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Publication Date

2016-10-01

DOI

10.1016/j.atherosclerosis.2016.06.048

Peer reviewed



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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ARTICLE INFO

Article history:

Received 29 February 2016

Received in revised form

23 June 2016

Accepted 29 June 2016

Available online 19 July 2016

Keywords:

LDL subclasses

HDL subclasses

Apolipoproteins

apoC-III

Obesity

Metabolic syndrome

Fructose

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 $11.7 \pm 4.0\%$ to $3.8 \pm 0.5\%$ 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 \AA ($p = 0.008$). 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.

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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 to 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 with 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 $11.7 \pm 4.0\%$ to $3.8 \pm 0.5\%$, 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 \pm 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

Table 1
Lipid and lipoprotein analyses mean \pm SD and median (range) on Day 0 and 10.

| | n | Day 0* | Day 10* | b-coefficient(Unadjusted change) [95% CI] | p value | b-Coefficient (adjusted change) [95% CI] | p value |
|---|---------------------|--|--|---|---------|---|---------|
| Triglyceride ^{b**} (mmol/L) | 43 | 1.4 \pm 0.9 1.2 (0.8, 1.6) | 1.0 \pm 0.5 0.9 (0.6, 1.3) | -50.5% (-65.7, -28.5) | <0.001 | -46.0% (-62, -25) | 0.02 |
| LDL-cholesterol ^b (mmol/L) | 43 | 2.4 \pm 0.6 2.3 (0.9, 1.8) | 2.1 \pm 0.6 1.9 (1.7, 2.5) | -0.3 (-0.4, -0.1) | <0.001 | -0.3 (-0.4, -0.1) | <0.001 |
| HDL- cholesterol ^b (mmol/L) | 43 | 1.2 \pm 0.2 1.1 (0.9, 1.3) | 1.0 \pm 0.2 1.0 (0.9, 1.5) | -0.1 (-0.14, -0.06) | <0.001 | -0.1 (-0.2, -0.09) | <0.001 |
| LDL-1 ^{***} (%)*** | 37 | 30.1 \pm 10.7 27 (23–36) | 26.5 \pm 9.3 26 (20–33) | -25.9% (-40.3, -8.2) | 0.008 | -25.5% (-40, -7) | 0.009 |
| LDL-2 [†] (%) | 37 | 16.5 \pm 13.5 13 (8–21) | 11.8 \pm 8.3 11 (5–17) | | 0.15 | | 0.15 |
| LDL-3 (%) | 10 ^{&} | 1.9 \pm 1.7 1.25 (0.9, 2.3) | 0.6 \pm 0.7 0.55 (0, 1.2) | -1.3 (-2.6, -0.06) | 0.04 | -1.3 (-2.6, -0.3) | 0.04 |
| LDL size (Angstroms) | 37 | 271.3 \pm 3.1 271.2 (269.7–273.5) | 272.2 \pm 2.5 272.1 (270.7–273.6) | +0.87 (+0.25, +1.49) | 0.008 | +0.87 (+0.24, +1.51) | 0.008 |
| Small HDL (%)*** | 37 | 14.6 \pm 6.1 15.2 (10.2–19.4) | 11.8 \pm 5.5 10.9 (7.9–15.1) | -2.79 (-4.22, -1.36) | <0.001 | -2.73 (-4.19, -1.28) | 0.001 |
| Intermediate HDL (%)*** | 37 | 58.5 \pm 5.2 58.3 (54.8–62.7) | 58.7 \pm 5.7 59.8 (54.8–62.7) | +0.27 (-1.21, +1.75) | 0.71 | +0.27 (-1.22, +1.77) | 0.72 |
| Large HDL (%)*** | 37 | 26.8 \pm 7.9 26.4 (21.6–33.5) | 29.3 \pm 7.7 29.3 (23.6–35.2) | +2.48 (+0.25, +4.71) | 0.03 | +2.42 (+0.17, +4.67) | 0.04 |
| APO-AI ^{**} (mg/dl) | 30 | 120 \pm 61 104.5 (74.5–149) | 95 \pm 50 81 (58–109) | -42.6% (-67.9, +2.9) | 0.06 | -43% (-68, +3) | 0.06 |
| APO-B ^{**} (mg/dl) | 37 | 78 \pm 24 75 (63–90) | 66 \pm 24 67 (75–51) | -32.9% (-44.4, -19.1) | <0.001 | -32% (-45, -19) | <0.001 |
| APO-CII ^{**} (mg/dl) | 37 | 8.7 \pm 3.7 7.9 (6.4–10.0) | 8.3 \pm 4.2 7.5 (5.4–10.2) | -14% (-31.9, +8.5) | 0.20 | -15% (-32, +9) | 0.19 |
| APO-CIII ^{**} (mg/dl) | 37 | 8.7 \pm 3.5 8.0 (7.1–9.9) | 6.5 \pm 2.6 5.7 (4.6–7.2) | -48.9% (-60.1, -34.7) | <0.001 | -49% (-61, -34) | <0.001 |
| | n | Day 0* | Day 10* | b-Coefficient (Unadjusted change) [95% CI] | p value | b-Coefficient (Adjusted change) [95% CI] | p value |
| APO-E ^{**} (mg/dl) | 37 | 4.6 \pm 2.3 4.1 (3.3–5.2) | 3.6 \pm 1.1 3.3 (2.8–6.7) | 38.3% (-52.1, -20.4) | <0.001 | -38% (-52, -19) | <0.001 |
| PON activity lactonase (U/L) | 37 | 51.7 \pm 16.3 51.3 (37.9–62.72) | 46.8 \pm 15.1 45.2 (36.44–58.63) | -4.96 (-6.41, -3.51) | <0.001 | -4.93 (-6.43, -3.43) | <0.001 |
| PON mass mg/ml | 19 | 23.6 \pm 6.2 23.95 (18.9–29.05) | 22.1 \pm 5.4 22.3 (18.02–26.08) | -1.48 (-2.89, -0.06) | 0.04 | -1.44 (-2.92, +0.04) | 0.06 |
| Non-HDL cholesterol (mmol/L) | 43 | 3.0 \pm 0.7 2.8 (2.4–3.3) | 2.5 \pm 0.7 2.5 (2.0–3.0) | -0.4 (-0.6, -0.3) | <0.001 | -0.4 (-0.6, -0.3) | <0.001 |
| Total cholesterol/HDL ratio ^{**} | 43 | 3.7 \pm 0.8 3.62 (3.13–4.12) | 3.5 \pm 0.7 3.40 (2.97–3.90) | -10.6% (-18.7, -1.7) | 0.02 | -10.3% (-18.7, -1.2) | 0.03 |
| Triglyceride/HDL ratio ^{**} | 43 | 3.1 \pm 2.5 2.64 (1.49–3.62) | 2.4 \pm 1.4 2.24 (1.23–3.22) | -38.6% (-59.1, -7.8) | 0.02 | -38% (-58.0, -7.0) | 0.02 |

Statistical significance $p < 0.05$ after adjustment for weight change by repeated measures ANCOVA.

* Data are expressed as mean \pm SD and median (range).

** Parameters not normally distributed and log transformed for analysis only, mean change and 95% CI are reported as percent change.

*** According to manufacturer's reported as % of total lipid AUC.

**** According to manufacturer's reported as % of total HDL lipid AUC.

^b Data previously reported in Lustig et al. [18].

[&] Only 10/37 had LDL3 fractions.

[†] Non-parametric Kruskal-Wallis, statistical significance $p < 0.05$.

[‡] Coefficient of determination for univariate regression analysis between change in lipid parameters and change in weight.

decreased by 38% ($p < 0.02$). None of these outcomes were affected by change in weight.

Analysis of LDL subclasses showed that 10 out of 37 children exhibited a sd-LDL fraction on Day 0. Fig. 1A depicts a typical LDL subclass profile of one of these children. Fructose restriction eliminated or reduced the sd-LDL fraction in 8/10 of these participants on Day 10. The changes in sd-LDL strongly correlated with the changes in LDL2 (Pearson correlation coefficient $r = 0.85$, $p < 0.002$). The overall LDL size in our cohort increased by 0.87 nm ($p < 0.008$), indicating a general shift from smaller LDL particles to larger ones. ApoB, which correlates with the number of LDL particles, decreased by 32% ($p < 0.001$).

The reduction in fasting TG [18] was accompanied by significant changes in apolipoprotein profile. ApoC-III was reduced ($p < 0.001$). ApoC-II did not decrease significantly. ApoE decreased ($p < 0.001$). Changes in TG correlated with changes in apoC-III ($r = 0.57$,

$p < 0.001$). These changes are consistent with a reduction in the number and/or size of VLDL particles as well as a qualitative change in their apolipoprotein profile toward a less atherogenic phenotype.

The previously reported slight decrease in HDL cholesterol [18] was paralleled by a trend toward decreased apoA-I, although there was a large splay. This was accompanied by a mild decrease in PON1 mass and lactonase activity. These quantitative changes were associated with qualitative modifications in the distribution of HDL subclasses. Small HDL (HDL₃) decreased by 2.73% ($p < 0.001$) while large HDL increased by 2.43% ($p = 0.04$) of total HDL, respectively. These data are consistent with a redistribution of HDL particles [23,24].

3.3. Correlation of changes in lipoprotein profiles with insulin resistance

Table 2 shows the correlations between the changes in lipid

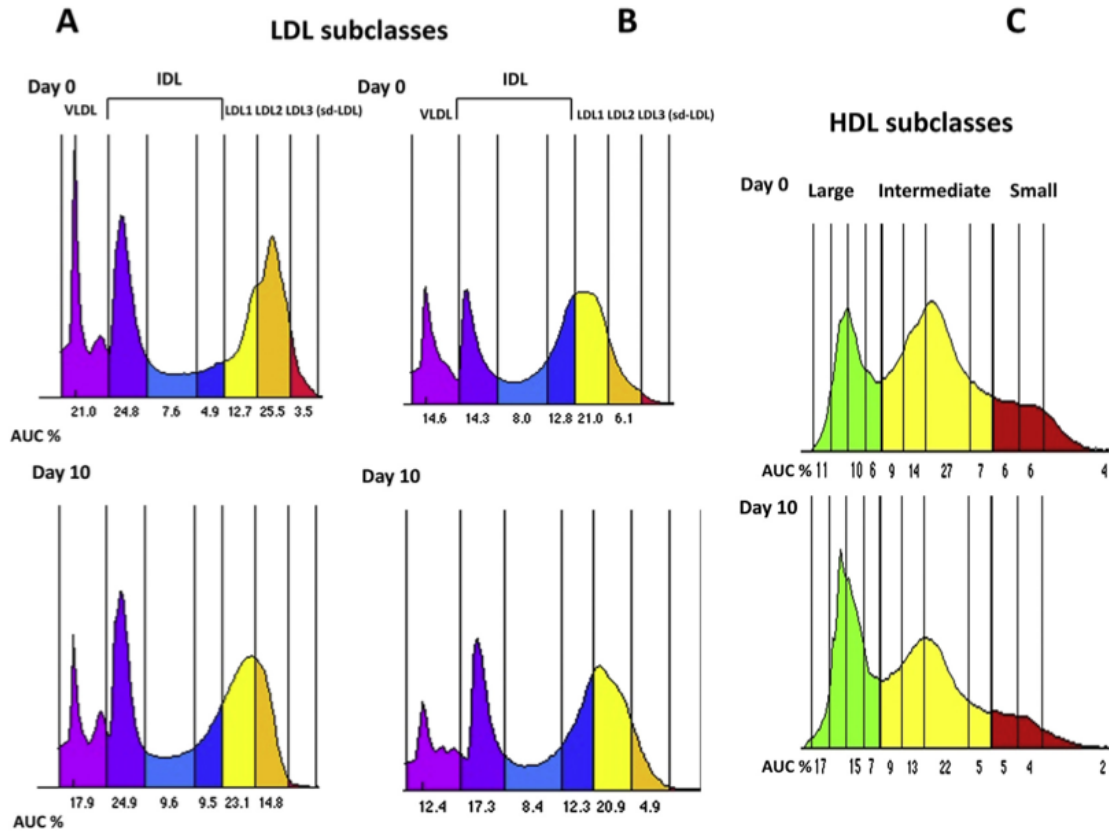


Fig. 1. LDL and HDL subclasses at Day 0 and Day 10. (A) Typical profiles of a participant with high sd-LDL. (B) Typical profile of a participant with low sd-LDL. (C) Typical HDL subclasses profile. Note (A and B) shift to the left for LDL subclasses (increase in size) as well as reduced sd-LDL levels and (C) reduced small HDL, mirrored by higher large HDL.

outcomes and previously reported changes in fasting levels of insulin, fasting C-peptide, and HOMA-IR. Most changes in fasting lipids, apolipoproteins, and ratios correlated strongly with changes in fasting parameters of insulin resistance. Of note, changes in VLDL-associated apoC-III and -C-II showed the strongest correlations. Weight change was not significant as a covariate in any of the repeated measures ANCOVAs.

Table 2
Correlation coefficients of day 0 to day 10 changes in lipids and apolipoproteins versus changes in insulin resistance measures.

| | Delta | | |
|------------------------|-----------------|-------------------|-----------------|
| | HOMA-IR | Fasting C-peptide | Fasting insulin |
| ApoA-I | -0.12 | 0.08 | -0.14 |
| Apo B | 0.43 (0.01) | 0.51 (0.001) | 0.41 (0.01) |
| ApoC-II | 0.45 (0.005) | 0.54 (<0.001) | 0.48 (0.003) |
| ApoC-III | 0.40 (0.01) | 0.43 (0.01) | 0.41 (0.01) |
| Apo E | 0.38 (0.02) | 0.54 (<0.001) | 0.40 (0.01) |
| Triglyceride | 0.31 (0.04) | 0.50 (<0.001) | 0.34 (0.03) |
| Total cholesterol/HDL* | 0.25 | 0.29 (0.06) | 0.36 (0.02) |
| Triglyceride/HDL | 0.31 (0.04) | 0.50 (<0.001) | 0.34 (0.03) |
| LDL size* | 0.03 | -0.15 | 0.05 |

Spearman correlation coefficients are reported if not otherwise noted.
*Pearson correlation coefficient.
p < 0.05 considered statistically significant. *p* value reported in parenthesis if < 0.1.

4. Discussion

This study provides new data demonstrating that short-term isocaloric fructose restriction in children with obesity and MetS can improve lipoprotein profiles compatible with a reduction of risk factors for cardiovascular disease; that is, reduction in TG, apoB, apoC-II, apoC-III, apoE, reduction in LDL-C with increase in LDL size, reduction of small HDL, and lowering of the TG/HDL ratio. The most dramatic reduction was seen for apoC-III, which is associated with the pathogenesis of atherogenic hypertriglyceridemia [10,25–27]. These improvements in fasting lipid profiles correlate significantly with the changes in parameters of insulin resistance.

The deleterious effect of dietary fructose on lipid profiles (high TG and smaller LDL particle size) has been observed in epidemiological studies [5,7,28,29]. Interventions increasing dietary fructose consumption in adults document worsening lipid profiles [17]. To better demonstrate a primary effect unrelated to energy intake or weight change, we substituted dietary added fructose calorie-for-calorie with glucose (in starch) so as to retain equivalence for both calories, total carbohydrate content, and body weight [18].

Our intervention reduced fasting VLDL levels, as indicated by significant reduction in TG [18], and reductions in apolipoproteins B, C-II, C-III and E. In contrast, in a study of adolescents subjected to acute weight loss (one month), reductions in TG levels of 31% required commensurate weight loss of 6% [30]. Five of our participants with severe hypertriglyceridemia on Day 0 (TG = 2.26–4.78 mmol/L) had reduced TG levels by 9–75% on Day 10, while 8 participants with moderate hypertriglyceridemia (TG = 1.69–2.25 mmol/L) had reduced TG levels by 11–76% on Day 10. Interestingly, the changes in VLDL were not only quantitative, but qualitative as well. Indeed, the most striking of all the changes

was the reduction in apoC-III ($p < 0.001$), even more so than apoC-II (– NS). Stanhope et al. recently demonstrated an increase in ApoC-III with added fructose administration over 2 weeks [17]. Conversely, we reduced ApoC-III after just 9 days of isocaloric fructose restriction. In addition to its potent inhibitory actions on lipoprotein lipase (LPL) activity, ApoC-III exerts multiple pro-atherogenic effects; e.g., facilitation of hepatic VLDL assembly and secretion, and inhibition of non-LPL-mediated removal of TG-rich particles and remnants [10]. Epidemiologic studies show direct associations between elevated apoC-III levels and CVD [25,27,31]. For instance, both individuals with heterozygous mutations as well those with as polymorphisms in apoC-III are characterized by a favorable lipid profile and improved cardiovascular health [26]. ApoC-III is thus postulated to be a novel therapeutic target for residual CVD risk reduction that could be even more appropriate in patients with MetS [10,25,27].

The most clinically useful measure of remnants and LDL size is the TG/HDL-C ratio [32–34]. Isocaloric fructose restriction reduced the TG/HDL-C ratio by 38%. The reduction in LDL-C [18] was also associated with a concomitant increase in LDL particle size as shown in subclass analysis. The prevalence of sd-LDL in children has not been thoroughly described in the literature. One study shows the general prevalence of the presence of sd-LDL is 7.5% in children under 10 years of age [35]. In our cohort, 25% of the subjects evidenced the presence of sd-LDL, and in these subjects, isocaloric fructose restriction reduced sd-LDL levels by two-thirds.

Our findings are also in agreement with previous data showing that fructose consumption correlates inversely with LDL particle size in other pediatric populations [29].

Fasting HDL-C exhibited a mild reduction of 4% in our participants [18] together with a trend toward reduced apoA-I (as well as the lower apoC-II and apoE noted above, which in part circulate on HDL). Although this may seem paradoxical vis-à-vis TG reduction, it must be remembered that the HDL-C hypothesis is an epidemiological concept that has gone out of favor mechanistically [36–45]. In this analysis, the small decrease in HDL-C was associated with a shift in HDL subclasses characterized by reduced HDL₃ and increased HDL₂, which correlate with the decline in both TG and TG/HDL ratio.

Our data are in agreement with reports showing a dose-dependent slight increase in HDL-C when sugar-containing beverages are added to a regular diet in adults [17].

The overall changes in total lipids as well as apolipoproteins and lipoprotein subclasses, together with their correlation with various measures of metabolic improvement (glucose, HOMA-IR), suggest a plausible unifying mechanism for our findings:

- Fructose restriction lowers *de novo* lipogenesis, resulting in less hepatic TG production, less export of VLDL, and less conversion to sd-LDL.
- *ApoC-III* gene expression is regulated by glucose directly through carbohydrate response element binding protein (ChREBP), which also mediates the induction of key lipogenic enzymes by glucose [31]. *ApoC-III* gene expression is thereby regulated in an opposite manner by insulin (negatively) and glucose (positively).
- As reported previously [18], our intervention decreased glucose levels, thereby reducing this positive input; and also decreased insulin resistance, thereby increasing the negative input.
- Increased levels of VLDL1 may alter the composition of HDL, leading to the formation of smaller and denser HDL, which was also reversed by our intervention. Our data are in agreement with the postulated key role of apoC-III in the dyslipidemia of insulin resistance associated with hyperglycemia.

Our pediatric subjects with MetS were high consumers of sugar, and it remains to be determined whether these beneficial changes in lipoprotein profiles also occur in adult populations or those with lower habitual fructose intake. We chose to reduce sugar intake to 4% of total calories, similar to guidelines recommended by the WHO study of comparable duration showed that there is a dose-dependent negative impact of added fructose (from 0 to 12.5% of total calories) on fasting and more so on postprandial lipid profiles [17,46]. Together with previous results in adults from our group [4], we believe that our findings portend a likelihood for adults with average consumption to evidence improvement in their atherogenic profiles with fructose reduction.

We proffer several strengths of this study. Rather than studying large acute oral fructose administration in normal participants, or the addition of fructose to the diet [4,17], we instead assessed restriction of added dietary fructose in children with MetS to determine whether their lipoprotein profile would change — an endpoint with clinical relevance and with few chances for artifact. To reduce systematic bias, we maintained investigator blinding on all data until final statistical analysis. The changes are important even in the fasting conditions, and postprandial changes might be even more pronounced.

As limitations, we grant that, although unintended, there was minor (1%) weight loss; however, that weight loss occurred in the fat-free mass compartment, and would not be expected to contribute to metabolic improvement. Moreover, a comprehensive study on weight loss and cardiometabolic profiles in children showed that improvement occurred only in those children whose BMI lowered by 20% in a year, with a magnitude far less than what we demonstrated with virtually no weight loss [47]. The short-term intervention period could be considered a limitation; on the other hand, it shows how rapidly dietary change can bring about amelioration of metabolic dysfunction. Other limitations are that we did not measure LDL particle number, or HDL or VLDL subclasses by NMR spectroscopy or ion mobility assays, and we did not measure prebeta-HDL. However, this does not detract from the significant substantial changes we report on lipoprotein profiles.

In conclusion, we show for the first time that short-term isocaloric fructose restriction in children with obesity and MetS results in changes in lipoprotein profiles compatible with reduction of critical risk factors for CVD; i.e. reduced TG, LDL-C, lower apo-B, apo-CIII, apo-E, fewer sd-LDL, increased LDL size, and lower TG/HDL ratio. Notably, the highest consistent reduction was seen for apoC-III, a risk factor associated with hypertriglyceridemia. The changes in these lipid patterns correlated significantly with changes in insulin resistance. The improvement in atherogenic dyslipidemia was dependent on fructose restriction specifically and was independently of its calories or its effects on weight. Further research is warranted to assess whether dietary fructose restriction can impact MetS dyslipidemia in adults, and whether such effects are sustainable long-term.

Conflict of interest

The authors declared they do not anything to disclose regarding conflict of interest with respect to this manuscript.

Financial support

NIH (R01DK089216), UCSF CTSI (NCATS–UL1-TR00004), and Touro University.

Trial registration

Metabolic Impact of Fructose Restriction in Obese Children,

<https://www.clinicaltrials.gov/ct2/show/NCT01200043?term=NCT01200043&rank=1>.

Acknowledgments

The authors would like to thank all the participants and parents/caregivers who volunteered for this study. Thanks is also given to all the UCSF Clinical and Translational Sciences Institute (CTSI) Pediatric and Adult CRS Staff (Jean Addis, Sarah Fuerstenau, Erin Matsuda, Grace Mausisa, Abigail Sobejana, Grady Kimes, Erin Miller, Raquel Herrera, Tamara Williamson, John Duda, Caitlin Sheets) who participated in this study, as well as the Bionutrition staff, Jennifer Culp and Monique Schloetter, who planned and prepared the food for this study. A special thank you to Drs. Emily Perito and Patrika Tsai. We also thank Arianna Pham, Davis Tang, Ari Simon, Moises Velasco-Alin, and Karen Pan. Special acknowledgment is given to Drs. Zea Malawa and Tami Hendriksz who helped recruit patients. Thanks to Laurie Herraiz, RD, who helped design and implement the protocol. And our greatest thanks is given to our wonderful WATCH clinic coordinators, who helped screen patients and implement this protocol, including Rachel Lipman, CPNP, Kelly Jordan (medical student at Tufts), Sally Elliott (medical student at UCLA), and Katrina Koslov, PhD (medical student at UCLA).

Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.atherosclerosis.2016.06.048>.

References

- J.M. Sorof, D. Lai, J. Turner, T. Poffenbarger, R.J. Portman, Overweight, ethnicity, and the prevalence of hypertension in school-aged children, *Pediatrics* 113 (2004) 475–482.
- R. Kohen-Avramoglu, A. Theriault, K. Adeli, Emergence of the metabolic syndrome in childhood: an epidemiological overview and mechanistic link to dyslipidemia, *Clin. Biochem.* 36 (2003) 413–420.
- F. Bacha, R. Saad, N. Gungor, J. Janosky, S.A. Arslanian, 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.* 88 (2003) 2534–2540.
- J.M. Schwarz, S.M. Noworolski, M.J. Wen, A. Dyachenko, J.L. Prior, M.E. Weinberg, et al., Effect of a high-fructose weight-maintaining diet on lipogenesis and liver fat, *J. Clin. Endocrinol. Metab.* 100 (2015) 2434–2442.
- V.S. Malik, F.B. Hu, Fructose and cardiometabolic health: what the evidence from sugar-sweetened beverages tells us, *J. Am. Coll. Cardiol.* 66 (2015) 1615–1624.
- J.J. DiNicolantonio, J.H. O'Keefe, S.C. Lucan, Added fructose: a principal driver of type 2 diabetes mellitus and its consequences, *Mayo Clin. Proc.* 90 (2015) 372–381.
- K.L. Stanhope, J.M. Schwarz, P.J. Havel, Adverse metabolic effects of dietary fructose: results from the recent epidemiological, clinical, and mechanistic studies, *Curr. Opin. Lipidol.* 24 (2013) 198–206.
- Tg, Hdl Working Group of the Exome Sequencing Project NHL, I. Blood, J. Crosby, G.M. Peloso, P.L. Auer, et al., Loss-of-function mutations in apoC3, triglycerides, and coronary disease, *N. Engl. J. Med.* 371 (2014) 22–31.
- A.B. Jorgensen, R. Frikke-Schmidt, B.G. Nordestgaard, A. Tybjaerg-Hansen, Loss-of-function mutations in apoC3 and risk of ischemic vascular disease, *N. Engl. J. Med.* 371 (2014) 32–41.
- M. Hassan, ApoC3: triglycerides do matter, *Glob. Cardiol. Sci. Pract.* 2014 (2014) 241–244.
- T. Geach, Genetics: apoC3 mutations lower cvd risk, *Nat. Rev. Cardiol.* 11 (2014) 496.
- R.M. Krauss, Lipoprotein subfractions and cardiovascular disease risk, *Curr. Opin. Lipidol.* 21 (2010) 305–311.
- K.R. Feingold, C. Grunfeld, M. Pang, W. Doerrler, R.M. Krauss, LDL subclass phenotypes and triglyceride metabolism in non-insulin-dependent diabetes, *Arterioscler. Thromb.* 12 (1992) 1496–1502.
- M.A. Austin, J.L. Breslow, C.H. Hennekens, J.E. Buring, W.C. Willett, R.M. Krauss, Low-density lipoprotein subclass patterns and risk of myocardial infarction, *JAMA* 260 (1988) 1917–1921.
- S.S. Martin, A.A. Khokhar, H.T. May, K.R. Kulkarni, M.J. Blaha, P.H. Joshi, et al., HDL cholesterol subclasses, myocardial infarction, and mortality in secondary prevention: the lipoprotein investigators collaborative, *Eur. Heart J.* 36 (2015) 22–30.
- A. Kontush, M. Lindahl, M. Lhomme, L. Calabresi, M.J. Chapman, W.S. Davidson, Structure of hdl: particle subclasses and molecular components, *Handb. Exp. Pharmacol.* 224 (2015) 3–51.
- K.L. Stanhope, V. Medici, A.A. Bremer, V. Lee, H.D. Lam, M.V. Nunez, et al., A dose-response study of consuming high-fructose corn syrup-sweetened beverages on lipid/lipoprotein risk factors for cardiovascular disease in young adults, *Am. J. Clin. Nutr.* 101 (2015) 1144–1154.
- R.H. Lustig, K. Mulligan, S.M. Noworolski, V.W. Tai, M.J. Wen, A. Erkin-Cakmak, et al., Isocaloric fructose restriction and metabolic improvement in children with obesity and metabolic syndrome, *Obes. (Silver Spring)* 24 (2016) 453–460.
- Board FaN, Dietary reference intakes: energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein, and amino acids, *Macronutrients* (2005). Institute of Medicine, fnic.nal.usda.gov.
- Guideline: Sugars Intake for Adults and Children, 2015.
- D.R. Matthews, J.P. Hosker, A.S. Rudenski, B.A. Naylor, D.F. Treacher, R.C. Turner, Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man, *Diabetologia* 28 (1985) 412–419.
- A. Gugliucci, R. Caccavello, K. Kotani, N. Sakane, S. Kimura, Enzymatic assessment of paraoxonase 1 activity on HDL subclasses: a practical zymogram method to assess HDL function, *Clin. Chim. Acta* 415 (2013) 162–168.
- G. Cavigiolio, B. Shao, E.G. Geier, G. Ren, J.W. Heinecke, M.N. Oda, The interplay between size, morphology, stability, and functionality of high-density lipoprotein subclasses, *Biochemistry* 47 (2008) 4770–4779.
- M. Hersberger, A. von Eckardstein, Modulation of high-density lipoprotein cholesterol metabolism and reverse cholesterol transport, *Handb. Exp. Pharmacol.* (2005) 537–561.
- A. Qamar, S.A. Khetarpal, A.V. Khera, A. Qasim, D.J. Rader, M.P. Reilly, Plasma apolipoprotein C-III levels, triglycerides, and coronary artery calcification in type 2 diabetics, *Arterioscler. Thromb. Vasc. Biol.* 35 (2015) 1880–1888.
- W.T. Gibson, Beneficial metabolic phenotypes caused by loss-of-function apoC3 mutations, *Clin. Genet.* 87 (2015) 31–32.
- S.J. Bernele Moens, J.C. van Capelleveen, E.S. Stroes, Inhibition of apoC-III: the next pcsk9? *Curr. Opin. Lipidol.* 25 (2014) 418–422.
- K.L. Stanhope, P.J. Havel, Fructose consumption: recent results and their potential implications, *Ann. N. Y. Acad. Sci.* 1190 (2010) 15–24.
- I. Aeberli, M.B. Zimmermann, L. Molinari, R. Lehmann, D. l'Allemand, G.A. Spinaz, et al., Fructose intake is a predictor of LDL particle size in overweight schoolchildren, *Am. J. Clin. Nutr.* 86 (2007) 1174–1178.
- J.P. Hobkirk, R.F. King, I. Davies, N. Harman, P. Gately, P. Pemberton, et al., The metabolic inter-relationships between changes in waist circumference, triglycerides, insulin sensitivity and small, dense low-density lipoprotein particles with acute weight loss in clinically obese children and adolescents, *Pediatr. Obes.* 9 (2014) 209–217.
- S. Caron, A. Verrijken, I. Mertens, C.H. Samanez, G. Mautino, J.T. Haas, et al., Transcriptional activation of apolipoprotein C-III expression by glucose may contribute to diabetic dyslipidemia, *Arterioscler. Thromb. Vasc. Biol.* 31 (2011) 513–519.
- A. Sonmez, M.I. Yilmaz, M. Saglam, H.U. Unal, M. Gok, H. Cetinkaya, et al., The role of plasma triglyceride/high-density lipoprotein cholesterol ratio to predict cardiovascular outcomes in chronic kidney disease, *Lipids Health Dis.* 14 (2015) 29.
- J. Armato, G. Reaven, R. Ruby, Triglyceride/high-density lipoprotein cholesterol concentration ratio identifies accentuated cardio-metabolic risk, *Endocr. Pract.* (2015) 1–18.
- G.L. Vega, C.E. Barlow, S.M. Grundy, D. Leonard, L.F. DeFina, Triglyceride-to-high-density-lipoprotein-cholesterol ratio is an index of heart disease mortality and of incidence of type 2 diabetes mellitus in men, *J. Investig. Med.* 62 (2014) 345–349.
- D.S. Freedman, B.A. Bowman, J.D. Otvos, S.R. Srinivasan, G.S. Berenson, Levels and correlates of LDL and VLDL particle sizes among children: the Bogalusa heart study, *Atherosclerosis* 152 (2000) 441–449.
- M.V. Holmes, F.W. Asselbergs, T.M. Palmer, F. Drenos, M.B. Lanktree, C.P. Nelson, et al., Mendelian randomization of blood lipids for coronary heart disease, *Eur. Heart J.* 36 (2015) 539–550.
- B.F. Voight, G.M. Peloso, M. Orho-Melander, R. Frikke-Schmidt, M. Barbalic, M.K. Jensen, et al., Plasma HDL cholesterol and risk of myocardial infarction: a mendelian randomisation study, *Lancet* 380 (2012) 572–580.
- B.M. Bishop, Systematic review of cetp inhibitors for increasing high-density lipoprotein cholesterol: where do these agents stand in the approval process? *Am. J. Ther.* 22 (2015) 147–158.
- N.E. Miller, Cetp inhibitors and cardiovascular disease: time to think again, *F1000Res.* 3 (2014) 124.
- C. Gebhard, D. Rhainds, J.C. Tardif, HDL and cardiovascular risk: is cholesterol in particle subclasses relevant? *Eur. Heart J.* 36 (2015) 10–12.
- A. Gugliucci, T. Menini, Paraoxonase 1 and HDL maturation, *Clin. Chim. Acta* 439 (2015) 5–13.
- A. Gugliucci, R. Caccavello, H. Nassar, W. Abu Ahmad, R. Sinnreich, J.D. Kark, Low protective PON1 lactonase activity in an arab population with high rates of coronary heart disease and diabetes, *Clin. Chim. Acta* 445 (2015) 41–47.
- M. Aviram, J. Vaya, Paraoxonase 1 activities, regulation, and interactions with atherosclerotic lesion, *Curr. Opin. Lipidol.* 24 (2013) 339–344.
- K. Kotani, N. Sakane, Y. Sano, K. Tsuzaki, Y. Matsuoka, K. Egawa, et al., Changes on the physiological lactonase activity of serum paraoxonase 1 by a diet

- intervention for weight loss in healthy overweight and obese women, *J. Clin. Biochem. Nutr.* 45 (2009) 329–334.
- [45] Y. Yang, B. Yan, M. Fu, Y. Xu, Y. Tian, Relationship between plasma lipid concentrations and HDL subclasses, *Clin. Chim. Acta* 354 (2005) 49–58.
- [46] K.L. Stanhope, A.A. Bremer, V. Medici, K. Nakajima, Y. Ito, T. Nakano, et al., Consumption of fructose and high fructose corn syrup increase postprandial triglycerides, LDL-cholesterol, and apolipoprotein-B in young men and women, *J. Clin. Endocrinol. Metab.* 96 (2011) E1596–E1605.
- [47] T. Reinehr, W. Andler, Changes in the atherogenic risk factor profile according to degree of weight loss, *Arch. Dis. Child.* 89 (2004) 419–422.