Isocaloric Fructose Restriction and Metabolic Improvement in Children with Obesity and Metabolic Syndrome

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Objective: Dietary fructose is implicated in metabolic syndrome, but intervention studies are confounded by positive caloric balance, changes in adiposity, or artifactually high amounts. This study determined whether isocaloric substitution of starch for sugar would improve metabolic parameters in Latino ($n = 27$) and African-American ($n = 16$) children with obesity and metabolic syndrome.

Methods: Participants consumed a diet for 9 days to deliver comparable percentages of protein, fat, and carbohydrate as their self-reported diet; however, dietary sugar was reduced from 28% to 10% and substituted with starch. Participants recorded daily weights, with calories adjusted for weight maintenance. Participants underwent dual-energy X-ray absorptiometry and oral glucose tolerance testing on Days 0 and 10. Biochemical analyses were controlled for weight change by repeated measures ANCOVA.

Results: Reductions in diastolic blood pressure (-5 mmHg; $P = 0.002$), lactate (-0.3 mmol/L; $P < 0.001$), triglyceride, and LDL-cholesterol (-46% and -0.3 mmol/L; $P < 0.001$) were noted. Glucose tolerance and hyperinsulinemia improved ($P < 0.001$). Weight reduced by 0.9 ± 0.2 kg ($P < 0.001$) and fat-free mass by 0.6 kg ($P = 0.04$). *Post hoc* sensitivity analysis demonstrates that results in the subcohort that did not lose weight ($n = 10$) were directionally consistent.

Conclusions: Isocaloric fructose restriction improved surrogate metabolic parameters in children with obesity and metabolic syndrome irrespective of weight change.

Introduction

Chronic diseases such as non-alcoholic fatty liver disease (NAFLD) and type 2 diabetes (T2DM) now occur in children, an age group that had never previously manifested such pathologies. In addition, dyslipidemia and hypertension, two risk factors for cardiovascular disease (CVD), are now common in childhood (1,2). While these diseases clearly exhibit higher prevalence in children with obesity, they nonetheless occur in those with normal weight (3). Furthermore, the prevalence of diabetes is higher than obesity prevalence in some countries, such as India, Pakistan, and China (4), suggesting that calories alone do not explain this phenomenon. It has been hypothesized that changes in dietary composition associated with the Western diet are responsible for biochemical alterations which promote insulin resistance and foment these diseases, known collec-

tively as metabolic syndrome (5). Fructose has attracted particular concern, due to several unique metabolic and neuroendocrine properties: 1) it is metabolized almost exclusively in the liver (6); 2) it serves as a substrate for *de novo* lipogenesis and drives hepatic triglyceride (TG) synthesis and accumulation (7,8); 3) it engages in non-enzymatic fructation and reactive oxygen species formation which causes cellular dysfunction (9); 4) it does not suppress the hunger hormone ghrelin, resulting in excessive consumption (10); and 5) it stimulates the nucleus accumbens resulting in increased reward and continued ingestion (11). Short-term studies demonstrate that excessive oral fructose increases serum TG and visceral fat more than does its isomer glucose (12). However, previous clinical studies of orally administered fructose on surrogate markers of metabolic syndrome were confounded by the administration of excessive

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or pharmacologic doses and by the inability to isolate the metabolic effects of fructose from either its caloric content or its effects on weight gain and adiposity.

Instead, we assessed the effects of dietary sugar restriction with iso-caloric substitution of starch (complex carbohydrate) on metabolic parameters in children with obesity with high habitual added sugar consumption who evidenced co-morbidity, so as to obviate concerns of dose, caloric equivalence, or effects on adiposity.

Methods

This study was approved by the UCSF Committee on Human Research and the Touro University Institutional Review Board, and listed as NCT01200043 on ClinicalTrials.gov. We restricted recruitment to Latino youth (who are known to be at higher risk for dyslipidemia and NAFLD) (13), and African-American youth (who are known to be at higher risk for T2DM and hypertension) (14), and who identified as high habitual sugar consumers (>15% sugar and >5% fructose).

Participants were identified primarily through the UCSF Weight Assessment for Teen and Child Health (WATCH) Clinic, an inter-disciplinary obesity clinic dedicated to targeting metabolic dysfunction rather than caloric balance (15). Eligibility criteria included: 1) ages 8-18 years; 2) obesity (body mass index (BMI) z-score $\geq +1.8$); and 3) at least one other co-morbidity, including hypertension (systolic blood pressure (BP) >95th percentile for age and sex), hypertriglyceridemia (TG >95th percentile for age and sex), impaired fasting glucose (Hemoglobin A_{1c} >6.0 or fasting glucose >5.55 mmol/L), hyperinsulinemia (fasting insulin >90 pmol/L or HOMA-IR >4.3), alanine aminotransferase (ALT) >40 U/L, or severe acanthosis nigricans. Exclusion criteria included: known diabetes, steroid medication use, any medication that affected insulin secretion or resistance, alcohol use, pregnancy, or neuroactive medications. Participants were recruited during initial clinic visits or from referrals from the community. Appropriate consent and assent were obtained in writing at the time of screening. Participants filled in food frequency questionnaires (FFQ) (16,17) and were interviewed by a dietitian, from which their baseline macronutrient profiles (percent of calories as fat, protein, and carbohydrate; and fiber content as g/1000 kcal) were identified. We estimated energy requirements using formulas published by the Institute of Medicine (IOM) for weight maintenance in overweight boys and girls, ages 3 through 18 (18). After the first 17 participants were studied, seven were noted to have lost >2% in weight, so caloric targets for each participant were increased by 10% thereafter.

Participants and guardians were told to continue their usual home diets until they came fasting to the UCSF Pediatric Clinical Research Center on Day 0, where anthropometric measurements were recorded. Blood pressure was obtained by automated monitor after a 15 min rest period. Fasting blood samples were obtained through a saline lock. An oral glucose tolerance test (OGTT) was performed by administering 75 g glucose, and blood was drawn for glucose and insulin levels at 0, 30, 60, 90, and 120 min. Whole-body dual-energy X-ray absorptiometry (DXA) scanning was performed to assess bone, fat, and fat-free mass. All participants were provided a floor scale with instructions on how to collect, record,

and report their weight each day, as well as store and prepare the study diet, and record their daily food intake for the following 9 days. Participants were sent home with 9 days of food (in three separate installments) prepared by the UCSF Clinical Research Service (CRS) Bionutrition Core to provide adequate calories to maintain their body weight. The menu was planned to restrict added sugar, while substituting other carbohydrates such as those in fruit, bagels, cereal, pasta, and bread so that the percentage of calories consumed from carbohydrate was consistent with their baseline diet, but total dietary sugar and fructose were reduced to 10% and 4% of total calories, respectively. Additional food items were provided when weight loss was observed during daily fasting weight checks which were reported to the study dietitian each morning. Additional individualized instructions for maintaining weight stability were provided by phone, email or text, and at food pickup or delivery. On Day 10, participants returned with their final record of dietary intake and fasting blood specimens, OGTT, and DXA were repeated. Any additional or missed foods that were recorded on the diet checklists were added or subtracted from the total study diet intake calculation. The caloric and nutrient content of the study diet assigned and after intervention was calculated using the nutrient analyses software Pro-Nutra 3.4 (Viocare) with USDA standard reference database 23.

Fasting clinical chemistries were measured in the UCSF clinical laboratory. All other specimens were processed and frozen for subsequent batch analysis. Plasma with sodium fluoride and potassium oxalate was used to measure for glucose and lactate concentrations (YSI 2300 Stat plus, Yellow Springs, OH). Serum insulin concentrations were measured by chemiluminescence on a Siemens Immulite 2000 XPI platform, fasting lipids on a Beckman DXC-600 by blanked timed endpoint, and high-density lipoprotein cholesterol (HDL-C) by homogeneous immunoinhibition (Trinity Biotech) at Pennington Biomedical Research Center (Baton Rouge, LA).

Data are expressed as mean $\pm$ SD. Normal distributions were tested by histogram, box-plot, q-norm plot, and Shapiro Wilk tests. To compare weight and DXA variables between Day 0 and Day 10, paired t-tests were used. Each analyte was evaluated for normality; when normal, repeated measures analysis of covariance (ANCOVA) was performed on each biochemical parameter to control for weight change, and separate regression analysis was performed to obtain the β-coefficient (mean difference adjusted for weight change, with 95% confidence intervals). When data were not normally distributed, log transformation was performed to achieve normal distribution, and then the data were subjected to repeated measures ANCOVA. Resultant β-coefficients were converted back to the raw data scale for each parameter to reflect percent change in mean differences adjusted for weight change, with 95% confidence intervals. When data were not normally distributed and could not be log transformed, Kruskal–Wallis non-parametric testing was instead used for analysis. We also performed univariate regression analysis to investigate the association between change in weight and change in metabolic analytes; r^2 values are reported to assess the change in the variance of each analyte versus change in weight. To assess the impact of the demographic variables (sex, age, Tanner stage, race/ethnicity), we re-ran the repeated measures ANCOVA models with each included as a single covariate, and with all included as multiple covariates in one model. For the glucose and insulin levels from the OGTT, we compared values at each time point using paired (Day 10 vs. Day 0) t-tests. *Post hoc* sensitivity analysis was performed for the 10 participants who did not lose weight during the study. All statistical tests

TABLE 1 Anthropometric and DXA measurements (mean ± SD) on Day 0 and 10 ($n = 43$)

	Day 0	Day 10	Mean change [95% CI]	P value
Weight (kg)	93.0 ± 22.1	92.1 ± 22.2	−0.9 [−1.3, −0.6]	0.001
Body mass index (kg/m^2)	35.6 ± 6.4	35.2 ± 6.5	−0.4 [−0.6, −0.2]	<0.001
Fat mass (kg)	43.9 ± 13.8	43.6 ± 14.2	−0.3 [−0.8, +0.1]	0.17
Fat-free mass (kg)	48.3 ± 9.4	47.6 ± 8.9	−0.6 [−1.2, −0.1]	0.04
Bone mass[a] (kg)	2.7 ± 0.5	2.7 ± 0.5	0 [−0.08, +0.05]	0.63

Paired t-test, statistical significance $P < 0.05$.
[a]Bone mass excludes the one participant who underwent BOD-POD analysis instead of DXA due to excessive weight ($n = 42$).

were considered significant at $P < 0.05$ based on two-tailed tests. All analyses were conducted with STATA version 12.1 (StataCorp, College Station, TX).

Results

Day 0 and Day 10 clinical and anthropometric parameters are listed in Table 1. Fifty-two Latino and African-American participants were recruited. Two participants were found to be ineligible, five did not arrive for their Day 0 visit, and two completed Day 0 but did not return on Day 10, and are excluded from this analysis. We analyzed 43 pairs (27 Latino, 16 African-American, 16M, 27F) of baseline and 10-day post-intervention data (42 pairs for OGTT). The mean age of our cohort was 13.3 ± 2.7 years, with BMI z-score 2.4 ± 0.3. Pubertal status was Tanner 1 in five, Tanner 2-3 in 16, and Tanner 4-5 in 22 participants.

We attempted to match each participant's macronutrient intake profile during the 9-day intervention to their baseline diet. After adjustments for both uneaten and supplementary foods, the mean self-reported intake of the study diet was 29 ± 6 kcal/kg with a macronutrient profile of 51 ± 3% carbohydrate, 16 ± 1% protein, and 33 ± 3% fat (16% saturated, 9% polyunsaturated, 13% monounsaturated). *Post hoc* analysis showed that, compared with the baseline macronutrient distribution determined by FFQ, the total percentage of carbohydrate intake on the study diet decreased by 4%, protein increased by 2%, and there was no change in percentage calories from fat. Within the carbohydrate fraction, dietary sugar intake reduced from 27.7 ± 8.3% to 10.2 ± 1.7%, and fructose from 11.7 ± 4.0% to 3.8 ± 0.5%, of daily calories. The consumption of dietary fiber of necessity increased from a daily mean of 9.3 ± 2.2 g/1,000 kcal to 11.7 ± 1.3 g/1,000 kcal. This study diet profile is consistent with recommendations by the IOM for macronutrients (18) and the World Health Organization for dietary sugar intake (19). This "child-friendly" study diet included various no- or low-sugar added processed foods including turkey hot dogs, pizza, bean burritos, baked potato chips, and popcorn that were purchased at local supermarkets.

Despite intensive efforts to maintain each participant's body weight at baseline levels, weight decreased by 0.9 ± 0.2 kg (1%, $P = 0.001$) over the 10 days of intervention. Of the 43 participants, consumption of the study diet ranged from 75% to 115% of calories assigned; 33 reported that they were unable to consume all of the food provided for weight maintenance. Individual weight curves and

the mean pattern of weight change (Figure 1) suggests that weight loss occurred within the first 4 days, with subsequent return toward baseline and stabilization thereafter, arguing for acute water loss and against persistent caloric deficit as the cause of the weight change. Comparison of DXA data (Table 1) demonstrated that fat and bone mass did not change significantly during the 10-day study period, although fat-free mass reduced by 0.6 kg ($P = 0.04$).

All subsequent physiologic and biochemical analyses that were normally distributed, either before or after log transformation, were adjusted for weight change by repeated measures ANCOVA. Our results did not differ when we controlled for sex, age, Tanner stage, and/or race/ethnicity. Weight change itself was not significant as a covariate in any of the repeated measures ANCOVAs. Aspartate aminotransferase (AST) was not normally distributed and was analyzed by Kruskal–Wallis testing.

Systolic BP did not change ($P = 0.42$) over the 10 days. However, diastolic BP decreased signficantly by 4.9 mmHg ($P = 0.002$). Heart rate (HR) tended to decline non-significantly by 2.8 bpm ($P = 0.12$). Interestingly, uric acid increased over the 10 days of intervention by 17.8 μmol/L ($P = 0.001$).
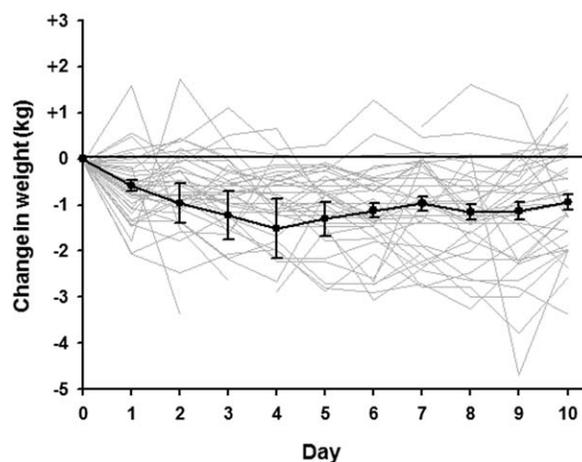

Figure 1 Change in weight from baseline in the 43 participants over the 10 days of study. Individual weight change curves are in light gray, while means ± SEM for the entire cohort are in black.
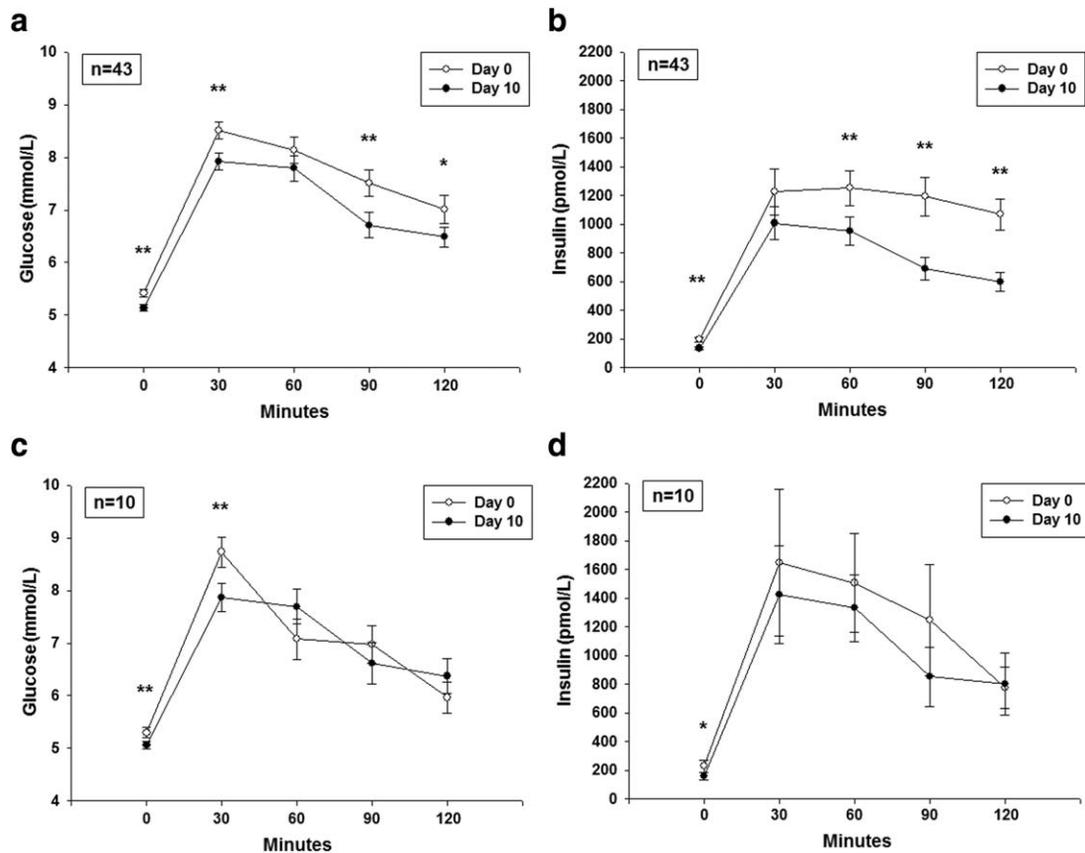
Figure 2 (a) Glucose and (b) insulin responses (mean ± SEM) to OGTT on Day 0 and 10 for all 43 participants. (c) Glucose and (d) insulin responses to OGTT on Day 0 and 10 for the 10 participants in the *post hoc* sensitivity analysis who gained weight during the study interval. *$P < 0.05$, paired *t*-test (Day 10 vs. Day 0) at each individual time point. **$P < 0.01$, paired *t*-test (Day 10 vs. Day 0) at each individual time point.

Glucose and insulin responses to OGTT are shown in Figures 2a,b. Fasting glucose decreased by 0.3 mmol/L ($P < 0.001$), while glucose area under the curve (AUC) decreased by 7.3% or 67.2 mmol/L/120 min ($P = 0.001$). Fasting insulin decreased by 53% ($P < 0.001$), and HOMA-IR decreased by 58% ($P < 0.001$). Peak insulin decreased by 56% ($P < 0.001$) and insulin AUC reduced by 57% ($P < 0.001$), implying reduction in hyperinsulinemia.

The results of other biochemical analyses are shown in Table 2. Fasting TG levels decreased by 46% ($P = 0.002$), low-density lipoprotein cholesterol (LDL-C) decreased by 0.3 mmol/L ($P < 0.001$), and HDL-C reduced by 0.1 mmol/L ($P < 0.001$). Serum free fatty acids increased by 0.12 mmol/L ($P < 0.001$), suggesting increases in peripheral lipolysis and increases in flow of fatty acid for oxidation. Alanine aminotransferase (ALT) tended to decline non-significantly by 13% ($P = 0.09$), while AST declined significantly by 3.6 U/L ($P = 0.02$ by Kruskal–Wallis). Fasting lactate on Day 0 was 1.2 ± 0.4 mmol/L, and decreased by 0.3 mmol/L, ($P < 0.001$), and lactate AUC decreased by 19.5% or 31.2 mmol/L/120 min ($P < 0.001$).

To provide assurance that the effects of sugar restriction were not due exclusively to the modest weight loss evidenced during the study, we performed univariate regression between the change in weight versus the change in metabolic analytes. We saw no relation-ship other than a positive association between change in glucose AUC and change in weight ($P = 0.045$). Furthermore, we analyzed the 10 participants who did not lose weight over the 10 days in a separate *post hoc* sensitivity analysis, and the results were directionally consistent as compared with the entire cohort (Table 3). Notably, hyperinsulinemia significantly improved in this subcohort as well (Figure 2d).

Discussion

Epidemiological studies have linked dietary fructose consumption, either as sucrose or high-fructose corn syrup, with the various comorbidities of metabolic syndrome, including CVD, T2DM, and NAFLD (4,20,21). However, proof of causation has been difficult to establish for four reasons. First, long-term randomized controlled trials of dietary fructose consumption are difficult because in real world settings, there is no integrated biomarker for dietary fructose or measure of compliance (22). Second, short-term experimental protocols feature an excessive dose of oral fructose (23). Third, recall bias underestimating sugar consumption is the norm in epidemiologic studies (24); therefore using recall data in order to conduct externally controlled studies becomes problematic. Fourth,

TABLE 2 Biochemical measurements (mean ± SD) on Day 0 and 10 ($n = 43$)

	Day 0	Day 10	β-coefficient (adjusted change) [95% CI]	P value	$r^{2\ a}$
Heart rate (beats/min)	83.1 ± 10.7	80.1 ± 11.3	−2.8 [−6.5, +0.9]	0.13	0.001
Systolic blood pressure (mmHg)	122.6 ± 10.5	121.1 ± 9.9	−1.4 [−4.9, +2.1]	0.43	0.002
Diastolic blood pressure (mmHg)	68.8 ± 8.9	63.7 ± 7.5	−4.9 [−8.1, −1.8]	0.003	0.03
Mean arterial blood pressure (mmHg)	86.7 ± 7.7	82.9 ± 7.3	−2.8 [−6.4, +0.8]	<0.001	0.01
Fasting glucose (mmol/L)	5.4 ± 0.5	5.1 ± 0.4	−0.3 [-0.4, −0.2]	<0.001	0.04
Glucose AUC (mmol/L/120 min)	911.9 ± 130.9	845.3 ± 130.4	−67.2 [−105.5, −28.9]	<0.001	0.09
Peak glucose during OGTT (mmol/L)	9.1 ± 1.4	8.3 ± 1.3	−0.8 [−1.2, −8.0]	<0.001	0.03
Fasting lactate (mmol/L)	1.2 ± 0.4	0.9 ± 0.3	−0.3 [−0.5, −0.2]	<0.001	0.001
Lactate AUC (mmol/L/120 min)	160.0 ± 34.5	129.0 ± 34.5	−31.2 [−41.9, −20.5]	<0.001	0.01
Fasting insulin (pmol/L)[b]	195.6 ± 115.2	135.6 ± 63.0	−53% [−65, −36]	<0.001	0.07
Insulin AUC (pmol/L/120 min)[b]	131760 ± 81240	89580 ± 53280	−57% [−71, −36]	<0.001	0.07
HOMA-IR[b]	7.9 ± 4.8	5.2 ± 2.6	−58% [−70, −43]	<0.001	0.07
Peak insulin during OGTT (pmol/L)[b]	1645.2 ± 1020.0	1172.8 ± 786.8	−56% [−69, −36]	<0.001	0.09
AST (U/L)[c]	27.4 ± 14.1	23.8 ± 8.9		0.02	0.04
ALT (U/L)[b]	28.9 ± 22.8	26.7 ± 19.6	−13% [−25, +0.2]	0.09	0.02
BUN (mmol/L)	3.5 ± 0.9	3.6 ± 1.1	0.1 [−0.2, +0.4]	0.56	0.06
Creatinine (μmol/L)	53.0 ± 8.8	53.0 ± 8.8	0 [−0.9, +2.7]	0.41	0.001
Fasting uric acid (μmol/L)	315.2 ± 53.5	333.1 ± 53.5	+17.8 [+8.3, +32.1]	0.001	0.08
Fasting triglycerides (mmol/L)[b]	1.4 ± 0.9	1.0 ± 0.5	−46% [−62, −25]	0.002	0.08
Fasting LDL-cholesterol (mmol/L)	2.4 ± 0.6	2.1 ± 0.6	−0.3 [−0.4, −0.1]	<0.001	0.003
Fasting HDL-cholesterol (mmol/L)	1.2 ± 0.2	1.0 ± 0.2	−0.1 [−0.2, −0.1]	<0.001	0.05
Fasting free fatty acids (mmol/L)	0.6 ± 0.2	0.7 ± 0.2	+0.1 [+0.1, +0.2]	<0.001	0.07

Statistical significance $P < 0.05$ after adjustment for weight change by repeated measures ANCOVA.
[a]Coefficient of determination for univariate regression analysis between change in biochemical parameters and change in weight.
[b]Parameters not normally distributed and log transformed for analysis only; mean change and 95% CI are reported as percent change.
[c]Non-parametric Kruskal–Wallis, statistical significance $P < 0.05$.

investigators routinely conflate the metabolic detriment of the fructose molecule with its caloric equivalence or with its effects on adiposity, either of which are assumed to be the intermediate cause of the pathology (25).

To circumvent these issues, we instead chose to evaluate whether short-term isocaloric restriction of dietary fructose in children with obesity and metabolic syndrome would mitigate metabolic pathology. However, to demonstrate a primary effect, we had to substitute dietary added sugar (glucose-fructose) calorie-for-calorie with dietary starch in order to maintain equivalence for both calories, carbohydrate content, and weight. We anticipated that a 9-day fructose restriction interval would be sufficient, based on previous work by our group in healthy adults demonstrating changes in liver fat within 7 days of isocaloric fructose restriction (8).

Fructose has been suggested to increase BP (26) by enhancing sympathetic activity (27), decreasing urinary sodium excretion (28), increasing gut sodium absorption (29), and increasing uric acid (the endogenous inhibitor of endothelial nitric oxide synthase) (30). Fructose has been associated with both systolic and diastolic BP increase in children (31,32). Our participants' diastolic BP declined significantly. A reduction in diastolic BP suggests decreased volume status which would normally trigger a compensatory increase in HR to maintain cardiac output. Our participants' weight loss occurred during the first 4 days (suggesting water loss) and then returned toward baseline (Figure 1). However, the non-significant decline in HR suggested that the diastolic BP reduction was not due to changes in volume status. In addition, blood urea nitrogen and creatinine did not change (Table 2). Interestingly, our participants' uric acid levels increased, despite the significant reduction in diastolic BP. We cannot attribute this increase to hemoconcentration, protein intake, or weight loss.

Fasting glucose and glucose AUC improved, implying improved glucose tolerance. Fasting, peak, and insulin AUC reduced, implying enhanced insulin sensitivity. These improvements were unrelated to calories or weight change.

We also documented improvement in fasting serum lipids. TG were reduced on the fructose-restricted diet, consistent with previously reported declines in _de novo_ lipogenesis and very-low-density lipoprotein (VLDL) production and release from the liver (8,33). LDL-C reduced consistent with VLDL reduction. Fasting free fatty acids increased, consistent with peripheral lipolysis.

Fasting lactate and lactate AUC decreased after fructose restriction. Although clinical norms for lactate in children vary, high lactate is seen in patients with decreased mitochondrial number or throughput, e.g., those with ischemia or anoxia, cancer (due to the Warburg

TABLE 3 Sensitivity analysis of the 10 children who did not lose weight; measurements (mean ± SD) on Day 0 and 10

	Day 0	Day 10	β-coefficient (adjusted change) [95% CI]	P value	$r^{2\,a}$
Fasting glucose (mmol/L)	5.3 ± 0.3	5.1 ± 0.2	−0.2 [−0.4, −0.1]	0.01	0.31
Glucose AUC (mmol/L/120 min)	854.7 ± 74.4	836.4 ± 95.5	−18.3 [−79.6, +42.2]	0.51	0.11
Peak glucose during OGTT (mmol/L)	8.7 ± 0.9	8.0 ± 0.9	−0.7 [−1.3, −0.2]	0.01	0.19
Fasting lactate (mmol/L)	1.3 ± 0.5	0.9 ± 0.4	−0.5 [−0.8, −0.1]	0.01	0.56
Lactate AUC (mmol/L/120min)	161.9 ± 34.2	128.5 ± 28.1	−33.4 [−58.2, −8.6]	0.01	0.09
Fasting insulin (pmol/L)[b]	228.6 ± 141.0	159.6 ± 76.2	−54% [−76, −11]	0.04	0.30
Insulin AUC (pmol/L/120 min)[b]	151080 ± 112620	123180 ± 74400	−32% [−67, +43]	0.24	0.01
HOMA-IR[b]	9.0 ± 5.7	5.9 ± 2.9	−58% [−78, −21]	0.03	0.25
Peak insulin during OGTT (pmol/L)[b]	2019.0 ± 1399.2	1614.0 ± 1129.8	−41% [−71, +21]	0.08	0.08
AST (U/L)[c]	25.9 ± 6.9	21.1 ± 3.9		0.08[c]	0.001
ALT (U/L)[b]	25.2 ± 13.1	22.5 ± 11.5	−21% [−51, +24]	0.42	0.001
BUN (mmol/L)	3.3 ± 0.6	3.7 ± 0.9	+0.4 [−0.1, +0.8]	0.11	0.002
Creatinine (μmol/L)	53.0 ± 8.8	53.0 ± 8.8	+ 8.8 [−3.5, +6.2]	0.61	0.25
Fasting uric acid (μmol/L)	315.2 ± 65.4	327.1 ± 47.6	+11.9 [−5.9, +29.7]	0.14	0.04
Fasting triglycerides (mmol/L)[b]	1.2 ± 0.4	0.9 ± 0.4	−33% [−69, +55]	0.30	0.17
Fasting LDL-cholesterol (mmol/L)	2.1 ± 0.5	1.9 ± 0.4	−1.2 [−0.4, +0.1]	0.26	0.04
Fasting HDL-cholesterol (mmol/L)	1.1 ± 0.2	1.1 ± 0.2	−0.05 [−0.14, +0.04]	0.26	0.01
Fasting free fatty acids (mmol/L)	0.5 ± 0.2	0.6 ± 0.1	+0.1 [−0.04, +0.17]	0.19	0.15

Statistical significance $P < 0.05$ after adjustment for weight change by repeated measures ANCOVA.
[a]Coefficient of determination for univariate regression analysis between change in biochemical parameters and change in weight.
[b]Parameters not normally distributed and log transformed for analysis only; mean change and 95% CI are reported as percent change.
[c]Non-parametric Kruskal–Wallis, statistical significance $P < 0.05$.

effect) (34), or in those with mitochondrial encephalomyopathy (Kearns-Sayre, MELAS) (35). Fasting lactate and lactate AUC reduced significantly either through decreased lactate production or increased lactate clearance. ALT, a marker of liver fat, did not decline significantly. However, AST, a marker of liver mitochondrial integrity (36,37), declined significantly by Day 10. While each of these indices are indirect, the simultaneous reduction of AST, lactate, and TG suggests that hepatic mitochondria may be capable of improved disposal of pyruvate. We proffer the testable hypothesis that excessive dietary fructose causes hepatic mitochondrial overload which results in metabolic syndrome, and that individual manifestations of metabolic syndrome may be due to organ-specific mitochondrial overload.

This study manifests several strengths. Rather than studying excessive acute oral fructose administration in normal participants, or the addition of fructose to a normal caloric allotment (12,38), we instead evaluated restriction of added dietary sugar in children with metabolic syndrome to see whether their metabolic dysfunction would resolve—an endpoint with clinical relevance, and with little chance for charges of artifact. If our participants had been non-compliant with the dietary regimen, it would only have diluted our findings. To reduce systematic bias, we maintained investigator blinding on all data until final statistical analysis.

However, there are some limitations to our paradigm. Although inclusion of a separate external control group would have been optimal, it would have presented novel challenges of its own, such as: 1) if subjects under- or over-estimated their baseline fructose con-

sumption, then providing them their reported daily fructose content would be problematic; 2) altering each subject's diet while trying to maintain the baseline fructose content would require changes in liquid versus solid, which may also result in caloric change, altered absorption, and altered satiety; and 3) our participants were all patients in an obesity program. We did not believe that maintaining fructose at the same level, even within a study, is commensurate with the message that the change in macronutrient composition is important for their health, and in order to use the study as an "educational moment." Furthermore, others have looked longitudinally at children with obesity over time without any intervention, but still within the confines of a study, and had seen no changes in metabolic outcomes (39). Rather, each participant served as his or her own control. Our paradigm of dietary sugar and fructose restriction, which included mid-study dietary adjustments to compensate for weight loss, resulted in a 4% decrease in percentage of calories from carbohydrate, a 2% increase in percentage of calories from protein, and a small increase in dietary fiber, which could have reduced macronutrient absorption (18), flux of fructose to the liver, and also increased satiety. Recognizing that consumption data by recall is routinely underestimated (24), we made every effort to maintain our participants' baseline weight throughout the 10-day study interval, and even increased the caloric allotment partway through the cohort, yet a decline of 0.9 ± 0.2 kg was noted during the 10 days. Furthermore, this reduction was documented by a 0.6 kg change in fat-free mass on DXA. One potential concern is that the weight loss over the 10-day study interval was a manifestation of unintended caloric deficit, and that this weight loss alone resulted in metabolic improvement. Although we cannot determine

whether this weight loss was muscle or water or combination thereof, the temporal pattern of weight change argues against persistent caloric deficit (Figure 1); and it is unlikely that a reduction of this magnitude in either compartment would improve metabolic health. To control for weight loss: 1) regression of change in metabolic analytes (except for glucose AUC) versus change in weight showed no significance (data not shown); 2) all analytes (except AST) were adjusted for changes in weight by repeated measures ANCOVA (Table 2); and 3) sensitivity analysis on the subcohort who gained weight (Table 3; Figures 2c,d) demonstrated directionally equivalent metabolic improvement, especially in hyperinsulinemia, suggesting that the effects were primarily due to fructose restriction rather than weight loss.

Our econometric analysis ascertained that sugar meets the Bradford Hill criteria for causation for diabetes, including dose, duration, directionality, and precedence (4). This study bolsters this assertion, and supports change in public health policy regarding sugar intake and food labeling.

Conclusion

Concerns surrounding the role of sugar consumption in chronic disease have previously focused on its caloric equivalence and its role in fomenting increases in weight. Furthermore, previous clinical studies have relied upon excessive sugar administration, which introduces experimental artifact. This study mitigates all three of these concerns by intervening in children who are already sick with metabolic syndrome and by adjusting for effects of calories, weight gain, and adiposity. This study argues that the health detriments of sugar, and fructose specifically, are independent of its caloric value or effects on weight. Further studies will be required to determine whether sugar restriction alone can impact metabolic syndrome in adults and whether such effects are short-lived or long-term.O

References

1. Sorof JM, Lai D, Turner J, Poffenbarger T, Portman RJ. Overweight, ethnicity, and the prevalence of hypertension in school-aged children. *Pediatrics* 2004;113:475-482.

2. Kohen-Avramoglu R, Theriault A, Adeli K. Emergence of the metabolic syndrome in childhood: an epidemiological overview and mechanistic link to dyslipidemia. *Clin Biochem* 2003;36:413-420.

3. Schwimmer JB, Deutsch R, Rauch JB, Behlung C, Newbury R, Lavine JE. Obesity, insulin resistance, and other clinicopathological correlates of pediatric nonalcoholic fatty liver disease. *J Pediatr* 2005;143:500-505.

4. Basu S, Yoffe P, Hills N, Lustig RH. The relationship of sugar to population-level diabetes prevalence: an econometric analysis of repeated cross-sectional data. *PLoS One* 2013;8:e57873.

5. Weiss R, Bremer AA, Lustig RH. What is metabolic syndrome, and why are children getting it? In: Braaten D (ed). Year in Diabetes and Obesity, 2012. *Ann NY Acad Sci* 2013;1281:123-140.

6. Kim HS, Paik HY, Lee KU, Lee HK, Min HK. Effects of several simple sugars on serum glucose and serum fructose levels in normal and diabetic subjects. *Diabetes Res Clin Pract* 1988;4:281-287.

7. Lim JS, Mietus-Snyder M, Valente A, Schwarz JM, Lustig RH. The role of fructose in the pathogenesis of NAFLD and the metabolic syndrome. *Nat Rev Gastroenterol Hepatol* 2010;7:251-264.

8. Schwarz JM, Noworolski SM, Wen MJ, et al. Effect of a high-fructose weight-maintaining diet on lipogenesis and liver fat. *J Clin Endocrinol Metab* 2015;100:2434-2442.

9. Schalkwijk CG, Stehouwer CD, van Hinsbergh VW. Fructose-mediated non-enzymatic glycation: sweet coupling or bad modification. *Diabetes Metab Res* 2004;20:369-382.

10. Van Name M, Giannini C, Santoro N, et al. Blunted suppression of acyl-ghrelin in response to fructose ingestion in obese adolescents: the role of insulin resistance. *Obesity* 2015;23:653-661.

11. Page KA, Chan O, Arora J, et al. Effects of fructose vs glucose on regional cerebral blood flow in brain regions involved with appetite and reward pathways. *JAMA* 2013;309:63-70.

12. Stanhope KL, Schwarz JM, Keim NL, et al. Consuming fructose-, not glucose-sweetened beverages increases visceral adiposity and lipids and decreases insulin sensitivity in overweight/obese humans. *J Clin Invest* 2009;119:1322-1334.

13. Louthan MV, Theriot JA, Zimmerman E, Stutts JT, McClain CJ. Decreased prevalence of nonalcoholic fatty liver disease in black obese children. *J Pediatr Gastroenterol Nutr* 2005;41:426-429.

14. Bacha F, Saad R, Gungor N, Janosky J, Arslanian SA. Obesity, regional fat distribution, and syndrome X in obese black versus white adolescents: race differential in diabetogenic and atherogenic risk factors. *J Clin Endocrinol Metab* 2003;88:2534-2540.

15. Madsen KA, Garber AK, Mietus-Snyder ML, et al. A lifestyle intervention for pediatric obesity: efficacy and behavioral and biochemical predictors of response. *J Pediatr Endocrinol Metab* 2009;22:805-814.

16. Neuhouser ML, Lilley S, Lund A, Johnson DB. Development and validation of a beverage and snack questionnaire for use in evaluation of school nutrition policies. *J Am Diet Assoc* 2009;109:1587-1592.

17. Cullen KW, Watson K, Zakeri I. Relative reliability and validity of the Block Kids Questionnaire among youth aged 10 to 17 years. *J Am Diet Assoc* 2008;108:862-866.

18. Food and Nutrition Board. *Dietary Reference Intakes: Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids (Macronutrients)*. Washington, D.C.: Institute of Medicine; 2005.

19. *Guideline: Sugars intake for adults and children*. Geneva: World Health Organization; 2015.

20. Yang Q, Zhang Z, Edward Gregg E, Flanders WD, Merritt R, Hu FB. Added sugar intake and cardiovascular diseases mortality among U.S. adults. *JAMA Int Med* 2014;174:516-524.

21. Ouyang X, Cirillo P, Sautin Y, et al. Fructose consumption as a risk factor for non-alcoholic fatty liver disease. *J Hepatol* 2008;48:993-999.

22. Hedrick VE, Dietrich AM, Estabrooks PA, Savla J, Serrano E, Davy BM. Dietary biomarkers: advances, limitations and future directions. *Nutr J* 2012;11:109.

23. Stanhope KL, Havel PJ. Endocrine and metabolic effects of consuming beverages sweetened with fructose, glucose, sucrose, or high-fructose corn syrup. *Am J Clin Nutr* 2008;88:1733S-1737S.

24. Rangan A, Allman-Farinelli M, Donohoe E, Gill T. Misreporting of energy intake in the 2007 Australian Children's Survey: differences in the reporting of food types between plausible, under- and over-reporters of energy intake. *J Hum Nutr Diet* 2014;27:450-458.

25. Sievenpiper JL, de Souza RJ, Mirrahimi A, et al. Effect of fructose on body weight in controlled feeding trials: a systematic review and meta-analysis. *Ann Int Med* 2012;156:291-304.

26. Fiaschi E, Baggio B, Favaro S, et al. Fructose-induced hyperuricemia in essential hypertension. *Metabolism* 1977;26:1219-1223.

27. Brito JO, Ponciano K, Figueroa D, et al. Parasympathetic dysfunction is associated with insulin resistance in fructose-fed female rats. *Braz J Med Biol Res* 2008;41: 804-808.

28. Rebello T, Hodges RE, Smith JL. Short-term effects of various sugars on antinatriuresis and blood pressure changes in normotensive young men. *Am J Clin Nutr* 1983;38:84-94.

29. Singh AK, Amlal H, Haas PJ, et al. Fructose-induced hypertension: essential role of chloride and fructose absorbing transporters PAT1 and Glut5. *Kidney Int* 2008;74: 438-447.

30. Johnson RJ, Segal MS, Sautin Y, et al. Potential role of sugar (fructose) in the epidemic of hypertension, obesity and the metabolic syndrome, diabetes, kidney disease, and cardiovascular disease. *Am J Clin Nutr* 2007;86:899-906.

31. Nguyen S, Choi HK, Lustig RH, Hsu CY. Sugar sweetened beverages, serum uric acid, and blood pressure in adolescents. *J Pediatr* 2009;154:807-813.

32. Kell KP, Cardel MI, Bohan Brown MM, Fernandez JR. Added sugars in the diet are positively associated with diastolic blood pressure and triglycerides in children. *Am J Clin Nutr* 2014;100:46-52.

33. Faeh D, Minehira K, Schwarz JM, Periasami R, Seongsu P, Tappy L. Effect of fructose overfeeding and fish oil administration on hepatic *de novo* lipogenesis and insulin sensitivity in healthy men. *Diabetes* 2005;54:1907-1913.

34. Seyfried TN, Shelton LM. Cancer as a metabolic disease. *Nutr Metab* 2010;27.

35. Treem WR, Sokol RJ. Disorders of the mitochondria. *Semin Liver Dis* 1998;18:237-253.

36. Kojima H, Sakurai S, Uemura M, Fukui H, Morimoto H, Tamagawa Y. Mitochondrial abnormality and oxidative stress in nonalcoholic steatohepatitis. *Alcohol Clin Exp Res* 2007;31:S61-S66.

37. Liangpunsakul S, Lai X, Ross RA, et al. Novel serum biomarkers for detection of excessive alcohol use. *Alcohol Clin Exp Res* 2015;39:556-565.

38. Maersk M, Belza A, Stødkilde-Jorgensen H, et al. Sucrose-sweetened beverages increase fat storage in the liver, muscle, and visceral fat depot: a 6-mo randomized intervention study. *Am J Clin Nutr* 2012;95:283-289.

39. Uysai Y, Wolters B, Knop C, Reinehr T. Components of the metabolic syndrome are negative predictors of weight loss in obese children with lifestyle intervention. *Clin Nutr* 2014;33:620-625.