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Effects of Dietary Fructose Restriction on Liver Fat, De Novo Lipogenesis, and Insulin Kinetics in Children With Obesity

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BACKGROUND & AIMS: Consumption of sugar is associated with obesity, type 2 diabetes mellitus, nonalcoholic fatty liver disease, and cardiovascular disease. The conversion of fructose to fat in liver (de novo lipogenesis [DNL]) may be a modifiable pathogenetic pathway. We determined the effect of 9 days of isocaloric fructose restriction on DNL, liver fat, visceral fat (VAT), subcutaneous fat, and insulin kinetics in obese Latino and African American children with habitual high sugar consumption (fructose intake >50 g/d). **METHODS:** Children (9−18 years old; n = 41) had all meals provided for 9 days with the same energy and macronutrient composition as their standard diet, but with starch substituted for sugar, yielding a final fructose content of 4% of total kilocalories. Metabolic assessments were performed before and after fructose restriction. Liver fat, VAT, and subcutaneous fat were determined by magnetic resonance spectroscopy and imaging. The fractional DNL area under the curve value was measured using stable isotope tracers and gas chromatography/mass spectrometry. Insulin kinetics were calculated from oral glucose tolerance tests. Paired analyses compared change from day 0 to day 10 within each child. **RESULTS:** Compared with baseline, on day 10, liver fat decreased from a median of 7.2% (interquartile range [IQR], 2.5%−14.8%) to 3.8% (IQR, 1.7%−15.5%) ($P < .001$) and VAT decreased from 123 cm^3 (IQR, 85−145 cm^3) to 110 cm^3 (IQR, 84–134 cm^3) ($P < .001$). The DNL area under the curve decreased from 68% (IQR, 46%–83%) to 26% (IQR, 16%−37%) ($P < .001$). Insulin kinetics improved ($P < .001$). These changes occurred irrespective of baseline liver fat. **CONCLUSIONS:** Short-term (9 days) isocaloric fructose restriction decreased liver fat, VAT, and DNL, and improved insulin kinetics in children with obesity. These findings support efforts to reduce sugar consumption. ClinicalTrials.gov Number: NCT01200043.

Keywords: Dietary Treatment; NAFLD; Pediatric; Overweight.

High dietary sugar consumption is associated with nonalcoholic fatty liver disease (NAFLD) and excess visceral adipose tissue (VAT),[1–3] which are in turn linked to type 2 diabetes mellitus (T2DM), dyslipidemia, and cardiovascular disease in adults and children.[4–6] NAFLD occurs when hepatic lipid concentration (from peripheral lipolysis or synthesis of new fat by hepatic de novo lipogenesis [DNL]) exceeds the combined rates of hepatic lipid oxidation and export.[7,8] Studies have linked visceral and/or liver fat with metabolic dysfunction, including insulin resistance and T2DM,[9–11] and NAFLD is a predictor of type 2 diabetes.[12,13] Recently, a survey in 675 children with biopsy-proven NAFLD showed that 30% had T2DM or prediabetes.[14]

The link between consumption of sugar, especially fructose, and accumulation of ectopic fat is not well understood, but recent studies suggest that fructose stimulates DNL,[2,15] which may drive the accumulation of liver and/or visceral fat.[7,16] Fructose has been shown to specifically increase carbohydrate response element-binding protein,[17] a transcription factor that induces 3 enzymes of DNL—adenosine triphosphate citrate lyase, acetyl-CoA carboxylase, and fatty acid synthase. We recently demonstrated that in weight-stable healthy men, high fructose intake for a 9-day period was associated with higher DNL and liver fat, compared with a diet with identical energy and macronutrient intake, but in which complex carbohydrate (starch) was substituted for sugar.[18] We provided evidence linking fructose-driven DNL with liver fat and demonstrated that short-term reduction in fructose intake was consistently associated with lower levels of liver fat and rates of DNL, even in the absence of weight loss.

In the current study, we hypothesized that short-term fructose restriction in children with obesity and metabolic syndrome who habitually consume high levels of fructose would reduce liver fat and hepatic DNL without change in energy intake or weight. We studied 41 Latino and

Abbreviations used in this paper: **AUC, area under the curve; CISI, Composite Insulin Sensitivity Index; DNL, de novo lipogenesis; IQR, interquartile range; ISR, insulin secretion rate; L/W, lipid/water; MR, magnetic resonance; NAFLD, non-alcoholic fatty liver disease; OGTT, oral glucose tolerance test; SAT, subcutaneous adipose tissue; T2DM, type 2 diabetes mellitus; VAT, visceral adipose tissue.**

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African-American children with high levels of self-reported sugar intake, feeding them diets that featured isocaloric substitution of starch for most sugar for 9 days, resulting in a reduction in total sugar content from 28% to 10%, and fructose from 12% to 4% of total energy intake. In separate publications from this study,[19,20] we reported improvements in glycemia, fasting lipoproteins, blood pressure, and other clinical parameters. Here, we report the effects of isocaloric fructose restriction on liver fat, hepatic DNL, VAT, and subcutaneous adipose tissue (SAT), and their relation to changes in insulin kinetics.

Methods

Study Design and Population

We recruited non-diabetic African-American and Latino children with obesity and metabolic syndrome who identified as high habitual sugar consumers (>15% sugar, >5% fructose) based on a food frequency questionnaire and interview by a dietitian.[19] As described elsewhere,[19] eligibility criteria included age 8−18 years, body mass index *z*-score ≥1.8, and at least 1 of the following: systolic blood pressure >95th percentile for age and sex, fasting triglycerides >150 mg/dL, alanine aminotransferase >40 U/L, fasting glucose 100−125 mg/dL, fasting insulin >15 μIU/mL, homeostatic model assessment of insulin resistance >4.3,[21] and severe acanthosis nigricans. This study protocol was approved by the Institutional Review Boards of the University of California, San Francisco (approval 10-03473) and Touro University-California (approval M-0609) and is registered with ClinicalTrials.gov (NCT01200043). Informed written consent/assent were obtained before formal screening was initiated. Comprehensive metabolic assessments were performed before (day 0) and after (day 10) a 9-day dietary intervention.

Metabolic Assessments

Participants and their guardians were instructed to continue their usual home diets and other routines before the study. On days 0 and 10, after fasting at least 8 hours, participants underwent metabolic studies at the University of California San Francisco Pediatric Clinical Research Center (Figure 1). Weight and vital signs were measured and urine pregnancy testing was performed in female participants. Body composition was measured by whole-body dual-energy x-ray absorptiometry (GE/Lunar Prodigy, Madison WI). A 2-hour 75-g oral glucose tolerance test (OGTT) was performed, with glucose, insulin, and C-peptide measurements at 0, 30, 60, 90, and 120 minutes. Fasting glucose and insulin, and their respective areas under the curve (AUC) are reported elsewhere.[19]

Tracer/Feeding Study

Upon completion of the OGTT, an 8-hour stable isotope tracer/feeding study to measure postprandial DNL was initiated (Figure 1) using liquid meals containing sodium [1-^{13}C]-acetate (Cambridge Isotope Laboratories, Cambridge, MA). After an initial double-sized meal, single-sized meals were fed every half-hour for 8 hours. Altogether, the meals provided 67% of estimated daily energy requirement (15% protein, 35% fat, 50% carbohydrate) and 5−7 g of the acetate tracer. On day 0, the fructose content of the liquid meals ranged from 12% to 18% of energy intake, depending on self-reported usual intake; on day 10, the fructose content was reduced to 4% of energy intake, but overall energy and carbohydrate content matched that of the day 0 test meals. In both cases, the remainder of carbohydrate was provided primarily as glucose polymer. Blood samples were drawn on K_2EDTA before the first test meal and every hour thereafter, processed, and frozen at −80°C.

Magnetic Resonance Imaging and Spectroscopy

During the tracer/feeding study, participants underwent an magnetic resonance (MR) exam on a 3-Tesla scanner (GE Healthcare, Waukesha, WI) to measure liver fat, VAT, and SAT. For the liver fat measures, MR spectroscopy was obtained from a 200-mL single voxel (64 acquisitions water-suppressed, 8 acquisitions unsuppressed, with a repetition time of 2500 ms and an echo time of 30 ms), similar to prior reports.[22,23] Spectra were automatically phase-, frequency-, motion-, and T2 relaxation-time−corrected (using in-house derived formulas for $T2_{water} = -12.4 \times L/W + 31.3$ ms, and $T2_{lipids} = 23.1 \times L/W + 58.5$ ms; where L/W is the MR measured lipids/water at echo time = 30 ms).[23] Quality was visually confirmed. MR liver fat fractions were calculated from the corrected MR measures of CH_2 and CH_3 lipids and of water as the total lipids / (total lipids + water).

VAT and SAT volumes were semi-automatically generated based on either water-suppressed gradient-recalled echo images or on the fat images generated from iterative decomposition and echo asymmetry with least-squares estimation (IDEAL) MR images (10-mm-thick) at the disc between lumbar vertebrae 3 and 4. Regions of interest for VAT and SAT were determined by a single reader using a threshold-based contour mapping algorithm written in-house in IDL (Exelis Visual Information Solutions, Inc., Boulder, CO) followed by a manual alteration, as needed.

Outpatient Feeding and Follow-up

Upon completion of the metabolic assessments on day 0, participants were discharged to home with 3 days of food and detailed instructions. They returned at 3-day intervals to pick up food for a total of 9 days. On day 10, all day 0 assessments were repeated. As described previously,[19] the University of California, San Francisco Clinical Research Service Bionutrition Core designed individualized menus for each child and provided all food. Study diets restricted sugar and fructose intake to 10% and 4% of total energy intake, respectively, by substituting an equal number of calories from starch to match overall proportional carbohydrate consumption in each participant's self-reported usual diet.[19] Total energy content was estimated using Institute of Medicine formulas for weight maintenance in overweight boys and girls[24] and adjusted if weight changed >2% during outpatient feeding.

De Novo Lipogenesis and Insulin Kinetics

Samples collected during the tracer/feeding studies underwent ultracentrifugation to isolate triglyceride-rich lipoproteins (density 1.006), and the palmitate from the triglyceride-rich lipoprotein-triglyceride fraction was analyzed