Low to moderate sugar-sweetened beverage consumption impairs glucose and lipid metabolism and promotes inflammation in healthy young men: a randomized controlled trial[1–4]

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ABSTRACT

Background: Sugar-sweetened beverages (SSBs) have unfavorable effects on glucose and lipid metabolism if consumed in high quantities by obese subjects, but the effect of lower doses in normal-weight subjects is less clear.

Objective: The aim was to investigate the effects of SSBs consumed in small to moderate quantities for 3 wk on LDL particle distribution and on other parameters of glucose and lipid metabolism as well as on inflammatory markers in healthy young men.

Design: Twenty-nine subjects were studied in a prospective, randomized, controlled crossover trial. Six 3-wk interventions were assigned in random order as follows: 600 mL SSBs containing *1*) 40 g fructose/d [medium fructose (MF)], *2*) 80 g fructose/d [high fructose (HF)], *3*) 40 g glucose/d [medium glucose (MG)], *4*) 80 g glucose/d [high glucose (HG)], *5*) 80 g sucrose/d [high sucrose (HS)], or *6*) dietary advice to consume low amounts of fructose. Outcome parameters were measured at baseline and after each intervention.

Results: LDL particle size was reduced after HF by −0.51 nm (95% CI: −0.19, −0.82 nm) and after HS by −0.43 nm (95% CI: −0.12, −0.74; $P < 0.05$ for both). Similarly, a more atherogenic LDL subclass distribution was seen when fructose-containing SSBs were consumed (MF, HF, and HS: $P < 0.05$). Fasting glucose and high-sensitivity C-reactive protein (hs-CRP) increased significantly after all interventions (by 4–9% and 60–109%, respectively; $P < 0.05$); leptin increased during interventions with SSBs containing glucose only (MG and HG: $P < 0.05$).

Conclusion: The present data show potentially harmful effects of low to moderate consumption of SSBs on markers of cardiovascular risk such as LDL particles, fasting glucose, and hs-CRP within just 3 wk in healthy young men, which is of particular significance for young consumers. This trial was registered at clinicaltrials.gov as NCT01021969. *Am J Clin Nutr* 2011;94:479–85.

INTRODUCTION

Sugar-sweetened beverages (SSBs) are the most commonly consumed caloric beverages and the leading source of added sugars in the United States and in many other Western countries (1). The development of the obesity epidemic in the United States runs in parallel to the increase in free fructose consumption as a result of the introduction of high-fructose corn syrup as a beverage sweetener (2). Whether there is a causal relation between the 2 events remains unclear. There is, however, strong epidemiologic evidence that suggests a causal relation between fructose intake and metabolic disorders (3, 4). Furthermore, a recent cross-sectional study among US adults showed a significant correlation between caloric sweetener consumption, such as sucrose and fructose, and dyslipidemia (5). In addition, SSB consumption was found to increase the risk of type 2 diabetes in the Health Professionals Follow-Up Study (6).

SSB consumption has also been associated with a risk of coronary heart disease (CHD). In the Nurses' Health Study it was shown that women who consumed ≥2 SSBs/d had a 35% greater risk of developing CHD than did those who consumed <1 SBB/mo (7). This relation remained significant even when additional adjustments were made for body mass index (BMI), energy intake, and incident diabetes, implying that the effect of SSBs on CHD is not entirely mediated by these factors. Furthermore, previous studies have shown an association between SSB consumption and increased markers of inflammation (8, 9).

The effects of fructose consumption in humans and animals have been comprehensively reviewed (10–14). In animals, strong evidence exists that consumption of diets high in fructose results in increased de novo lipogenesis, dyslipidemia, insulin resistance, and obesity. Although the evidence is more limited in humans,

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a recent study showed an unfavorable effect of high fructose consumption on de novo lipogenesis, dyslipidemia, insulin resistance, and obesity in overweight and obese adults (15). Short-term feeding trials have provided evidence that very high fructose doses led to increases in de novo lipogenesis, blood triglycerides, and hepatic insulin resistance (10, 12, 16). Indeed, most studies to date have used very high fructose doses that provide 25–60% of total daily energy (17), an amount that corresponds to the sugar provided by 1.7–4 L SSB consumption/d, which may not always reflect real-life intake. In a cross-sectional study in schoolchildren we have previously shown that decreased LDL particle size is associated with consumption of <10 g fructose/d (3). In these children, inflammatory markers related to obesity were also studied (18), and reduced LDL particle size was significantly associated with C-reactive protein (CRP) concentrations (I Aeberli, unpublished data, 2007).

Even though commercially available SSBs are usually sweetened either with sucrose or with high-fructose corn syrup, it is important to understand the effect of the contained monosaccharides. Therefore, the aim of the present study was to investigate the effect of 5 different SSBs containing fructose, glucose, or sucrose, in amounts likely to be consumed in everyday life and over a limited time period, on lipid and glucose metabolism with a particular focus on LDL particle size and inflammatory markers in healthy young men.

SUBJECTS AND METHODS

Study design

The entire study consisted of a baseline examination followed by 6 different interventions in random sequence. Each of the interventions lasted 3 wk and was directly followed by an examination in our clinic. Thereafter, a washout period of a minimum of 4 wk was implemented before the beginning of the next intervention. The first subject included underwent the baseline examination in July 2007, and the last subject completed the study in May 2010. During 5 of the 6 interventions, subjects were supplied with SSBs containing different sugars in different concentrations as follows: *1*) 40 g fructose/d [medium fructose (MF)], *2*) 80 g fructose/d [high fructose (HF)], *3*) 40 g glucose/d [medium glucose (MG)], *4*) 80 g glucose/d [high glucose (HG)], or *5*) 80 g sucrose/d [high sucrose (HS)]. The drinks were provided in containers of 200 mL each, and the subjects had to consume 3 containers (=600 mL)/d. The sugar concentrations of the drinks were 66.5 and 133.5 g/L for the medium and high concentrations, respectively. Subjects were advised to consume the drinks together with the 3 main meals. To assess compliance, subjects were asked to return beverages not consumed on the day of the metabolic ward stay. The drinks were produced by the Nestlé Product Technology Center (PTC) in Konolfingen, Switzerland, under good manufacturing practice conditions and according to our instructions. Before their use in the study, the drinks underwent quality control at the PTC. During the study, the sugar content of the drinks was monitored. The sixth intervention consisted of dietary advice aimed at reducing free fructose intake [low fructose (LF)]. Subjects received a written manual as well as oral instructions regarding which foods contain free fructose and should therefore be avoided. The main items listed were as follows: fruit (maximum of 1 portion/d), fructose-rich vegetables, honey, dried fruits, and products sweetened with honey, fructose, or glucose-fructose syrup. The order of the 6 different interventions was randomly assigned to the subjects (physical randomization), and the study was carried out in a double-blind manner with intention-to-treat analysis of the data.

Subjects

Twenty-nine healthy, normal-weight male volunteers [BMI (in kg/m^2) between 19 and 25, age between 20 and 50 y] living in the region of Zurich, Switzerland, were included in this study. Subjects were recruited through advertisements at the various universities in Zurich. Written informed consent was obtained from all subjects before entering the study. The study was approved by the Ethics Committee of the University Hospital Zurich and registered under clinicaltrials.gov (NCT01021969). Volunteers taking regular medication or who consumed SSBs with a total content of >60 g carbohydrates/d were not included in the study.

Protocol

At baseline and after each intervention, all subjects were examined at the University Hospital Zurich between 0700 and 0930 after a 12-h overnight fast. Furthermore, subjects were asked not to engage in strenuous physical activity during the day before the examination. On arrival, weight was determined to the nearest 100 g by using a digital balance (WB 100 P; Tanita, Hoofddorp, Netherlands), and height was measured to the nearest 0.5 cm by using a wall-mounted stadiometer. BMI was calculated as weight (kg)/height $(m)^2$. Waist and hip circumference were determined by using a nonstretchable measuring tape. Percentage body fat was measured by bioelectrical impedance (AKERN BIA 101; AKERN, Pontassieve, Italy) in a supine position. Blood pressure was measured by using an automated device (Omron M6 upper arm blood pressure monitor; Omron Electronics AG, Rotkreuz, Switzerland) after a 15-min rest in a supine position. Venous as well as finger-stick blood samples were taken by the study nurse in the fasted state. After blood collection, subjects underwent a standard oral-glucose-tolerance test. The study nurse taking the anthropometric data was blinded to the interventions.

In the week before each examination, all subjects completed a 3-d (2 weekdays and one weekend day) weighed food record (19). During those 3 d all foods and drinks consumed had to be weighed on a digital kitchen balance whenever possible and, if not possible, amounts had to be documented in standard kitchen measures to allow quantitative estimation of dietary intake. Subjects were asked not to change their usual eating habits during the days of recording.

The individual 3-d food records of each subject were carefully checked at the day of the examination to ensure completeness and comprehensibility. The data were then entered into a nutrition software system [EBISpro for Windows 8.0 (Swiss version); Dr J Erhardt, University of Hohenheim, Germany] by one investigator (IA) to convert the amount of food eaten into individual nutrients. The 3-d energy and nutrient intakes were averaged to obtain a mean daily energy and nutrient intake for each subject.

Free fructose and free glucose refer to fructose and glucose that is contained in the food as monosaccharide, whereas total fructose and total glucose refer to both the monosaccharide and

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the part derived from the disaccharide sucrose (50% fructose and 50% glucose).

Laboratory analysis

Blood glucose was directly measured from whole-blood samples from the finger stick (both fasting and 2-h samples) by using plasma referenced reflection photometry (Reflotron Sprint; Roche, Basel, Switzerland). The venous blood samples were centrifuged, and the serum and plasma were either directly processed (lipid profile) or stored at −20°C for further analysis. Triglycerides, cholesterol, and free fatty acids were measured in fresh serum with a Roche MODULAR by enzymatic reactions (triglyceride GPO-PAP and cholesterol CHOP-PAP; Roche Diagnostics, Mannheim, Germany), with a Roche INTEGRA by a homogenous enzymatic color reaction (HDL cholesterol plus third generation; Roche Diagnostics) and with a Konelab (Free Fatty Acids; Thermo Scientific, Dreieich, Germany). Also in fresh serum, aspartate aminotransferase and alanine aminotransferase were determined by enzymatic reaction. From frozen serum, C-peptide was measured by using radioimmunoassay (RIA) (IRMA-C-PEP; CIS Bio International, Bagnols-sur-Cèze Cedex, France), leptin was measured by using ELISA (EZHL-80 SK; Linco Research Inc, St Charles, MO), adiponectin and high-sensitivity CRP (hs-CRP) were also measured by using ELISA (DRP300 and DCRP00; R&D Systems, Minneapolis, MN), and ghrelin was measured by using RIA (R90; Mediagnost, Reutlingen, Germany). LDL size and subclasses were determined in frozen samples. For analysis of LDL size and subclasses, nondenaturing polyacrylamide gradient gel electrophoresis of plasma was performed and analyzed as described elsewhere (20–22). LDL subclass distribution as a percentage of total LDL was calculated as previously described (20).

Insulin resistance was estimated from fasting glucose and C-peptide concentrations by using a computer-based homeostasis model assessment system (HOMA2-IR) provided by the Oxford Center for Diabetes, Endocrinology, and Metabolism (http://www.dtu.ox.ac.uk/homa). This approach has been previously validated for studies with a similar design (23).

Statistical analysis

Statistical analysis was performed by using the statistical package PASW 18.0 for Windows (SPSS Inc, Chicago, IL). All variables were checked for normal distribution before data analysis. Data are expressed as arithmetic means ± SDs for normally distributed variables and as geometric means ± SDs for nonnormally distributed data. Nonnormally distributed data were log-transformed, and further analysis was carried out with the transformed data. According to the intention-to-treat design of the study, all subjects (completers and noncompleters) were included in the final analysis. The effect of the interventions as well as their order on anthropometric and metabolic parameters were examined by using multiple linear regression (as described in reference 24), with baseline parameters and between-patient differences controlled for. Post hoc Bonferroni correction was applied to account for multiple comparisons. For the comparison of the different interventions to baseline, a correction factor of 6 was used (6 interventions), whereas between interventions (HG compared with HF compared with HS), a corrections factor of 2 was used. Considering a P value <0.05 to be significant ($\alpha = 0.05$) and a P value <0.01 to be highly significant ($\alpha = 0.01$), the resulting α levels were $\alpha^* = 0.0083$ and $\alpha^{**} = 0.0017$ for comparison to baseline and $\alpha^{\dagger} = 0.025$ and $\alpha^{\dagger\dagger} = 0.005$ for comparison between high sugar interventions. All α levels discussed in Results were Bonferroni-corrected if necessary.

RESULTS

A total of 29 subjects were randomly assigned. The mean study duration from baseline measurements until the end of the last intervention was 44 wk (range: 10–90 wk). The mean (±SD) age of the subjects at baseline was 26.3 ± 6.6 y. The important anthropometric and biochemical data are summarized in **Table 1**. Whereas a significant change in BMI and weight was observed only in the MG intervention, waist-to-hip ratio was significantly higher in all interventions containing fructose (range: 0.92 ± 0.05 to 0.93 ± 0.05) compared with baseline (0.92 ± 0.06) ($P < 0.0083$). When only the interventions with high sugar doses (HG, HF, and HS) were compared, a significantly higher percentage body fat in the HF intervention than in the HG intervention (15.7 ± 3.2% compared with 15.1 ± 3.4%, $P < 0.005$) and a significantly higher waist circumference in the HS intervention than in the HG intervention (82.9 ± 5.5 compared with 82.6 ± 5.6 cm, $P \leq 0.005$) were observed.

LDL particle size decreased significantly only after the HF (−0.51 ± 0.80 nm) and HS (−0.43 ± 0.81 nm) interventions compared with baseline ($P < 0.0083$). Both fructose interventions (MF and HF) and HS significantly decreased the large LDL I subclass ($P < 0.0083$), whereas there was no significant increase in small, dense particles. When only HG, HS, and HF interventions where compared, there was a significant difference of the LDL I subclass between the HG and the 2 fructose-containing interventions (**Figure 1**). The traditional lipid profile parameters (total, LDL, and HDL cholesterol and triglycerides) did not change.

hs-CRP concentrations increased significantly after all of the interventions, with the highest value after the HF intervention (430.1 ± 1697.2 ng/mL, $P < 0.0017$) compared with baseline (205.6 ± 430.7 ng/mL). No significant changes were observed in the adipokines analyzed (Table 1), with the exception of leptin, which increased significantly after the 2 interventions containing glucose only: MG (+0.25 ± 1.38 ng/mL, $P < 0.0083$) and HG (+0.63 ± 0.93 ng/mL, $P < 0.0017$). Liver function did not change after any of the interventions.

Fasting glucose increased significantly after all of the interventions (range: 4.64 ± 0.50 to 4.84 ± 0.30 mmol/L) compared with baseline (4.45 ± 0.45 mmol/L) ($P < 0.0083$). With regard to HOMA2 IR and C-peptide, there was an increase after the HF intervention, although it was not significant after Bonferroni correction (Table 1).

Intakes of energy, the different sugars, macronutrients, fibers, and β-carotene at baseline and during each of the 6 interventions are described in **Table 2**. Dietary intake of the different sugars varied widely according to the specific interventions. Despite those variations, total energy intake did not differ significantly between the baseline and any of the 6 interventions. Mean energy intake during all interventions was 2507 kcal/d. The different interventions thus provided 6.5% (MF and MG) and 13.1% (HF, HG, and HS) of total daily energy in the form of

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TABLE 1
Anthropometric measures, LDL size and subclasses, high-sensitivity C-reactive protein (hs-CRP), adipokines, glucose metabolism, and liver function tests of the 29 subjects at baseline and after the 6 different interventions[1]

	Baseline	MF	MG	HF	HG	HS	LF
Weight (kg)[2]	73.7 ± 8.8	73.9 ± 9.1	74.3 ± 9.1*	73.8 ± 8.9	73.6 ± 9.2	74.4 ± 8.6	74.1 ± 8.7
BMI (kg/m^2)[2]	22.4 ± 1.9	22.4 ± 2.0	22.6 ± 2.0*	22.4 ± 1.9	22.4 ± 1.9	22.5 ± 1.8	22.4 ± 1.8
Body fat (%)[2]	15.4 ± 3.1	15.2 ± 3.1	15.5 ± 3.2	15.7 ± 3.2††	15.1 ± 3.4	15.2 ± 3.2	15.4 ± 2.8
Body fat (kg)[2]	11.6 ± 3.4	11.5 ± 3.4	11.7 ± 3.4	11.8 ± 3.4	11.3 ± 3.6	11.5 ± 3.3	11.6 ± 3.2
Waist circumference (cm)[2]	82.5 ± 5.7	82.3 ± 5.5	82.7 ± 5.7	82.6 ± 5.8	82.6 ± 5.6	82.9 ± 5.5††	82.5 ± 5.4
Waist-to-hip ratio[2]	0.92 ± 0.06	0.93 ± 0.05**	0.92 ± 0.05	0.93 ± 0.05**	0.92 ± 0.05	0.92 ± 0.05**	0.91 ± 0.05
Systolic blood pressure (mm Hg)[2]	125.9 ± 8.5	125.5 ± 8.6	126.5 ± 8.5	124.9 ± 8.8	123.6 ± 7.6	126.9 ± 8.7	124.2 ± 9.3
Diastolic blood pressure (mm Hg)[2]	74.1 ± 8.5	72.0 ± 6.3	72.0 ± 6.2	74.2 ± 9.1	71.5 ± 5.6	72.7 ± 7.0	73.0 ± 7.7
LDL size (nm)[2]	27.71 ± 0.76	27.62 ± 1.38	27.30 ± 0.85	27.24 ± 0.82*	27.61 ± 1.30	27.27 ± 1.04*	27.51 ± 0.67
LDL subclasses (%)[2]							
I	34.3 ± 8.3	31.7 ± 7.6*	31.8 ± 7.4	30.2 ± 7.3**,†	33.0 ± 7.4	30.0 ± 7.4 **,††	32.7 ± 7.3
IIa	16.4 ± 3.2	16.2 ± 3.7	17.0 ± 3.2	17.2 ± 3.6	16.3 ± 2.9	17.2 ± 3.4	17.7 ± 2.9
IIb	15.4 ± 2.4	16.5 ± 2.7*	17.0 ± 3.0**	17.6 ± 2.8**	16.1 ± 2.7	17.6 ± 3.2**	16.3 ± 2.6
IIIa	12.0 ± 2.8	12.9 ± 3.5	12.7 ± 3.8	13.4 ± 4.1*	13.0 ± 3.6	13.6 ± 4.0**	12.3 ± 2.0
IIIb	5.4 ± 1.1	6.0 ± 1.8	5.6 ± 1.2	5.8 ± 1.2	5.9 ± 1.2	5.9 ± 1.2	5.4 ± 1.1
IVa	7.7 ± 1.8	8.3 ± 2.2	7.4 ± 1.7	7.6 ± 1.3	7.5 ± 1.6	7.7 ± 1.6	7.5 ± 1.6
IVb	8.8 ± 2.8	8.5 ± 2.0	8.4 ± 2.3	8.1 ± 1.7	8.1 ± 2.0	8.0 ± 1.9	8.1 ± 2.3
hs-CRP (ng/mL)[3]	205.6 ± 430.7	374.6 ± 1182.1**	394.4 ± 1258.4**	430.1 ± 1697.2**	390.1 ± 2336.2**	422.4 ± 1393.4**	329.1 ± 662.0*
Leptin (ng/mL)[3]	1.21 ± 1.68	1.31 ± 1.36	1.44 ± 1.29*	1.75 ± 1.83	1.83 ± 1.96 **	1.48 ± 1.34	1.24 ± 0.80
Adiponectin (μg/mL)[3]	6.44 ± 7.69	7.39 ± 4.39	7.40 ± 2.37	7.64 ± 2.95	7.77 ± 2.76	7.57 ± 10.00	7.61 ± 12.09
Ghrelin (pg/mL)[3]	718 ± 414	728 ± 359	780 ± 365	789 ± 415	734 ± 324	750 ± 377	692 ± 320
Fasting glucose (mmol/L)[2]	4.45 ± 0.45	4.70 ± 0.32**	4.81 ± 0.30**	4.64 ± 0.50*	4.68 ± 0.33**	4.76 ± 0.34**	4.84 ± 0.30**
Postprandial glucose (2 h) (mmol/L)[2]	5.09 ± 0.94	4.73 ± 0.58	4.97 ± 0.60	4.84 ± 0.61	4.97 ± 0.59	5.21 ± 0.66	5.38 ± 0.73
C-peptide (pmol/L)[3]	128 ± 223	115 ± 206	92 ± 363	157 ± 251	103 ± 197	102 ± 189	113 ± 218
HOMA2 IR[3]	0.27 ± 0.48	0.25 ± 0.45	0.20 ± 0.78	0.34 ± 0.52	0.22 ± 0.42	0.22 ± 0.41	0.26 ± 0.47
Free fatty acids (μmol/L)[3]	319.2 ± 180.1	306.6 ± 170.1	307.6 ± 217.6	296.9 ± 184.7	327.6 ± 175.9	307.9 ± 164.7	332.1 ± 169.5
AST (U/L)[2]	26 ± 6	27 ± 6	28 ± 7	27 ± 8	28 ± 6	29 ± 9	28 ± 7
ALT (U/L)[2]	23 ± 7	25 ± 11	26 ± 10	25 ± 9	25 ± 10	25 ± 13	23 ± 8

[1] MF, moderate fructose (40 g fructose/d); MG, moderate glucose (40 g glucose/d); HF, high fructose (80 g fructose/d); HG, high glucose (80 g glucose/d); HS, high sucrose (80 g sucrose/d); LF, low fructose (dietary advice to consume low free fructose); AST, aspartate aminotransferase; ALT, alanine aminotransferase; HOMA2 IR, computer-based homeostasis model assessment system (provided by the Oxford Center for Diabetes, Endocrinology, and Metabolism; http://www.dtu.ox.ac.uk/homa). *Significantly different from baseline on the basis of multiple linear regression with Bonferroni correction for multiple comparisons ($P < \alpha$*; 0.0083, corresponds to $P < 0.05$ after correction). **Significantly different from baseline on the basis of multiple linear regression with Bonferroni correction for multiple comparisons ($P < \alpha$**; 0.0017, corresponds to $P < 0.01$ after correction). †Significantly different from HG intervention on the basis of multiple linear regression with Bonferroni correction for multiple comparisons ($P < \alpha^{\dagger}$; 0.025, corresponds to $P < 0.05$ after correction). ††Significantly different from baseline on the basis of multiple linear regression with Bonferroni correction for multiple comparisons ($P < \alpha^{\dagger\dagger}$; 0.005, corresponds to $P < 0.01$ after correction).

[2] Values are arithmetic means ± SDs.

[3] Values are geometric means ± SDs.

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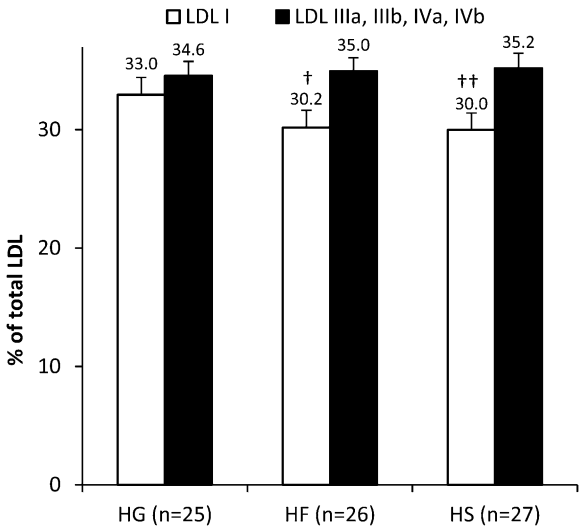

FIGURE 1. LDL subclasses according to mean (±SEM) percentage of total LDL after consumption of high glucose (HG; 80 g glucose/d), high fructose (HF; 80 g fructose/d), or high sucrose (HS; 80 g sucrose/d). LDL subgroup I represents the largest particles, whereas subgroups IIIa, IIIb, IVa, and IVb represent the smaller, dense particles. †Significantly different from the HG intervention on the basis of multiple linear regression with Bonferroni correction for multiple comparisons ($P < \alpha^{\dagger}$; 0.25, corresponds to $P < 0.05$ after correction). ††Significantly different from the HG intervention on the basis of multiple linear regression with Bonferroni correction for multiple comparisons ($P < \alpha^{\dagger\dagger}$; 0.005, corresponds to $P < 0.01$ after correction).

a mono- or disaccharide. As shown in Table 2, carbohydrate intake increased in all SSB-providing interventions, even though the increase was not significant in the MF intervention. With regard to protein and fat intake, a decrease was observed in the SSB-providing interventions, even though this was not always significant (Table 2). The LF intervention was not associated with any significant changes in carbohydrate, protein, or fat intake.

No significant change in the intake of dietary fiber was seen after the various interventions; however, a strong trend toward a decrease was seen in the LF group (18.7 ± 5.4 g, $P = 0.015$). β-Carotene intake decreased after all interventions and was the lowest after the LF intervention. To assess whether the washout period of 4 wk was sufficient to allow a return of anthropometric and metabolic parameters to baseline, we analyzed the influence of the position (eg, first position to sixth position) at which a specific intervention was placed on these parameters. There was a significant increase in waist-to-hip ratio and in fasting glucose from the first intervention to the last intervention, with a rise in waist-to-hip ratio from 0.92 ± 0.06 to 0.93 ± 0.05 ($P = 0.001$) and in fasting glucose from 4.45 ± 0.45 to 4.83 ± 0.31 mmol/L ($P = 0.004$).

Five of the 29 subjects did not complete the study for various reasons (2 moved away, 1 was afraid of developing caries, 1 changed work and could not come for examinations, 1 did not indicate a reason). The 6 interventions were completed by 26 (MF), 25 (MG), 26 (HF), 25 (HG), 27 (HS), and 26 (LF) subjects. Of a total of 9765 containers, 16 (0.16%) were returned not consumed.

DISCUSSION

This is the first study to show adverse effects of low to moderate consumption of fructose- glucose-, and sucrose-containing beverages over a period of only 3 wk on LDL size and other parameters of lipid and glucose metabolism as well as on inflammatory response in healthy young men. Even with lower doses (40 g sugar/d), which provided just 6.5% of daily energy in the form of SSBs, adverse effects could be observed with regard to LDL particle size and distribution, waist-to-hip ratio, fasting glucose, and inflammatory markers. Previous studies in humans have also shown negative effects of high fructose consumption, mainly with regard to dyslipidemia and insulin resistance, but these studies provided ≥25% of daily energy in the form of fructose, amounts which are rarely consumed in everyday life (15, 16).

In the present study, waist-to-hip ratio increased significantly compared with baseline after the fructose and sucrose but not after the glucose interventions. Although the change in waist-to-hip ratio within 3 wk was small, we think that this finding is still worrisome since it shows the effects of only 3 wk of consumption of SSBs in young healthy men. This is in accordance with the

TABLE 2
Dietary intake of the 29 subjects at baseline and after different interventions[1]

	Baseline	MF	MG	HF	HG	HS	LF
Energy (kcal/d)	2329 ± 523	2431 ± 671	2505 ± 382	2468 ± 559	2533 ± 518	2596 ± 576	2340 ± 714
Free fructose (g/d)	16.1 ± 9.0	54.9 ± 10.1**	16.8 ± 8.8	91.6 ± 8.6**	16.9 ± 9.0	13.4 ± 9.6	7.2 ± 6.8**
Total fructose (g/d)	48.4 ± 23.0	85.0 ± 26.0**	49.4 ± 22.0	115.9 ± 18.9**	46.6 ± 19.7	78.6 ± 21.3**	33.7 ± 24.1*
Free glucose (g/d)	14.0 ± 6.8	12.4 ± 6.4	54.3 ± 6.2**	11.4 ± 6.2	92.9 ± 5.8**	14.0 ± 13.6	8.0 ± 7.1
Total glucose (g/d)	46.3 ± 21.0	42.5 ± 23.6	86.8 ± 19.5**	35.8 ± 16.7	122.7 ± 17.9**	79.2 ± 21.2**	34.5 ± 24.3
Sucrose (g/d)	64.6 ± 33.9	60.1 ± 39.1	65.1 ± 34.2	48.6 ± 25.5	59.5 ± 28.2	130.4 ± 30.3**	53.1 ± 37.3
Carbohydrates (% of energy)	48.4 ± 6.7	50.9 ± 6.0	52.5 ± 9.1*	54.9 ± 5.0**	56.5 ± 4.8**	54.8 ± 6.2**	45.8 ± 7.5
Protein (% of energy)	15.3 ± 2.5	14.4 ± 2.6	13.1 ± 3.1**	13.5 ± 2.2*	12.8 ± 2.5**	13.0 ± 1.8**	16.3 ± 2.6
Fat (% of energy)	36.5 ± 6.3	34.7 ± 6.6	31.9 ± 5.9**	31.5 ± 4.7**	30.5 ± 4.1**	32.2 ± 5.9	38.0 ± 7.1
Fiber (g/d)	22.1 ± 7.6	19.9 ± 6.4	22.3 ± 7.4	20.0 ± 6.3	19.9 ± 5.5	21.4 ± 7.6	18.7 ± 5.4
Carotene (mg/d)	4.6 ± 4.6	2.8 ± 1.9*	3.2 ± 2.0*	2.8 ± 1.9*	2.6 ± 1.2**	2.9 ± 2.1**	2.2 ± 2.3**

[1] All values are arithmetic means ± SDs. MF, moderate fructose (40 g fructose/d); MG, moderate glucose (40 g glucose/d); HF, high fructose (80 g fructose/d); HG, high glucose (80 g glucose/d); HS, high sucrose (80 g sucrose/d); LF, low fructose (dietary advice to consume low free fructose). *Significantly different from baseline on the basis of multiple linear regression with Bonferroni correction for multiple comparisons ($P < \alpha^{*}$; 0.0083, corresponds to $P < 0.05$ after correction). **Significantly different from baseline on the basis of multiple linear regression with Bonferroni correction for multiple comparisons ($P < \alpha^{**}$; 0.0017, corresponds to $P < 0.01$ after correction).

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findings of Stanhope et al (15), whose results suggested that fructose consumption may specifically promote lipid deposition in visceral adipose tissue, particularly in men, whereas glucose consumption appears to favor lipid deposition in subcutaneous adipose tissue. Furthermore, in accordance with this finding, a significant increase in leptin concentrations was seen only in the interventions that did not contain any fructose but only glucose. This is also in agreement with previous studies showing that leptin production is higher in subcutaneous adipocytes compared with omental adipocytes (25, 26).

Even though we found no effect of any of the different diets on the traditional lipid profile, a reduction in large, buoyant LDL particles (LDL I) was observed during interventions containing fructose (MF, HF, and HS). In addition, when comparing the effects of 80 g glucose, 80 g fructose, and 80 g sucrose, it was shown that large LDL particles decreased significantly after the HF and HS intervention compared with the HG intervention. Interestingly, LDL size decreased during the HF and HS intervention. Those findings are in line with a meta-analysis investigating the effect of fructose interventions (providing $\leq$100 g fructose/d) in which no adverse effect on triglycerides was observed (27). Moreover, we were able to confirm our previous findings in overweight children (3) in normal-weight individuals, namely that fructose intake is inversely associated with LDL particle size. Even though we found the strongest effect on LDL particle size after the HF diet, the sucrose diet also led to a significant reduction, indicating that this sugar may be harmful with regard to lipid metabolism. This is consistent with a recent study that showed that high-fructose and high-glucose diets led to similar increases in VLDL triacylglycerols (28). A study by Stanhope et al (15) found that both LDL and small dense LDL increased with fructose- but not with glucose-sweetened beverages; however, this was in a population of overweight/obese adults and sugar amounts were higher (25%).

Subclinical inflammation has been shown to be a key factor in the development of insulin resistance and cardiovascular disease (29, 30). Fructose can activate inflammatory pathways such as nuclear transcription factor κB in animal models (31). Sorensen et al (9) found in a 10-wk intervention study that CRP concentrations increased in the sucrose group although they decreased in the sweetener group. Furthermore, Schulze et al (8) showed a positive association between dietary patterns high in SSBs and markers of inflammation in an observational study. In the present study we were able to show for the first time to our knowledge that SSB consumption markedly increases hs-CRP concentrations in humans.

We found elevated fasting glucose concentrations after all interventions. Stanhope et al (15) observed in a population of overweight/obese adults that fasting glucose increased with fructose- but not with glucose-sweetened beverages. These discrepancies may, as described above, well be due to differences in the study protocol. In the present study, there was an increase albeit not significant in both C- peptide and HOMA2-IR after the fructose interventions. Thus, 3-wk consumption of SSBs seems to be sufficient to induce subtle changes in glucose metabolism that may over the long term lead to insulin resistance. Several previous studies have found effects on fasting glucose or insulin resistance after the ingestion of high-fructose but not high-glucose diets (12, 15, 32, 33), although the results have not been always consistent (28, 33). However, the amounts of sugar used in the studies mentioned above were considerably higher compared with those in our study. In the present study, an increase in fasting glucose was also found after the LF intervention. A potential explanation for this observation is that dietary patterns may have been altered during the LF intervention toward a diet with a high glycemic load, indicated by lower fiber and β-carotene consumption.

We found differential effects between glucose and fructose on waist-to-hip ratio (reflecting visceral fat deposition) and on LDL subfraction distribution, both suggesting a more detrimental effect of fructose compared with glucose. These differences may be due, at least in part, to the fact that although calorically identical to glucose intake, fructose metabolism differs considerably from that of glucose (2, 34). For example, fructose, unlike glucose, has been shown to increase de novo lipogenesis (15). Moreover, the rate of hepatic uptake of fructose from the portal circulation is greater than the rate of glucose uptake; furthermore, fructose metabolism bypasses phosphofructokinase, and thus is not under the regulatory control of insulin (34).

The main limitation of the present study is that the 3-wk intervention period may not have been long enough to observe significant effects in parameters such as lipoprotein concentrations, insulin resistance, adipokines, body weight, and blood pressure, which were previously described by others (15, 35–37). However, it should be pointed out that the studies showing such associations either used very high amounts of SSBs ($\geq$25% of the total daily energy requirements) or had a nonrandomized design. In addition, the crucial aim of this study was to mimic sugar dosages comparable to those used in commercially available SSBs, thereby allowing us to draw clinically relevant conclusions. Daily consumption of 4–8 dL of SSBs for 3 wk (eg, during vacations) is much more relevant and closer to real life than is a 2- to 3-mo consumption of several liters of SSBs. However, it still has to be clearly pointed out that 4 of the 5 SSB interventions used in the current study had a composition not generally found in commercially available SSBs because they contained either only fructose or only glucose. Thus, even though the amounts of sugar used mimicked a real-life situation, the sugar composition itself did not. However, for the further development of new products, especially with regard to healthier diets, it is of utmost importance to understand the effects of the different sugar components both individually and combined.

Another limitation is the possibility of carryover effects between the different interventions as well as the time difference between measurements at baseline and after the interventions. To limit this possible bias, a randomly assigned order of the interventions was chosen and a washout period of $\geq$4 wk was included in the study protocol. Furthermore, the effects of the interventions themselves were separated from the effects of the order of the interventions by multiple linear regression. This analysis indeed showed differences of 2 parameters—waist-to-hip ratio and fasting glucose—between the first and the last intervention that were independent of the type of the intervention. Although the interventions lasted only 3 wk and the washout periods 4 wk, the adverse changes produced by SSBs seem to have accumulated over the entire study period.

In conclusion, this study clearly shows that consumption of SSBs, even in moderate amounts, has adverse effects on lipid and glucose metabolism as well as on the inflammatory status of healthy young men. It also shows that even though there are differential effects caused by the different sugars, all of them

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seem to be detrimental to some extent. Therefore, not only does chronic consumption of high amounts of SSBs in predisposed subjects increase cardiovascular risk markers but it appears that only a few weeks of moderate consumption in healthy young men is sufficient to increase these risk markers.

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