The association between baseline fructose consumption and baseline biomarkers of inflammation in a randomized controlled feeding trial.

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Abstract

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Background: High fructose intake, especially through sugar-sweetened beverage consumption, has been associated with increased risk of chronic diseases, such as obesity, type 2 diabetes mellitus, cardiovascular disease, hypertension, and cancer. Biomarkers of inflammation are useful indicators of low-grade chronic inflammation caused by chronic diseases.

Objective: To examine the association between baseline fructose consumption and baseline biomarkers of inflammation in the Carbohydrates and Related Biomarkers (CARB) Study participants.

Methods: Data are from the CARB Study conducted at the Fred Hutchinson Cancer Research Center (FHCRC), with healthy adult participants. Baseline 12-hour fasting serum samples were collected to measure biomarkers of inflammation, which were high-sensitivity C-reactive protein (*hs*-CRP), serum amyloid A (SAA), and interleukin-6 (IL-6). At baseline, participants completed 3-day food records and food frequency questionnaires (FFQ) to estimate their dietary intake. Each participant also received a full-body dual-energy X-ray absorptiometry (DXA) scan to assess

percent body fat. Participants were then stratified into two subgroups, based on their percent body fat.

Statistical Analysis: Ordinary least squares (OLS) linear regression models were utilized to quantify the association between fructose consumption and biomarkers of inflammation. Effect modification assessment was performed to examine the difference between associations of fructose consumption and biomarkers in the two subgroups. Adjustments were made for participants' age, sex, percent body fat, glycemic load, and energy intake.

Results: Eighty participants were included in this analysis. Participants with high percent body fat (>32% for female and >25% for male) tended to be older and more likely to be female than those with low percent body fat (<32% for female and <25% for male). On average, participants with low percent body fat consumed more energy, fructose, glucose, added sugar, sweetened soft drinks, and had higher glycemic load diets than participants with high percent body fat. Using both univariate- and multivariate-adjusted models, there was no significant associations between fructose consumption and biomarkers of inflammation.

Conclusion: In CARB Study participants, we found no significant association between fructose consumption and *hs*-CRP, SAA, or IL-6 concentrations. Further investigation of this association could be designed with a larger sample size and greater difference in fructose consumption among participants.

Introduction

High dietary glycemic load, achieved through consumption of high glycemic index foods, such as refined carbohydrates, sugar sweetened beverages, overripe fruits, and/or canned fruits packed in syrup, is associated with an increased risk of chronic diseases, such as type 2 diabetes mellitus, gallbladder disease, and breast cancer (1). Neuhouser et al. and Runchey et al. have previously shown through the randomized, controlled Carbohydrates and Related Biomarkers (CARB) Study that high-glycemic load diets increased serum concentrations of high-sensitivity C-reactive protein (*hs*-CRP), insulin, and insulin-like growth factor 1 (IGF-1), and decreased serum concentrations of adiponectin in overweight/obese adults (2, 3). In another study, Hartman et al. showed that legume-rich diets, which have low glycemic load and high fiber content, reduced hepatic fat content and improved insulin sensitivity, and in turn, improved biomarkers of inflammation and insulin resistance (4).

Fructose, a five-carbon monosaccharide, is a major sugar in fruits and some sugar-sweetened beverages; it has a different digestion, absorption, and metabolism than those of glucose. This difference in metabolism results in fructose contributing less to glycemic index than glucose does (5). Fructose favors hepatic de novo lipogenesis while increasing hepatic insulin resistance, hepatic fat content, and visceral adiposity (6). Moreover, fructose does not stimulate insulin secretion from pancreatic β-cells (7). Fructose metabolism starts with phosphorylation by fructokinase. While glucose metabolism is regulated via feedback inhibition by cytosolic citrate and ATP, fructokinase is not limited by that mechanism. In addition, fructose can facilitate triglyceride production by providing its carbon atom. As a

result, there is an unregulated production of triglycerides in the hepatocyte if large amounts of fructose are consumed (8).

It has been growing interest to researchers that high fructose intake, especially through sugar-sweetened beverage consumption, is associated with increased risk of chronic diseases, such as obesity, type 2 diabetes mellitus, cardiovascular disease, hypertension, and cancer (6, 9-11). Experimental studies have also supported a relationship between high fructose intake and increased disease risk, indicated by elevated levels of inflammatory biomarkers. A three-week randomized controlled trial found that daily consumption of sugar sweetened beverages containing 40 g fructose increased mean serum *hs*-CRP concentrations by 95% (374.6±1182.1 vs. 205.6±430.7 ng/mL at baseline; $P<0.01$) in healthy young men (12). Further, another study showed that elderly patients with chronic kidney disease, after consuming a low-fructose diet for six weeks, had a reduction of *hs*-CRP concentration. This one-arm crossover feeding study with 28 patients at stage 2 and 3 of chronic kidney disease reduced participants' fructose consumption by 80% during the intervention period, and achieved a significant decrease in participants' mean *hs*-CRP concentration (3.3±4.5 vs. 4.3±4.9 mg/L at baseline; $P<0.01$) (13).

With fructose consumption increasing by 26% of average national consumption over the past three decades, due to the increased intake of high-fructose corn syrup in soft drinks and other beverages (14), it is important to understand the relationship of fructose consumption to potential health risks. The primary aim of this study is to examine the association between fructose consumption and biomarkers of inflammation in the CARB Study participants. Secondly, this study is aimed to examine the difference between the associations of the predictor and outcomes in adults with normal body weight and overweight/obese body weight.

Methods

This analysis is a cross-sectional observation study investigating the baseline data of the CARB Study. Data are from CARB Study conducted at the Fred Hutchinson Cancer Research Center (FHCRC). The CARB study was a randomized, crossover, feeding trial conducted between June 2006 and July 2009. Eighty-two healthy, nonsmoking men (*n=41*) and women (*n=41*), aged 18 to 45 years, were recruited from the Seattle area through advertisement by the FHCRC. Half of the participants were normal weight (BMI >18.5 and < 25 kg/m^2), and the other half of participants were overweight or obese (BMI ≥ 28 and ≤40 kg/m^2). Upon recruitment, each participant's height and weight were measured to the nearest 0.5 cm and 0.5 kg, respectively. Exclusion criteria were the following: 1) having physician-diagnosed diseases that required dietary restriction, such as diabetes, renal disease, cardiovascular disease, and metabolic diseases, etc.; 2) pregnant, lactating, or planning on becoming pregnant; 3) using hormones and anti-inflammatory medications; 4) using tobacco; 5) drinking more than two alcoholic drinks per day; 6) having food allergies and restrictive eating; and 7) having impaired blood glucose (fasting blood glucose ≥5.6 mmol/L), which were measured as part of the pre-screening eligibility (2, 3).

Each participant received a full-body dual-energy X-ray absorptiometry (DXA) scan using a GE Lunar DPX-Pro densitometer to assess percent body fat. Because the parent analyses discovered misclassification of some of the individuals' weight statuses when based on BMI alone (2, 3), we used percent body fat from DXA to characterize adiposity in this analysis. Participants were stratified into two subgroups based on percent body fat measured by DXA; low percent body fat was

defined as <32% for female and <25% for male and high percent body fat was defined as >32% for female and >25% for male (15).

On the first day of the study, baseline 12-hour fasting serum samples were collected to measure biomarkers of inflammation, which were high-sensitivity C-reactive protein (*hs*-CRP), serum amyloid A (SAA), interleukin-6 (IL-6), as the outcomes of this analysis. All serum samples were processed and stored in -80°C until analysis. High sensitivity latex-enhanced nephelometry was utilized to measure concentrations of *hs*-CRP and SAA, and ELISA was used to analyze concentrations of IL-6. The lowest limits of quantification was 0.2 mg/L for *hs*-CRP and 0.8 mg/L for SAA (2). Individuals with *hs*-CRP greater than 10 mg/L were excluded since this level is correlated with acute infection (16). No lowest or highest limits of quantification were identified for IL-6 (2).

At baseline, participants completed three-day food records and food frequency questionnaires (FFQ) to estimate their habitual energy intake for the interventional portion of the study. Food records required participants to record food or beverage intake at the time of consumption over a period of three days. FFQs asked participants to recall the consumption frequency of foods listed in the questionnaires over a three-month period. Dietary intake data, gathered from food records and FFQs, were then analyzed using the Nutrition Data System for Research (NDSR) database. The NDSR software calculated the amount of each nutrient as foods were entered into the database by trained staff. For this analysis we used the baseline dietary information from food records, such as fructose consumption, energy intake, glycemic load, and sweetened soft drink intake. Participants' fruit and vegetable intake information was obtained from FFQs since it was not available in the food records dataset.

Ordinary least squares (OLS) linear regression models were utilized to quantify the association between fructose consumption and outcomes. Robust standard error was used to obtain 95% confidence intervals and P-values for each estimate of association. All tests were two-sided and statistical significance was defined as $p<0.05$. Adjustments were made for participants' age, sex, percent body fat, glycemic load, and energy intake to control for confounders in the statistical models, as these variables can affect the biomarkers of inflammation. For the second aim of the analysis, we performed effect modification models to assess the difference between associations of the predictor and outcomes in low and high percent body fat subgroups. Statistical analyses were performed using R (version 3.1.2).

Results

Characteristics of Participants at Baseline

Biomarkers of inflammation were obtained from 80 of the 82 study participants. Two data points were excluded due to missing information on outcome measures and diet. There were 31 participants in the low percent body fat subgroup, and 49 in the high percent body fat subgroup (Table 1). Participants with high percent body fat were older than those with low percent body fat ($P = 0.008$). Participants in the high percent body fat subgroup were more likely to be female ($P = 0.02$) (Table 1).

Participants' Diets

Information on participants' habitual intake of specific diet components is shown in Table 2. The average daily fructose consumption was 23g. On average, participants with low percent body fat consumed more energy, fructose, glucose,

added sugar, sweetened soft drinks, and had higher glycemic load than participants with high percent body fat; however, these differences were not statistically significant. Moreover, the nutrient dataset from the FFQs showed that the average fruit consumption was 2.6 servings and vegetable consumption was 1.9 servings per day in the study population. Participants with low percent body fat consumed more fruits (P = 0.50) and vegetables (P = 0.25) than those in the high percent body fat subgroup.

Biomarkers of Inflammation

The CARB study participants had average serum concentrations of 2.1 mg/dL *hs*-CRP, 4.5 mg/dL SAA, and 2.1 pg/mL IL-6. Participants in the high percent body fat subgroup had higher levels of *hs*-CRP (P = 0.74), SAA (P = 0.72), and IL-6 (P = 0.003) than those in the low percent body fat group (Table 1).

Using both univariate- and multivariate-adjusted models, we did not detect significant associations between fructose consumption and biomarkers of inflammation (Table 3). Among participants with the same age, sex, percent body fat, energy intake, and glycemic-load diet, those who consumed one more gram of fructose in their diet on average tended to have 0.02 mg/dL higher *hs*-CRP (P=0.26; 95% CI = -0.02~0.06), 0.03 mg/dL higher SAA (P=0.61; 95% CI=-0.10~0.17), and 0.004 pg/mL lower IL-6 (P=0.87; 95% CI=-0.05~0.04) (Table 3).

Table 4 delineates the association of fructose consumption and outcomes in two subgroups: low percent body fat and high percent body fat. Results from the effect modification assessment showed that the associations of the two subgroups were not statistically different from one another for *hs*-CRP (P=0.65), SAA (P=0.43), and IL-6 (P=0.077). In addition, in the subgroup analysis, there were inverse associations between fructose consumption and both SAA (estimate=-0.035; P=0.61)

and IL-6 concentrations (estimate=-0.046; P=0.036) in participants with low percent body fat; however, only the inverse association for IL-6 was statistically significant (Table 4).

Exploratory Analysis

We conducted an exploratory analysis investigating the association between sweetened soft drink intake and each biomarker, considering sodas are a major source of fructose in the American diet. While there was a suggestion that each additional daily soft drink serving was associated with a 0.68 mg/dL increase in *hs*-CRP, the association was not statistically significant (P=0.103). Moreover, there was no significant relationship of soft drink consumption with either of the other biomarkers of interest (IL-6 and SAA) (Table 5).

Discussion

The primary aim of this analysis was to examine the association of baseline fructose consumption and baseline biomarkers of inflammation among CARB study participants. In the 80 healthy participants, we found no significant association between fructose consumption and *hs*-CRP, SAA, or IL-6 concentrations. Moreover, when we examined the association between fructose consumption and the outcomes in two subgroups stratified by percent body fat, there was only one significant inverse association for IL-6 in the low percent body fat subgroup (Table 4). However, the inverse association between fructose consumption and IL-6 may be too small to be clinically meaningful.

Despite findings from epidemiological studies showing a positive association between fructose consumption and chronic inflammation, our study did not find

such relationship. One possible explanation could be the lack of power of the secondary analysis due to small sample size. The CARB study was conducted primarily to examine the effect of glycemic load of diets on biomarkers of inflammation through a randomized controlled design. Conducting a secondary analysis in these 80 participants at baseline has limited power to detect associations between the predictor and outcomes. Another reason could be confounders. Participants with high percent body fat tended to consume less energy, sugar, and sweetened soft drink than those in the low percent body fat subgroup did, though the differences were not statistically significant (Table 2). It is possible that participants in the low percent body fat subgroup engaged in higher levels of physical activity, and thus consumed more energy than those in the high percent body fat subgroup. Therefore, physical activity level would be a confounder. It must be noted that the sample utilized in this analysis was not a population-based sample and that the results might not be generalizable to the general population.

As suggested by many of the animal and human studies on the association between fructose consumption and biomarkers of inflammation, body weight or body fat may be a mediator in this relationship (17). High fructose consumption is associated with decreased circulating insulin and leptin, which are essential long-term regulators of energy balance (17). Leptin, a hormone that inhibits hunger, is mediated by insulin and produced in adipose tissue (17). The reduced stimulation of insulin and production of leptin could lead to increased energy intake, which ultimately contributes to weight gain and obesity. Moreover, with increased fructose consumption favoring hepatic de novo lipogenesis and contributing to increased triglyceride production, there is an increase in body fat, leading to higher risk of obesity (6). Obesity has been demonstrated to contribute to a pro-inflammatory state that can lead to increased low-grade chronic inflammation (18). Controlling for

percent body fat in the statistical models in this analysis could have interrupted the pathway where body fat status was a mediator between fructose intake and biomarkers of inflammation, thus reducing the association between them. To test the association between fructose consumption and biomarkers, with the assumption that body weight was a mediator, we conducted a sensitivity analysis without adjusting for percent body fat. Still, we did not detect any statistically significant association (*hs*-CRP: estimate=0.003, P-value=0.870, 95% CI=-0.037~0.043; SAA: estimate=-0.003, P-value=0.968, 95% CI=-0.137~0.132; IL-6: estimate=-0.002, P-value=0.442, 95% CI=-0.070~0.031).

The dietary intake pattern of our study participants may have affected the association between the predictor and outcomes. On average, the participants were consuming 2.6 servings of fruits and 1.9 servings of vegetables per day. In 2000, the mean consumption of fruits and vegetables in the United States was 3.37 servings per day (19). The average consumption of fruits and vegetables in our participants exceeded the national average intake. A secondary analysis of two interventional studies, the Fruit and Vegetable Randomized Intervention Trial and the Aging and Dietary Intervention Trial, showed that higher fruit and vegetable intake (≥5 servings/day) lowered SAA levels, but not CRP and IL-6, in hypertensive and older adults (20). The change in concentrations of inflammatory biomarkers may be able to explain that the reason for a lack of significant association in this analysis was the beneficial effects exerted by relatively high fruit and vegetable intake. Considering the well-known benefits of fruit and vegetable intake in chronic disease prevention, there could be protective effects of these foods against chronic inflammation.

Underreporting of sugar intake and overreporting of fruit and vegetable consumption in the food records and FFQs may also affect the capacity to detect an association between dietary intakes and the biomarkers of inflammation. In this

analysis, participants with high percent body fat, on average, had lower glycemic-load diets and consumed less energy, glucose, fructose, and sweetened soft drinks than those with low percent body fat did (Table 2). As a result, mis-estimation of dietary intake or mis-reporting was suspected. In this case, the underreporting or overreporting could have reduced the strength of association between fructose consumption and outcomes, leading to insignificant results in this analysis.

Moreover, it is possible that the outcome variables under examination in this analysis were not responsive to fructose metabolism. For instance, one interventional study of 31 participants, 40 to 72 years of age, showed that fructose-sweetened beverage consumption (25% of energy requirements) for 10 weeks increased some of the biomarkers of inflammation but not others; fructose consumption increased E-selectin, plasminogen activator inhibitor-1 (PAI-1), and monocyte chemoattractant protein-1 (MCP-1) levels, but not *hs*-CRP and IL-6 (21).

There is emerging evidence from animal models showing that high fructose consumption may be a potential risk factor for some chronic diseases, such as type 2 diabetes mellitus and metabolic syndrome (10). In rodent studies, fructose doses of 35% of total energy intake induced development of metabolic syndrome (insulin resistance, elevated triglycerides, and high blood pressure) within four to six weeks (22, 23). Even fructose doses as low as 15% of energy intake were able to induce insulin resistance after 15 months in rats, comparing to rats fed with the same amount of starch, by percentage of total energy intake (24). This dose percentage is comparable to the average of 12% of total energy intake from fructose in the US population (25). However, fructose intake was only attributed to about 5% of energy intake in the CARB study population at baseline, which might be too low to exert an effect on biomarkers of inflammation.

While rodent models showed consistent evidence of fructose being a risk factor, there have been conflicting results in human studies (26). One of the reasons may be that, in intervention studies in humans, the induction of metabolic syndrome and other chronic diseases depended on the dosage of fructose and duration of consumption (9). A randomized, controlled trial of 74 healthy men found that an additional 200g fructose per day for two weeks was able to induce the development of metabolic syndrome; there was a significant increase in fasting serum triglycerides, blood pressure, BMI, and significant decrease in high-density lipoprotein cholesterol and insulin sensitivity (28). In another intervention study of seven healthy men fed with an average of 216g ± 12g fructose per day (3 g fructose per kg of body weight) for six days, insulin resistance occurred in the liver and adipose tissue, as suggested by significant increase in endogenous glucose production. However, whole-body insulin-stimulated glucose disposal remained unaffected, suggesting muscle insulin sensitivity was not altered by six days of high fructose overfeeding (27). It was not surprising that there was no association detected between fructose consumption and biomarkers of inflammation in our analysis, since the average fructose intake of the participants was about 23g per day.

Lastly, the exploratory analysis was conducted to investigate the sources of fructose and their relationship with inflammatory changes. Since late 1960s, intakes of high fructose corn syrup (HFCS) and sucrose have increased to become dominant sources of sweetener in the American diet, contributing to a 26% increase in fructose consumption (11). HFCS is the major sweetener used in soft drinks and other sugar-sweetened beverages (17). Meanwhile, epidemiological studies have found an increase in the prevalence of obesity taking place at the same period as the rise in fructose consumption, indicating that fructose from sugar-sweetened beverages may be a critical factor for obesity (17). However, in this analysis, there was no significant

association between sweetened soft drink consumption and biomarkers of inflammation, likely due to the small sample size.

In conclusion, we did not detect a statistically significant association between fructose consumption and biomarkers of inflammation in healthy, non-smoking adults with normal, over, and obese body weight. Further investigation of this association could be designed with a larger sample size and greater difference in fructose consumption among participants.

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Tables

Table 1 Characteristics of participants by body fat status, at baseline

Characteristics	All participants (n=80)[1]	Low % body fat (n=31)[2]	High % body fat (n=49)[3]	Difference (P-value)
Age, years[4]	29.6 (8.2)	27.6 (7.8)	30.9 (8.2)	0.008
# of female[5]	40 (50%)	10 (32.3%)	30 (61.2%)	0.02
% Body Fat[4]	32.8 (11.9)	21.2 (6.9)	39.7 (8.3)	<0.01
BMI, kg/m^2 [4]	27.4 (5.9)	22.2 (2.0)	30.2 (5.2)	<0.01
hs-CRP, mg/dL[4]	2.1 (4.4)	1.9 (5.8)	2.3 (3.4)	0.74
SAA, mg/dL[4]	4.5 (7.3)	4.1 (8.8)	4.8 (6.6)	0.72
IL-6, pg/mL[4]	2.1 (2.4)	1.2 (1.0)	2.6 (2.9)	0.003

[1] Two participants were excluded due to missing information on biomarkers and diet.
[2] Low % body fat is defined as <32% for female and <25% for male.
[3] High % body fat is defined as >32% for female and >25% for male.
[4] Mean (SD).
[5] Number of female (%)

Table 2 Baseline dietary pattern from 3-day food record and food frequency questionnaire

Diet Characteristics	**All participants (n=80)**	**Low % body fat (n=31)[2]**	**High % body fat (n=49)[3]**	**Difference (P-value)**
Energy, kcal[1]	2269 (746)	2325 (799)	2232 (714)	0.60
Fructose, g[1]	23 (13)	26 (14)	21 (12)	0.11
Glucose, g[1]	25 (14)	29 (15)	23 (12)	0.071
Glycemic load[1, 4]	224 (83)	233 (93)	217 (76)	0.42
Added sugar, g[1]	68 (39)	71 (35)	66 (41)	0.57
Sweetened soft drinks, serving[1, 5]	0.3 (0.7)	0.4 (0.6)	0.3 (0.7)	0.72
Fruit intake, serving[1]	2.6 (3.1)	2.8 (2.5)	2.3 (3.3)	0.50
Vegetable intake, serving[1]	1.9 (1.5)	2.1 (2.0)	1.6 (0.8)	0.25

[1] Mean (SD)
[2] Low % body fat is defined as <32% for female and <25% for male.
[3] High % body fat is defined as >32% for female and >25% for male.
[4] Glycemic load with bread reference
[5] A serving is considered 8 fl. oz.

Table 3 Association between fructose consumption and outcomes across all participants from adjusted and unadjusted effect modification models

Outcomes	Adjusted estimate[1]	95% CI	P-value	Unadjusted estimate	95% CI	P-value
hs-CRP, mg/dL	0.023	(-0.017, 0.063)	0.26	0.029	(-0.006, 0.063)	0.10
SAA, mg/dL	0.034	(-0.099, 0.168)	0.61	-0.003	(-0.087, 0.082)	0.95
IL-6, pg/mL	-0.004	(-0.050, 0.043)	0.87	0.015	(-0.019, 0.049)	0.38

[1] Adjusted for age, sex, % body fat, total energy intake, and glycemic load

Table 4 Association of fructose consumption and outcomes from adjusted effect modification models, stratified by body fat status

	Low % body fat			High % body fat			Difference
Outcomes	**Adjusted estimate[1]**	**95% CI**	**P-value**	**Adjusted estimate[1]**	**95% CI**	**P-value**	**P-value**
hs-CRP, mg/dL	0.003	(-0.006, 0.063)	0.94	0.017	(0.030. 0.064)	0.47	0.65
SAA, mg/dL	-0.035	(-0.169, 0.099)	0.61	0.039	(-0.150, 0.227)	0.69	0.43
IL-6, pg/mL	-0.046	(-0.089, -0.003)	0.04	0.015	(-0.053, 0.083)	0.67	0.08

[1] Adjusted for age, sex, body fat mass status, total energy intake, and glycemic load

Table 5 Exploratory analysis on the association of sweetened soft drink consumption and biomarkers of inflammation

Outcomes	Adjusted estimate[1]	95% CI	P-value
hs-CRP, mg/dL	0.681	(-0.137, 1.498)	0.10
SAA, mg/dL	0.786	(-1.560, 3.133)	0.51
IL-6, pg/mL	-0.015	(-0.674, 0.644)	0.97

[1] Adjusted for age, sex, fructose consumption, glycemic load of diet, body fat mass status