietary fat intake (8). However, whether dietary carbohydrate restriction was more effective than the currently recommended low-calorie, low-fat diet for the reduction of hepatic triglyceride was not addressed.

The present study was designed to build on our prior report and compare the effectiveness of carbohydrate and calorie restriction at reducing hepatic triglyceride content in individuals with hepatic steatosis after a similar degree of weight loss. The primary endpoint of the study was the change in hepatic triglyceride.

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content after 2 wk of dietary intervention, as determined by proton magnetic resonance spectroscopy (1H-MRS).

SUBJECTS AND METHODS

Participants

Eighteen consecutive patients who were evaluated by a single hepatologist (JDB) at The University of Texas Southwestern Medical Center for elevated liver function tests and found to have a diagnosis of NAFLD were asked to participate in the present study. All subjects had a negative viral hepatitis serologies, transferrin saturation <40%, normal z1-antitrypsin and ceruloplasmin concentrations, negative autoimmune liver disease markers (nuclear antibody, mitochondrial antibody, smooth muscle antibody, liver-kidney microsomal antibody), and no history of medication use associated with hepatic steatosis. On the basis of the interview with the treating physician, all subjects either abstained from alcohol or consumed <5 alcoholic beverages annually. Liver biopsy samples were obtained from 14 of 18 subjects as part of their clinical evaluation. None of the subjects were cirrhotic or had laboratory or radiographic evidence of portal hypertension. All subjects had stable weight in the 6 mo preceding enrollment, and none were actively engaged in a weight-loss program.

The protocol and consent form were approved by the Institutional Review Board of The University of Texas Southwestern Medical Center, and all participants provided written informed consent before enrollment.

Design

At enrollment, all subjects underwent 1H-MRS to determine liver triglyceride content and were assigned to either a low-carbohydrate or low-calorie diet in a semirandom manner to achieve equivalence between groups. Before the diet was initiated, all subjects underwent a teaching session with the Clinical and Translational Research Center (CTRC) dietitian. After completing the 2-wk dietary intervention, the subjects underwent a repeat 1H-MRS for the measurement of liver triglyceride content and had their respiratory quotient measured with a Delta Trak II indirect calorimeter (Sensormedics, Yorba Linda, CA).

Subjects assigned to a low-carbohydrate diet were instructed to limit carbohydrate intake to <20 g/d (9). They initiated this diet on their own for the first 7 d of the study, keeping a detailed dietary record. This was done to increase compliance and to add heterogeneity by allowing the subjects to individualize their diet with respect to protein and fat intake. The final 7 d was provided to subjects as frozen meals prepared by the CTRC kitchen in accordance with the energy intake and food preferences documented in the dietary record.

Subjects assigned to a low-calorie diet were asked to continue their regular diet for 4 d before the study and to record their food intake. On the basis of this dietary record, the CTRC kitchen prepared all meals for the 14 d of the study in accordance with the dietary composition and food preferences recorded by the individuals, but reduced in energy to 1200 kcal/d for women and to 1500 kcal/d for men following current recommendations for the treatment of NAFLD (10).

Blood samples

Blood was collected into heparin-treated tubes and centrifuged immediately to isolate plasma from red blood cells. Plasma glucose, cholesterol, triglyceride, HDL cholesterol, and aminotransferases were measured by using a Vitros 250 spectrophotometric analyzer (Ortho-Clinical Diagnostics, Rochester, NY). A commercially available enzyme-linked immunosorbent assay kit was used to measure insulin (Millipore, Billerica, MA). Other routine chemistry values were determined by a commercial laboratory (Quest Diagnostics, Madison, NJ).

MRS

Localized 1H-NMR spectra of the liver were acquired while the subjects were in the supine position by using a 1.5-T Gyroscan Intera MR system (Philips Medical Systems, Amsterdam, Netherlands) as previously described (11). Sagittal, coronal, and axial slices through the right lobe of the liver were acquired, and a 27-cm3 volume of interest was positioned, avoiding major blood vessels, intraparenchymal bile ducts, and the lateral margins of the liver. After the system was tuned and shimmed, spectra were collected by using a Q-body coil for radiofrequency transmission and signal reception and a double-echo PRESS sequence with interpulse delay (Tr = 3 s) and echo time (Te = 25 ms). The hepatic triglyceride content is expressed as a percentage of the methylene resonance referenced to the combined signal from both methylene groups and water (12, 13). Resonance areas were determined by using the 1-D NMR processor in ACD/Laboratories 9.0 (Advanced Chemistry Development, Toronto, Canada).

Statistical analysis

Statistical analyses were performed by using SigmaPlot 11.0 (Systat Software Inc, Chicago, IL). Differences between the 2 groups were evaluated by using unpaired t tests (means) or Mann-Whitney rank sums tests (medians). Differences between 2 repeated measures were analyzed by using the paired t test. Proportions were evaluated with Fisher’s exact test. Comparison of repeated measures between groups was performed by using 2-factor repeated-measures analysis of variance. Correlations between variables were determined by using the Spearman rank-sum test. Statistical significance was set at P < 0.05.

RESULTS

Subjects

The study consisted of 18 subjects who were equally divided between carbohydrate or calorie restriction. Subjects tended to be middle-aged and were primarily female (Table 1). The 2 dietary groups were matched with regard to age, sex, weight, and body mass index (BMI; in kg/m2). Fasting lipid profiles were also similar. Serum aminotransferase concentrations were similarly elevated in both treatment groups, as was fasting plasma glucose. The hepatic triglyceride content was elevated in all subjects (>5.6%) (11), with a similar degree of elevation in both treatment groups.

Intervention

All subjects completed their assigned diet and experienced weight loss (−4.3 ± 1.4 kg; P < 0.001). Despite differences in macronutrient composition (Table 2), weight loss was similar between the low-carbohydrate and low-calorie groups (Table 1). Plasma alanine aminotransferase (ALT) and fasting glucose
concentrations did not change significantly in either group, whereas plasma triglycerides and aspartate aminotransferase (AST) fell after 2 wk of weight loss. Plasma ketones were significantly higher (469 ± 171 compared with 1164 ± 548, P = 0.016) and respiratory quotients were significantly lower (0.89 ± 0.07 compared with 0.78 ± 0.04, P < 0.001) in the low-carbohydrate group. Posttreatment plasma insulin concentrations were similar between the groups (P = 0.191).

Aside from a single low-calorie subject, hepatic triglyceride content decreased in both groups (absolute reduction: −9 ± 8%, P < 0.001; relative reduction: −42 ± 23%, P < 0.001). Despite comparable overall weight loss, however, the decrease in hepatic triglyceride content was greater in the low-carbohydrate than in the low-calorie group (Table 1).

**Correlations**

No significant relation was observed between the degree of weight loss and the proportion of protein, fat, or carbohydrate in the diet. However, a significant association was observed between both dietary fat and carbohydrate intake and the magnitude of reduction in hepatic triglyceride content (r = 0.643, P = 0.004; r = −0.606, P = 0.008, respectively). No relation between changes in hepatic triglycerides during weight loss and the proportion of dietary protein was apparent (P = 0.378). The magnitude of reduction in liver triglycerides was also highly correlated with posttreatment plasma total ketone concentration and respiratory quotient (r = 0.755, P = 0.006, and r = −0.797, P < 0.001, respectively). Other posttreatment metabolic variables, such as plasma concentrations of cholesterol, triglycerides, glucose, insulin, and transaminases, showed no relation with reduction in liver triglyceride content.

**DISCUSSION**

In the current study, hepatic triglyceride content was measured in 18 subjects with NAFLD by using $^1$H-MRS before and 2 wk after obtaining equal weight loss via 2 dietary interventions that differed in macronutrient composition. A major finding of this study was that, regardless of dietary intervention, the intrahepatic triglyceride content rapidly decreased by ≈42% in NAFLD subjects after a ≈4.3% weight loss over 2 wk. However, given a similar degree of weight loss, the low-carbohydrate diet resulted in significantly greater intrahepatic triglyceride reduction than did the low-calorie diet (Table 1). The magnitude of liver triglyceride reduction during weight loss in all subjects was closely associated

**TABLE 1**

Characteristics of subjects before and after weight loss

<table>
<thead>
<tr>
<th></th>
<th>Low-calorie diet (n = 9)</th>
<th>Low-carbohydrate diet (n = 9)</th>
<th>P value $^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
<td>Before</td>
</tr>
<tr>
<td>Age (y)</td>
<td>47 ± 12 $^a$</td>
<td>—</td>
<td>42 ± 11</td>
</tr>
<tr>
<td>Sex ratio (F:M)</td>
<td>6:3</td>
<td>—</td>
<td>7:2</td>
</tr>
<tr>
<td>Liver biopsy</td>
<td>NAFLD activity score</td>
<td>4.3 ± 1.8</td>
<td>5.8 ± 1.6</td>
</tr>
<tr>
<td></td>
<td>Fibrosis stage</td>
<td>1.3 ± 1.4</td>
<td>0.8 ± 0.9</td>
</tr>
<tr>
<td></td>
<td>BMI (kg/m$^2$)</td>
<td>34 ± 9</td>
<td>33 ± 9</td>
</tr>
<tr>
<td>Body weight</td>
<td>Absolute (kg)</td>
<td>96 ± 21</td>
<td>92 ± 20</td>
</tr>
<tr>
<td></td>
<td>Fractional (%)</td>
<td>100</td>
<td>96 ± 1</td>
</tr>
<tr>
<td></td>
<td>Total cholesterol (mg/dL)</td>
<td>207 ± 29</td>
<td>204 ± 53</td>
</tr>
<tr>
<td></td>
<td>Triglycerides (mg/dL)</td>
<td>154 ± 65</td>
<td>115 ± 46</td>
</tr>
<tr>
<td></td>
<td>AST (UL)</td>
<td>56 ± 28</td>
<td>15 ± 4</td>
</tr>
<tr>
<td></td>
<td>ALT (UL)</td>
<td>77 ± 49</td>
<td>81 ± 45</td>
</tr>
<tr>
<td></td>
<td>AST:ALT ratio</td>
<td>0.8 ± 0.2</td>
<td>0.2 ± 0.1</td>
</tr>
<tr>
<td>Fasting glucose (mg/dL)</td>
<td>122 ± 55</td>
<td>106 ± 21</td>
<td>113 ± 34</td>
</tr>
<tr>
<td>Hepatic TG content (%)</td>
<td>Absolute</td>
<td>19 ± 10</td>
<td>14 ± 7</td>
</tr>
<tr>
<td></td>
<td>Fractional (%)</td>
<td>100</td>
<td>72 ± 23</td>
</tr>
</tbody>
</table>

$^1$ ALT, alanine aminotransferase; AST, aspartate aminotransferase; NAFLD, nonalcoholic fatty liver disease; TG, triglyceride.

$^2$ Analysis by 2-factor repeated-measures ANOVA unless otherwise indicated.

$^a$ Mean ± SD (all such values).

$^b$ Unpaired t test.

$^c$ Fisher’s exact test.

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**TABLE 2**

Characteristics of diets

<table>
<thead>
<tr>
<th></th>
<th>Low-calorie diet (n = 9)</th>
<th>Low-carbohydrate diet (n = 9)</th>
<th>P value $^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy intake (kcal/d)</td>
<td>1325 ± 180</td>
<td>1553 ± 517</td>
<td>0.229</td>
</tr>
<tr>
<td>Diet composition</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein (%)</td>
<td>16 ± 3</td>
<td>33 ± 4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fat (%)</td>
<td>34 ± 6</td>
<td>59 ± 7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Carbohydrate (%)</td>
<td>50 ± 4</td>
<td>8 ± 5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Protein (g/d)</td>
<td>53 ± 12</td>
<td>121 ± 34</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fat (g/d)</td>
<td>49 ± 9</td>
<td>105 ± 44</td>
<td>0.002</td>
</tr>
<tr>
<td>Carbohydrate (g/d)</td>
<td>169 ± 33</td>
<td>26 ± 8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fat intake (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saturated</td>
<td>42 ± 8</td>
<td>37 ± 4</td>
<td>0.134</td>
</tr>
<tr>
<td>Monounsaturated</td>
<td>37 ± 2</td>
<td>38 ± 6</td>
<td>0.634</td>
</tr>
<tr>
<td>Polyunsaturated</td>
<td>18 ± 7</td>
<td>15 ± 4</td>
<td>0.221</td>
</tr>
</tbody>
</table>

$^1$ All values are means ± SDs.

$^2$ Analysis by unpaired t test.
with the proportion of carbohydrate and fat in the diet, which indicated that macronutrient composition is an independent determinant of the fate of intrahepatic triglycerides during the initial period of negative energy balance. Indeed, the observed relation between both plasma ketones and respiratory quotient suggests that the fate of hepatic triglycerides during dietary weight loss is oxidative disposal—a process that occurs to a greater degree during carbohydrate restriction.

Regardless of dietary macronutrient composition, weight loss effectively reduced liver fat. However, our results indicate that there is a metabolic advantage to dietary carbohydrate restriction for the short-term reduction of liver triglyceride content. This advantage appears to be related to enhanced lipid oxidation mandated by reduced carbohydrate intake (14). Both calorie and carbohydrate restriction led to a dramatic increase in plasma ketone concentrations, consistent with enhanced ketogenesis and hepatic mitochondrial β-oxidation. However, ketone concentrations were significantly higher in carbohydrate-restricted individuals. Likewise, whole-body lipid oxidation, as assessed by respiratory quotient, was also significantly higher in the low-carbohydrate group. Taken together, these data indicate that the reduction in hepatic triglycerides observed after 2 wk of weight loss is associated with enhanced hepatic and whole-body lipid oxidation and that this enhancement occurred to a greater degree during carbohydrate restriction.

Currently, it is speculated that mitochondrial dysfunction is a key feature of NAFLD; however, the nature of this dysfunction remains unclear. Both in vitro and in vivo studies have found no difference in hepatic mitochondrial oxidative capacity between subjects with NAFLD and control subjects, whereas defects in oxidative phosphorylation may be present in nonalcoholic steatohepatitis (NASH) (15–19). Plasma ketone concentrations have been shown to be higher in subjects with NAFLD and NASH (15), which is indirect evidence of increased hepatic fatty acid oxidation. However, an alternative interpretation could involve impaired mitochondrial oxidation of ketones in peripheral tissue (20). There is also direct evidence that hepatic oxidation of long-chain fatty acids is elevated in obese insulin-resistant individuals (21), but similar studies in persons with NAFLD have not been performed. The rapid reduction in liver triglycerides in the present study, in association with enhanced lipid oxidation, suggests that mitochondrial oxidative capacity in NAFLD subjects is intact but perhaps mismatched relative to the lipid load during weight-stable conditions. Such a rapid response would be unlikely in the setting of an intrinsic defect in mitochondrial structure and function (16, 22). A harmonious explanation for this observation is substrate-level inhibition of β-oxidation, possibly consequent to increased hepatic de novo lipogenesis. This explanation is supported by the strong inverse relation between the magnitude of hepatic triglyceride reduction and dietary carbohydrate intake, perhaps because of the primary role of dietary carbohydrate in lipid synthesis.

Since our initial report (8) and another report (23), it has been unclear whether the effect of a low-carbohydrate diet on hepatic triglycerides was specific to the diet or a more general feature of weight loss. Although we have shown here a metabolic advantage of carbohydrate restriction, the increased effectiveness of this diet in reducing liver triglycerides cannot be extrapolated beyond the 2-wk period of observation. The only other study similar to ours was carried out by Kirk et al (24) in 22 subjects. They showed that carbohydrate restriction reduced hepatic triglycerides more than did calorie restriction after 48 h of negative energy balance (−2% weight); however, the 2 dietary interventions were equally effective by ≈11 wk of negative energy balance (−7% weight). Most studies that have examined dietary intervention in NAFLD have focused on calorie restriction, with or without fitness training, and have typically lasted ≥3 mo (10). Within the surgical literature, several studies have examined the effect of short-term calorie restriction (≈500–800 kcal/d for 2–12 wk) on hepatic fat due to the operative difficulties encountered as a consequence of hepatomegaly and reduced intraabdominal space. Consistent with the dramatic reduction in hepatic triglycerides in our study, 80% of the reduction in liver volume occurred in the first 2 wk of calorie restriction and was maximal by 8 wk (25). Likewise, the hepatic triglyceride content was reduced by 40% after 4 wk and by 43% after 12 wk (25, 26). Taken together, these data suggest that a pool of hepatic triglycerides is rapidly mobilized during energy restriction, especially in the absence of dietary carbohydrate. The remainder appears to be mobilized more slowly; gastric bypass patients were shown to achieve postoperative reductions in hepatic triglycerides of ≈60% and ≈90% only after 6 and 12 mo, respectively (27).

A surprising finding was that plasma AST was most responsive to the reduction in hepatic triglycerides, decreasing 37 ± 1 U/L after 2 wk (P < 0.001). Conversely, despite the reduction in liver fat, plasma ALT concentrations remained unchanged. Most subjects (16 of 18) were followed clinically at an average follow-up interval of 3.2 ± 1.3 mo after study completion. Although the posttreatment weight of the study population was unchanged at follow-up (P = 0.149), plasma ALT concentrations decreased significantly (from 88 ± 34 to 33 ± 16 U/L; P < 0.001), with normalization in 79% of subjects. The reason for this differential response of transaminases is unclear but may be related to differences in enzyme half-life, enhanced sinusoidal clearance, changes in release from the cytosolic and/or mitochondrial compartments, or changes in hepatic metabolic activity (28, 29). However, the decline in AST may prove to be clinically relevant to monitor the response during initial weight reduction in subjects with NAFLD.

Interpretation of the present study results is subject to limitations. This study was not designed to determine the therapeutic efficacy of the diets in NAFLD. Rather, the primary goal was to assess the physiologic response of persons with NAFLD to negative energy balance in the presence and near absence of dietary carbohydrate. As a result, postintervention liver biopsy samples were not performed, precluding our ability to comment on histologic improvement. Additionally, the results presented may be subject to referral bias because of the demographics of the study population (primarily middle-aged women).

In conclusion, 2 wk of dietary intervention resulting in a reduction in body weight of ≈4.3% reduced the hepatic triglyceride content by ≈42% in subjects with a clinical diagnosis of NAFLD. However, dietary carbohydrate restriction was significantly more effective at reducing liver triglycerides than was calorie restriction. Our data suggest that this was due to enhanced lipid disposal via hepatic and whole-body oxidation. Additionally, the increase in lipid oxidation in these subjects suggests that mitochondrial oxidative capacity is attenuated in NAFLD during weight-stable conditions. Of the serum transaminases, only AST...
decreased in parallel with the reduction in liver triglycerides. Despite reductions in the liver triglyceride content, ranging from 30% to 50% in these subjects, improvements in serum ALT concentrations were not initially apparent.

We thank the staff of the CTRC at the University of Texas Southwestern Medical Center and Jay D Horton for critical reading of the manuscript.

The authors’ responsibilities were as follows—JDB: designed and conducted the research, analyzed the data, performed the statistical analysis, wrote the manuscript, and had primary responsibility for the final content; JAB: analyzed the data and provided the essential materials; TR: conducted the research; SS: analyzed the data and provided essential materials; and SCE designed the research, analyzed the data, provided the essential materials, and wrote the manuscript. The authors had no conflicts of interest to report.

REFERENCES