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Effects of a carbohydrate-restricted diet on hepatic lipid content in adolescents with non-alcoholic fatty liver disease: A pilot, randomized trial

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Summary

Background: Non-alcoholic fatty liver disease (NAFLD) has emerged as the most common form of liver disease among adolescents in industrialized countries. While lifestyle intervention remains the hallmark treatment for NAFLD, the most effective dietary strategy to reverse NAFLD in children is unknown.

Objective: The objective of this study was to determine the effects of a moderately CHO-restricted diet (CRD) vs fat-restricted diet (FRD) in adolescents with NAFLD on reduction in liver fat and insulin resistance.

Methods: Thirty-two children/adolescents (age 9-17) with obesity and NAFLD were randomized to a CRD (<25:25:>50% energy from CHO:protein:fat) or FRD (55:25:20) for 8 weeks. Caloric intakes were calculated to be weight maintaining. Change in hepatic lipid content was measured via magnetic resonance imaging, body composition via dual energy X ray absorptiometry and insulin resistance via a fasting blood sample.

Results: Change in hepatic lipid did not differ with diet, but declined significantly (−6.0 ± 4.7%, $P < .001$ only within the CRD group. We found significantly greater decreases in insulin resistance (HOMA-IR, <.05), abdominal fat mass ($P < .01$) and body fat mass ($P < .01$) in response to the CRD vs FRD.

Conclusion: These findings suggest that consumption of a moderately CHO-restricted diet may result in decreased hepatic lipid as well as improvements in body composition and insulin resistance in adolescents with NAFLD even in the absence of intentional caloric restriction. Larger studies are needed to determine whether a CHO-restricted diet induces change in hepatic lipid independent of change in body fat.

KEYWORDS
hepatic lipid, insulin resistance, NAFLD, nutrition, paediatric obesity

1 | INTRODUCTION

For childrens and adolescents aged 2 to 19 years, the prevalence of obesity has reached 17% and affects nearly 13 million children in the United States.[1,2] In parallel with the rate of childhood obesity, the occurrence of non-alcoholic fatty liver disease (NAFLD) is increasing among children and adolescents. NAFLD has emerged as the most common cause of paediatric chronic liver disease with the incidence reaching 40% in children with obesity.[3-5] NAFLD refers to a spectrum of liver diseases ranging from simple fat infiltration to non-alcoholic

steatohepatitis, fibrosis and cirrhosis. The pathogenesis of NAFLD in children is not fully understood, but is thought to involve complex interactions between alterations in nutrient metabolism, insulin resistance and the onset of inflammation in multiple organ systems.[6] While the principal existing therapies target the metabolic disorders associated with fatty liver, no treatment currently exists to directly reverse hepatic fat infiltration.[7] In adolescents with NAFLD, early intervention is crucial to reduce the risk of progression to advanced stages of the disease in adulthood.[7] Understanding the effectiveness of lifestyle interventions as long-term solutions in treating this condition in child and adolescent populations is critical.

In general, treatment of childhood NAFLD currently focuses on several areas that include weight reduction via dietary intervention, increased physical activity, lifestyle modifications and management of various disease-specific components.[6,8,9] Other available options to manage obesity include behavioural intervention, pharmacological therapies and bariatric surgery.[10] However, there are limited data from randomized controlled trials (RCTs) to support the effectiveness of dietary recommendations to induce meaningful reductions in hepatic fat infiltration in children.[11] Specifically, the recommendation of calorie restriction for weight loss may not be optimal for reversal of fatty liver in a paediatric population. RCTs examining the effects of a lifestyle intervention with emphasis on weight loss and exercise on paediatric NAFLD have had limited success possibly due to the difficulty in adhering to long-term physical exercise and calorically restrictive regimens. Evidence from rodent models and studies in adults have shown that reducing intake of carbohydrate sources such as added sugars, high glycemic grains and fructose may be the most effective approach to reverse fatty liver by significantly reducing hepatic de novo lipogenesis (DNL).[12] Limiting hepatic DNL would reduce the accrual of hepatic lipids and simultaneously enhance their disposal via mitochondrial β-oxidation.[13,14] Data are needed to determine the optimal diet for the selective depletion of hepatic lipid without the need for surgery and severe caloric restriction in a paediatric population with NAFLD.

Given this, the primary objective of this study was to compare the effects of an individualized weight-maintaining CHO-restricted diet (CRD) vs a standard, fat-restricted diet (FRD) in children and adolescents with NAFLD on reduction in hepatic lipid content and insulin resistance using a family-based intervention with a 2-week feeding phase and 6-week free-living phase.

2 | RESEARCH DESIGN AND METHODS

2.1 | Participants

Thirty-two males and females with obesity and NAFLD were recruited from Children's Hospital of Alabama, University of Alabama at Birmingham via electronic medical record search and advertisement in the hepatology and endocrinology clinics from January 2016 to December 2017. Inclusion/exclusion criteria have been reported.[15] In brief, inclusion criteria were children ages 9 to 17 years, body mass index (BMI) *z*-score >85th percentile, diagnosis of NAFLD (ALT >45 and/or perfusely echogenic liver via ultrasound) and sedentary (<2 hours/week of intentional exercise, and agreed to maintain their level of activity throughout the study). Exclusion criteria included those with diabetes, unwilling to follow the prescribed diets, recent weight change (±10 lbs. in previous year), history of eating disorder, digestive diseases, major liver dysfunction, current/recent smoker, current use of oral corticosteroids (>5 days/month) and using medications for treatment of psychosis or manic-depressive illness. Participants were informed of the experimental design, and oral and written consent was obtained. The study was approved by the Institutional Review Board for Human Use at UAB. The trial is registered on clinicaltrials.gov (NCT02787668).

2.2 | Study design

The study design was a randomized two-arm, parallel dietary intervention. Participants were randomized to either 8-weeks of a CRD or a FRD.[15] All participants underwent screening at the UAB Department of Nutrition Sciences to confirm eligibility. Dual-energy X-ray absorptiometry (DXA), indirect calorimetry and fasting blood draw were performed on all participants at baseline and following completion of the diet intervention at the UAB Department of Nutrition Sciences. Magnetic resonance imaging (MRI) was performed on all participants at baseline and follow-up at the Civitan International Neuroimaging Laboratory at UAB Highlands hospital. Following completion of baseline testing, participants were assigned to a diet using a block randomization scheme, and the condition assignments were placed in sealed envelopes that were not opened until a specific participant was assigned. Based on sample size calculations determined using Browning et al,[16] we expected a −12.0% absolute decrease in hepatic triglyceride content in the treatment group (low carbohydrate) compared to −5% in the control group (low fat). With a SD of 15, alpha of 0.05, a sample size of 16 in each group, we would have 80% power to detect difference of 4.4%. Because this was a diet intervention study, it was not possible for participants or all study personnel to be blinded to group assignment; however, study personnel involved in analysis of main outcomes were blinded to group assignment.

2.3 | Diets

This was an 8-week family-based diet intervention with a 2-week modified controlled feeding phase where all groceries were provided to the family followed by a 6-week free living phase. Parents and guardians were encouraged to adopt the prescribed diet for the entire family, as family involvement can predict successful adherence to diet.[17,18] All aspects of the diet prescription and intervention have been previously reported.[15] In brief, participants received 14-day meal plans, along with groceries specific to their diet assignment and recipes at the initial diet instruction meeting. A grocery delivery service was used to minimize the initial burden of shopping on the family, and study personnel coordinated with participants to choose a time for

grocery pick up or delivery. This intervention also consisted of biweekly, diet specific, individual and group counselling sessions led by the study's registered dietitian (RD). Individual meetings with the RD focused on diet instruction, meal planning, goal setting and a review of nutritional resources. The RD encouraged adherence to the diets by using behavioural strategies that have shown to be useful in childhood weight management, such as goal setting, review of food journals and counselling modification based on the participant's readiness to change.[17,18] Diet specific group classes included topics such as label reading, meal planning, healthy substitutions, mindful eating and other relevant topics important for dietary adherence.

The CRD diet was designed to minimize intake of refined CHO sources such as added sugars, high glycemic grains and fructose and provided ≤25% energy from CHO, 25% energy from protein and ≥50% energy from fat. CHO sources were primarily derived from leafy greens and non-starchy vegetables. Additional CHO sources included in the diet prescription were nuts and nut butters, unsweetened yoghurt and low-glycemic fruits such as apples and berries. Limited amounts of whole grains. Legumes, root vegetables and 'treats' like dark chocolate were permitted. Protein sources included meat, fish, eggs, poultry and whey protein if appropriate. Saturated fat intake was limited to <10% total energy/day. Other permitted fat sources included olive oil, walnut oil and other sources of poly and monounsaturated fatty acids. A multivitamin was also encouraged to ensure all micronutrient requirements were met.

The FRD was comprised of low sugar, high quality foods with low energy density, which is the standard of care in the dietary management of children with NAFLD.[8,19] This diet was based on the USDA MyPlate Daily Food Plan for teenagers with 55:25:20% energy from CHO:protein:fat. Participants were asked to avoid consuming foods high in fat such as fried foods, butter, cream cheese and bacon, whereas fruits, vegetables (starchy and non-starchy), whole grains, poultry, lean meats and low-fat dairy products were permitted.

2.4 | Outcome measures

2.4.1 | Hepatic lipid content

At baseline and week 8, 3-point M Dixon MRI was performed to assess liver fat.[20,21] The 3-point M Dixon techniques uses chemical-shift principles to estimate resonant frequencies of water and methylene groups of triglyceride fatty acid chains. Water- and fat-suppressed images obtained from 3-point M Dixon technique were used to assess hepatic lipid percentage by identifying three regions of interest (ROI) free of artifacts and blood vessels. The signal intesity (SI) of the three ROIs, which is based on tissue densities, was averaged and used to calculate the hepatic fat fraction (fat SI/fat SI + water SI).

2.4.2 | Body composition

Body composition was estimated using DXA in the Nutrition and Obesity Research Center (NORC)/Diabetes Research Center (DRC) core facility. Participants were asked to wear light clothing and lie flat on their backs with arms by their sides during the DXA scan (iDXA; GE Healthcare Lunar, Madison, Wisconsin). Total and regional (within the trunk and leg) fat, bone and lean mass were estimated at baseline and week 8. Girls of childbearing age were required to complete a urine pregnancy test prior to DXA scans. Any female with a positive pregnancy test result was excluded from participating in the study.

2.4.3 | Blood draw and serum analyte analyses

Blood draws at baseline and week 8 were conducted following an overnight fast. Samples were centrifuged, aliquotted and sera stored

TABLE 1 Baseline characteristics of study participants

Variable	FRD(n = 16)	CRD(n = 16)
Age at screening, mean (SD), y	14.5 (2.6)	14.2 (2.1)
Race/ethnicity, No. (%)		
Non-Hispanic, white	9 (56)	14 (88)
Hispanic	7 (44)	1 (6)
Asian	0 (0)	1 (6)
Sex, No. (%)		
Female	7 (44)	9 (56)
Male	9 (56)	7 (44)
Anthropometrics, mean (SD)		
Height, cm	166.4 (13.5)	165.6 (10.4)
Weight, kg	107.1 (25.6)	99.6 (25.2)
Body mass index	38.4 (7.3)	35.9 (6.7)
z score	2.45 (0.3)	2.45 (0.2)
Blood pressure, mean (SD), mm Hg		
Systolic	119.1 (12.4)	119.1 (9,5)
Diastolic	76.9 (8.1)	74.7 (5.3)
Lipids, mean (SD), mm/dL, fasting		
Total cholesterol	179.9 (36.6)	166.2 (28.5)
Low-density lipoprotein	102.1 (30.1)	90.7 (23.0)
High-density lipoprotein	51.4 (7.8)	51.8 (9.4)
Triglycerides	132.0 (51.7)	111.2 (37.9)
Insulin and glucose, mean (SD), fasting		
Glucose, mg/dL	82.3 (8.7)	87.8 (5.5)
Insulin, μIU/mL	28.6 (17.3)	34.3 (20.7)
HOMA-IR	7.5 (5.6)	7.5 (5.0)
Liver enzymes, mean (SD), fasting		
Alanine aminotransferase, U/L	57.7 (36.6)	49.8 (27.3)
Aspartate aminotransferase, U/L	35.5 (18.4)	32.9 (19.6)
γ-Glutamyl transpeptidase, mg/dL	25.1 (12.0)	25.6 (20.5)
Hepatic fat fraction, mean (SD), %	12.4 (9.6)	18.6 (9.0)
Resting energy expenditure, mean (SD), kcal	1933.7 (380.0)	1999.5 (369.5)

Abbreviations: CRD, CHO-restricted diet; FRD, fat-restricted diet.

at −85°C; concentrations of serum-derived analytes were assayed at the DRC Core Laboratory and the UAB Outreach Laboratory. Glucose, total cholesterol, HDL-cholesterol and triglycerides were measured using a SIRRUS analyzer (Stanbio Laboratory, Boerne, Texas); LDL-C was calculated using the method of Friedewald.[22] The total cholesterol-to-HDL-C ratio was calculated; a ratio of 5 to 1 or lower is the recommended target range, with an optimum ratio of 3·5 to 1. Insulin was assayed by immunofluorescence on a TOSOH AIA-II analyzer (TOSOH Corp., South San Francisco, California); intra-assay CV of 1·5% and interassay CV of 4·4%. High-sensitivity C-reactive protein (CRP) was assessed by turbidometric methods by using a SIRRUS analyzer (Stanbio Laboratory), with reagents obtained from Pointe Scientific and minimum detectable concentrations was 0.05 mg/L with a mean intra-assay CV of 7.49% and interassay CV of 2.13%. HOMA-IR was calculated using measurements for fasting glucose and fasting insulin.[23] Subjects were instructed to avoid strenuous physical activity the day prior to testing, and to avoid all physical activity on the morning of testing.

2.4.4 | Resting energy expenditure

Resting energy expenditure was determined after an overnight fast, by indirect calorimetry (Vmax ENCORE 29N Systems, SensorMedics Corporation, Yorba Linda, California) in UAB's NORC Metabolism Core. A clear, plastic, canopy hood was placed over the head and shoulders, and expired air was collected for 20 minutes after a 10-minutes equilibration period. Carbon dioxide production and oxygen consumption were measured continuously during this time.

2.4.5 | Dietary intake and adherence

Participants were asked to complete 3-day food diaries (two weekdays, one weekend day) at week 4 of the intervention. Food records were analyzed using NDSR Software (Version 2012, Nutrition Coordinating Center, University of Minnesota, Minneapolis, Minnesota) in order to quantify average nutrient intake.

TABLE 2 Baseline and 8-week body composition and hepatic lipid outcomes by diet group

		FRD	CRD	*P*for diet	*P*for diet adjusted for change in total fat
Weight (kg)	Baseline	109.0 (24.3)	102.0 (24.8)		
	Week 8	108.6 (23.6)	99.6 (25.6)		
	Mean difference	−0.4	−3.0*	0.06	0.48
BMI	Baseline	37.7 (5.8)	36.8 (6.0)		
	Week 8	37.6 (5.5)	35.7 (6.2)		
	Mean difference	−0.1	−1.1*	0.05	0.74
BMI *z* score	Baseline	2.45 (0.3)	2.45 (0.2)		
	Week 8	2.43 (0.3)	2.37 (0.3)		
	Mean difference	−0.02	−0.08**	0.03	0.80
Total fat (kg)	Baseline	49.8 (1.4)	46.1 (1.5)		
	Week 8	49.9 (1.4)	43.6 (1.5)		
	Mean difference	0.1	−2.5**	0.01	-
Total lean (kg)	Baseline	51.6 (11.6)	48.8 (11.8)		
	Week 8	51.5 (11.3)	48.1 (11.8)		
	Mean difference	−0.1	−0.7	0.36	0.36
Abdominal fat (kg)	Baseline	27.1 (7.5)	26.2 (9.0)		
	Week 8	27.1 (7.4)	24.6 (9.5)		
	Mean difference	0.0	−1.5**	0.02	0.97
Leg fat (kg)	Baseline	17.1 (5.2)	14.8 (4.8)		
	Week 8	17.1 (5.1)	14.0 (4.8)		
	Mean difference	0.0	−0.8**	0.02	0.80
HFF (%)	Baseline	12.4 (9.6)	18.6 (9.0)		
	Week 8	10.6 (8.5)	12.6 (6.6)		
	Mean difference	−1.8	−6.0***	0.12	0.46

Abbreviations: BMI, body mass index; CRD, CHO-restricted diet; FRD, fat-restricted diet.

Note: Data presented as mean (SD).

*P < .05, **P < .01, ***P < .001 for paired t test; P for effect of diet, results from ANCOVA (8-week outcome adjusted for baseline); P for the effect of diet, results from ANCOVA (8-week outcome adjusted for baseline and change in fat mass).

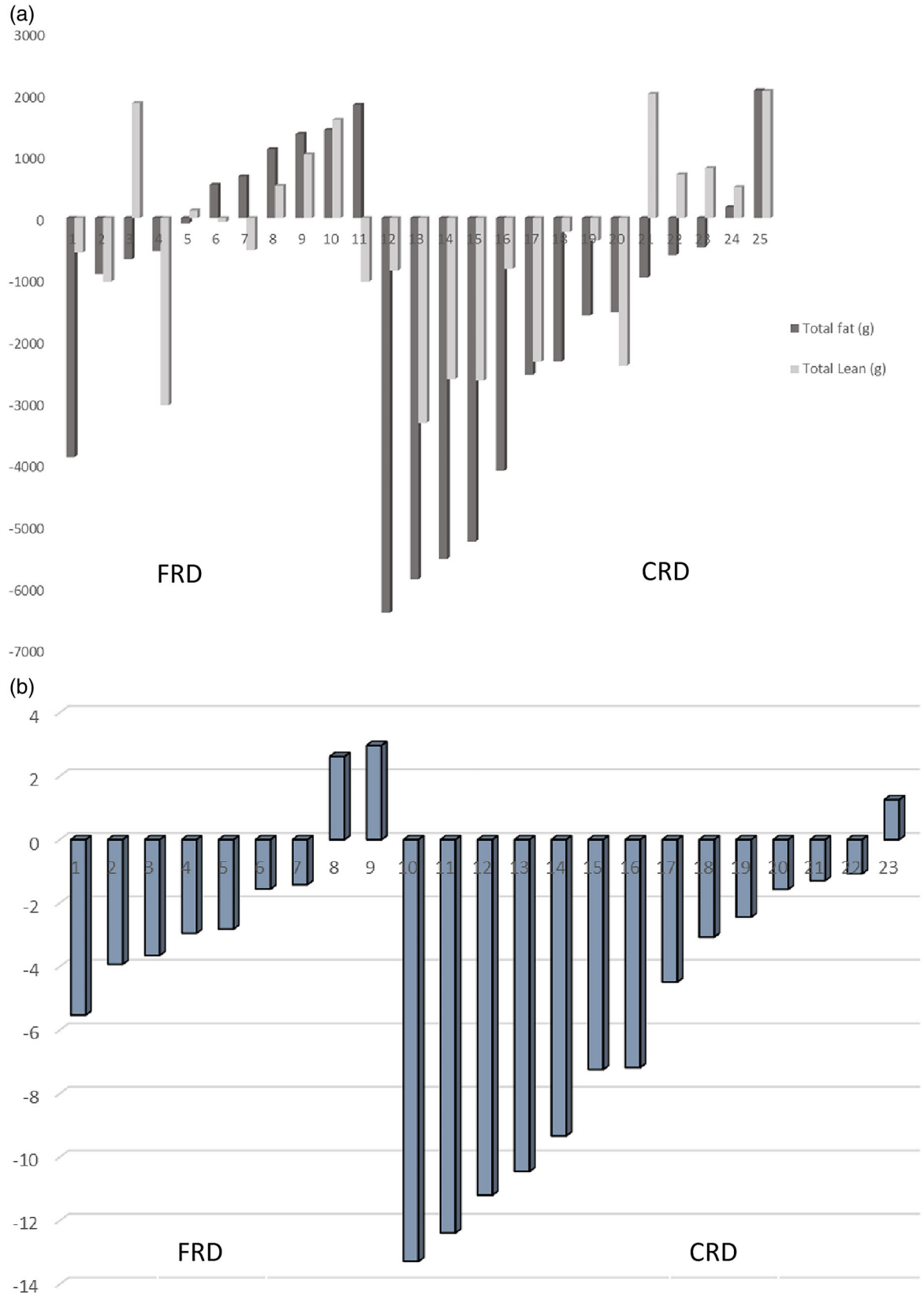

FIGURE 1 Individual changes in body composition (A) and hepatic lipid content (B) in response to the FRD and CRD. CRD, CHO-restricted diet; FRD, fat-restricted diet

2.5 | Statistical analysis

Descriptive statistics were computed by diet assignment. Statistical tests were two-sided, with an alpha level of 0.05 denoting statistical significance. Analyses were performed using SAS (version 9.2 SAS Institute, Inc, Cary, North Carolina). Paired *t* tests were used to determine the difference in baseline and post-intervention hepatic fat fraction, body composition and serum analyte variables by diet group. Analysis of covariance determined the effect of diet (adjusted for baseline) on post-intervention variables. Further adjustments were made for change in total body fat mass to determine the independent effect of diet on selective depletion of hepatic lipid content, changes in insulin sensitivity, liver enzymes and other hormones. Independent *t* tests were used to determine group differences in dietary intake.

3 | RESULTS

Thirty-two adolescent females and males completed baseline testing and were randomized to a diet group (16 to the CRD and 16 to the FRD). Eight participants discontinued the intervention for various reasons unrelated to the study (2 in the CRD group and 5 in the FRD group). Twenty-five participants completed baseline and follow-up testing (two participants were unable to undergo follow-up MRI scanning). As shown in Table 1, the study participants were primarily non-Hispanic white or Hispanic with an average age of 14 years in each group. All participants were above the 95th percentile of BMI-for-age at baseline and after the 8-week intervention. Although diets were designed to be weight maintaining, participants lost weight. On average, percent change in body weight tended to be greater in the CRD group compared to the FRD group (−2.4% vs −0.4%, $P = .06$). The CRD group also experienced a 3.0% decrease in BMI, whereas the FRD group experienced a 0.3% ($P < .05$).

Changes in body composition and hepatic fat fraction by diet group are shown in Table 2 and Figure 1. Within the CRD, there were significant reductions in weight, BMI, BMI *z* score, total fat mass, abdominal fat and leg fat. The CRD group experienced a 5.5% reduction in total fat mass, while the FRD group had a 0.1% increase ($P < .01$ for effect of diet). Change in BMI, BMI *z* score, abdominal fat and leg fat were significantly greater in the CRD vs FRD group ($P < .05$ for effect of diet). Individual changes in HFF% are shown in Figure 1B. Figure 2A shows that, on average, the CRD experienced a 32% decrease in HFF from the average baseline value with an absolute decrease of 6.2% ($P < .001$ for paired *t* test), but this decrease was not significantly greater than an absolute decrease of 1.0% in the FRD group ($P = .12$ for effect of diet). There were no significant between group differences in change in total lean mass.

Changes in metabolic and hormonal outcomes by diet group are shown in Table 3. Within-diet analyses showed that following the CRD, there were significant decreases in ALT and AST. Between diet analyses showed that, compared to the FRD group, the CRD group experienced significantly greater decreases in fasting insulin and HOMA-IR (Figure 2B). There were no significant changes or between-group differences in fasting glucose, hsCRP, total cholesterol, LDL-C, HDL-C, triglycerides or GGT. After adjustment for change in total fat, change in HOMA-IR remained significantly greater in the CRD group compared to the STD group.

Table 4 shows the average participant self-reported dietary intake at the mid-point of the 8-week intervention, 2 weeks after the end of the grocery delivery phase. There were no significant differences in the average total calories, grams of protein, grams of total fat or grams of saturated fat consumed per day between the CRD and FRD diet groups. There was a significant difference in glycemic load, total grams of carbohydrate, total grams of sugar and grams of added sugar consumed per day between the two groups. The CRD group consumed 29:24:47% energy from CHO:protein:fat and the FRD group consumed 48:19:33% energy from CHO:protein:fat.

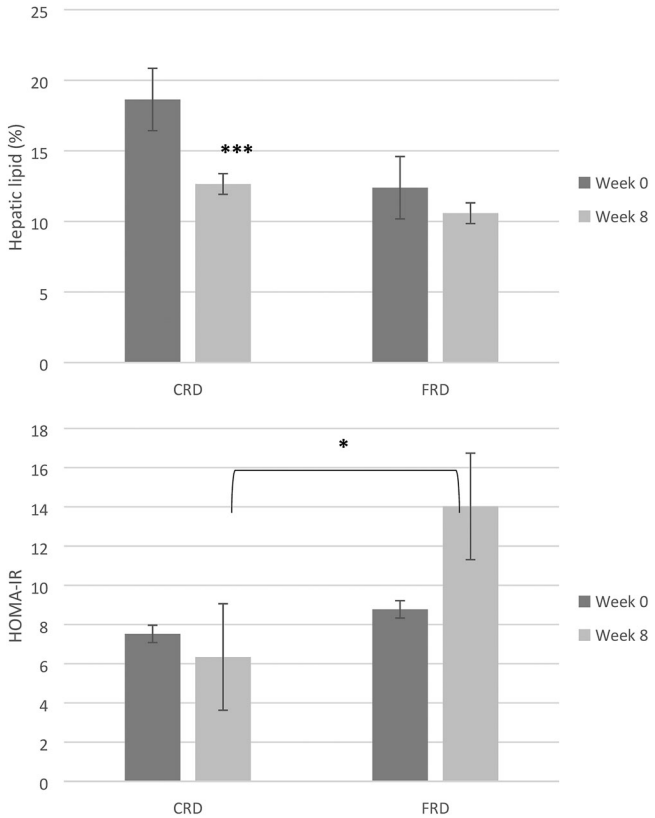

FIGURE 2 Mean baseline and week 8 hepatic lipid content (A) and HOMA-IR (B) by diet group. Percent hepatic lipid was significantly reduced following the CRD (*** indicates $P < .001$) and change in HOMA-IR was significantly different between the CRD and the FRD (* indicates $P < .05$). CRD, CHO-restricted diet; FRD, fat-restricted diet

4 | DISCUSSION

To our knowledge, this is the first randomized trial using a family-based feeding design to compare the effects of CHO-restricted vs FRD on change in liver fat in an ethnically diverse group of adolescent

TABLE 3 Baseline and 8-week metabolic outcomes by diet group

		FRD	CRD	*P*for diet	*P*for diet adjusted for change in total fat
Glucose	Baseline	85.8 (7.8)	83.2 (9.1)		
	Week 8	90.4 (12.2)	86.0 (7.0)		
	Mean difference	2.6	2.8	0.27	0.93
Insulin	Baseline	34.8 (24.8)	35.6 (20.7)		
	Week 8	61.4 (44.9)	29.8 (17.5)		
	Mean difference	21.0	−5.8	0.03	0.03
HOMA-IR	Baseline	8.8 (5.3)	7.5 (5.0)		
	Week 8	14.0 (10.5)	6.3 (3.7)		
	Mean difference	5.3	−1.18	0.03	0.25
CRP	Baseline	3.6 (3.4)	4.0 (4.3)		
	Week 8	5.1 (5.6)	5.0 (6.3)		
	Mean difference	1.6	1.0	0.77	0.16
Cholesterol	Baseline	178.1 (37.2)	177.1 (33.5)		
	Week 8	168.8 (24.9)	164.3 (26.4		
	Mean difference	−12.3	−12.8	0.77	0.66
LDL	Baseline	103.3 (31.4)	98.3 (22.6)		
	Week 8	88.4 (18.9)	88.8 (19.6)		
	Mean difference	−17.9	−9.5	0.61	0.40
HDL	Baseline	50.3 (8.6)	52.1 (8.7)		
	Week 8	50.9 (9.1)	50.4 (10.5)		
	Mean difference	−0.1	−1.7	0.57	0.56
Triglycerides	Baseline	122.7 (49.4)	133.6 (57.6)		
	Week 8	124.1 (40.8)	125.8 (48.4)		
	Mean difference	1.4	−7.9	0.84	0.89
ALT, U/L	Baseline	59.3 (37.8)	65.7 (54.3)		
	Week 8	52.9 (40.5)	42.7 (27.7)		
	Mean difference	−5.8	−23.6*	0.15	0.65
AST, U/L	Baseline	30.4 (13.5)	48.4 (39.2)		
	Week 8	28.1 (17.8)	27.4 (13.1)		
	Mean difference	−2.8	−20.7*	0.43	0.83
GGT, mg/dL	Baseline	24.4 (10.6)	25.8 (16.8)		
	Week 8	23.3 (10.3)	26.0 (18.2)		
	Mean difference	−0.9	0.1	0.73	0.25

Abbreviations: CRD, CHO-restricted diet; CRP, C-reactive protein; FRD, fat-restricted diet.

Note: Data presented as mean (SD).

*$P < .05$, **$P < .01$, ***$P < .001$ for paired *t* test; *P* for effect of diet, results from ANCOVA (8-week outcome adjusted for baseline); *P* for the effect of diet, results from ANCOVA (8-week outcome adjusted for baseline and change in fat mass).

boys and girls with NAFLD. Despite prescribing an energy-balanced diet, the CRD group had greater decreases in weight and adipose tissue than the FRD group. The CRD group also experienced greater decreases fasting insulin and insulin resistance than the FRD group. Change in hepatic lipid did not differ with diet, but declined significantly (−32%) only within the CRD group. In summary, these data suggest that recommendation of a CRD to adolescents with NAFLD and obesity results in favorable changes in body composition, depletion of liver fat and improvement in glucose metabolism.

Current guidelines for treatment of paediatric NAFLD focus on several areas that include weight reduction via dietary intervention, increased physical activity, lifestyle modifications and management of various disease-specific components.[6,8,9] While there are no pharmacological therapies to directly treat NAFLD, they may be prescribed to treat other aspects of the disease such as insulin resistance and dyslipidemia. Dietary interventions recommended from the North American Society for Pediatric Gastroenterology, Hepatology and Nutrition (NASPGHN) and the American Association for the Study of

TABLE 4 Self-reported dietary intake by diet group

	CRD	FRD	*P*
Total energy (kcal)			
	1598.6 ± 165.8	1710.3 ± 234.5	.70
CHO			
% kcal	29.2 ± 2,9%	47.9 ± 4.1%	<.01
g/day	112.6 ± 22.4	211.0 ± 31.6	<.05
Protein			
%kcal	23.5 ± 1,4%	19.1 ± 2.0%	.09
g/day	93.7 ± 10.1	75.0 ± 14.3	.30
Fat			
%kcal	47.2 ± 2.2%	33.1 ± 3.1%	<.01
g/day	87.3 ± 9.2	64.5 ± 12.9	.17
Saturated Fat			
%kcal	15.5 ± 0.77%	11.5 ± 1.1%	<.001
g/day	28.9 ± 3.4	22.5 ± 4.9	.29
Sugar			
Total g/day	40.4 ± 13.7	94.3 ± 19.4	<.05
Added g/day	17.4 ± 11.8	63.1 ± 16.6	<.05
Glycemic load			
	57.6 ± 13.0	114.5 ± 18.4	<.05

Abbreviations: CRD, CHO-restricted diet; FRD, fat-restricted diet.
Note: Data reported as mean ± SD. Results are average intakes from 3-day food records (two-week days and one weekend day) completed after 4 weeks in the study.

Liver Diseases (AASLD) practice guidelines include avoidance of sugar sweetened beverages; consumption of healthy, well-balanced diet, moderate to high intensity exercise daily and less than 2 hours/day of screen time for adolescents and children older than 2 years of age.[10] Consumption of whole fruits, vegetables and dietary fibre is promoted in addition to portion control education, understanding food labelling and encouraging eating regular meals to avoid grazing. While these recommendations may be important for the prevention of paediatric NAFLD, there are limited data from RCTs to support the effectiveness of these recommendations to induce meaningful reductions in hepatic fat infiltration and insulin resistance in children and teenagers.

The dietary recommendation of calorie restriction for weight loss may not be optimal for reversal of fatty liver in a paediatric population. RCTs examining the effects of a lifestyle intervention with emphasis on weight loss and exercise on paediatric NAFLD have had limited success possibly due to the difficulty in adhering to long-term physical exercise and calorically restrictive regimens. Our data suggest that the recommendation of energy restriction may be unnecessary in effectively treating paediatric NAFLD and significant reductions in liver fat can occur in a relatively short period of time. In only 8 weeks, adolescents consuming a CRD lost close to one third of their baseline liver fat. Despite measuring the participants resting energy expenditure and feeding/prescribing a weight maintaining diet, participants in the CRD group lost more fat mass than those following the FRD. Whether the significant reduction in liver fat in the CRD group could be explained by negative energy balance and loss of fat mass is not clear. Nonetheless, the effects were only observed in the CRD, suggesting that the clinical recommendation of CHO-restriction may result in spontaneous calorie restriction and improvements in disease course in a paediatric population. Further, our findings support the results from a recent feeding study in adolescent Hispanic boys with NAFLD showing a significant decrease in hepatic lipid content in response to a low 'free sugar' diet.[24] In that study, participants consumed fewer total calories in the low sugar diet group compared to the 'usual diet' group, limiting the ability to determine the effects of reducing sugar intake on liver fat independent of reducing caloric intake. Regardless, the recommendation of reducing sugar and CHO intake alone without counting calories may significantly improve liver fat in adolescents.

A CHO-restricted dietary approach may reduce liver fat by targeting several key pathways that lead to lipid deposition in the liver. Sugars and highly processed carbohydrates, particularly if they contain fructose, stimulate high insulin secretion and hepatic DNL.[12] Saturated fatty acids produced from this process are selectively deposited in the liver as ectopic lipid. Thus, reduction in both the substrate (glucose/fructose) and the stimulus (insulin) for hepatic DNL with a CHO-restricted diet should result in reduced accumulation and improved export of hepatic fat perhaps leading to depletion of hepatic lipid content. Furthermore, elevated postprandial glucose leads to the production of free radicals, oxidative stress and inflammation.[25] The elevation in insulin secretion that accompanies this glycemic response stimulates peripheral adipose tissue expansion that may lead to inflammation and ultimately an impaired capacity for storage in adipocytes.[26] The resultant fatty acid 'overflow' is thought to promote hepatic lipid deposition.[27] High circulating insulin and glucose engage glycolysis and upregulate malonyl-coA at the expense of fatty acid transport and beta-oxidation. Taken together, it seems likely that the CHO-restricted diet reduces hepatic lipid deposition and promotes the return of hepatic insulin sensitivity by reducing DNL, thus permitting lipid oxidation.

We proposed to test this hypothesis in the present study by examining the effect of carbohydrate restriction vs fat restriction in the context of a weight maintaining diet prescription. Although hepatic lipid was decreased with the CRD in the present study, the change was not greater than that observed with the FRD. Furthermore, the CRD group tended to lose more weight, and lost greater fat mass, than the FRD group, making it difficult to isolate an independent contribution of diet composition. Thus, it is not clear whether carbohydrate restriction has effects on hepatic lipid that are independent of weight loss. Further studies with larger sample sizes are needed to explore the effects of a CHO-restricted diet during weight maintenance on hepatic lipid content. In addition, it would be interesting to determine if longer-term interventions can achieve greater decreases in hepatic lipid content, placing adolescents with NAFLD in the non-disease range (<5% hepatic lipid).

According to the food records, the two interventions resulted in notably different macronutrient intakes. During the intervention, the participants in the CRD group consumed on average 29% of their

daily calorie intake from CHO and 47% from fat, whereas the FRD group consumed 47% from CHO and 29% from fat. Protein intake was slightly greater in the CRD group though not significantly different and %kcal from fat was significantly greater in the CRD group. These study results demonstrate the beneficial health effects that can be achieved with a proportionally higher fat, moderately CHO-restricted diet in adolescents with NAFLD and obesity.

There are limitations and strengths to our study. Our goal was to design the interventions so that participants maintained body weight through the intervention. Because the participants in the CRD group lost more body fat than the FRD group, we cannot draw conclusions regarding the effects of the CHO-restricted diet on change in liver fat independent of fat loss among adolescents with NAFLD. We did not provide all food for the families for the entire duration of the study and only have data from self-reported dietary intake using food records at the midpoint of the study. Self-reported dietary intake may result in under reporting and not accurately reflect actual intake. It is a limitation of this pilot study that objective measurement of dietary adherence and more frequent assessment of dietary intake were not included. Provision of all food could have increased dietary adherence, study visit attendance and/or participant retention. Because of feasibility issues, we did not blind participants or study staff to diet assignment with the resultant potential for bias. However, staff performing MRI, DXA and serum analysis were blinded to diet assignment and the interventions measurements were performed as objectively as possible. The short duration of the study prevents us from determining the durability of the dietary intervention in reversing NAFLD long term.

In conclusion, the CHO-restricted diet reduced liver fat over 8 weeks in adolescents with NAFLD; however, our sample size may not have been large enough to determine whether this decrease in liver fat was due to a unique effect of CHO restriction. Depleting liver fat may be critical for reducing the risk of disease progression and risk of other metabolic diseases as children and adolescents age into adulthood. Larger studies are needed to determine whether a CHO-restricted diet induces change in hepatic lipid independent of change in body fat.

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CONFLICT OF INTEREST

The authors have no conflicts to disclose.

AUTHOR CONTRIBUTIONS

A.G. provided study design and oversight, data analysis and manuscript preparation; M.P. and S.D. provided study implementation, data management and input into manuscript preparation; T.S., A.A., M.B., S.M. and B.G. helped with study design and implementation and provided input into manuscript preparation; A.A. conducted nutrition data analysis and reviewed the manuscript.

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