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Short-term isocaloric fructose restriction lowers apoC-III levels and yields less atherogenic lipoprotein profiles in children with obesity and metabolic syndrome

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ABSTRACT

Background and aims: Dietary fructose may play a role in the pathogenesis of metabolic syndrome (MetS). In a recently published study of obese children with MetS, we showed that isocaloric fructose restriction reduced fasting triglyceride (TG) and LDL-cholesterol (LDL-C). In these ancillary analyses, we tested the hypothesis that these effects were also accompanied by improved quantitative and qualitative changes in LDL and HDL subclasses and their apolipoproteins; as well as change in VLDL, particularly apoC-III.

Methods: Obese children with MetS (n = 37) consumed a diet that matched self-reported macronutrient composition for nine days, with the exception that dietary fructose was reduced from 11.7 ± 4.0% to 3.8 ± 0.5% of daily calories and substituted with glucose (in starch). Participants underwent fasting biochemical analyses on Days 0 and 10. HDL and LDL subclasses were analyzed using the Lipoprint HDL and LDL subfraction analysis systems from Quantimetrix.

Results: Significant reductions in apoB (78 ± 24 *vs.* 66 ± 24 mg/dl) apoC-III (8.7 ± 3.5 *vs.* 6.5 ± 2.6 mg/dl) and apoE (4.6 ± 2.3 *vs.* 3.6 ± 1.1 mg/dl), all $p < 0.001$) were observed. LDL size increased by 0.87 Å ($p = 0.008$). Small dense LDL was present in 25% of our cohort and decreased by 68% ($p = 0.04$). Small HDL decreased by 2.7% ($p < 0.001$) and large HDL increased by 2.4% ($p = 0.04$). The TG/HDL-C ratio decreased from 3.1 ± 2.5 to 2.4 ± 1.4 ($p = 0.02$). These changes in fasting lipid profiles correlated with changes in insulin sensitivity.

Conclusions: Isocaloric fructose restriction for 9 days improved lipoprotein markers of CVD risk in children with obesity and MetS. The most dramatic reduction was seen for apoC-III, which has been associated with atherogenic hypertriglyceridemia.

1. Introduction

Dyslipidemia and hypertension, two risk factors for cardiovascular disease (CVD), are now common in childhood in association with non-alcoholic fatty liver disease (NAFLD) and type 2 diabetes (T2DM) [1–3]; the cluster of diseases often referred to as metabolic syndrome (MetS). Changes in dietary composition associated with the Western Diet may be at the root of the biochemical alterations which promote MetS and associated atherogenic dyslipoproteinemia. Fructose is a key suspect since: a) its consumption has increased concurrently with incidence of MetS conditions; b) it is metabolized almost exclusively in the liver, where it stimulates *de novo* lipogenesis to drive hepatic triglyceride (TG) synthesis [4–7]; and c) it contributes to hepatic insulin resistance [4].

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Hepatic lipid accumulation is linked to overproduction of large VLDL1 particles rich in apolipoprotein C-III (apoC-III) [8–11]. Results from two large-scale Mendelian randomization studies are highly suggestive for a causal association between apoC-III levels and CVD [8–11].

VLDL1 levels are associated with small dense LDL (sd-LDL) levels [12–14] Increased levels of VLDL1 may alter the composition of HDL as well, leading to the formation of small HDL [15,16].

Fructose consumption is associated with dyslipoproteinemia, as demonstrated by a recent study by Stanhope et al. in which serum concentrations of non-HDL-C, LDL-C, apoB, and apoC-III levels increased in a dose-dependent manner in young adults consuming beverages providing up 25% of calories as high fructose corn syrup (HFCS) for 2 weeks [17]. We recently reported the effects of a controlled dietary intervention study of isocaloric substitution of starch for sugar on metabolic parameters in children with obesity and metabolic co-morbidities [18]. When dietary fructose was reduced from an average of 12%–4% of total caloric intake, reduction in fasting TG levels of 0.4 mmol/L, 46% ($p < 0.002$), LDL-C by 0.3 mmol/L ($p < 0.001$), and HDL-C by 0.1 mmol/L ($p < 0.001$) were observed within 10 days.

The present ancillary analyses, using stored fasting serum samples from the aforementioned study, tested the hypothesis that these effects were associated qualitative changes in VLDL catabolism compatible with a reversal of changes induced by insulin resistance (i.e. improved quantitative and qualitative changes in LDL and HDL subclasses), and changes in apolipoproteins, particularly apoC-III.

2. Materials and methods

This study was approved by the UCSF and the Touro University Institutional Review Boards, and listed as NCT01200043 on ClinicalTrials.gov. As described in detail in the report of the parent study [18], we recruited Latino and African-American children with obesity who were high habitual sugar consumers (>15% sugar and >5% fructose) and had at least one metabolic co-morbidity.

After completion of baseline metabolic testing, including collection of fasting serum samples, participants were provided with nine days worth of food prepared by the UCSF Clinical Research Service (CRS) Bionutrition Core to provide sufficient calories to maintain their body weight. The menu was prearranged so that the percentage of calories consumed from all carbohydrate sources was consistent with their baseline diet but fructose was reduced from 11.7 ± 4.0% to 3.8 ± 0.5%, of daily calories. This intervention study diet profile is consistent with recommendations by the IOM for macronutrients [19] and the World Health Organization for dietary sugar intake [20].

On Day 10, all assessments performed at baseline were repeated.

Fasting standard clinical analytes were measured as reported in the previous paper [18]. HOMA-IR was calculated from fasting insulin and glucose levels [21]. The new analyses reported in this paper were:

1) apolipoproteins measured by ELISA kits from Abcam, USA: Apolipoprotein A-I (APOA-I) Human Simple Step ELISA Kit ab189576; Apolipoprotein C-II Human ELISA Kit ab168549; APOC-III Human ELISA Kit ab154131; Apolipoprotein E (APOE) Human ELISA Kit ab108813; and Apolipoprotein B (APOB) Human SimpleStep ELISA Kit ab190806.
2) human paraoxonase-1 mass was measured by ELISA, rd191279200R, BioVendor, USA. The PON1 lactonase activity was kinetically measured using dihydroxycoumadin DHC as a substrate at 37 °C as described previously [22].
3) HDL and LDL subclasses were analyzed using the Lipoprint HDL and LDL subfraction analysis systems from Quantimetrix (Redondo Beach, CA, USA) according to the manufacturer's instructions.

2.1. Statistical analysis

Data are expressed as mean ± SD when normally distributed or median and 95% confidence interval when not normally distributed. Normal distributions were tested by histogram, box-plot, q-norm plot, and Shapiro-Wilk tests. Repeated measures analysis of covariance (ANCOVA) was performed on each biochemical parameter to control for weight change, and a separate regression analysis was done to obtain the beta-coefficient (mean difference adjusted for weight change, with 95% confidence intervals) if the analyte was normally distributed. When data were not normally distributed, log-transformation was performed to achieve normal distribution and then the data were subjected to repeated measures ANCOVA. The beta-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 log-transformed to achieve normal distribution. Kruskal-Wallis non-parametric testing was used for analysis when log-transformation did not yield a normal distribution. We ruled out the effect of minor change in weight by conducting univariate regression analysis to investigate the association between change in each metabolic analyte *versus* change in weight. To measure the influence of demographic variables (sex, age, Tanner stage, race/ethnicity), we re-ran the same analysis with each included as a single covariate to the model, and with all included as multiple covariates in one model. All statistical tests 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, USA).

3. Results

3.1. Characteristics of participants

As reported previously [18], 43 Latino and African-American participants were assessed in the parent study. Due to paired sample availability in this ancillary study, we ran supplemental assays in 37 participants. The mean age in this subgroup 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.

Analysis of DXA data established that fat and bone mass did not change significantly during the 10-day study period, although fat-free mass reduced by 0.6 kg ($p = 0.04$). Despite efforts to sustain each participant's body weight at baseline-, average weight decreased by 1% ($p < 0.001$) over the intervention [18]. Consequently, all physiologic and biochemical analyses that were normally distributed, either before or after log-transformation, were adjusted for weight change by repeated measures ANCOVA. These results did not differ when controlled for sex, age, Tanner stage, and/or race/ethnicity (data not shown).

3.2. Lipids, lipoprotein subclasses, and apolipoproteins

The results of lipid and lipoprotein analyses are shown in Table 1. 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 decreased by 0.1 mmol/L ($p < 0.001$). The TC/HDL-C ratio decreased by 11% ($p < 0.03$), and the TG/HDL-C ratio

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