Moderate Amounts of Fructose Consumption Impair Insulin Sensitivity in Healthy Young Men: A randomized controlled trial

Aeberli, Isabelle ; Hochuli, Michel ; Gerber, Philip A ; Sze, Lisa ; Murer, Stefanie B ; Tappy, Luc ; Spinas, Giatgen A ; Berneis, Kaspar

DOI: https://doi.org/10.2337/dc12-0540

Moderate amounts of fructose consumption impair insulin sensitivity in healthy young men – a randomized controlled trial

Isabelle Aeberli, PhD*, Michel Hochuli, MD PhD*, Philip A. Gerber, MD MSc, Lisa Sze MD, Stefanie B. Murer MSc, Luc Tappy MD, Giatgen A. Spinas MD, Kaspar Berneis, MD

Division of Endocrinology, Diabetes, and Clinical Nutrition, University Hospital Zurich, Zurich, Switzerland (IA, MH, PAG, SBM, GAS, KB); Human Nutrition Laboratory, Institute of Food, Nutrition and Health, ETH Zurich, Zurich, Switzerland (IA, SBM); Division of Endocrinology and Diabetes, Kantonsspital St. Gallen, St. Gallen, Switzerland (LS); Competence Center for Systems Physiology and Metabolic Diseases, Zurich, Switzerland (PAG, GAS); Faculty of Biology and Medicine, Department of Physiology, University of Lausanne, Lausanne, Switzerland (LT); Zurich Center for Integrative Human Physiology, Zurich, Switzerland (KB)

* these authors contributed equally to this article

Running title: Fructose impairs insulin sensitivity

Word count: 4056**, No of figures:** 1**; No of tables:** 3

Address of correspondence:

Prof. Dr. Kaspar Berneis, MD

Division of Endocrinology, Diabetes, and Clinical Nutrition

University Hospital Zurich

8091 Zurich

Switzerland

Phone: +41 44 255 3585

Fax: +41 44 255 4447

e-mail: kaspar.berneis@usz.ch

Abstract

Objective Adverse effects of hypercaloric, high fructose diets on insulin sensitivity and lipids in human subjects have been shown repeatedly. The implications of fructose in amounts close to usual daily consumption however, have not been well studied. The study aim was to assess the effect of moderate amounts of fructose and sucrose compared to glucose on glucose and lipid metabolism.

Research Design and Methods Nine healthy, normal weight male volunteers (age 19-25 years) were studied in this double blind, randomized cross-over trial. All subjects consumed four different sweetened beverages (600 ml/day) for three weeks each: medium fructose (MF, 40 g/day), high fructose (HF), high glucose (HG) and high sucrose (HS) (each 80 g/day). Euglycemic-hyperinsulinemic clamps with [6,6]-$^{2}H_2$ glucose labeling were used to measure endogenous glucose production. Lipid profile, glucose and insulin were measured in fasting samples.

Results Hepatic suppression of glucose production during the clamp was significantly lower after HF (59.4±11.0%) compared to HG (70.3±10.5%, $p<0.05$), while fasting glucose, insulin and C-peptide did not differ between the interventions. Compared to HG, both LDL cholesterol and total cholesterol were significantly higher after MF, HF and HS and free fatty acids were significantly increased after MF but not after the two other interventions ($p<0.05$). Subjects' energy intake during the interventions did not differ significantly from baseline intake.

Conclusion This study clearly shows that moderate amounts of fructose and sucrose significantly alter hepatic insulin sensitivity and lipid metabolism compared to similar amounts of glucose.

Trial registration: This trial has been registered at ClinicalTrials.gov under the registration Nr. NCT01021969.

Introduction

In the USA, the consumption of fructose has increased by more than 25% between 1970 and 1997 as the total sugar intake of the population rose (1). During the same time period, the prevalence of obesity has risen dramatically, paralleling the increase in fructose consumption and the introduction of high fructose corn syrup (2). Whether there is a causal relationship between those developments, however, remains unclear. Total fructose consumption from natural and added sources, estimated from food disappearance data, was estimated to be 97 g/person/day in 1997 in the US (1) and 83 g/person/day in 1998 in Switzerland (3).

In both epidemiological and intervention studies fructose and other caloric sweeteners have shown detrimental effects on health. In a cross-sectional study in US adults, for example, the consumption of caloric sweeteners was associated with increased dyslipidemia (4) and in the Health Professional Follow-up Study high intakes of sugar-sweetened beverages (SSB) were found to increase the risk for type 2 diabetes (5). Intervention trials have provided evidence that high to very high fructose doses lead to increases in *de novo* lipogenesis, blood triglycerides and hepatic insulin resistance (6-8). But not all of these studies found consistent effects for all parameters. In the study by Le et al., where 1.5 g of fructose/kg body weight were consumed over a period of four weeks, fasting lipids as well as glucose were affected, while insulin resistance as determined by a euglycemic-hyperinsulinemic clamp did not change. However, in this study only fructose was tested without comparison to other sugars (6). Furthermore, in most of these studies relatively high amounts of fructose were consumed, reaching up to 25% of total energy intake. In a recent intervention study in healthy Swiss men we have found adverse effects of low to moderate amounts of fructose, but also glucose and sucrose on fasting glucose and inflammatory markers, while only fructose containing beverages seemed to affect LDL particle size negatively. Even though fasting glucose was

altered, none of the interventions showed any effect on glucose tolerance, nor on indices of insulin sensitivity calculated during an oral glucose tolerance test (9).

The aim of the present study was therefore to assess the effect of moderate amounts of fructose and sucrose compared to the same amounts of glucose specifically on hepatic insulin sensitivity, but also lipid profiles of healthy human subjects using euglycemic-hyperinsulinemic clamps with [6,6]-$^{2}H_2$ labeled glucose.

Subjects and Methods

Study Design

The study consisted of four different interventions in random sequence. Each of the interventions lasted three weeks and was directly followed by an examination in our clinic. Thereafter, a wash-out period of a minimum of four weeks was implemented before the beginning of the next intervention. The first subject started the study in February 2009 and the last subject completed the study in March 2011. During each intervention subjects were supplied with SSB containing different sugars in different concentrations: 40 g fructose/day (medium fructose, MF), 80 g fructose/day (high fructose, HF), 80 g glucose/day (high glucose, HG), and 80 g sucrose/day (high sucrose, HS). The drinks were provided in containers of 200 ml each, with blinded content, and the subjects had to consume three drinks (=600 ml) per day. The sugar concentrations of the drinks were 66.5 g/l and 133.5 g/l for the medium and high concentrations, respectively. Subjects were advised to consume the drinks together with the three main meals. To assess compliance subjects were asked to return beverages not consumed on the day of visit to the metabolic ward. The drinks were produced by the Nestlé Product Technology Center (PTC) in Konolfingen, Switzerland, under good manufacturing practice (GMP) conditions and according to our instructions. Prior to their use

in the study the drinks underwent quality control at the PTC. During the study, sugar content of the drinks was monitored and found to be stable. The order of the four 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. The random allocation of the order of interventions was carried out by a co-worker not otherwise involved in the study. Participants as well as the nurse taking the anthropometric measurements and the laboratory technicians were blinded to the order of interventions.

Subjects

Nine healthy, normal weight male volunteers (BMI between 20 and 24 kg/m^2, age between 21 and 25 years) living in the region of Zurich, Switzerland, were included in this study. Subjects were recruited through advertisements at the Universities in Zurich by IA. Written informed consent was obtained from all subjects prior to entering the study. The study was approved by the Ethics Committee of the University Hospital Zurich and registered under ClinicalTrials.gov (NCT01021969). Sample size calculation was based on an estimated difference in hepatic suppression between 2 interventions of 10% with a standard deviation of 6% (alpha level 0.016 after Bonferroni correction for 3 comparisons) and determined a samples size of 9 volunteers to be sufficient. Volunteers were eligible for the study if they were male, had a normal BMI (19-25 kg/m^2), were healthy and 20 to 50 years old. Volunteers taking regular medication or consuming SSB with a total content of more than 60g of carbohydrates per day were not included in the study.

Protocol

One day prior to each examination, subjects were asked not to engage in strenuous physical activity. On the examination day they were asked to present at the Clinical Trials unit of the University Hospital Zurich at 7.30 am after a 12 hour overnight fast. Upon arrival, weight was

determined to the nearest 100 g using a digital balance (WB 100 P, Tanita, Hoofddorp, The Netherlands) and height was measured to the nearest 0.5 cm using a wall mounted stadiometer at the first examination. BMI was calculated as weight (kg)/height $(m)^2$. Waist and hip circumference were determined using a non-stretchable measuring tape. Percent body fat (%BF) was measured by bioelectrical impedance (AKERN BIA 101, AKERN, Pontassieve, Italy) in supine position.

Blood pressure was measured using an automated device (Omron M6, upper arm blood pressure monitor) after a 15 min rest in supine position. Following this, with the subjects resting quietly in a bed, an indwelling catheter was inserted into the vein of the right arm for blood sampling. Another indwelling catheter was inserted into an antecubital vein of the left arm for the infusion of glucose, insulin and the tracer ([6,6]-$^2H_2$ glucose). In the fasted state, blood samples were collected for the measurement of glucose, insulin, C-peptide, lipid profile, and leptin. Following blood sampling, a primed continuous infusion of [6,6]-$^2H_2$ glucose was administered during 5h to determine endogenous glucose production (bolus of 2 mg/kg over 10 minutes, followed by a continuous rate of 0.02 mg/kg/min). After 180 min of tracer equilibration, a hyperinsulinemic-euglycemic clamp was started for the following 120 min. Insulin was infused continuously (bolus of 60 mU/m^2/min insulin for 3 minutes followed by continuous rate of 15 mU/m^2/min). A relatively low insulin infusion rate with incomplete suppression of hepatic glucose production was chosen to reveal differences in insulin sensitivity in our generally insulin sensitive study group, and based on our previous experiences (10). Blood samples were collected every 5 minutes during the clamp to monitor blood glucose concentrations, and glucose (20 % wt/vol) was infused at variable rates to keep the blood glucose euglycemic (around 4.5 mmol/l) (Table 2). The 20% glucose infusion contained 1.2 % [6,6]-$^2H_2$ glucose to maintain a constant plasma D2-glucose tracer/trace ratio (TTR) during the clamp. Blood was drawn at time points 150, 165 and 180 min of the 3h

tracer equilibration (baseline) period, and at 60, 90, 105 and 120 min of the 2h clamp period for the determination of tracer concentrations (TTR = tracer/tracee ratio of [6,6]-$^{2}H_2$ glucose). Glucose kinetics were calculated as described previously (11) at near steady state at the end of the tracer equilibration baseline period (150 – 180min), and during the last 30 min of the clamp (mean values from time points given above). Total glucose flux (Ra), endogenous glucose production rate (EGP), glucose metabolic clearance rate (Rd divided by the glucose concentration, *i.e.* insulin mediated glucose disposal, a standard parameter of whole body insulin sensitivity) and % hepatic suppression of glucose production (a parameter of hepatic insulin sensitivity) were calculated as follows:

Ra = F / TTR, with F being the rate of tracer infusion; EGP = Ra – glucose infusion rate; glucose metabolic clearance rate = Rd / glucose concentration = Ra / glucose concentration; Hepatic suppression = $100\%*((EGP_{basal} - EGP_{clamp})/ EGP_{basal})$.

In the week prior to each examination as well as before the start of the first intervention, all subjects filled in a three day (two weekdays and one weekend day) weighed food record (12). During those three days all foods and drinks consumed had to be weighed on a digital kitchen scale 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 three day food records of each subject were carefully checked at the day of the examination to ensure completeness and comprehensibility. Data was then entered into a nutrition software system (EBISpro for Windows 8.0 (Swiss version), Dr. J. Erhardt, University of Hohenheim, Germany) to convert the amount of food eaten into individual nutrients. Three day 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, while total fructose and total glucose refer to both the monosaccharides and the part derived from the disaccharide sucrose (50% fructose and 50% glucose).

The primary outcome measure of this trial was the change in insulin sensitivity, determined as the hepatic glucose suppression during the euglycemic-hyperinsulinemic clamp after fructose and sucrose interventions compared to glucose. Secondary outcome measures were changes fasting concentrations of lipids as well as glucose, insulin and C-peptide as well as changes in anthropometric measures.

Laboratory Analysis

Blood glucose was directly measured from whole blood samples (both fasting and during the clamp) using an automated enzymatic method (YSI 2300, YSI Life Sciences, Yellow Springs, USA). The remaining 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 on Roche MODULAR by enzymatic reactions (Triglyceride GPO-PAP and cholesterol CHOP-PAP; Roche Diagnostics, Mannheim, Germany), on Roche INTEGRA by a homogenous enzymatic color reaction (HDL-cholesterol plus 3rd generation; Roche Diagnostics, Mannheim, Germany) and on Konelab (Free Fatty Acidy, Thermo Scientific, Dreieich, Germany). From frozen serum, C-peptide was measured using RIA (IRMA-C-PEP, CIS bio international, Bagnols-sur-Cèze Cedex, France), leptin using ELISA (EZHL-80 SK, Linco Research, Inc., St. Charles, USA). Plasma [6,6]-$^{2}H_{2}$ glucose enrichment (tracer / tracee ratio ;TTR) was measured by gas chromatography-mass spectrometry (Hewlett-Packard Instruments, Palo Alto, CA) as described elsewhere (13).

Statistical Analysis

Statistical analysis was performed using the statistical package SPSS 19.0 (SPSS inc, IBM, Chicago, Illinois, USA). All variables were checked for normal distribution prior to data analysis. Data are expressed as arithmetic mean ± SD for normally distributed variables and as geometric mean ± SD for non-normally distributed data. Non-normally distributed data were log-transformed and further analysis carried out with the transformed data. According to the intention to treat design of the study, all subjects (completers and non-completers) were included in the final analysis. The effect of the interventions as well as of the order of the interventions on anthropometric and metabolic parameters was examined using multiple linear regression (as described in (14)), always controlling for between-patient differences. Post-hoc Bonferroni correction was applied to account for multiple comparisons. In the main analysis the three other interventions were compared to the glucose intervention, and thus a correction factor of 3 (3 interventions) was used. For the dietary intake all 4 interventions were compared to baseline, and thus a correction factor of 4 was used. A p-value of <0.05 after correction was considered significant.

Results

A total of 9 subjects participated in the study. Except for one, who was not able to finish the last intervention (MF) because he moved abroad, all subjects completed all four interventions. Mean age of the subjects at baseline was 22.8 ± 1.7 years and their anthropometric characteristics after each of the interventions are shown in **Table 1**. Compared to the HG intervention, body weight, BMI, body fat and waist circumference were slightly but significantly lower after the HF intervention ($p<0.05$, GLM with bonferroni correction for 3

comparisons). Body weight and BMI were also significantly lower after the MF intervention compared to HG ($p<0.01$).

The results of the euglycemic hyperinsulinemic clamps after each intervention are shown in **Table 2**. The hepatic suppression of glucose production during the clamp was significantly lower after the HF intervention compared to HG ($p=0.015$), as also shown by the higher ratio of endogenous production during the clamp / baseline ($p=0.009$), while there was no difference between HG and MF or HS (compare **Figure 1**). This shows a significant decrease in hepatic insulin sensitivity after relatively small amounts of daily fructose consumption. In contrast, no significant differences between diets were seen in glucose metabolic clearance rate, *i.e.* insulin mediated glucose clearance, which is a parameter of whole body insulin sensitivity. Mean glucose levels during the baseline measurements and the clamp were kept in the same range.

Also shown in Table 1 are the fasting metabolic characteristics (glucose, insulin, C-peptide, lipids) of the subjects after each intervention. Fasting levels of glucose, insulin and C-peptide did not differ significantly between HG and any of the other interventions. Compared to the HG intervention, both LDL cholesterol and total cholesterol were significantly higher after the MF, HF and HS interventions ($p<0.05$). Furthermore, the free fatty acid concentration was increased after MF compared to HG ($p=0.033$), with a trend towards higher values after HF, and HS, albeit not significant. No differences were seen between the interventions for HDL cholesterol or triglycerides.

Compared to HG (2.02 ± 2.28 ng/ml) leptin concentrations were significantly lower after MF (1.26 ± 1.22 ng ml, $p=0.012$) and HF (1.37 ± 2.54 ng/ml, $p=0.012$), while the difference to HS (1.71 ± 2.99 ng/ml) was not significant.

Dietary intake was assessed at baseline as well as after each of the 4 interventions. The consumption of energy, macronutrients, fibers as well as the different sugars are shown in **Table 3** Energy intake as well as the % energy from fat, carbohydrates and protein did not differ significantly between HG and any of the other interventions, neither did fiber intake. However, protein intake was significantly lower in all interventions except for HS compared to baseline, while fat intake was significantly lower in the MF and the HS interventions, again compared to baseline. Carbohydrate intake was higher in the HF, HG and HS interventions compared to baseline, but the differences were not significant. The consumption of the individual sugars varies according to the interventions.

Discussion

This study has investigated the effect of sweetened beverages containing fructose or sucrose compared to those containing glucose and has resulted in two important findings: 1) Compared to HG, suppression of endogenous glucose production is reduced after the HF diet during the euglycemic hyperinsulinemic clamp, indicating reduced hepatic insulin sensitivity after the HF diet; 2) after all fructose containing diets (MF, HF and HS), both total and LDL-cholesterol were elevated compared to HG.

Glucose and fructose have a similar caloric content, but intermediary fructose metabolism has unique features. After a dietary fructose load, fructose is rapidly cleared from the plasma and efficiently metabolized in the liver in an insulin independent manner (2). While glucose metabolism via hexokinase and glycolysis is tightly regulated by the energy status of the cell and insulin levels, fructose metabolism via fructokinase bypasses these regulatory steps. Hence, rapid breakdown of fructose into trioses leads to high fluxes through the downstream steps of the glycolytic pathway, generating e.g. precursors and substrates for de novo

lipogenesis. Fructose ingestion also affects lipid metabolism via enhanced and extended activity of regulator proteins (e.g. SREBP-1c for de novo lipogenesis) (1, 8, 15, 16).

Several studies have shown that supplementation with high amounts of fructose, associated with excess energy intake, induce features similar to those encountered in the metabolic syndrome. The most striking effect is an increase in fasting and post-prandial triglycerides, which can be explained by a stimulation of hepatic de novo lipogenesis (8, 17), a stimulation of VLDL-TG secretion, and a decreased VLDL-TG clearance (18, 19). In addition several studies have reported a mildly impaired hepatic insulin sensitivity, as indicated by an increase in fasting hepatic glucose production or by a blunted suppression of glucose production during hyperinsulinemia, or a deposition of ectopic fat in liver cells(7, 17, 20).

In the present study, we have observed that, even with relatively small amounts of daily fructose consumption, there was a significant decrease in hepatic insulin sensitivity. This could be documented by using insulin clamp at low insulin infusion rates, which incompletely suppressed hepatic glucose production. In addition care was taken to have a long tracer infusion time prior to measurement to avoid erroneous results linked to incomplete tracer equilibration, which may explain why similar results had not been observed with former experiments (6). This clearly indicates that hepatic insulin sensitivity is exquisitely sensitive to fructose intake. The mechanisms underlying these effects remain unknown, but may involve a stimulation of gluconeogenesis and increased glycogen stores, or may be related to hepatic lipotoxicity.

In contrast to this impaired hepatic insulin sensitivity, whole body (presumably essentially muscle) insulin sensitivity was not significantly altered by fructose-containing drinks. This is consistent with other studies having used higher amounts of fructose (7, 17), but may appear at odds with the observation that high fructose intakes can impair glucose tolerance. This strongly suggests that this impaired glucose tolerance is explained by impaired suppression of

hepatic glucose output rather than by muscle insulin resistance, at least with short term high fructose diets. It remains however possible that fructose administration over longer periods of time may also alter muscle insulin sensitivity, possibly through a progressive deposition of ectopic fat in skeletal muscle, as shown by Le et al. (7).

In contrast to other studies (6, 21), we did not observe a significant increase in plasma triglyceride concentrations. This is most likely related to the relatively low amount of fructose administered in the present study. Based on a meta-analysis (22) fasting triglyceride concentrations increase with daily fructose intake above 100g/day, ie somewhat higher than used in the present experiments. However, interestingly, we have found an increase in total and LDL-cholesterol concentrations after the HF, MF, and HS diets compared to HG. Similarly, the study by Bantle et al. revealed differences in total and LDL-cholesterol between fructose and glucose diets after a study duration of 4 weeks, but they were no longer significant after 6 weeks, which was the study endpoint. At this point the only parameter which did differ between the glucose and fructose diets was triglyceride concentration in men (21). However, despite similar amounts of fructose and glucose, the study population in this trial was not necessarily comparable to the one in the present study. While in our study all subjects were aged between 20 and 25 years, in the study by Bantle et al. half of the subjects were aged above 40 years. Further, the mean BMI of our healthy volunteers was 22.3 kg/m2 at baseline, while it was 24.7 and 25.8 kg/m2 in the subjects below and above 40 years of age respectively in the study by Bantle et al (21). Following along the same line, another recent study investigating the effect of different sugars on lipid metabolism reported results comparable to those we found, despite methodological differences (25% of energy requirements given as SSB, duration 2 weeks). This group observed increased concentrations of LDL-cholesterol, but also of 24-hour triglyceride area under the curve (a parameter we did

not assess) after fructose and high fructose corn syrup but not after glucose consumption while fasting triglycerides were similar after all interventions (23).

A special feature of the present study is that it provided a direct comparison of the effects of fructose-containing drinks to glucose alone. Only few studies have performed such a direct comparison. Stanhope et al. found an increase in fasting glucose and a decrease in insulin sensitivity after a 10-week intervention with fructose containing beverages but not after glucose containing beverages (8). However, in this study, the energy provided by the fructose and glucose beverages accounted for 25% of total energy intake. In our study diminished hepatic insulin sensitivity could be seen despite a considerably lower amount of sugar given (15% of baseline energy intake) and a much shorter study duration. This indicates that already relatively low amounts of fructose over a short period of time may negatively affect glucose metabolism even in healthy lean subjects. In disagreement to our results, recent studies by Silbernagel et al. and Ngo Sock et al. found similar effects of high glucose and high fructose diets with regard to insulin sensitivity determined by oral glucose tolerance test (24) or to intrahepatic fat content (20); There was, however, a significant increase in fasting triglyceride concentrations with fructose only.

Compared to the glucose intervention, we found significantly lower weight and BMI following the MF and HF intervention and significantly lower body fat and waist circumference after HF only. Even though the differences were relatively small, the finding was consistent over the different anthropometric measurements and could not be explained by higher energy intake during the HG intervention or by reduced physical activity. One previous study comparing weight and fat changes after high fructose and high glucose diets found that, even though overall weight gain was similar, fructose induced more gain in intra-abdominal adipose tissue, while glucose led to increased subcutaneous adipose tissue (8). The duration of the interventions in this study, however, was 10 weeks, and 25% of energy requirements were

provided in the form of sugar. We did not distinguish between intra-abdominal and subcutaneous adipose tissue in our study and can therefore not be sure what the changes we have observed were attributable to. However, it has been shown in previous studies, that leptin is mainly secreted in subcutaneous adipose tissue (25, 26). Thus, the increased secretion of leptin after HG compared to HF and MF we have observed also points towards an increase in subcutaneous adipose tissue after this intervention. The small sample size in our study and the relatively short study duration may have blunted other changes seen in previous studies.

A limitation of the present study may be the relatively short duration of the interventions and the moderate amount of sugars given. However, our aim was to study the effect of the different sugars in amounts that are likely to be consumed in normal life. And the fact that we did see certain effects already at this level and after three weeks seems to justify our decision. Another limitation is the lack of baseline measurements prior to each of the interventions. Still, based on the complexity of the method used and the already high subject burden, we decided against them. However, to control for possible baseline differences we used a randomized cross-over design and controlled for the order of interventions in the statistical analysis.

In conclusion, this study shows that, with regard to glucose metabolism and specifically hepatic insulin sensitivity, fructose, even in moderate amounts, seems to be more harmful than the same amount of glucose. Furthermore, all fructose-containing drinks (including sucrose) showed significant effects on the lipid profile when compared to glucose. On the other hand, anthropometric measurements pointed towards higher adiposity after the glucose intervention, even though differences were small. Thus, even when consumed in moderate amounts and over a limited period of time, SSB, especially those containing fructose, can result in alterations of hepatic glucose metabolism and lipid profile in healthy young men, which may possibly be associated with increased cardiometabolic risk. Further research will

be needed to better understand the underlying mechanisms, specifically with regard to the lipid metabolism, and also to understand other influencing factors such as age, gender or genetic predisposition.

Acknowledgements

Our thanks go to Valeria Meyer, University Hospital Zurich, Switzerland, the study nurse assisting in the entire project and to Cornelia Zwimpfer, University Hospital Zurich, Switzerland, and Valentine Rey, University of Lausanne, Switzerland, for expert laboratory and technical work. Furthermore we would like to thank all the subjects participating in this study and the Nestlé Product and Technology Centre in Konolfingen, Switzerland, for providing the study drinks.

Funding

The study was funded by the Swiss National Science Foundation (grant 32003B-119706 to KB) and the Vontobel Foundation (GAS).

Neither of the funding organizations was involved in the design or conduct of the study, the collection, management, analysis and interpretation of the data or the preparation, review or approval of the manuscript.

Contribution of the authors:

KB and IA: designed the research; IA, MH, PAG, LS, SBM, and KB: conducted the research; IA, MH, PAG, LS, SBM and KB: analyzed data or performed statistical analysis; IA, MH, PAG, LT, GAS, and KB: wrote the manuscript; all authors have read and edited the manuscript; KB: had primary responsibility for final content of the manuscript.

IA had full access to the all data in the study and takes responsibility for the integrity of the data analysis.

Conflict of interest

None of the authors have any conflict of interest with regard to this manuscript.

References

1. Elliott SS, Keim NL, Stern JS, Teff K, Havel PJ. Fructose, weight gain, and the insulin resistance syndrome. Am J Clin Nutr 2002;76(5):911-22.
2. Bray GA, Nielsen SJ, Popkin BM. Consumption of high-fructose corn syrup in beverages may play a role in the epidemic of obesity. Am J Clin Nutr 2004;79(4):537-43.
3. Eidgenössische Ernährungskommission. Kohlenhydrate in der Ernährung. In: Keller U, ed. Zurich: Bundesamt für Gesundheit, 2009.
4. Welsh JA, Sharma A, Abramson JL, Vaccarino V, Gillespie C, Vos MB. Caloric Sweetener Consumption and Dyslipidemia Among US Adults. J Am Med Assoc 2010;303(15):1490-7.
5. de Koning L, Malik VS, Rimm EB, Willett WC, Hu FB. Sugar-sweetened and artificially sweetened beverage consumption and risk of type 2 diabetes in men. Am J Clin Nutr 2011. doi: ajcn.110.007922.
6. Lê KA, Faeh D, Stettler R, et al. A 4-wk high-fructose diet alters lipid metabolism without affecting insulin sensitivity or ectopic lipids in healthy humans. Am J Clin Nutr 2006;84(6):1374-9.
7. Lê KA, Ith M, Kreis R, et al. Fructose overconsumption causes dyslipidemia and ectopic lipid deposition in healthy subjects with and without a family history of type 2 diabetes. Am J Clin Nutr 2009;89(6):1760-5. doi: DOI 10.3945/ajcn.2008.27336.
8. Stanhope KL, Schwarz JM, Keim NL, et al. Consuming fructose-sweetened, not glucose-sweetened, beverages increases visceral adiposity and lipids and decreases insulin sensitivity in overweight/obese humans. J Clin Invest 2009;119(5):1322-34. doi: Doi 10.1172/Jci37385.
9. Aeberli I, Gerber PA, Hochuli M, et al. Low to moderate sugar-sweetened beverage consumption impairs glucose and lipid metabolism and promotes inflammation in healthy young men: a randomized controlled trial. Am J Clin Nutr 2011;94(2):479-85. doi: 10.3945/ajcn.111.013540.
10. Berneis K, Ninnis R, Haussinger D, Keller U. Effects of hyper- and hypoosmolality on whole body protein and glucose kinetics in humans. American Journal of Physiology-Endocrinology and Metabolism 1999;276(1):E188-E95.
11. Vella A, Rizza RA. Application of isotopic techniques using constant specific activity or enrichment to the study of carbohydrate metabolism. Diabetes 2009;58(10):2168-74. doi: 10.2337/db09-0318.
12. Gibson RS. Principles of Nutritional Assessment. 2. ed. New York: Oxford University Press, 2005.
13. Schneiter P, Gillet M, Chiolero R, Jequier E, Tappy L. Hepatic nonoxidative disposal of an oral glucose meal in patients with liver cirrhosis. Metabolism: clinical and experimental 1999;48(10):1260-6.
14. Senn S. Crossover Trials in Clinical Research. 2nd ed. Chichester, UK: Wiley Publishing, 2002.
15. Dekker MJ, Su Q, Baker C, Rutledge AC, Adeli K. Fructose: A highly lipogenic nutrient implicated in insulin resistance, hepatic steatosis and metabolic syndrome. Am J Physiol Endocrinol Metab 2010;299(5):E685-94. doi: ajpendo.00283.2010.
16. Stanhope KL, Havel PJ. Fructose consumption: recent results and their potential implications. Ann Ny Acad Sci 2010;1190:15-24. doi: DOI 10.1111/j.1749-6632.2009.05266.x.

17. Faeh D, Minehira K, Schwarz JM, Periasamy R, Park S, Tappy L. Effect of fructose overfeeding and fish oil administration on hepatic de novo lipogenesis and insulin sensitivity in healthy men. Diabetes 2005;54(7):1907-13.
18. Parks EJ. Dietary carbohydrate's effects on lipogenesis and the relationship of lipogenesis to blood insulin and glucose concentrations. Br J Nutr 2002;87 Suppl 2:S247-53.
19. Chong MF, Fielding BA, Frayn KN. Mechanisms for the acute effect of fructose on postprandial lipemia. Am J Clin Nutr 2007;85(6):1511-20.
20. Ngo Sock ET, Le KA, Ith M, Kreis R, Boesch C, Tappy L. Effects of a short-term overfeeding with fructose or glucose in healthy young males. Br J Nutr 2010;103(7):939-43. doi: 10.1017/S0007114509992819.
21. Bantle JP, Raatz SK, Thomas W, Georgopoulos A. Effects of dietary fructose on plasma lipids in healthy subjects. Am J Clin Nutr 2000;72(5):1128-34.
22. Livesey G, Taylor R. Fructose consumption and consequences for glycation, plasma triacylglycerol, and body weight: meta-analyses and meta-regression models of intervention studies. Am J Clin Nutr 2008;88(5):1419-37. doi: DOI 10.3945/ajcn.2007.25700.
23. Stanhope KL, Bremer AA, Medici V, et al. Consumption of fructose and high fructose corn syrup increased postprandial triglycerides, LDL-cholesterol and apolipoprotein-B in young men and women. J Clin Endocrinol Metab 2011;96(10):E1596-E605.
24. Silbernagel G, Machann J, Unmuth S, et al. Effects of 4-week very-high-fructose/glucose diets on insulin sensitivity, visceral fat and intrahepatic lipids: an exploratory trial. Br J Nutr 2011;106(1):79-86. doi: Doi 10.1017/S000711451000574x.
25. Russell CD, Petersen RN, Rao SP, et al. Leptin expression in adipose tissue from obese humans: depot-specific regulation by insulin and dexamethasone. Am J Physiol Endocrinol Metab 1998;38(3):E507-E15.
26. Van Harmelen V, Reynisdottir S, Eriksson P, et al. Leptin secretion from subcutaneous and visceral adipose tissue in women. Diabetes 1998;47(6):913-7.

Table 1 Anthropometric characteristics as well as fasting glucose, insulin, C-peptide and lipid concentrations of all subjects after each of the four 3-week interventions

	HG	MF	HF	HS
N	9	8	9	9
Anthropometrics				
Weight (kg) [1]	74.1 ± 7.1	72.0 ± 7.7[3]	72.3 ± 6.9[3]	73.4 ± 7.1
Height (m) [1]	1.80 ± 0.08	1.80 ± 0.08	1.80 ± 0.08	1.80 ± 0.08
BMI (kg/m^2) [1]	22.8 ± 1.4	22.3 ± 1.5[3]	22.3 ± 1.3[3]	22.6 ± 1.1
Waist circumference (cm) [1]	83.3 ± 6.2	83.1 ± 6.1	82.9 ± 6.1[3]	83.3 ± 5.8
Body fat (%)[1]	16.8 ± 2.8	16.7 ± 2.5	15.8 ± 2.2[3]	16.3 ± 2.0
Body fat (kg) [1]	12.5 ± 3.3	12.2 ± 3.0	11.3 ± 2.0[3]	12.1 ± 2.5
Fasting glucose metabolism				
Fasting glucose (mmol/l) [1]	4.24 ± 0.26	4.29 ± 0.33	4.33 ± 0.256	4.32 ± 0.28
Insulin (pmol/l) [1]	105.4 ± 36.2	89.6 ± 22.0	113.8 ± 37.3	105.1 ± 20.4
C-peptide (pmol/l) [1]	548.9 ± 127.5	499.4 ± 86.4	535.6 ± 119.2	512.2 ± 93.6
Lipids				
Total cholesterol (mmol/l)[1]	3.48 ± 0.69	3.65 ± 0.59[3]	3.72 ± 0.68[3]	3.76 ± 0.77[3]
HDL-C (mmol/l)[2]	1.16 ± 0.23	1.18 ± 0.21	1.21 ± 0.28	1.22 ± 0.23
LDL-C (mmol/l)[2]	1.85 ± 0.57	2.03 ± 0.54[3]	2.02 ± 0.57[3]	2.08 ± 0.68[3]

Triglycerides (mmol/l)[2]	0.77 ±0.40	0.65 ± 0.58	0.79 ± 0.41	0.71 ± 0.31
Free fatty acids (µmol/l)[1]	419.3 ± 236.1	578.6 ± 230.1[3]	457.0 ± 117.3	506.0 ± 269.8

[1] arithmetic mean ± SD

[2] geometric mean ± SD

[3] significantly different compared to HG ($p<0.05$, multiple linear regression with bonferroni correction for 3 comparisons)

correction for 3 comparisons)

HG: 80 g glucose/day, MF: 40 g of fructose/day, HF: 80 g of fructose/day, HS: 80 g of sucrose per day

Table 2 Glucose metabolism during the clamp in all subjects after each of the four 3-week interventions

	HG	MF	HF	HS
N	9	8	9	9
Glucose concentrations				
Glucose Baseline (BL)[1](mmol/l)	4.30 ± 0.23	4.17 ± 0.17	4.34 ± 0.28	4.48 ± 0.50
Glucose Clamp[2](mmol/l)	4.50 ± 0.19	4.40 ± 0.40	4.71 ± 0.42	4.51 ± 0.28
Hepatic suppression				
Clamp (%)	70.3 ± 10.5	72.3 ± 21.9	59.4 ± 11.0[3]	72.7 ± 12.1
Endogenous glucose production rate (EGP)				
Clamp (□mol/kg*min)	3.15 ± 1.17	3.49 ± 2.06	4.17 ± 1.37	3.25 ± 1.00
BL (□mol/kg*min)	10.53 ± 0.99	10.42 ± 1.01	10.23 ± 1.52	10.64 ± 1.10
Clamp/BL	0.30 ± 0.10	0.31 ± 0.18	0.41 ± 0.11[3]	0.30 ± 0.07
Metabolic clearance rate (glucose)				
Clamp (ml/kg*min)	5.53 ± 1.43	5.60 ± 2.25	5.71 ± 2.58	5.67 ± 1.62
BL (ml/kg*min)	2.52 ± 0.22	2.53 ± 0.31	2.41 ± 0.30	2.47 ± 0.42

Clamp/BL	2.20 ± 0.55	2.28 ± 1.04	2.37 ± 0.98	2.29 ± 0.45
Total Flux (R_a)				
Clamp (□mol/kg*min)	24.6 ± 6.05	24.2 ± 7.71	26.1 ± 10.50	25.1 ± 6.66
BL (□mol/kg*min)	10.7 ± 1.00	10.6 ± 1.02	10.4 ± 1.53	10.8 ± 1.11
Clamp/BL	2.30 ± 0.56	2.33 ± 0.87	2.56 ± 1.00	2.31 ± 0.49

arithmetic mean ± SD (all values)

[1] mean value at the end of the tracer equilibration period (baseline) at time points 150, 165, 180min

[2] mean value at the end of the 2h clamp period at timepoints 90, 105 and 120 min.

[3] significantly different compared to HG ($p<0.05$, multiple linear regression with bonferroni correction for 3 comparisons)

Glucose kinetics are calculated in near steady state at the end of the tracer equilibration period (= baseline period, BL) and the clamp period as described in ‘materials and methods’.

HG: 80 g glucose/day, MF: 40 g of fructose/day, HF: 80 g of fructose/day, HS: 80 g of sucrose per day

Table 3 Dietary intake (mean ± SD) of all subjects at baseline and after each of the four 3-week interventions

	Baseline	HG	MF	HF	HS
N	9	9	8	9	9
Energy (kcal/d)	2108 ± 469	2187 ± 497	1830 ± 766	2338 ± 335	2141 ± 349
% Carbs	49 ± 8.5	54 ± 5.3	47 ± 18.5	56 ± 5.0	54 ± 6.1
% Protein	17 ± 3.5	14 ± 1.7[1]	13 ± 5.4[1]	13 ± 2.0[1]	16 ± 3.4
% fat	34 ± 6.9	31 ± 5.1	29 ± 11.7[1]	31 ± 4.3	30 ± 5.8[1]
Free fructose (g/d)	14.9 ± 6.5	7.8 ± 6.3[1]	51.2 ± 5.9[1,2]	88.2 ± 4.8[1,2]	9.5 ± 4.9
Total fructose (g/d)	40.3 ± 15.4	27.7 ± 11.2[1,2]	77.3 ± 13.9[1,2]	110.2 ± 8.8[1,2]	71.5 ± 17.3[1,2]
Free glucose (g/d)	13.8 ± 5.8	89.3 ± 4.7[1,2]	7.8 ± 3.9[2]	7.2 ± 3.5[1,2]	8.2 ± 4.0[2]
Total glucose (g/d)	39.2 ± 14.9	109.2 ± 7.8[1,2]	33.9 ± 14.7[1,2]	29.2 ± 8.3[1,2]	68.5 ± 16.1[1,2]
Sucrose (g/d)	50.8 ± 23.4	39.6 ± 14.9	52.2 ± 27.4	44.0 ± 15.1	117.1 ± 21.4[1,2]
Fibers (g/d)	22.7 ± 11.0	17.5 ± 8.4	18.3 ± 5.9	19.8 ± 7.0	16.5 ± 5.9

Ariithmetic mean ± SD (all values)

[1] significantly different compared to baseline ($p<0.05$, multiple linear regression with bonferroni correction for 4 comparisons)

[2] significantly different compared to HG ($p<0.05$, multiple linear regression including MF, HF, HG and HS with bonferroni correction for 3 comparisons)

HG: 80 g glucose/day, MF: 40 g of fructose/day, HF: 80 g of fructose/day, HS: 80 g of sucrose per day

Figure legend

Figure 1 Hepatic suppression of glucose production (%) after three weeks consumption of different sugar sweetened beverages (HG: 80 g glucose/day, MF: 40 g fructose/day, HF: 80 g fructose/day, HS: 80 g sucrose/day). * significantly different from HG, $p<0.05$. Values are means ± 1 SD.