Consumption of fructose and high fructose corn syrup increase postprandial triglycerides, LDL-cholesterol, and apolipoprotein-B in young men and women

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Abbreviated Title: Sugar-sweetened beverages & CVD risk factors

Key Terms: High fructose corn syrup, cardiovascular disease risk factors, postprandial triglyceride, low density lipoprotein, apolipoprotein B

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Precis: Consumption of HFCS-sweetened beverages for 2 weeks at 25%E increased risk factors for cardiovascular disease comparably to fructose and more than glucose in young adults.

Word Count: 3600
Abstract:
Context: The American Heart Association Nutrition Committee recommends women and men consume no more than 100 and 150 kcal of added sugar/day, respectively, whereas the Dietary Guidelines for Americans, 2010 suggests a maximal added sugar intake of 25% or less of total energy.
Objective: To address this discrepancy, we compared the effects of consuming glucose, fructose or high fructose corn syrup (HFCS) at 25% of energy requirements (E) on risk factors for cardiovascular disease.
Participants, Design and Setting, Intervention: 48 adults (Age:18-40 years; BMI:18-35 kg/m²) resided at the Clinical Research Center for 3.5 days of baseline testing while consuming energy-balanced diets containing 55%E complex carbohydrate. For 12 outpatient days they consumed usual ad libitum diets along with 3 servings/day of glucose, fructose, or HFCS-sweetened beverages (n=16/group), which provided 25%E requirements. Subjects then consumed energy-balanced diets containing 25%E sugar-sweetened beverages/30%E complex carbohydrate during 3.5 days of inpatient intervention testing.
Main Outcome Measures: 24-h TG AUC, fasting plasma LDL and apolipoprotein B (apoB) concentrations.
Results: 24-h TG AUC was increased compared with baseline during consumption of fructose (+4.7±1.2 mmol/Lx24h, P=0.0032) and HFCS (+1.8±1.4 mmol/Lx24h, P=0.035), but not glucose (-1.9±0.9 mmol/Lx24h, P=0.14). Fasting LDL and apoB concentrations were increased during consumption of fructose (LDL:+0.29±0.082 mmol/L, P=0.0023; apoB:+0.093±0.022 g/L, P=0.0005) and HFCS (LDL:+0.42±0.11 mmol/L, P<0.0001; apoB:+0.12±0.031 g/L, P<0.0001), but not glucose (LDL:+0.012±0.071 mmol/L, P=0.86; apoB:+0.0097±0.019 g/L, P=0.91).
Conclusions: Consumption of HFCS-sweetened beverages for 2 weeks at 25%E increased risk factors for cardiovascular disease comparably to fructose and more than glucose in young adults.
Introduction: In epidemiological studies, consumption of sugar and/or sugar-sweetened beverages has been linked to the presence of unfavorable lipid levels (1-5), insulin resistance (6, 7), fatty liver (8, 9), type 2 diabetes (10-12), cardiovascular disease (13) and metabolic syndrome (14). We have recently reported that consumption of fructose-sweetened beverages at 25% of energy requirements (E) increased visceral adipose deposition and de novo lipogenesis, produced dyslipidemia, and decreased glucose tolerance/insulin sensitivity in older, overweight/obese men and women, while consumption of glucose-sweetened beverages did not (15). Since the commonly consumed sugars, sucrose and high fructose corn syrup (HFCS), are composed of 50-55% fructose, these results provide a potential mechanistic explanation for the associations between sugar consumption and metabolic disease. However, the adverse metabolic effects of fructose consumption observed in the older, overweight/obese population (15) may not occur in a younger, leaner population. Authors of 3 recent reviews have concluded that long-term sugar intakes as high as 25-50%E have no adverse effects with respect to components of metabolic syndrome (16) and that fructose consumption up to 140 grams/day does not result in biologically relevant increases of fasting or postprandial triglycerides (TG) in healthy, normal weight (17) or overweight or obese (18) humans. These reviews (16, 17) are cited in the Report of the Dietary Guidelines Advisory Committee on the Dietary Guidelines for Americans 2010, released June of 2010, in which a maximal intake level of 25% or less of total energy from added sugars is suggested (19). However, in August of 2009, the American Heart Association Nutrition Committee recommended that women consume no more than 100 kcal/day and men consume no more than 150 kcal/day of added sugar (20). This equates to differences between the 2 guidelines of 400 kcal/d for women consuming 2000 kcal/d and 525 kcal/d for men consuming 2500 kcal/d. To address this discrepancy, we compared the effects of consuming 25%E as glucose, fructose or HFCS for 2 weeks on risk factors for cardiovascular disease in young adults.

Methods:

The subjects who participated in this study are a subgroup of participants from an ongoing 5-year NIH-funded investigation in which a total of 8 experimental groups (n=25/group) will be studied. The
objectives include comparing the metabolic effects of fructose, glucose, and HFCS consumption at 25% E, and to compare the metabolic effects of fructose and HFCS consumption at 0%, 10%, 17.5% and 25% E. The results reported in this paper are from the first 48 subjects to complete the study protocol in the experimental groups consuming 25% E as glucose, fructose, or HFCS (n=16/group). Participants were recruited through an internet listing (Craigslist.com) and underwent telephone and in-person interviews with medical history, complete blood count, and serum biochemistry panel to assess eligibility. Inclusion criteria included age 18-40 years and BMI 18-35 kg/m² with a self-report of stable body weight during the prior six months. Exclusion criteria included: diabetes (fasting glucose >125 mg/dl), evidence of renal or hepatic disease, fasting plasma TG >400 mg/dl, hypertension (>140/90 mm Hg), surgery for weight loss. Individuals who smoked, habitually ingested more than two alcoholic beverages/day, exercised more than 3.5 hours/week at a level more vigorous than walking, or used thyroid, lipid-lowering, glucose-lowering, anti-hypertensive, anti-depressant, or weight loss medications were also excluded. The UC Davis Institutional Review Board approved the experimental protocol for this study, and subjects provided written informed consent to participate.

For the 5 weeks prior to study, subjects were asked to limit daily consumption of sugar-containing beverages to one 8-oz serving of fruit juice. Fifty-five subjects were enrolled in the experimental groups consuming 25% E as glucose, fructose, or HFCS. Four subjects withdrew due to unwillingness to comply with the study protocol (2 in the HFCS group, 2 prior to group assignment) and 2 were withdrawn due to medical conditions not apparent during screening (HFCS and glucose group). The samples from one subject (HFCS group) who completed the study protocol were not analyzed because of illness during the 24-h serial blood collection. The experimental groups were matched for gender (9 men, 7 women/group), BMI, fasting TG, cholesterol, HDL and insulin concentrations. The subjects and UC Davis Clinical Research Center (CCRC) and technical personnel were blinded to the sugar assignments.
This was a parallel-arm, diet intervention study with 3 phases: (1) a 3.5-day inpatient baseline period during which subjects resided at the CCRC; (2) a 12-day outpatient intervention period; (3) a 3.5-day inpatient intervention period at the CCRC. During days 2 and 3 of the baseline and intervention inpatient periods, subjects consumed energy-balanced meals consisting of conventional foods. Daily energy requirements were calculated by the Mifflin equation (21) with adjustment of 1.3 for activity on the days of the 24-h serial blood collections, and adjustment of 1.5 for the other days. The baseline diet contained 55%E mainly as complex carbohydrate, 30% fat, and 15% protein. The intervention inpatient meals were as identical as possible to baseline meals, excepting the carbohydrate component consisted of 25%E as glucose-, fructose-, or HFCS-sweetened beverages and 30%E as complex carbohydrate. Sugar-sweetened beverages were provided to subjects as three daily servings consumed with meals, and were flavored with an unsweetened drink mix (Kool-aid®, Kraft). The timing of inpatient meal service and the energy distribution were: Breakfast-09:00h (25%); Lunch-13:00h (35%); Dinner-18:00h (40%).

During the 12-day outpatient phase of the study, subjects were provided with and instructed to drink 3 servings of sugar-sweetened beverage/day (one/meal), to consume their usual diet, and to not consume other sugar-containing beverages, including fruit juice. To monitor compliance, the sugar-sweetened beverages contained a biomarker (riboflavin), which was measured fluorimetrically in urine samples collected at the time of beverage-pickup. These measurements indicated that the 3 groups of subjects were comparably compliant.

Twenty-four hour serial blood collections were conducted on the 3rd day of the baseline (0 wk) and intervention (2 wk) inpatient periods. Three fasting blood samples were collected at 08:00, 08:30, 09:00h. Twenty-nine postprandial blood samples were collected at 30-60 minute intervals from 09:30 until 08:00h the next morning. Additional 6 ml samples were collected at the fasting time-points, 08:00, 08:30, 09:00h, and also at 22:00, 23:00, 24:00h, the period during which TG concentrations peaked during our
previous study (15). The additional plasma from the 3 fasting samples was pooled, as was that from the 3
late-evening postprandial samples; multiple aliquots of each pooled sample were stored at -80°C.

**Analyses:** Primary outcomes include fasting TG, 24-h TG incremental area under the curve (AUC), late-
evening postprandial TG concentrations, and fasting LDL, non-HDL-cholesterol (-C), apolipoprotein
(apo)B concentrations, and the apoB/apoAI ratio. Secondary outcomes include body weight, fasting HDL,
postprandial LDL, non-HDL-C, apoB, remnant lipoprotein-cholesterol and -TG (RLP-C & RLP-TG), and
fasting and postprandial small dense LDL-cholesterol (sdLDL-C). **Fasting concentrations, 24-h AUCs,**
and post-meal peaks for glucose and insulin, and HOMA-IR are presented in the online
supplement. Fasting measures were conducted on samples collected or pooled from the 8:00, 8:30, 9:00h
time-points and postprandial measures were conducted on samples collected or pooled from the 22:00,
23:00, 24:00h time-points. Lipid and lipoprotein concentrations (total cholesterol, HDL, TG, apoB,
apoA1) were determined with a Polychem Chemistry Analyzer (PolyMedCo, Inc.). LDL concentrations
were determined by direct homogenous assay using detergents (Denka Seiken) (22) and sdLDL-C
concentrations were quantified using the sdLDL-C-EX"SEIKEN" homogeneous assay kit (Denka Seiken,
Tokyo) (23). RLP concentrations were quantified with an immunoseparation assay (24). **Glucose was**
measured with an automated glucose analyzer (YSI, Inc.), and insulin by radioimmunoassay
(Millipore).

The incremental 24-h area AUC was calculated for TG, **glucose and insulin** by the trapezoidal method.
**Glucose and insulin post-meal peaks were assessed as the mean amplitudes of the three post-meal peaks; specifically the peak post-meal value minus the pre-meal value was averaged for breakfast, lunch and dinner for each subject.** The absolute change (Δ from 2 wk when 25%E sugar/30%E
complex carbohydrate was consumed compared with 0 wk when 55%E complex carbohydrate was
consumed) for each outcome was analyzed with SAS 9.2 (SAS, Cary, NC) in a mixed procedures (PROC
MIXED) model with sugar and gender as factors, and BMI, the change (2 wk – 0 wk) in body weight
(ΔBW), and outcome concentration at baseline (outcome_b) as continuous covariables. ΔBW and outcome_b were removed if they did not improve the precision of the model. Significant differences (P < 0.05) among the three sugars were identified by the Tukey’s multiple comparisons test. Outcomes that were significantly affected by 2 weeks of glucose, fructose or HFCS consumption were identified as least squares means (LS means) of the change significantly different than zero. **Primary outcomes were also analyzed with BMI as a factor (BMI <25 m/kg^2 vs >25 m/kg^2).** Data are presented as mean ± SEM.

**Results:** There were no significant differences among the 3 experimental groups in anthropomorphic (Table 1) or outcome measures at baseline (Tables 2,3,S1). Body weight (Table 3) and blood pressure (data not shown) were not affected by 2 weeks consumption of glucose, fructose or HFCS.

**Primary outcomes – Comparing glucose, fructose and HFCS with complex carbohydrate consumption:** Table 2 presents the primary outcomes during consumption of complex carbohydrate at baseline (0wk) and at the end of the 2-week sugar interventions. The 24-h TG profiles during baseline and the end of the 2-week intervention are shown in **Figure 1A-C.** The 24-h TG AUC (**Figure 2A**) was significantly increased compared with baseline (LS means of Δ different than zero) in subjects consuming fructose (+4.7±1.2 mmol/L x 24 h, P=0.0032) and HFCS (+1.8±1.4 mmol/L x 24 h, P=0.035), while it tended to decrease during consumption of glucose (-1.9±0.9 mmol/L x 24 h, P=0.14). The consumption of all 3 sugars resulted in a late-evening TG peak between 22:00-24:00 h that was not apparent when complex carbohydrate was consumed (**Figure 1A-C**). The late-evening peaks (**Figure 2B**) were significantly increased compared with baseline during consumption of fructose (+0.59±0.11 mmol/L, P<0.0001) and HFCS (+0.46±0.082 mmol/L, P<0.0001), but not by glucose (+0.22±0.10 mmol/L, P=0.077). All three sugars tended to increase fasting TG, but this was only significant in the group consuming glucose (**Figure 2C**). Fasting LDL-C concentrations (**Figure 3A**) were during consumption of fructose (+0.29±0.082 mmol/L, P=0.0023) and HFCS (+0.42±0.11 mmol/L, P<0.0001), but not glucose (+0.012±0.071 mmol/L, P=0.86). Similarly, fasting non-HDL-C (**Figure 3B**), apoB (**Figure 3C**) and the
apoB/apoAI ratio (Figure 3D) were all significantly increased in subjects consuming fructose (non-HDL-C:+0.29±0.066 mmol/L, P=0.0081; apoB:+0.093±0.022 g/L, P=0.0005; apoB/apoAI:+14.6±3.8%, P=0.0006) and HFCS (non-HDL-C:+0.55±0.14 mmol/L, P<0.0001; apoB:+0.12±0.031 g/L, P<0.0001; apoB/apoAI:+19.5±4.4%, P<0.0001) compared with baseline, but not in subjects consuming glucose (non-HDL-C:+0.055±0.080 mmol/L, P=0.49; apoB:+0.0097±0.019 g/L, P=0.90; apoB/apoAI:+1.9±2.5%, P=0.81).

Primary outcomes – Comparing glucose, fructose and HFCS consumption: The effects of the 3 sugars were significantly different (PROC MIXED 2-factor analysis with adjustment for BMI, ΔBW and outcome) for all primary outcomes except fasting TG (See effects of sugar P values in Table 2). The effects of HFCS compared with fructose consumption on all primary outcomes were not significantly different (P>0.05, Tukey’s). The increases in 24-h TG AUC (P=0.0068), late evening TG peaks (P=0.015), fasting apoB (P=0.037) and the apoB/apoAI ratio (P=0.028) were larger after fructose consumption compared with glucose consumption. The increases in 24-h TG AUC (P=0.034), fasting LDL (P=0.0083), non-HDL-C (P=0.0055), apoB (P=0.0056), and apoB/apoAI ratio (P=0.0034) were larger after HFCS consumption than glucose consumption.

BMI: While the statistical results presented in Table 1 and Figures 2 & 3 include adjustment for BMI, Online Figure S1 A-F presents the changes of the primary outcomes with subjects grouped as normal weight (BMI<25 kg/m²) or overweight/obese (BMI>25 kg/m²). The effect of BMI status was significant for the change of 24-h TG AUC (P=0.016) and late-evening TG peaks (P=0.019), but not for fasting TG (P=0.55, data not shown), LDL-C (P=0.30), non-HDL-C (P=0.93), apoB (P=0.62) and apoB/apoAI (P=0.51). Normal weight and overweight/obese subjects consuming HFCS had comparable absolute (Figure S1) and percent increases of late-evening TG (BMI<25 kg/m²:+46±11%; BMI>25 kg/m²:+31±6%), fasting LDL-C (BMI<25 kg/m²:+22±1%; BMI>25 kg/m²:+28±1%), non-HDL-C (BMI<25 kg/m²:+36±19%, BMI>25 kg/m²:+17±7), apoB (BMI<25 kg/m²:+17±
Secondary Outcomes – Comparing glucose, fructose and HFCS to complex carbohydrate consumption: Table 3 presents the secondary outcomes during consumption of complex carbohydrate at baseline and at the end of the 2-week sugar interventions. Fasting HDL concentrations were unaffected by consumption of the 3 sugar-sweetened beverages. Similar to the responses in the fasting state, subjects consuming fructose and HFCS had increased postprandial concentrations of LDL-C, non-HDL-C and apoB compared with baseline, while subjects consuming glucose did not. Fructose and HFCS consumption increased postprandial concentrations of RLP-C and RLP-TG compared with baseline, while consumption of glucose did not. Consumption of all 3 sugars increased fasting and postprandial sdLDL-C compared with baseline.

Secondary outcomes – Comparing glucose, fructose and HFCS consumption: The effects of the 3 sugars were significantly different (PROC MIXED 2-factor analysis with adjustment for BMI, ∆BW and outcome) for postprandial LDL, non-HDL-C, apoB, RLP-C and sdLDL-C (See effects of sugar P values in Table 3). The effects of HFCS compared with fructose consumption on all secondary outcomes were not significantly different (P > 0.05, Tukey’s). The increases in postprandial RLP-C were larger during consumption of fructose compared with glucose (P=0.044), and HFCS consumption caused larger increases in postprandial LDL (P=0.0024), non-HDL-C (P=0.0007), apoB (P=0.025), and sdLDL-C (P=0.014) (Tukey’s) than glucose consumption.

Glucose, insulin and HOMA-IR: The 24-h glucose and insulin profiles during baseline (0 wk) and at the end of the 2-week intervention are presented in Online Figures S2A-C and S3A-C, respectively. Compared with baseline, the 24-h glucose and insulin 24-h AUCs and the post-meal insulin peaks were significantly increased in subjects consuming glucose, significantly decreased in subjects
consuming fructose, and were unchanged in subjects consuming HFCS (Online Table S1). Post-meal glucose peaks were increased in subjects consuming glucose and HFCS. Fasting glucose concentrations were significantly decreased in subjects consuming glucose, while fasting insulin concentrations and HOMA-IR did not change significantly in any group.

Gender: While there were no significant sugar-gender interactions for any of the primary or secondary outcomes, men exhibited larger increases of fasting TG, non-HDL-C, apoB and sdLDL-C concentrations, and postprandial LDL, non-HDL-C and sdLDL-C concentrations in response to sugar consumption than women (See effects of gender $P$ values in Tables 2 & 3). However, postprandial TG responses; as assessed by the 24-h TG AUC, late-evening TG peaks, postprandial apoB and RLP–TG concentrations; were not different between genders. The subjects consuming glucose exhibited the most divergent gender responses, particularly in sdLDL-C. Fasting and postprandial sdLDL-C levels were increased compared to baseline by $+0.22 \pm 0.07 \text{ mmol/L} \ (P=0.0001)$ and $+0.24 \pm 0.05 \text{ mmol/L} \ (P<0.0001)$ respectively in men after glucose consumption, but were unchanged in women (fasting sdLDL-C: -0.004 $\pm$ 0.02 mmol/L, $P=0.61$; postprandial sdLDL-C: $+0.006 \pm 0.019 \text{ mmol/L}, \ P=0.69$).

Discussion: The current study provides evidence that postprandial TG and fasting and postprandial concentrations of LDL, non-HDL-C, apoB, and the apoB/apoAI ratio – established risk factors for coronary heart disease (25) – are significantly increased in response to 2-weeks consumption of 25% of energy requirements as fructose and HFCS, but not glucose, in younger, normal weight and overweight subjects. In contrast, and as was observed in older subjects (15), fasting TG concentrations were increased in subjects consuming glucose, but not in those consuming fructose-containing sugars. The differential effects of fructose and glucose consumption on fasting and postprandial TG responses in subjects from both studies suggest that fasting TG concentrations are not a reliable indicator of the adverse changes in postprandial TG and other lipid/lipoprotein risk factors induced by fructose consumption. There is growing evidence linking increases of postprandial TG concentrations with proatherogenic conditions.
It is important to note that for both the current and previous study (15), the differential effects of fructose and HFCS compared to complex carbohydrate on the 24-h TG profile were most marked in the late evening, approximately 4 and 6 hours after dinner. Studies investigating the relationship between this late-evening peak and proatherogenic changes would be of interest, as would investigations into the sources of the TG that contributes to these peaks (DNL, diet, or fatty acids derived from adipose lipolysis).

To our knowledge this is the first study to directly compare the effects of sustained consumption of HFCS with 100% fructose and glucose-sweetened beverages. This comparison is important because it would seem likely that the effects of HFCS-sweetened beverages on circulating lipids and lipoproteins would be less than those of pure fructose-sweetened beverage, because they contain 45% less fructose. And indeed, the postprandial TG and RLP responses exhibited the expected pattern based on the fructose content of the sugars, with increases being greatest in subjects who consumed 145±4 g fructose/day from beverages, lowest in subjects who consumed 144±5 g glucose/day and 0 g fructose/day from beverages, and intermediate in subjects who consumed HFCS-sweetened beverages providing 64±2 g glucose/day and 79±3 g fructose/day. However, the changes of fasting and postprandial concentrations of LDL, non-HDL-C, apoB, and the apoB/apoAI ratio in subjects consuming HFCS were significantly larger compared with subjects consuming glucose, and tended to be higher compared with subjects consuming pure fructose. More studies are needed to confirm this unexpected pattern and to determine if it is a result of a synergistic effect of consuming fructose and glucose in combination. Additional studies are also needed to determine if the substantial increases, seen after just 2 weeks, are further aggravated with longer term consumption of HFCS-sweetened beverages.

Compared with baseline, post-meal glucose and insulin responses (indexed as 24-h AUC and post-meal peaks) were mainly increased during glucose consumption, decreased during fructose consumption, and unchanged during HFCS consumption. This pattern is expected, and further
supports our data indicating that the adverse effects associated with chronic consumption of sugar-
sweetened beverages result from the specific effects of fructose (29), rather than from increased
circulating glucose and insulin excursions (i.e., glycemic index (GI)) (30-32). While consumption of
fructose increased fasting glucose and insulin concentrations in 2 weeks and decreased insulin
sensitivity by 17% in 10 weeks (15), in the current study HOMA-IR was unchanged after 2-weeks
consumption of fructose, HFCS or glucose. This may be related to the subjects in the current study
being younger and leaner (28±7 years; 25.5±4.0 kg/m^2) than the subjects in the previous study (54±8
years; 29.1±2.9 kg/m^2). In study by Le and colleagues, inclusion of fructose with an energy-balanced
diet for 4 weeks in young, normal weight men (24.7 ± 1.3 years; ~22 kg/m^2) increased fasting
glucose levels, but other indices of insulin sensitivity were unaffected (33). However it was recently
reported that consumption of fructose or glucose (150 g/d) for 4 weeks lowered insulin sensitivity
and increased HOMA-IR in subjects of similar age and BMI (31±9 years; 25.9±2.2 kg/m^2) (34).

As would be expected based on the evidence that both increasing age and post-menopausal status result in
augmented postprandial lipid responses in women (35), more significant gender differences in lipid
outcomes were observed in these younger subjects in the current study than in the older subjects
previously studied (15). With the exception of postprandial TG, apoB and RLP-C and RLP–TG, younger
men exhibited larger lipoprotein responses after 2 weeks of sugar consumption than younger women. The
comparable responses in postprandial TG and apoB concentrations and the significantly different fasting
TG and apoB responses between the genders suggest that rates of VLDL secretion may be similar
between men and women, whereas rates of VLDL clearance are different. This is supported by kinetic
studies, which demonstrate that women have higher TG-rich lipoprotein and LDL-apoB fractional
catabolic rates than men, while production rates are comparable (36, 37).

The greater effect of glucose consumption on sdLDL-C levels in younger men compared with younger
women represents the most marked difference between the current and our previous lipid results, which
showed older men and women were comparably non-responsive to consumption of glucose (15). The increase of fasting sdLDL-C concentrations compared with baseline in younger men consuming glucose was unexpected, as they did not exhibit increases in fasting LDL and apoB concentrations.

The added sugar component of the typical US diet consists of nearly equal amounts of HFCS and sucrose (38), therefore it is a limitation of this study that we did not also investigate the effects of sucrose consumption. However, we expect that the effects of sucrose would be comparable to those of HFCS because its composition (50% glucose/50% fructose) is very similar to the composition of the HFCS used for this study (45% glucose/55% fructose). This is supported by results from a crossover study in which subjects consumed standardized diets containing 5, 18, or 33% of energy as sucrose, each for 6 weeks. Compared with the 5% sucrose diet, LDL concentrations increased by 17% on the 18% sucrose diet and by 22% on the 33% sucrose diet (39).

Self-reported intake data suggest that 13% of the US population consumes >25% of energy from added sugar (40). Importantly, the current results provide evidence that sugar consumption at this level increases risk factors for cardiovascular disease within 2 weeks in young adults, thus providing direct experimental support for the epidemiological evidence linking sugar consumption with dyslipidemia (1-5) and cardiovascular disease (13). They contradict the conclusions from recent reviews that sugar intakes as high as 25-50% of energy have no adverse long-term effects with respect to components of the metabolic syndrome (16) and that fructose consumption up to 140 grams/day does not result in a biologically relevant increase of fasting or postprandial TG in healthy, normal weight (17) or overweight or obese (18) humans. Additionally they provide evidence that the maximal upper limit of 25% of total energy requirements from added sugar, suggested by the Dietary Guidelines for Americans 2010 (19), may need to be re-evaluated.
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References:


consumption of sugar-sweetened drinks and fruit juice in middle and older-aged adults. J Nutr 137:2121-2127


http://www.cnpp.usda.gov/DGAs2010-DGACReport.htm


30. **Ding EL, Malik VS** 2008 Convergence of obesity and high glycemic diet on compounding diabetes and cardiovascular risks in modernizing China: An emerging public health dilemma. Global Health 4:4

31. **Hu FB, Malik VS** 2010 Sugar-sweetened beverages and risk of obesity and type 2 diabetes: epidemiologic evidence. Physiol Behav 100:47-54


Table 1: Subjects' baseline anthropomorphic and metabolic parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Glucose (n=16)</th>
<th>Fructose (n=16)</th>
<th>HFCS (n=16)</th>
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<tr>
<td>Age (year)</td>
<td>27.0±7.2</td>
<td>28.0±6.8</td>
<td>27.8±7.6</td>
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<tr>
<td>Weight (kg)</td>
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<td>76.8±10.4</td>
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<td>BMI (kg/m2)</td>
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<td>25.4±3.8</td>
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<td>Waist circumference (cm)</td>
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<td>Body fat (%)</td>
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<td>Total cholesterol (mmol/L)</td>
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<td>1.2±0.4</td>
<td>1.2±0.4</td>
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<tr>
<td>Insulin (pmol/L)</td>
<td>97.9±30.4</td>
<td>102.8±86.4</td>
<td>89.1±31.6</td>
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*P* > 0.05 for differences among groups at baseline for all parameters, PROC MIXED ANOVA

Mean±SD
Table 2: Primary outcomes during consumption of complex carbohydrates at 0wk and during consumption of glucose-, fructose- or HFCS-sweetened beverages at 2wk

<table>
<thead>
<tr>
<th>Primary outcomes</th>
<th>Glucose</th>
<th></th>
<th>Fructose</th>
<th></th>
<th>HFCS</th>
<th></th>
<th>Effects</th>
<th></th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>24-h TG AUC&lt;sup&gt;1&lt;/sup&gt;</td>
<td>5.6±1.1</td>
<td>3.6±1.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.9±1.5</td>
<td>7.6±1.9**&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.8±1.4</td>
<td>5.5±1.7&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>Sugar</td>
<td>0.0058</td>
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<tr>
<td>(mmol/L*24h)</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>Gender</td>
<td>0.13</td>
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<td></td>
<td></td>
<td>BMI</td>
<td>0.0033</td>
<td></td>
</tr>
<tr>
<td>Late-evening TG&lt;sup&gt;2&lt;/sup&gt;</td>
<td>1.3±0.2</td>
<td>1.5±0.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.2±0.1</td>
<td>1.8±0.2****&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.3±0.2</td>
<td>1.8±0.2****&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>Sugar</td>
<td>0.016</td>
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<tr>
<td>(mmol/L)</td>
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<td>Gender</td>
<td>0.40</td>
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<td>BMI</td>
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</tr>
<tr>
<td>Fasting TG&lt;sup&gt;3&lt;/sup&gt;</td>
<td>1.2±0.1</td>
<td>1.4±0.2**</td>
<td>1.2±0.1</td>
<td>1.3±0.1</td>
<td>1.3±0.1</td>
<td>1.4±0.1</td>
<td>Sugar</td>
<td>0.54</td>
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<td>(mmol/L)</td>
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<td>Gender</td>
<td>0.035</td>
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<td>BMI</td>
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<tr>
<td>Fasting LDL-C&lt;sup&gt;1&lt;/sup&gt;</td>
<td>2.6±0.2</td>
<td>2.6±0.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.1±0.2</td>
<td>2.4±0.2**&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>2.3±0.2</td>
<td>2.7±0.2****&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Sugar</td>
<td>0.0098</td>
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<td>(mmol/L)</td>
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<td></td>
<td>BMI</td>
<td>0.40</td>
<td></td>
</tr>
<tr>
<td>Fasting non-HDL-C&lt;sup&gt;1&lt;/sup&gt;</td>
<td>3.2±0.2</td>
<td>3.3±0.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.7±0.2</td>
<td>3.0±0.2**&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>2.9±0.2</td>
<td>3.4±0.2****&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Sugar</td>
<td>0.0077</td>
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<td>(mmol/L)</td>
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<td></td>
<td>Gender</td>
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<td>BMI</td>
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<tr>
<td>Fasting apoB&lt;sup&gt;1&lt;/sup&gt;</td>
<td>0.82±0.06</td>
<td>0.83±0.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.65±0.04</td>
<td>0.74±0.05****&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.73±0.05</td>
<td>0.85±0.06****&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Sugar</td>
<td>0.0051</td>
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<td>(g/L)</td>
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<td>BMI</td>
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</tr>
<tr>
<td>apoB/apoAI&lt;sup&gt;3&lt;/sup&gt;</td>
<td>0.70±0.06</td>
<td>0.70±0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.54±0.04</td>
<td>0.63±0.05**&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.60±0.06</td>
<td>0.71±0.07****&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Sugar</td>
<td>0.0031</td>
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<td>BMI</td>
<td>0.61</td>
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</tr>
</tbody>
</table>

<sup>1</sup>PROC MIXED 2-factor (sugar, gender) analysis with adjustment for BMI, ΔBW (2wk - 0wk) and outcome on absolute Δ (2wk vs 0wk)

<sup>2</sup>PROC MIXED 2-factor (sugar, gender) analysis with adjustment for BMI on absolute Δ (2wk vs 0wk)

<sup>3</sup>PROC MIXED 2-factor (sugar, gender) analysis with adjustment for BMI and ΔBW (2wk - 0wk) on absolute Δ (2wk vs 0wk)

*P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001 LS means of Δ different from zero

<sup>a</sup>Δ (2wk vs 0wk) significantly different from <sup>b</sup>Δ (2wk vs 0wk), Tukey's multiple comparison test

*P > 0.05 for differences among groups at baseline for all outcomes

Mean ± SEM
Table 3: Secondary outcomes during consumption of complex carbohydrates at 0wk and during consumption of glucose-, fructose- or HFCS-sweetened beverages at 2wk

<table>
<thead>
<tr>
<th>Secondary outcomes</th>
<th>Glucose 0wk</th>
<th>Glucose 2wk</th>
<th>Fructose 0wk</th>
<th>Fructose 2wk</th>
<th>HFCS 0wk</th>
<th>HFCS 2wk</th>
<th>Effects</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (kg)</td>
<td>76.8±3.5</td>
<td>77.2±3.7</td>
<td>76.8±2.6</td>
<td>76.7±2.6</td>
<td>74.3±3.7</td>
<td>74.7±3.7</td>
<td>Sugar</td>
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<td>Gender</td>
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<td></td>
<td>BMI</td>
<td>0.50</td>
</tr>
<tr>
<td>Fasting HDL (mmol/L)</td>
<td>1.2±0.1</td>
<td>1.2±0.1</td>
<td>1.2±0.1</td>
<td>1.1±0.1</td>
<td>1.2±0.1</td>
<td>1.2±0.1</td>
<td>Sugar</td>
<td>0.92</td>
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<td>Gender</td>
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<td>BMI</td>
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<tr>
<td>Postprandial (PP) LDL (mmol/L)</td>
<td>2.5±0.2</td>
<td>2.6±0.2a</td>
<td>2.0±0.2</td>
<td>2.3±0.2ab</td>
<td>2.1±0.2</td>
<td>2.7±0.2****b</td>
<td>Sugar</td>
<td>0.0033</td>
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<td></td>
<td></td>
<td>BMI</td>
<td>0.54</td>
</tr>
<tr>
<td>PP non-HDL-C (mmol/L)</td>
<td>3.0±0.2</td>
<td>3.2±0.2a</td>
<td>2.5±0.2</td>
<td>3.0±0.2****ab</td>
<td>2.6±0.2</td>
<td>3.4±0.2****b</td>
<td>Sugar</td>
<td>0.0012</td>
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<td></td>
<td></td>
<td>BMI</td>
<td>0.27</td>
</tr>
<tr>
<td>PP apoB (g/L)</td>
<td>0.78±0.05</td>
<td>0.83±0.05a</td>
<td>0.62±0.04</td>
<td>0.73±0.05ab</td>
<td>0.68±0.05</td>
<td>0.84±0.06****b</td>
<td>Sugar</td>
<td>0.031</td>
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<td>BMI</td>
<td>0.56</td>
</tr>
<tr>
<td>PP RLP-C (mmol/L)</td>
<td>0.17±0.02</td>
<td>0.19±0.02a</td>
<td>0.16±0.02</td>
<td>0.23±0.03****b</td>
<td>0.15±0.02</td>
<td>0.21±0.02****ab</td>
<td>Sugar</td>
<td>0.035</td>
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<td></td>
<td>BMI</td>
<td>0.034</td>
</tr>
<tr>
<td>PP RLP-TG (mmol/L)</td>
<td>0.34±0.07</td>
<td>0.44±0.06</td>
<td>0.35±0.06</td>
<td>0.58±0.08***</td>
<td>0.33±0.06</td>
<td>0.54±0.09***</td>
<td>Sugar</td>
<td>0.088</td>
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<td></td>
<td>BMI</td>
<td>0.012</td>
</tr>
<tr>
<td>Fasting sdLDL-C (mmol/L)</td>
<td>0.65±0.08</td>
<td>0.77±0.10a</td>
<td>0.47±0.04</td>
<td>0.59±0.06***</td>
<td>0.61±0.08</td>
<td>0.78±0.09****</td>
<td>Sugar</td>
<td>0.37</td>
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<td>PP sdLDL-C (mmol/L)</td>
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<td>0.79±0.10ab</td>
<td>0.48±0.04</td>
<td>0.64±0.07****ab</td>
<td>0.60±0.08</td>
<td>0.86±0.10****b</td>
<td>Sugar</td>
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<td>Gender</td>
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<td>BMI</td>
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</tr>
</tbody>
</table>

1PROC MIXED 2-factor (sugar, gender) analysis with adjustment for BMI on absolute Δ (2wk vs 0wk)
2PROC MIXED 2-factor (sugar, gender) analysis with adjustment for BMI, ΔBW (2wk - 0wk) and outcome on absolute Δ (2wk vs 0wk)
3PROC MIXED 2-factor (sugar, gender) analysis with adjustment for BMI and outcome on absolute Δ (2wk vs 0wk)
4PROC MIXED 2-factor (sugar, gender) analysis with adjustment for BMI and ΔBW (2wk - 0wk) on absolute Δ (2wk vs 0wk)

*P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001 LS means of Δ different from zero

Δ (2wk vs 0wk) significantly different from Δ (2wk vs 0wk), Tukey's multiple comparison test

P > 0.05 for differences among groups at baseline for all outcomes

Mean ± SEM
Figure Legends:

Figure 1: 24-h TG Profiles During Consumption of Complex Carbohydrate and During Consumption of Sugar-Sweetened Beverages. 24-h TG concentrations during consumption of energy-balanced baseline diet containing 55%E complex carbohydrate at 0wk and during consumption of energy-balanced intervention diet containing 30%E complex carbohydrate and 25%E glucose (1A), fructose (1B), or HFCS (1C) at 2wk. n = 16/group. Data are mean ± SEM.

Figure 2: Effects of Sugar-Sweetened Beverage Consumption on TG Concentrations. The change in 24-h TG AUC (2A), late-night TG (2B) and fasting TG concentrations (2C) compared to baseline after consuming 25% of energy requirements as glucose-, fructose- or HFCS-sweetened beverages for 2 weeks. $P < 0.05$, **$P < 0.01$, effect of sugar; 2-factor (sugar, gender) PROC MIXED analysis on ∆ with adjustment for BMI (2B), ∆BW (2C), and outcome at baseline (2A). $^*P<0.05$, **$P<0.01$, ****$P<0.0001$, LS means different from zero. ^A ∆ different from ^B ∆, Tukey’s. n = 16/group. Data are mean ± SEM.

Figure 3: Effects of Sugar-Sweetened Beverage Consumption on Risk Factors for Cardiovascular Disease. The change in fasting LDL (3A), non-HDL-C (3B), apoB concentrations (3C), apoB/apoA1 (3D) after consuming 25% of energy requirements as glucose-, fructose- or HFCS-sweetened beverages for 2 weeks. $^{**}P<0.01$, effect of sugar; 2-factor (sugar, gender) PROC MIXED analysis on ∆ with adjustment for BMI, ∆BW (3D) and outcome at baseline (3A,3B,3C). $^{**}P<0.01$, $^{***}P<0.001$, ****$P<0.0001$, LS means different from zero. ^A ∆ different from ^B ∆, Tukey’s. n = 16/group. Data are mean ± SEM.