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ORIGINAL CONTRIBUTION

The relationship between sugar-sweetened beverages and liver enzymes among healthy premenopausal women: a prospective cohort study

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Abstract
Purpose To prospectively assess the association between sugar-sweetened beverages (SSB), added sugar, and total fructose and serum concentrations of liver enzymes among healthy, reproductive-age women.
Methods A prospective cohort of 259 premenopausal women (average age 27.3 ± 8.2 years; BMI 24.1 ± kg/m^2) were followed up for up to two menstrual cycles, providing up to eight fasting blood specimens/cycle and four 24-h dietary recalls/cycle. Women with a history of chronic disease were excluded. Alanine and aspartate aminotransferases (ALT and AST, respectively) were measured in serum samples. Linear mixed models estimated associations between average SSB, added sugar, and total fructose intake and log-transformed liver enzymes adjusting for age, race, body mass index, total energy and alcohol intake, and Mediterranean diet score.
Results For every 1 cup/day increase in SSB consumption and 10 g/day increase in added sugar and total fructose, log ALT increased by 0.079 U/L (95 % CI 0.022, 0.137), 0.012 U/L (95 % CI 0.002, 0.022), and 0.031 (0.012, 0.050), respectively, and log AST increased by 0.029 U/L (−0.011, 0.069), 0.007 U/L (0.000, 0.014), and 0.017 U/L (0.004, 0.030), respectively. Women who consumed ≥1.50 cups/day (12 oz can) SSB versus less had 0.127 U/L (95 % CI 0.001, 0.254) higher ALT [percent change 13.5 % (95 % CI 0.1, 28.9)] and 0.102 (95 % CI 0.015, 0.190) higher AST [percent change 10.8 % (95 % CI 1.5, 20.9)].
Conclusions Sugar-sweetened beverages were associated with higher serum ALT and AST concentrations among healthy premenopausal women, indicating that habitual consumption of even moderate SSB may elicit hepatic lipogenesis.

Keywords Liver enzymes · Menstrual cycle · Fructose · Dietary sucrose · Sugar-sweetened beverages

Introduction

While added sugar consumption appears to be on the decline in the USA, average intakes continue to exceed recommendations [1]. Sugar-sweetened beverages (SSB) are the largest contributor of added sugars and fructose and the greatest single source of calories in the US diet, making up an estimated 9.3 % of total energy intake among American women aged 20–39 years [2]. Many non-beverage items are also high in added sugars, predominately in packaged foods, resulting in total mean intakes of 73.2 g/day among adult women, more than double the recommended limit of

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32–48 g/day for a moderately physically active woman, aged 19–30 years [3]. High-fructose corn syrup, which is approximately 50 % fructose, is the most common sweetener added to SSB, and unlike glucose alone, which can be readily used as a primary fuel source by all cells in the body, fructose is primarily metabolized in the liver where it promotes glycogen synthesis and de novo lipogenesis [4]. Thus, consumption of fructose in liquid form, such as found in SSB, may be particularly lipogenic as it is quickly absorbed into the bloodstream, rapidly accessing the liver via the portal vein and potentially leading to greater liver stress and damage compared with non-liquid intake [4].

Growing evidence indicates that high intake of SSB and added sugars contribute to the development of non-alcoholic fatty liver disease (NAFLD), a disease characterized by excess hepatic triglyceride content (steatosis), with the link thought to be driven, at least in party, by fructose-mediated increases in hepatic lipogenesis [5–8]. Hepatic steatosis may initiate hepatic insulin resistance, thereby promoting a subsequent cascade of related metabolic complications such as hyperlipidemia and impaired glucose tolerance. However, little research has assessed the effects of low to moderate intake of sweetened beverages and added sugars on the liver, particularly among women with no history of chronic disease. Therefore, the objective of this study was to determine the association between SSB, added sugar, and total fructose intake and serum concentrations of liver enzymes, as surrogate markers of hepatic metabolic function, among a healthy population of reproductive-age women.

Materials and methods

Study population

The BioCycle Study (2005–2007) was a prospective cohort study that followed 259 regularly menstruating women from western New York State for one ($n = 9$) or two ($n = 250$) menstrual cycles. The study size was based on power to detect differences in oxidative stress levels with endogenous reproductive hormone levels and antioxidants, the primary study outcome [9]. The study population, materials, and methods have been previously described in detail [9]. Briefly, study participants were between the ages of 18 and 44 with a self-reported BMI between 18 and 35 at screening and usual menstrual cycle length between 21 and 35 days for each cycle in the past 6 months. Women with a history of chronic disease such as heart disease, diabetes mellitus, cancer, inflammatory diseases, autoimmune, liver or kidney disease, thyroid disease, or any other endocrine dysfunction were excluded. Additionally, women with a history of chronic medication use including oral contraceptives, lipid lowering drugs, anti-hypertensive medications, and aspirin were excluded as were women taking antibiotics in the past 3 months. Women with a history of alcohol abuse were also excluded.

Among the 449 women screened for study participation, 319 met the eligibility criteria, 276 enrolled in the study, and 17 withdrew after enrollment. The University at Buffalo Health Sciences Institutional Review Board approved the study and served as the IRB designated by the National Institutes of Health for this study under a reliance agreement. Written informed consent was obtained from all participants.

Timing of clinic visits was individually determined, aided by an algorithm incorporating cycle length and data from a daily fertility monitor (Clearblue® Easy Fertility Monitor, Inverness Medical, Waltham, MA) and timed to occur during specific phases of the menstrual cycle corresponding to menses, mid-follicular phase, 3 days around the luteinizing hormone (LH) surge (expected ovulation), and the early, mid-, and late luteal phases [10]. Adherence to the study protocol was high, with 94 % of women completing at least seven of eight clinic visits per cycle.

Liver enzyme assessment

The complete metabolic profile, including liver enzymes alanine and aspartate aminotransferases (ALT and AST, respectively), was measured in morning fasting serum samples ($n = 3898$ total) collected at each clinic visit using a LX20 automated chemistry analyzer (Beckman, Brea, CA) at the Kaleida Center for Laboratory Medicine in Buffalo, New York. Coefficients of variation reported by the laboratory for ALT and AST tests were <5 %.

Dietary assessment

For each menstrual cycle under study, participants completed four 24-h dietary recalls (24HDR) coinciding with fasting blood specimen collection at the menses, mid-follicular, expected ovulation, and mid-luteal phase visits. Trained interviewers collected food and beverage consumption information from the participants. Nutrient and food/beverage data, including total energy (kcal), fiber (g), protein (g), alcohol (g), carbohydrates (g), fat (g), and sweetened and artificially sweetened (diet) beverages [8-oz cups (1 oz = 28 g; 1 cup = 237 mL)], were calculated using the Nutrition Data System for Research (NDSR, version 2005; Nutrition Coordinating Center (NCC), University of Minnesota, Minneapolis, MN) [11–14]. The NDSR takes into account brand and serving size of each item reported and then computes the daily nutrients, food sources, and food components consumed. Total fructose was calculated as the sum of free fructose and half the intake of added sugar

(which assumes 50 % of sugar intake is from fructose on average) [15, 16]. Average daily SSB intake in our study was calculated as the sum of sweetened sodas and sweetened fruit drinks for a given day. Mediterranean diet score was calculated for each cycle visit as previously described as a measure of overall diet quality [17]. 96 % of the participants completed four 24HDRs in at least one cycle.

Covariate assessment

Height and weight were measured by trained professionals using standard protocols at baseline in order to calculate BMI (kg/m^2). Physical activity was assessed at baseline using the long-form International Physical Activity Questionnaire (IPAQ) and categorized using total metabolic equivalent of task (MET) hours per week according to published guidelines [18]. Study participant demographics and lifestyle habits were self-reported at the enrollment visit using standardized questionnaires [9].

Statistical analysis

Average daily intake of SSBs, added sugar, and total fructose was determined for each woman over 1) each menstrual cycle and 2) the entire study period, with average daily consumption of SSB categorized by tertiles (0–0.11, >0.11–0.52, and ≥0.52 cups/day; 1 cup = 237 mL) and meaningful cut points (≥0.75 cups/day vs. less). We chose 0.75 cups/day (equivalent to a half a can of soda) as a meaningful cut point given that it represents half of the added sugars a healthy reproductive-age woman is recommended to consume [3] and is equivalent to the traditional 6.5 oz [177 g] soda serving size. Added sugar and fructose were categorized by tertiles.

Participant characteristics were compared between average SSB intake categories (0, >0–0.75, and >0.75 cups/day) using ANOVA and Fisher's exact tests for continuous and categorical variables, respectively. Variation in reported SSB, added sugar, and total fructose intake across the menstrual cycle was assessed using linear mixed models to account for repeated measures within women (both across the cycle and between cycles). Pair-wise comparisons were made between reported intake at menses, at follicular phase, at expected ovulation, and in the luteal phase of the menstrual cycle using the Tukey method to account for multiple comparisons.

We also used linear mixed models, with random intercepts and a direct product autoregressive correlation structure, to examine the effects of average SSB, added sugar, and total fructose intake on log-transformed ALT and AST concentrations. Exposures were assessed both continuously (per 1 cup increase in SSB or per 10 g increase in added sugar or total fructose) and categorically (using meaningful cut points 0, >0–0.75, and >0.75 cups/day of SSB and using tertiles for SSB, added sugar, and total fructose). Random intercepts were used to account for the baseline differences in ALT and AST levels between women. Results of the models are presented as log difference in ALT and AST relative to the lowest intake level for categorical assessment or per 1 cup increase in SSB or per 10 g increase in added sugar or total fructose. We also present percent change in geometric mean ALT and AST concentrations by categorical and continuous intake using the following formula: $([\exp \beta] - 1 \times 100\ \%)$, where β is the estimated log difference in ALT and AST concentrations relative to the lowest intake level for categorical assessment or per 1 cup increase in SSB or per 10 g increase in added sugar or total fructose. Adjusted models included factors determined a priori that are thought to be associated with sugar consumption and liver enzyme levels including age (years, continuous), race (white, black, other), BMI, and average cycle phase total energy intake (kcal/day), alcohol intake (g/day), and Mediterranean diet score (as an indicator of overall diet quality). 111 (2.8 %) of the 3898 serum samples were excluded from analyses due to observations missing data on total energy or Mediterranean diet score. No subgroup analyses were performed due to lack of pathophysiological justification.

While we hypothesized that habitual versus episodic consumption of SSBs, added sugar, and total fructose would be associated with liver enzyme concentrations, we additionally ran models with time-varying exposures (where sugar consumption was allowed to vary between phases of the menstrual cycle) to assess whether there were short-term effects. While we were limited in high SSB consumption among our study population, we also assessed the effects of SSB above daily recommended levels [>12 oz can of soda (1.5 cups) vs. less]. All statistical analyses were performed using SAS version 9.3 (SAS Institute, Cary, NC, USA).

Results

Participant characteristics and SSB intake

The average age of study participants was 27.3 years (SD 8.2) with a mean BMI of 24.1 kg/m^2 (SD 3.9) (Table 1). Twenty percent ($n = 51$) of women consumed on average >0.75 cups/day of SSB, more than half (56 %, $n = 145$) consumed >0–0.75 cups/day, and the remaining 24 % ($n = 63$) reported no SSB consumption during the study period. Geometric mean concentrations of ALT and AST for the women across the study period were 15.96 and 18.92 U/L, respectively. SSB consumption significantly differed by race with African-American women reporting higher intakes compared with Caucasian or other race

Table 1 Characteristics of women participating in the BioCycle Study by average sugar-sweetened beverage intake across the study period ($n = 259$ women)

	Average sugar-sweetened beverage intake				
	Total	0 cups/day	>0–0.75 cups/day	>0.75 cups/day	*P*
Participants [*n* (%)]	259	63 (24.3)	145 (56.0)	51 (19.7)	
Age (years)	27.3 ± 8.2	28.1 ± 8.8	27.2 ± 8.1	26.7 ± 7.8	0.63
Race [*n* (%)]					<0.001
Caucasian	154 (59.5)	43 (68.3)	90 (62.1)	21 (41.2)	
African-American	51 (19.7)	3 (4.8)	25 (17.2)	23 (45.1)	
Other	54 (20.9)	17 (27.0)	30 (20.7)	7 (13.7)	
Ethnicity					0.44
Non-Hispanic	248 (95.8)	62 (25.0)	138 (55.7)	48 (19.4)	
Hispanic	11 (4.3)	1 (9.1)	7 (63.6)	3 (27.3)	
BMI (kg/m^2)	24.1 ± 3.9	23.5 ± 3.5	24.2 ± 3.9	24.5 ± 4.1	0.39
Physical activity [*n* (%)]					0.94
Low	25 (9.7)	7 (11.1)	14 (9.7)	4 (7.8)	
Moderate	92 (35.5)	20 (31.8)	54 (37.2)	18 (35.3)	
High	142 (54.8)	36 (57.1)	77 (53.1)	29 (56.9)	
Diet					
Total energy (kcal)	1613.3 ± 367.3	1459.8 ± 329.7	1639.3 ± 380.7	1729.1 ± 313.9	<0.001
% calories from added sugar	14.1 ± 5.5	10.4 ± 4.7	13.9 ± 4.8	19.2 ± 4.2	<0.001
MDS	2.86 ± 0.95	3.16 ± 0.99	2.93 ± 0.93	2.29 ± 0.68	<0.001
Fiber (g/day)	13.6 ± 5.6	15.0 ± 6.5	14.1 ± 5.5	10.7 ± 3.2	<0.001
Total fructose (g/day)	35.4 ± 13.7	26.2 ± 10.5	34.7 ± 12.0	48.8 ± 11.6	<0.001
Protein (g/day)	62.7 ± 16.9	60.8 ± 16.6	63.6 ± 18.1	62.2 ± 13.9	0.54
Carbohydrates (g/day)	201.7 ± 50.3	179.2 ± 50.6	204.2 ± 48.8	222.4 ± 44.0	<0.001
Fat (g/day)	62.2 ± 19.4	55.9 ± 16.9	63.6 ± 20.6	65.9 ± 17.2	0.01
Artificially sweetened beverages (cups/day)	0.24 ± 0.49	0.39 ± 0.66	0.19 ± 0.41	0.21 ± 0.45	0.03
Alcohol (g/day)	2.8 ± 5.5	3.2 ± 6.5	2.8 ± 5.2	2.2 ± 4.8	0.67

1 cup = 237 mL. Sugar-sweetened beverages include sugar-sweetened sodas and sugar-sweetened fruit juices assessed by 24-h dietary recall. All dietary factors were averaged over up to eight 24-h dietary recalls. Differences in SSB category were calculated by using ANOVA for continuous data and Fisher's exact test for categorical data. No missing data for age, race, BMI, and physical activity. Among the 250 women completing two menstrual cycles, 190 (76 %) completed all eight recalls, 244 (97 %) completed seven recalls, 249 (99 %) completed at least five recalls, and all 250 women (100 %) completed at least four recalls. Among the nine women completing one cycle, seven (78 %) completed four recalls and all nine (100 %) completed at least three recalls. Values are mean ± SD unless otherwise reported. ALT and AST were log-transformed for normality for statistical analyses

MDS Mediterranean diet score

women (global $P < 0.001$). SSB consumption was also positively associated with total energy, total fructose, carbohydrate, and fat intake and inversely associated with fiber (all $P < 0.001$) and artificially sweetened soda ($P = 0.03$) intake. There was not a statistically significant difference in baseline BMI ($P = 0.39$), physical activity levels ($P = 0.94$), or average daily alcohol consumption among consumption categories ($P = 0.67$).

While neither added sugar nor total fructose intake varied over the menstrual cycle, SSB intake was significantly higher at estimated day of ovulation compared with menses, follicular, and luteal cycle phases (all $P < 0.05$) (Fig. 1).

Associations of SSB, added sugar, and total fructose intake and liver enzymes

High versus low SSB intake was associated with higher log ALT ($\beta = 0.073$ U/L, 95 % CI 0.009, 0.136; percent change 7.6 %; 95 % CI 0.9, 14.6) but not log AST ($\beta = -0.002$ U/L, 95 % CI −0.046, 0.042; percent change −0.2 %; 95 % CI −4.5, 4.3) after adjusting for age, race, BMI, total energy and alcohol intake, and Mediterranean diet score (Table 2). This equates to average ALT concentrations of approximately 16.13 U/L for high SSB consumers versus 14.98 U/L for low consumers but no significant differences in AST levels.

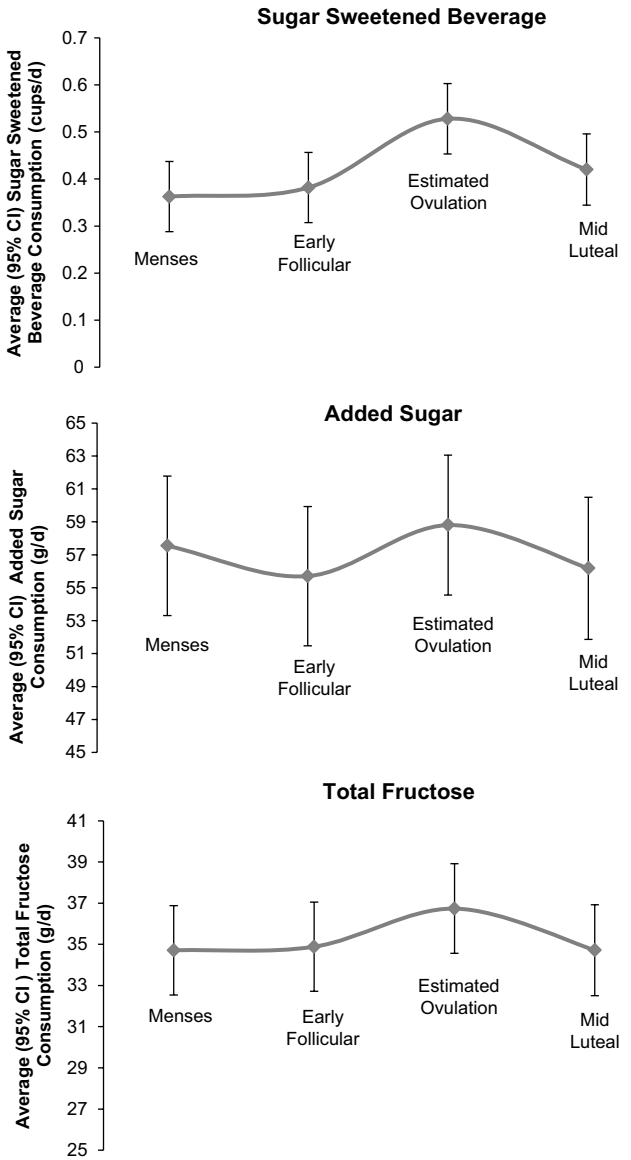

Fig. 1 Average sugar-sweetened beverage, added sugar, and total fructose intake consumption across the study period ($n = 259$ women were followed up for up to two menstrual cycles). Dietary assessment conducted via four 24-h dietary recalls per cycle conducted at clinic visits corresponding to menses, early follicular, estimated day of ovulation, and mid-luteal phase visits. Sugar-sweetened beverage intake was significantly higher at estimated day of ovulation compared with menses, follicular, and luteal cycle phases (all $P < 0.05$ via Tukey method for multiple comparisons). No significant differences in added sugar or total fructose intake over the menstrual cycle

High versus low added sugar was not significantly associated with higher log ALT ($\beta = 0.062$ U/L, 95 % CI −0.001, 0.126; percent change 6.4 %; 95 % CI −0.1, 13.4) nor higher log AST ($\beta = 0.031$ U/L, 95 % CI −0.013, 0.075; percent change 3.2 %; 95 % CI −1.3, 7.8). Similarly to SSB intake, high versus low total fructose intake was significantly associated with log ALT ($\beta = 0.079$ U/L, 95 % CI 0.017, 0.141; percent change 8.2 %; 95 % CI 1.71, 15.1) but not log AST ($\beta = 0.040$ U/L, 95 % CI −0.002, 0.083; percent change 4.1 %, 95 % CI −0.2, 8.7).

Continuous exposure assessment mirrored the categorical assessment. For each 1 cup/day increase in SSB, log ALT increased by 0.079 U/L, 95 % CI 0.022, 0.137 [percent change 8.2 % (95 % CI 2.2, 14.7)]. The association between 1 cup/day increased in SSB and log AST remained nonsignificant. For each 10 g/day increase in added sugar, log ALT and AST increased by 0.012 U/L, 95 % CI 0.002, 0.022 [percent change 1.2 % (95 % CI 0.2, 2.2)] and 0.007 U/L, 95 % CI 0.000, 0.014 [percent change 0.7 % (95 % CI 0.0, 1.4)], respectively, and for each 10 g/day increase in total fructose, ALT and AST increased by 0.031 U/L, 95 % CI 0.012, 0.050 [percent change 3.1 % (95 % CI 1.2, 5.1)] and 0.017 U/L, 95 % CI 0.004, 0.030 [percent change 1.7 % (95 % CI 0.4, 3.1)], respectively.

In regard to meaningful SSB cut points, while there were no significant associations for women who consumed on average ≥0.75 cups/day versus less, women who consumed ≥1.50 cups/day (12 oz can) SSB versus less had 0.127 U/L (95 % CI 0.001, 0.254) higher ALT [percent change 13.5 % (95 % CI 0.1, 28.9)] and 0.102 (95 % CI 0.015, 0.190) higher AST [percent change 10.8 % (95 % CI 1.5, 20.9)] concentrations after adjusting for age, race, BMI, total energy and alcohol intake, and Mediterranean diet score. Time-varying exposure analyses produced similar, albeit attenuated, results (data not shown).

Discussion

We observed that among healthy, predominately normal weight women, average SSB, added sugar, and total fructose intake were all associated with modest increases in serum concentrations of ALT and to a lesser extent AST. Our findings indicate that habitual exposure to SSB, added sugar, and most notably total fructose may impact hepatic lipogenesis even at moderate levels among young women without clinically apparently adverse health conditions. Our findings of increased ALT and AST for women consuming on average more than one 12 oz serving of soda per day indicate that consumption above recommended limits [3] may pose a public health risk among otherwise healthy women in regard to fatty liver disease but should be corroborated by additional research. Future investigation into the relationship between total fructose and additional cardiometabolic risk factors among reproductive-age women is also warranted.

Previous epidemiological research has shown increased liver fat content and increased rates of metabolic syndrome in response to heightened levels of sugar consumption among patients presenting with liver damage [19,

Table 2 Association between average consumption of sugar-sweetened beverages, total added sugar, and total fructose tertiles and log-transformed ALT and AST ($n = 259$ women)

	log ALT (U/L)		log AST (U/L)	
	β	95 % CI	β	95 % CI
Categorical				
SSB				
High (>0.52 cups/day)	0.073	(0.009, 0.136)	−0.002	(−0.046, 0.042)
Middle (>0.11–0.52 cups/day)	0.025	(−0.036, 0.086)	−0.023	(−0.065, 0.019)
Low (0–0.11 cups/day)	REF		REF	
Added sugar				
High (>65.8 g/day)	0.062	(−0.001, 0.126)	0.031	(−0.013, 0.075)
Middle (>44.4–65.8 g/day)	0.015	(−0.048, 0.078)	0.002	(−0.041, 0.046)
Low (0–44.4 g/day)	REF		REF	
Total fructose				
High (>40.4 g/day)	0.079	(0.017, 0.141)	0.040	(−0.002, 0.083)
Middle (>27.6–40.4 g/day)	0.035	(−0.027, 0.098)	0.058	(0.015, 0.1009)
Low (0–27.6 g/day)	REF		REF	
Continuous				
SSB (per 1 cup/day)	0.079	(0.022, 0.137)	0.029	(−0.011, 0.069)
Added sugar (per 10 g/day)	0.012	(0.002, 0.022)	0.007	(0.000, 0.014)
Total fructose (per 10 g/day)	0.031	(0.012, 0.050)	0.017	(0.004, 0.030)

1 cup = 237 mL. Analyses conducted using linear mixed models adjusting for age; race (white, black, other), body mass index (kg/m^2), energy (kcal) and alcohol (g) intake, and Mediterranean diet score. Of the 3898 serum samples, 111 (2.8 %) were excluded from analyses due to observations missing data on total energy intake or Mediterranean diet score

ALT alanine aminotransferase, *AST* aspartate aminotransferase, *SSB* sugar-sweetened beverages, *U/L* units per liter

20]. Similarly, previous intervention research has shown increased fat accumulation in the liver, skeletal muscle, and visceral adipose tissue in subjects consuming SSBs compared with milk, ASB, and water over a 6-month period [21]. Fructose consumption has also been shown to be associated with increased risk of NAFLD [6] and with increased histological severity of the disease [22]. This is the first study to assess these associations in a healthy population. Our results indicate that added sugar intake at levels similar to the US national average for adult females (14.1 % average percent of calories from added sugar among the women in our study compared to the US national average of 14.5 % among women ages 20–39) [23] can result in significant, albeit small, changes in serum liver enzyme concentrations among healthy women.

In adults with no obvious liver disease, unexplained elevation of ALT is more common in participants with metabolic syndrome [24] and is thought to reflect predominantly the presence of NAFLD [25]. In healthy individuals, baseline concentrations of serum transaminases are determined, at least in part, by genetic factors [26] many of which are associated with lipid metabolism, other metabolic pathways, and fatty liver pathogenesis. An association with fat is also supported by prospective studies, which have reported an association of short-term overload of carbohydrates (fructose or glucose) with increased hepatic triglyceride content and transaminases [27–29]. We cannot identify with certainty whether the observed variability in liver enzyme concentrations reflects variability in fat content, hepatic inflammation, genetic determinants of liver enzyme concentrations, or other factors; however, it is possible that an increase in liver fat underlies the elevation of transaminases seen with increased sugar intake, especially since there is a biologically plausible mechanism to explain that association. Whatever the mechanism, the small magnitude of change suggests that small increases in added sugar consumption are not associated with significant liver injury.

The BioCycle Study had several strengths including multiple repeated measures of dietary intake and liver biomarkers over the course of two menstrual cycles. The cohort consisted of women with no known reproductive disorders or underlying chronic disease, which is unique compared to other studies assessing dietary intake on liver enzymes. Although there may be some misclassification of dietary intake, we used multiple 24HDRs, which has been shown to be the gold standard tool for dietary assessment [30, 31]. Our prospective assessment of diet over the course of the study, versus using a single retrospective questionnaire at baseline, better assessed intake over relevant period

and helped to reduce intra-individual variability. Our findings were limited by the low amount of overall consumption of added sugar and soda intake, but allowed us to ascertain that alterations in liver enzymes may still occur at low to moderate intake levels. Our findings are additionally limited by reliance on ALT and AST to evaluate liver health/fat content. The accepted gold standard to evaluate liver disease is a liver biopsy, which was not feasible in the present study. Further, the magnitude of ALT does not necessarily correspond to the extent of liver damage [32]. However, within the BioCycle Study cohort of healthy women, such biomarkers were the most feasible method to quantify liver function [33].

In conclusion, our findings showed that among healthy women, higher levels of SSB, added sugar, and total fructose consumption were significantly associated with increased serum liver enzyme concentrations, indicating that habitual consumption of even moderate added sugar levels may elicit hepatic lipogenesis.

Conflict of interest All authors declare no conflicts of interest.

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