Dig Dis Sci (2007) 52:589–593
DOI 10.1007/s10620-006-9433-5

ORIGINAL ARTICLE

The Effect of a Low-Carbohydrate, Ketogenic Diet on Nonalcoholic Fatty Liver Disease: A Pilot Study

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Received: 14 March 2006 / Accepted: 8 May 2006 / Published online: 12 January 2007

Abstract Nonalcoholic fatty liver disease is an increasingly common condition that may progress to hepatic cirrhosis. This pilot study evaluated the effects of a low-carbohydrate, ketogenic diet on obesity-associated fatty liver disease. Five patients with a mean body mass index of 36.4 kg/m^2 and biopsy evidence of fatty liver disease were instructed to follow the diet (<20 g/d of carbohydrate) with nutritional supplementation for 6 months. Patients returned for group meetings biweekly for 3 months, then monthly for the second 3 months. The mean weight change was -12.8 kg (range 0 to -25.9 kg). Four of 5 posttreatment liver biopsies showed histologic improvements in steatosis ($P = .02$) inflammatory grade ($P = .02$), and fibrosis ($P = .07$). Six months of a low-carbohydrate, ketogenic diet led to significant weight loss and histologic improvement of fatty liver disease. Further research is into this approach is warranted.

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Keywords Nonalcoholic fatty liver disease · NASH · Low-carbohydrate ketogenic diet · Cirrhosis · Fatty liver

Nonalcoholic fatty liver disease (NAFLD) is a clinicohistopathologic entity with features that resemble alcohol-induced liver injury, but by definition, occurs in patients with little or no history of alcohol consumption [1]. The prevalence of NAFLD is estimated to be about 26% in the United States [2, 3]. The prevalence is higher in obese adults; steatosis has been found in 60–70% of obese patients, with steatohepatitis present in 18.5% [4]. In adults with class 3 obesity (body mass index [BMI] ≥ 40 kg/m^2), steatosis and steatohepatitis increase to 91% and 29%, respectively [5]. By extrapolating to the general US population, where 65% of adults are overweight or obese [6], NAFLD presents a significant public health problem.

Although an association between NAFLD and obesity clearly exists, the pathogenesis of NAFLD has not been fully elucidated. Triglyceride, the principle component of human fat tissue, may be overproduced in the liver during conditions of obesity, starvation, and total parenteral nutrition, as well as increased dietary intake of carbohydrate [7, 8]. Other potential causes of fatty liver include choline deficiency, inappropriate metabolism of fatty acids in hepatocytes, elevated levels of serum free fatty acids, or improper packaging of very-low-density lipoprotein cholesterol particles [9, 10]. One widely supported theory implicates insulin resistance as the key mechanism leading to hepatic steatosis, and some have proposed that insulin resistance also contributes mechanistically to the development of steatohepatitis [11, 12]. These theories are supported indirectly by observations from several pilot studies, which have demonstrated beneficial effects from insulin-sensitizing medications in patients with NAFLD [13–15].

Recent clinical studies have shown a low-carbohydrate ketogenic diet (LCKD) to be effective for the treatment of obesity and insulin resistance. We therefore hypothesized that an LCKD might lead to improvements in NAFLD [16–18]. The purpose of this pilot study was to assess the effects of an LCKD on both liver histology and serologic measures of liver function in patients with NAFLD.

Methods

Subjects

For this prospective, single-arm clinical pilot trial, patients with biopsy evidence of NAFLD were identified from the Duke Outpatient Clinics and screened for eligibility. Inclusion criteria were age 18–65, BMI ≥ 30 kg/m^2 (but not >40 kg/m^2), and abnormal aminotransferase levels (>1.5 fold over normal, on at least 2 separate occasions). Exclusion criteria were active alcohol consumption, history of excessive alcohol consumption (defined as intake >40 g/wk of ethanol), use of medications associated with steatosis or steatohepatitis, use of insulin-sensitizing medications, presence of a major medical condition, histologic diagnosis of cirrhosis, other causes of chronic liver disease, drug use, terminal illness, or pregnancy.

Procedures

Subjects satisfying the eligibility criteria met in a group treatment session every other week for the first 3 months, then once monthly for the final 3 months. The duration of each visit was about 2 hours. Subjects were instructed to follow an LCKD requiring intake of <20 g/d of carbohydrate [18]. The initial diet consisted of unlimited amounts of meats (beef, pork, chicken, turkey, fish, shellfish), unlimited eggs, cheese (4 oz/d), salad vegetables (2 cups/d), and low-carbohydrate vegetables (1 cup/d). There was no predetermined limit on the amount of caloric intake; patients were instructed to eat until hunger was relieved. Exercise was not part of the program, and was not formally assessed during the program. Dietary compliance was assessed via periodic food records provided by patients as well as by measurement of urine ketones (see below). Nutritional supplementation (multivitamin formula, essential oil formula, diet formula, chromium picolinate) was provided and intake was encouraged throughout the duration of the study. These supplements do not contain any known weight loss–inducing substances. Bouillon dissolved in hot water was recommended once or twice daily throughout the study [19].

Measures

Vital signs

Total body weight was measured using a bioelectric impedance scale (Tanita Corp., Arlington Heights, IL). Blood pressure was taken twice with an automated digital cuff (Omron Corp., Vernon Hills, IL) during each return visit after a subject had been seated for 3 minutes. The average of these 2 blood pressure measurements was used in the analysis.

Serum tests

At baseline and during weeks 10 and 24 of the study, a serum sample was obtained from each subject after fasting for 12 hours, which was analyzed for alanine aminotransferase (ALT), aspartate aminotransferase, total bilirubin (TB), hemoglobin A_{1C}, glucose, insulin, total cholesterol (TC), low-density lipoprotein cholesterol, high density lipoprotein cholesterol (HDL-C), and triglycerides.

Urine tests

As the intake of <20 g/d carbohydrate per day results in elevated excretion of urinary ketones, dietary compliance was assessed by measurement of ketone levels in urine samples collected from each patient during group meetings. Commercially available ketone test strips were used.

Liver histology and analysis

Liver biopsies were obtained percutaneously by a gastroenterologist using a Tru-Cut needle with ultrasound guidance. A core sample length of at least 20 mm was regarded as an acceptable specimen. Preintervention and postintervention biopsies were interpreted by the same pathologist masked to patient identification and timing of biopsy [20].

Steatosis was graded according to the following scale: none; minimal (sparse, spotty macrovesicular steatosis involving $<5\%$ of the acinar tissue); mild (macrovesicular and/or microvesicular steatosis involving $<33\%$ of the acinar tissue); moderate (macrovesicular and/or microvesicular steatosis involving 33–66% of the acinar tissue); and marked (macrovesicular and/or microvesicular steatosis involving $>66\%$ of the acinar tissue). Extent of necroinflammation was graded according to the following scale: none; minimal (a focus or rare foci of necrotic or ballooned hepatocytes, with or without Mallory-type coagulum, in association with acute or chronic inflammatory cells adjacent to hepatocytes distended by steatosis; architectural disarray may be present); mild (mild intra-acinar inflammatory cells with or without rare neutrophils in association with mild hepatocyte necrosis

and ballooning, with or without Mallory-type coagulum, adjacent to hepatocytes distended by steatosis; variable architectural disarray); moderate (moderate intra-acinar inflammatory cells with occasional neutrophils in association with moderate hepatocyte necrosis and ballooning, with or without Mallory-type coagulum, adjacent to hepatocytes distended by steatosis; moderate architectural disarray is usually present); or marked (intra-acinar inflammatory cells with neutrophils in association with marked hepatocyte necrosis and ballooning, with or without Mallory-type coagulum, adjacent to hepatocytes distended by steatosis; prominent architectural disarray is present).

The assessment of fibrosis in 15μm tissue sections was performed by staining with 0.1% Sirius red F3B dissolved in saturated picric acid followed by counterstaining with Fast Green (Sigma, St. Louis, MO). Collagen surface density was quantified using a computerized image analysis system (IMAGE 1, Universal Imaging Co., Version 4.18, Westchester, PA). The surface density of collagen was measured via video screen display at a magnification of 40 × or 160 × and expressed as a percent (the ratio of collagen surface area per total analyzed field surface area). Large portions of liver, including both central and portal areas, were analyzed at these magnifications. An average score taken from 10 random fields was used to generate a single score for each liver specimen. The 3 main outcomes were steatosis, steatohepatitis, and fibrosis, each of which was scored on the following scale: 0 (none), 1 (minimal), 2 (mild), or 3 (marked). Because the preintervention biopsy for patient 5 did not contain an adequate sample to assess stage of fibrosis, the pre–post comparison of fibrosis included 4 subjects.

Statistical analyses

The comparison of interest in this pre–post study design was the percent change from baseline to 24 weeks. A 2-tailed paired *t*-test was used for analysis of these outcome variables as well as for changes in preintervention to postintervention NAFLD scores in liver biopsies. $P<.05$ was used for statistical significance.

Informed consent

Patients read and signed informed consent prior to study participation. This study was approved by the Duke University Health System Institutional Review Board.

Results

Five patients completed the 6-month study. At baseline, mean weight was 102.2 kg (range, 61.8–130.0 kg) and average BMI was 36.4 kg/m^2. The mean age was 35.6 years (range, 24–50 years). Three subjects were female; 4 were Caucasian; 1 was Hispanic. All subjects had at least some college education. The mean change in weight was − 12.8 kg (range, 0 to − 25.9 kg) and the mean percentage change in weight was − 10.9% ($P=.036$). Systolic blood pressure was 130.3 mmHg (SD = 17.0) at baseline and 117.6 mmHg (SD = 12.9) at 6 months, resulting in an average reduction of 9.5% ($P=.006$). The mean diastolic blood pressure was 83.5 mmHg (SD = 8.3) at baseline and 77.5 mmHg (SD = 4.3) at 6 months, an average reduction of 6.3% ($P=.32$).

At baseline, all 5 liver biopsies showed steatosis, necroinflammation, and fibrosis (Table 1). At the conclusion of the study, 4 out of 5 posttreatment liver biopsies showed improvements in steatosis ($P=.02$) necroinflammatory grade ($P=.02$), and fibrosis ($P=.07$). The posttreatment biopsy of patient 5 had a worsening in necroinflammation, steatosis, and steatohepatitis. (This may be a consequence of dietary nonadherence as this patient did not lose weight and had only 1 urine sample with positive ketones during the 6-month study.)

Individual weights and results of serum tests are shown in Table 2. There were statistically significant reductions in average percentage changes for overall body weight (− 10.9%, $P=.036$; 95% CI, − 20.6% to − 1.1%), TB (− 20.0%; $P=.027$; 95% CI, − 35.6% to − 4.4%), and TC/HDL-C ratio (− 15.9%; $P=.040$; 95% CI, − 30.6% to − 1.2%). Although not statistically significant, there were reductions

Table 1 Effect of low-carbohydrate, ketogenic diet on liver biopsy histology

Patient		Body Weight (kg)	Steatosis	Necroinflammatory Grade	Centrilobular Fibrosis
1	Baseline	104.5	Moderate	Minimal	Mild
	Week 24	94.5	Minimal	None	None
2	Baseline	124.1	Marked	Moderate	Mild-moderate
	Week 24	103.2	Mild	None	None
3	Baseline	130.0	Marked	Moderate	Mild
	Week 24	104.1	Mild	None	Minimal
4	Baseline	90.9	Marked	Moderate	Mild
	Week 24	83.6	Mild	Minimal	None
5	Baseline	61.8	Mild	Minimal	Mild
	Week 24	61.8	Moderate	Moderate	Mild-moderate

Steatosis: $P=0.02$ for preintervention–postintervention change.

Necroinflammatory grade: $P=0.05$ for pre–post change.

Stage of fibrosis: $P = 0.07$ for preintervention–postintervention change.

Table 2 Effect of low-carbohydrate, ketogenic diet on individual weights and fasting serum tests

ID	Week	Weight (kg)	AST (U/L)	ALT (U/L)	TB (mg/dL)	HbA_{1c} (%)	Insulin (μIU/mL)	Glucose (mg/dL)	HOMA	TC (mg/dL)	TG (mg/dL)	HDL-C (mg/dL)	LDL-C (mg/dL)	TC/HDL (ratio)
1	1	104.5	48	100	0.6	4.0	14.0	82	51.0	207	314	41	103	5.0
	10	91.4	18	28	1.0	4.2	3.2	85	12.1	165	79	50	99	3.3
	24	94.5	21	24	0.5	4.6	8.6	91	34.8	208	163	59	116	3.5
2	1	124.1	23	31	–	5.3	17.5	–	–	213	–	40	–	5.3
	10	110.9	22	24	0.5	4.8	10.4	98	45.3	181	96	38	124	4.7
	24	103.2	21	24	0.7	5.0	7.6	99	33.4	222	102	44	158	5.0
3	1	130.0	27	36	0.5	6.0	–	89	–	197	241	40	109	4.9
	10	120.0	28	39	0.3	5.3	60.3	112	300.2	115	93	34	62	3.4
	24	104.1	47	43	0.4	5.3	44.3	100	198.2	134	103	37	76	3.6
4	1	90.9	25	56	1.0	5.3	10.4	103	47.6	254	143	43	182	5.9
	10	86.8	22	42	0.6	5.2	4.9	81	17.6	254	167	47	174	5.4
	24	83.6	22	33	0.9	5.4	3.7	89	14.6	265	202	52	173	5.1
5	1	61.8	43	83	0.3	5.0	10.0	83	36.9	215	191	42	135	5.1
	10	62.3	42	67	0.1	5.30	8.7	88	34.0	193	148	37	126	5.2
	24	61.8	52	66	0.2	5.3	15.3	85	57.8	192	184	39	116	4.9

Abbreviations: AST, amino aspartate transferase; ALT, alanine transferase; TB, total bilirubin; HbA_{1c}, hemoglobin A_{1c}; HOMA, homeostasis model of insulin resistance (glucose × insulin/22.5); TC, total cholesterol; TG, triglycerides; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; – missing data.

in average percent changes for ALT (-28.1%; $P = .144$; 95% CI, -71.2% to $+14.9\%$) and insulin ($-28.4.\%$; $P = .249$; 95% CI, -86.8% to $+30.1\%$). The diet was well tolerated, although 1 study subject experienced an episode of leg muscle cramping, which was relieved by daily consumption of bouillon.

Discussion

In this pilot study, 6 months of an LCKD led to significant improvements in liver histology in patients with NAFLD who adhered to the diet treatment. The magnitude of effect of the LCKD used in this study was large compared to other treatments for NAFLD [21, 22]. Comparison of preintervention–postintervention liver biopsies in patients 1–4 revealed improvements in steatosis and necroinflammatory grade. In addition, evidence of hepatic necroinflammation was absent in patients 1–3. Furthermore, fibrosis was reduced in these 3 patients over the 6-month regimen, which may represent a reversal of a cirrhotic-type process. In contrast, patient 5 had histologic worsening. However, this patient did not experience weight loss and did not comply with the diet protocol as revealed by only 1 positive sample of urinary ketones during the 6-month study period.

Given the absence of a comparison treatment in this study, we cannot determine whether the observed effects are specifically due to an LCKD or, more generally, to weight loss. However, decreased carbohydrate intake may have an independent beneficial effect on fatty liver [23]. High intake of monosaccharides like glucose and fructose, as well as the disaccharide sucrose, can lead to excessive hepatic lipogenesis and hypertriglyceridemia [24, 25], which may accelerate the onset of NAFLD. As both conditions are prevalent today, a reduction in sugar consumption may be a strategy to reduce NAFLD [23, 26, 27].

The improvement in NAFLD may have been a result of improvement in fasting insulin and glucose levels, which was observed in the subjects who experienced weight loss. Our findings support the theory that NAFLD may be a consequence of insulin resistance. If the observed benefits in this study are solely due to weight loss, the LCKD was nonetheless effective in this regard and provided patients with NAFLD a novel method to achieve therapeutic weight loss. Because 1 subject was not able to comply with the dietary restrictions despite frequent follow-up, efforts to enhance long-term adherence with this diet program are warranted.

The results of this pilot study are encouraging and should lead to larger studies to more accurately assess the usefulness and feasibility of using this type of dietary approach therapeutically for NAFLD. If an LCKD proves to be a safe, effective, and broadly applicable regimen with a high rate of patient compliance, then this diet may present physicians with a therapeutic option in the treatment of NAFLD.

Acknowledgment This study was partially funded by an unrestricted research grant from the Robert C. Atkins Foundation. Dr. Yancy was supported by a Veterans' Affairs Health Services Research Career Development Award (RCD 02-183-1)

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