Research Article
NAFLD and Alcohol-Related Liver Diseases

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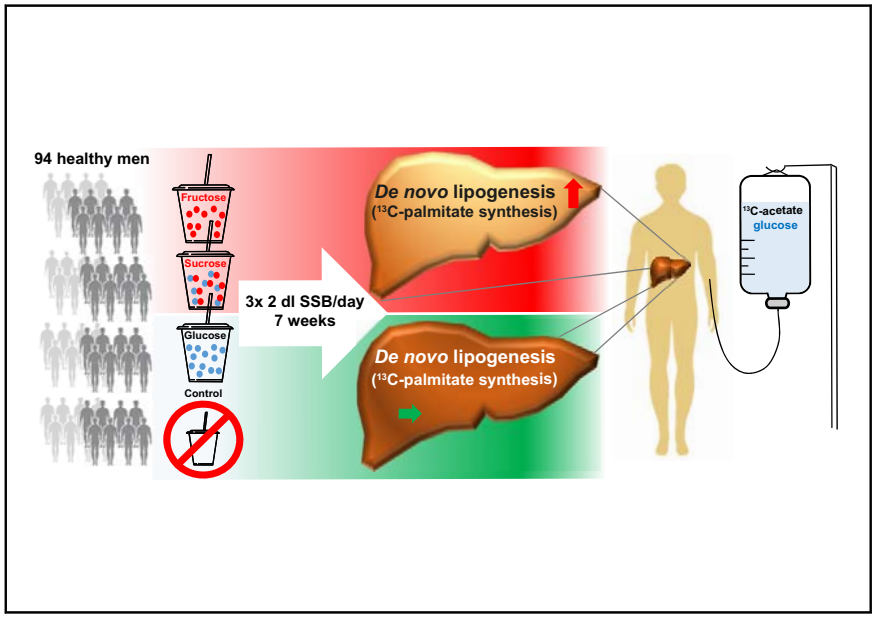
Graphical abstract



Highlights

- It is debated whether fructose drives the metabolic syndrome or non-alcoholic fatty liver disease.
- Fructose in a liquid form, within sugar-sweetened beverages, may impact liver metabolism.
- Herein, consumption of beverages containing fructose or sucrose increased hepatic lipogenesis.
- Increased hepatic lipogenic activity may promote long-term metabolic perturbations.

Authors

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Lay summary

This study investigated the metabolic effects of daily sugar-sweetened beverage consumption for several weeks in healthy lean men. It revealed that beverages sweetened with the sugars fructose and sucrose (glucose and fructose combined), but not glucose, increase the ability of the liver to produce lipids. This change may pave the way for further unfavorable effects on metabolic health.

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Fructose- and sucrose- but not glucose-sweetened beverages promote hepatic *de novo* lipogenesis: A randomized controlled trial

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Background & aims: Excessive fructose intake is associated with increased *de novo* lipogenesis, blood triglycerides, and hepatic insulin resistance. We aimed to determine whether fructose elicits specific effects on lipid metabolism independently of excessive caloric intake.

Methods: A total of 94 healthy men were studied in this double-blind, randomized trial. They were assigned to daily consumption of sugar-sweetened beverages (SSBs) containing moderate amounts of fructose, sucrose (fructose-glucose disaccharide) or glucose (80 g/day) in addition to their usual diet or SSB abstinence (control group) for 7 weeks. *De novo* fatty acid (FA) and triglyceride synthesis, lipolysis and plasma free FA (FFA) oxidation were assessed by tracer methodology.

Results: Daily intake of beverages sweetened with free fructose and fructose combined with glucose (sucrose) led to a 2-fold increase in basal hepatic fractional secretion rates (FSR) compared to control (median FSR %/day: sucrose 20.8 (p = 0.0015); fructose 19.7 (p = 0.013); control 9.1). Conversely, the same amounts of glucose did not change FSR (median of FSR %/day 11.0 (n.s.)). Fructose intake did not change basal secretion of newly synthesized VLDL-triglyceride, nor did it alter rates of peripheral lipolysis, nor total FA and plasma FFA oxidation. Total energy intake was similar across groups.

Conclusions: Regular consumption of both fructose- and sucrose-sweetened beverages in moderate doses – associated with stable caloric intake – increases hepatic FA synthesis even in a basal state; this effect is not observed after glucose consumption. These findings provide evidence of an adaptative response to regular fructose exposure in the liver.

Lay summary: This study investigated the metabolic effects of daily sugar-sweetened beverage consumption for several weeks in healthy lean men. It revealed that beverages sweetened with the sugars fructose and sucrose (glucose and fructose combined), but not glucose, increase the ability of the liver to produce lipids. This change may pave the way for further unfavorable effects on metabolic health.

Keywords: sugar; carbohydrate; liver; stable isotopes; lipid metabolism.
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Introduction

How dietary habits impact human health is a highly debated issue, as the incidence of obesity and associated diseases such as non-alcoholic fatty liver disease (NAFLD), type 2 diabetes and cardiovascular disease continues to increase.[1,2] Excessive energy intake from free sugars, and in particular from increased fructose intake, is associated with obesity, metabolic syndrome and NAFLD.[3,4] Moreover, evidence exists that high-fructose intake increases hepatic *de novo* lipogenesis and hepatic fat content and decreases hepatic insulin sensitivity independently from weight gain.[5] Even consumption of sugar-sweetened beverages (SSBs) containing moderate amounts of fructose for a few weeks changes the serum fatty acid (FA) profile and induces hepatic insulin resistance.[6,7]

Differences between hepatic fructose and glucose metabolism and fructose-specific mechanisms promoting metabolic disturbances are known.[8] Importantly, fructose-specific effects result from the fact that the liver plays the major role in fructose clearance.[9] Fructose consumption induces the hepatic master transcription factors that regulate the expression of lipogenic enzymes, *e.g.* fatty acid synthase and acetyl-CoA carboxylase, more effectively than glucose.[10–12] Increased hepatic lipogenic capacity, via upregulation of lipogenic gene expression, may be an important mechanism enhancing hexose disposal and supporting metabolic homeostasis in response to the uptake of large carbohydrate (CHO) loads.[13] Furthermore, it may enhance lipogenesis from microbiota-derived acetate.[14] Increased lipogenic capacity may not only be an acute cellular response to process large loads of CHOs/lipogenic substrates, but also a general metabolic adaptation to a diet rich in CHO over a prolonged period.[15] Thus, the amounts of CHO and possibly the type/composition of CHO in the diet modify substrate flux within the liver.

Apart from being a lipogenic substrate and an inducer for lipogenic gene expression in the liver, fructose may also affect other components of FA metabolism such as peripheral lipolysis and FA oxidation.[7,16] It may promote ectopic fat deposition in the liver and muscle, which is associated with insulin resistance.[17–19]

However, to date, it is neither known whether moderate amounts of sugar sustainably increase the flux of the FA synthesis pathway nor whether they dysregulate basal free fatty acid (FFA) delivery and oxidation. In particular, it is not known whether fructose exerts divergent effects on hepatic lipid metabolism when consumed alone or co-ingested with glucose, *i.e.* as sucrose or high-fructose corn syrup (HFCS). This is of importance because most commercially available SSBs are sweetened with HFCS (United States) or sucrose (Europe).

In this study, we investigated the metabolic effects of moderate fructose, sucrose and glucose intake – in a liquid form (as SSBs). Thus, the aim was i) to identify hexose-specific metabolic effects free from confounding factors, *i.e.* CHO overfeeding or differences in the degree of complexity or types of sugars and ii) to investigate the effects of fructose-containing SSBs, which possibly represent the most deleterious form of fructose administration as they are associated with incomplete intestinal catabolism allowing a high proportion of fructose to reach the liver.[20] First, we assessed whether a 6-week intervention with SSBs containing moderate, but biologically relevant amounts (80 g/day) of free fructose, fructose in combination with glucose (sucrose), glucose, or SSB abstinence, had a differential effect on hepatic FA synthesis, using the method of mass isotopomer distribution analysis (MIDA) (primary outcome). It was postulated that the greatest effects on basal hepatic lipogenic activity would be elicited by free fructose-containing SSBs, whereas intermediate effects would be elicited by sucrose-containing SSBs, and minor effects would be elicited by glucose SSB consumption. Secondly, effects on systemic FA flux were investigated measuring lipolysis and plasma FFA oxidation by stable isotope infusions (after 5-weeks of SSB interventions). Thirdly, we assessed the effects of SSB intake on macronutrient and caloric intake, and on anthropometry.

Materials and methods

Individuals and intervention

One-hundred and twenty-six healthy male volunteers (age 18-30 years) with BMI <24 kg/m^2 were recruited to this double-blind, randomized trial in the years 2013-2016. Study participation was limited to only 1 sex (males) as there is evidence for divergent metabolic effects of fructose on males and females. Furthermore, a body mass cut-off was defined to exclude individuals with potentially elevated liver fat content.[21,22] Eligibility was assessed by examination including medical history and blood biochemistry. Individuals with high SSB consumption (exceeding CHO 60 g/day) or more than 3 hours of physical activity per week were excluded from the study.

Sample size (n = 24 per group) was calculated based on previous studies showing changes in fractional *de novo* lipogenesis after fructose exposure.[23] Individuals were randomly assigned to 1 out of 4 dietary intervention groups by the Cantonal Pharmacy of Zurich (simple random allocation) and supplied with SSB (80 g sugar/day) containing fructose, sucrose or glucose, or no SSB (control) (Molkerei Biedermann AG, Bischofszell (provided SSB in coded containers), Swiss technology testing service, Dietikon (quality control)). As non-caloric sweeteners potentially affect human metabolism (*e.g.* appetite control, weight, microbiome composition), the present study did not use a placebo in the control group.[24,25] The study (NCT01733563) was approved by the ethical committee (Canton Zurich, Switzerland). Informed consent was obtained from all individuals and all procedures were performed in compliance with the guidelines of the Declaration of Helsinki.

Study design

After 4 weeks of SSB abstinence, individuals started a 7-week intervention with thrice daily consumption of a 2 dl SSB containing 13.3 g/dl of either fructose, sucrose or glucose with their regular meals, or continued SSB abstinence. At baseline and at the end of the study period (week 7), an oral glucose tolerance test (OGTT) was performed (Accu-Chek Dextrose O.G-T., Roche Pharma AG, 75 g). At week 5 and 6, respectively, tracer-based metabolic measurements were performed to assess plasma FFA oxidation (week 5), FA and triglyceride (TG) synthesis (week 6) and lipolysis (week 5 and 6) (Fig. S1 and Fig. 1). Individuals abstained from strenuous physical activity in the days before examinations. Examination started after a 12-hour overnight fast at the Clinical Trial Unit (University Hospital Zurich).

To assess compliance, individuals had to return empty SSB containers and not consumed SSBs and to keep SSB records. To evaluate the impact of SSBs on their dietary pattern, individuals had to keep 3-day food records before each examination day. Food records were analyzed using a software (EBISpro, University of Hohenheim, Hohenheim, Germany). Laboratory and anthropometric parameters were measured at each examination.

Metabolites and hormones

Blood glucose was measured from whole blood samples (BIOSEN C-line, EKF Diagnostic, Germany). Kits used in this study are indicated in the supplementary materials. TG, cholesterol and FFA were measured enzymatically in fresh serum. From frozen serum, C-peptide was measured using immunoradiometric assay, insulin using radioimmunoassay and leptin using ELISA. Insulin sensitivity/beta cell function and adipose tissue resistance were calculated as described previously.[26,27]

Anthropometry

Weight was determined using a digital balance accurate to 0.1 kg, and height was measured using a wall-mounted stadiometer. BMI was calculated as weight kg/height(m)^2. Waist and hip circumference were determined using a measuring tape. Body fat percentage was measured by bioelectrical impedance (AKERN BIA 101, Pontassieve, Italy). Blood pressure was measured using an automated device (Omron M6).

Metabolic studies

During examinations, individuals remained at rest with an indwelling catheter placed in an antecubital vein for tracer infusion, and a sampling catheter inserted in a vein of the contralateral arm. All infusates were prepared by the Cantonal Pharmacy of Zurich with tracers from Cambridge Isotope Laboratory, Inc. Arterialized blood was obtained applying heated hand technique.[28] Baseline blood and breath samples were drawn to measure natural $^{13}C/^2H$ enrichments.

Measurement of peripheral lipolysis and plasma FFA and total fat oxidation (week 5)

Lipolysis represented as the rate of appearance (Ra) of glycerol was assessed by [$^2H_5$]glycerol infusion and regular measurements of plasma [$^2H_5$]glycerol enrichment.[29] The tracer infusion protocols and blood samplings are indicated in Fig. 1A. Glycerol

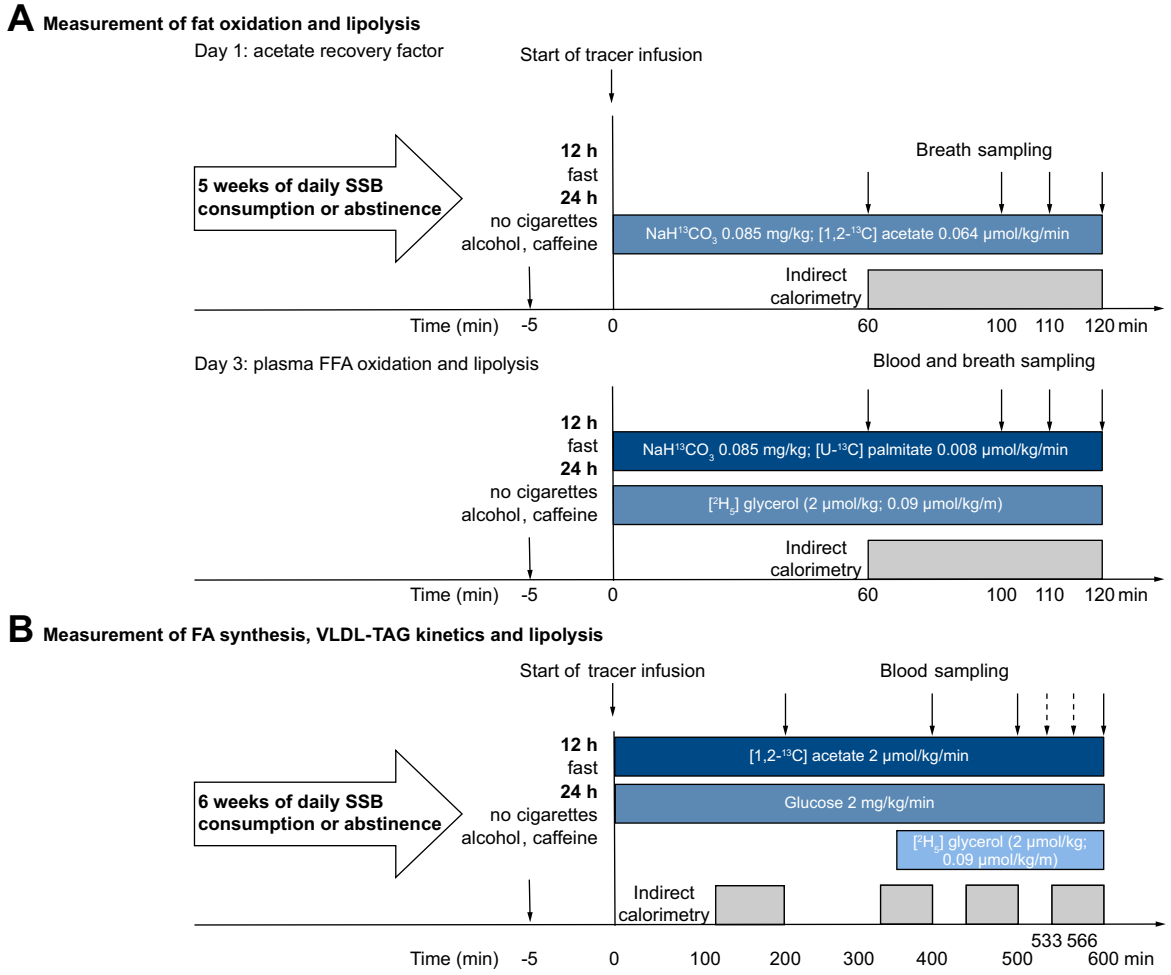

Fig. 1. Tracer examinations. (A) Day 1 for determination of the acetate recovery factor; day 3 for measurement of fat oxidation and lipolysis at week 5. (B) Measurement of FA synthesis, VLDL-TG kinetics and lipolysis at week 6. FA, fatty acid; TG, triglyceride.

derivatization/MS-analysis and calculations are described in the supplementary material.

Plasma FFA oxidation was assessed by [U-^{13}C]palmitate/albumin infusion and measurement of breath $^{13}CO_2$ enrichment and indirect calorimetry (Fig. 1A) (Ergostik, Geratherm Respiratory GmbH, Germany). MS-analysis and calculations are described in the supplementary material.

Measurement of FA, VLDL-TG synthesis/secretion and lipolysis (week 6)

Basal secretion of newly synthesized VLDL-palmitate was assessed by [1,2-^{13}C]acetate and glucose infusion and palmitate isotopomer distribution analysis (Fig. 1B). Sample preparation/derivatization and calculations are described in the supplementary material.

Simultaneously, secretion of newly synthesized VLDL-TG and lipolysis were assessed by primed constant [$^2H_5$]glycerol infusion (Fig. 1B). [$^2H_5$]glycerol enrichment in VLDL-TG was measured to assess TG synthesis/secretion. Plasma [$^2H_5$]glycerol enrichment was measured to assess the Ra of glycerol (reflecting lipolysis).[30] Sample analysis/derivatization and calculations are described in the supplementary materials.

sdLDL analysis

LDL size and subclasses were determined in frozen samples. For analysis of LDL size and subclasses, non-denaturing polyacrylamide gradient gel electrophoresis of plasma was performed and analyzed as described elsewhere.[31]

Statistics

Data were tested for normal distribution and presented accordingly as means ± standard deviations or as medians with interquartile ranges. SSB groups and the control group were compared by ANOVA testing (parametric data) or Kruskal-Wallis test (non-parametric data). When means or medians were significantly different between groups, appropriate *post hoc* tests were performed either with Tukey's or Dunn's multiple comparison's test or Mann-Whitney tests. In general, 2-tailed tests were performed. Only when 1-sided hypotheses were explicitly formulated in advance, 1-tailed tests were performed. Paired *t* test (parametric data) or Wilcoxon test (non-parametric data) were applied to compare parameters within 1 group (baseline *vs.* after intervention). The significance level was set at $p < 0.05$ and was adjusted for multiple comparisons by Bonferroni correction. Statistics were performed using GraphPad PRISM (Version 7.04)/IBM SSPS (Version 25).

Results

One-hundred and twenty-six healthy male volunteers were randomized to 4 different intervention groups, with either daily consumption of fructose-, sucrose- or glucose-sweetened beverages (80 g sugar/day), or SSB abstinence (control n = 31, glucose n = 32, fructose n = 32, sucrose n = 31). Individuals that completed the study (control n = 24, glucose n = 24, fructose n = 23, sucrose n = 23) were included in the analysis. Data from 22–24 individuals per group could be analyzed. The data from the remaining 1–3 individuals per group could not be completely collected during the study visits for technical reasons or incompliance with the study protocol. At baseline, the individuals were on average 22.7 ± 2.4 years old. Their mean body weight was 71.5 ± 7.7 kg and their BMI was normal (21.8 ± 1.6 kg/m^2).

Caloric intake and composition of diet

Total energy intake did not differ significantly between baseline and after SSB interventions (week 7) in any of the SSB groups (Table S1). Macronutrient composition varied according to the dietary intervention: SSB consumption significantly increased % caloric intake from CHOs. Absolute sugar intake (g/day) was increased according to the assigned interventions. SSB consumption decreased partially sugar intake from fruits (*i.e.* fructose and sucrose group). Percentage of caloric intake from complex CHOs was significantly reduced during the fructose and sucrose SSB interventions. Percent caloric intake from protein was significantly lowered in all SSB groups. Similarly, % caloric intake from fat was significantly lowered in the groups consuming SSBs containing fructose or glucose, and tended to be decreased in the sucrose group. SSB consumption increased *absolute* total CHO intake and partially decreased the absolute intake of other macronutrients (*i.e.* decreased fat intake in the glucose group and decreased protein intake in the sucrose group).

Anthropometry

The average body weight and percentage of body fat tended to increase during the SSB interventions in all groups (Table 1). However, this increase was only significant for the glucose SSB intervention (week 7 72.4 ± 6.6 kg *vs.* baseline 71.6 ± 6.8 kg, p = 0.009; 23.8 ± 4.8 % body fat *vs.* baseline 20.5 ± 5.4 % body fat, p = 0.007).

Vital parameters and laboratory parameters, glucose tolerance

Relevant vital and laboratory parameters are summarized in Table 1 and Table S2. Systolic and diastolic blood pressure slightly decreased during the study in all groups. Neither fasting plasma TG, glucose and insulin concentrations nor overall insulin (HOMA-IR) and adipose tissue insulin sensitivity (Adipo-IR) changed throughout the study. Furthermore, glucose tolerance assessed by an OGTT (75 g glucose) was not changed by the dietary interventions. Fasting leptin concentrations significantly increased in the sucrose (p = 0.019) and glucose (p = 0.033) group, but not in the fructose group (p = 0.291).

Concentrations, pool sizes, and distributions (% of plasma TG bound to VLDL) of plasma TGs and palmitate pool sizes after 6 weeks of dietary interventions are summarized in Table S3 (fasting state). There were no significant differences between the dietary intervention groups. Fatty acid profiles of VLDL-TGs are presented in Table S4 (fasting state). Overall, SSB interventions did not change FA profiles. There was only a significant decrease in oleic acid (C18:1n9) in the fructose group compared to the control group (p = 0.038). Accordingly, the saturation index C18:1n9/C18:0 was decreased in the fructose group compared to the control group (p = 0.030).

Synthesis and secretion of VLDL-bound palmitate and VLDL-TG (week 6)

We measured basal hepatic fractional and absolute secretion rates of newly synthesized VLDL-palmitate to assess the activity of the FA synthesis pathway during the infusion of 2 mg/kg/min glucose (which provided the lipogenic substrate). Palmitate accounts for 75–85% of all newly synthesized FAs by the liver and thus represents a suitable proxy for newly synthesized FAs.[30] The fractional secretion rate (FSR, defined as the fraction of the plasma VLDL-palmitate pool that is newly synthesized per unit of time) in the basal state was higher after both fructose and sucrose SSB interventions than after the glucose SSB intervention and control. Consumption of beverages containing fructose resulted in 2-fold increased basal FSR of newly synthesized FA compared to control (median FSR %/day: sucrose 20.8 (p = 0.0015); fructose 19.7 (p = 0.013); control 9.1) (Fig. 2A). In contrast, the same amounts of glucose did not change FSR (median of FSR %/day 11.0 p = 0.16).

Table 1. Anthropometric and vital parameters.

	Control (n = 24)		Glucose (n = 22)		Fructose (n = 23)		Sucrose (n = 23)	
	Baseline	Week 7	Baseline	Week 7	Baseline	Week 7	Baseline	Week 7
Weight[1] (kg)	70.4 ± 8.1	70.6 ± 8.0	71.6 ± 6.8	72.4 ± 6.6[A]	69.2 ± 7.7	69.5 ± 7.4	75.5 ± 7.3	76.00 ± 7.0
BMI (kg/m^2)[2]	21.0 (2.8)	21.3 (1.8)	22.0 (2.3)	22.4 (2.6)[A]	21.2 (2.3)	21.5 (2.4)[A]	22.9 (1.4)[B]	23.3 (2.0)[C]
WHR[1]	0.88 ± 0.03	0.89 ± 0.04	0.85 ± 0.04[B]	0.85 ± 0.04[C]	0.87 ± 0.05	0.87 ± 0.04	0.87 ± 0.04	0.88 ± 0.06
Body fat (%)[1]	21.0 ± 5.5	21.9 ± 4.2	20.5 ± 5.4	23.8 ± 4.8[A]	20.5 ± 5.5	21.7 ± 5.1	21.4 ± 6.8	22.5 ± 4.7
Muscle (%)[1]	56.7 ± 5.1	53.6 ± 3.7[A]	56.6 ± 4.9	54.0 ± 3.9[A]	56.3 ± 4.2	55.3 ± 5.1	55.5 ± 5.4	55.1 ± 4.3
Systolic blood pressure (mmHg)[1]	127.0 ± 10.7	122.9 ± 9.3	125.7 ± 9.0	125.6 ± 11.3	122.6 ± 8.8	121.5 ± 6.5	126.2 ± 7.2	123.1 ± 9.2
Diastolic blood pressure (mmHg)[1]	69.7 ± 10.1	66.1 ± 8.5	71.6 ± 8.6	66.7 ± 9.55	67.3 ± 11.8	65.2 ± 8.7	67.2 ± 8.1	63.8 ± 6.5[A]

SSB, sugar-sweetened beverage; WHR, waist-to-hip ratio.
[1]Arithmetic means ± SDs
[2]Medians (Interquartile range)
[A]Significant differences between baseline and after 7-weeks SSB interventions (p <0.05) (Paired t test or Wilcoxon)
[B]Significant differences between SSB intervention groups and control at baseline (p <0.05) (ANOVA with Tukey's multiple comparisons test or Kruskal-Wallis with Dunn's multiple comparison's test).
[C]Significant differences between SSB intervention groups and control after 7-weeks SSB interventions (p <0.05) (ANOVA with Tukey's multiple comparisons test or Kruskal-Wallis with Dunn's multiple comparison's test).

Table 2. Indirect calorimetry (Week 5), fasting condition.

	Control (n = 23)	Glucose (n = 24)	Fructose (n = 23)	Sucrose (n = 22)
REE (kcal/kg/min)	0.0229 ± 0.0130	0.02102 ± 0.0046	0.0194 ± 0.0038	0.0195 ± 0.0029
NPRQ	0.76 ± 0.09	0.76 ± 0.10	0.74 ± 0.08	0.76 ± 0.08
Fat oxidation (mg/kg/min)	1.45 ± 0.60	1.59 ± 0.83	1.56 ± 0.64	1.43 ± 0.69
CHO oxidation (mg/kg/min)	1.20 ± 0.88	1.13 ± 1.43	0.78 ± 0.77	1.10 ± 1.00

Arithmetic means ± SD
No significant differences between SSB interventions and control (ANOVA)
CHO, carbohydrate; NPRQ, non-protein respiratory quotient; REE, resting energy expenditure; SSB, sugar-sweetened beverage.

Similarly, absolute secretion rates of newly synthesized VLDL-palmitate, calculable from FSR and the VLDL-palmitate pool size, tended to be increased by the fructose intervention ($p = 0.055$) and were significantly increased by the sucrose SSB intervention ($p = 0.008$) compared to control in the basal state (Table S5). The total rate of secretion of VLDL-palmitate (*de novo* synthesized and preformed palmitate) also tended to be higher after the fructose and sucrose SSB interventions compared to control in the basal state, although this was below statistical significance. Parameters for calculation of the FSR of newly synthesized VLDL-palmitate are summarized in Table S5.

For hepatic TG synthesis and secretion, FA uptake from the plasma is of importance.[32] Thus, peripheral lipolysis, a source of FA for hepatic TG synthesis, was also measured. SSB consumption did not impact on basal peripheral lipolysis (Table S6).

We also measured basal fractional and absolute secretion rates of newly synthesized VLDL-TG with incorporated plasma glycerol. There were no differences of fractional or absolute rates of secretion of these VLDL-TGs between groups consuming SSB (for 6 weeks) and controls (Fig. 2B and Table S6).

Whole-body fuel use (week 5)

Resting energy expenditure (REE), total fat and CHO oxidation as well as non-protein respiratory quotient (NPRQ) were measured after 5 weeks of SSB interventions by indirect calorimetry. There were no differences regarding REE, total fat and CHO oxidation or NPRQ between the groups (Table 2). Energy expenditure ranged from 0.019 ± 0.004 kcal/kg per min to 0.023 ± 0.013/kg per min in the different groups. In the fasting state, total fat oxidation varied from 1.43 ± 0.69 to 1.59 ± 0.83 mg/kg per min and CHO oxidation from 0.78 ± 0.77 to 1.20 ± 0.88 mg/kg per min.

Fig. 3 shows the analysis of different components of FA metabolism. The basal Ra of glycerol (reflecting lipolysis) did not differ between the intervention groups (Fig. 3A). Neither basal rates of plasma FFA oxidation nor total FA oxidation differed between the groups (Fig. 3B,C). The percentage of infused U-13C-palmitate that was oxidized was not significantly different between the intervention groups (Fig. 3D).

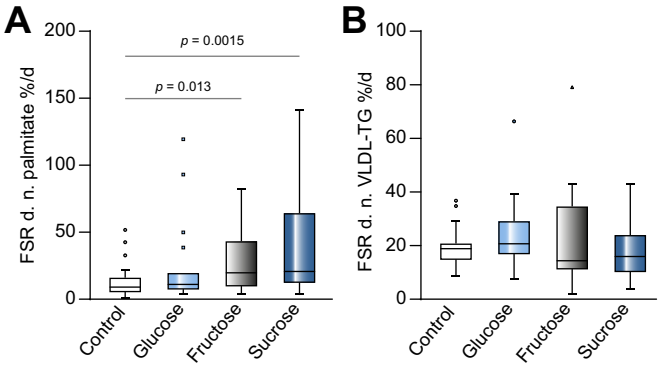

Fig. 2. FSR of newly synthesized palmitate and newly synthesized VLDL-TG containing plasma glycerol after 6-week SSB interventions. (A) FSR of newly synthesized palmitate are significantly increased in the fructose and sucrose group compared to the control group (fructose $p = 0.013$; sucrose $p = 0.0015$; glucose $p = 0.16$). Fructose n = 23; Glucose n = 23; Sucrose n = 23; Control n = 23. (B) FSR of newly synthesized TG are not significantly different between the SSB groups and control. Fructose n = 23; Glucose n = 23; Sucrose n = 22; Control n = 21. Kruskal-Wallis test for comparison of SSB intervention groups *vs.* control, Mann-Whitney test (1-tailed) for comparison of fructose *vs.* control and sucrose *vs.* control. Significance level $p = 0.017$ (Bonferroni corrected)). FSR, fractional secretion rates; SSB, sugar-sweetened beverage; TG, triglyceride.

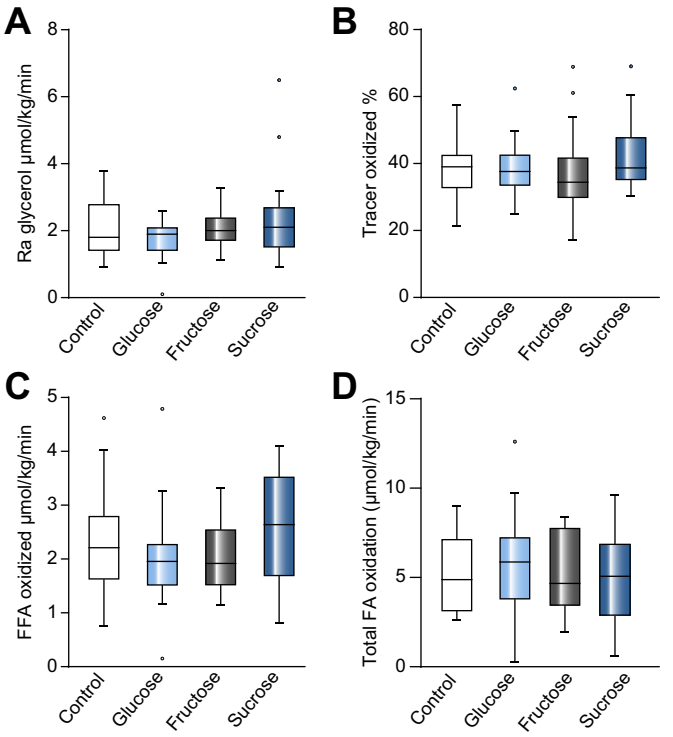

Fig. 3. Lipolysis, percentage infused U–13C-palmitate oxidized, oxidation of plasma FFA and total FA after 5-weeks SSB interventions. (A) Rate of appearance of glycerol (representing lipolysis). No significant differences between the groups. Fructose n = 23; Glucose n = 24; Sucrose n = 23; Control n = 23. (B) Percentage of infused tracer oxidized. No significant differences between the groups. Fructose n = 23; Glucose n = 24; Sucrose n = 23; Control n = 23. (C) Oxidation rates of plasma FFA. No significant differences between the groups. Fructose n = 22; Glucose n = 24; Sucrose n = 23; Control n = 23. (D) Total FA oxidation. No significant differences between the groups. Fructose n = 22; Glucose n = 24; Sucrose n = 24; Control n = 23. Kruskal-Wallis test for comparison of SSB intervention groups *vs.* control. FA, fatty acid; FFA, free fatty acid; SSB, sugar-sweetened beverage.

sdLDL

Large, buoyant LDL particles (subgroups I and IIa) tended to decrease at 7 weeks after all SSB interventions (Table S7); this decrease was significant in the sucrose intervention group, with a decrease of large LDL particles (subgroup I) by >13% ($p = 0.012$). Similarly, small, dense LDL particles tended to increase after all interventions. The increase was significant in the sucrose group (LDL particles of subgroup IIIa, $p = 0.031$).

Discussion

This study demonstrates that daily consumption of beverages containing moderate amounts (comparable to those provided by commercial soft drinks/fruit juices) of either fructose or sucrose, but not glucose, increases hepatic FA synthesis in healthy men in a basal state. SSB consumption (with *ad libitum* meals) influenced absolute macronutrient intake (*i.e.* decreased fat and protein intake) but did not increase total energy intake. Measurements of FA synthesis applying the MIDA approach revealed that consumption of fructose or the fructose-glucose disaccharide sucrose (3 times 2 dl SSB containing 13.3 g sugar/dl) increased the FSR of newly synthesized palmitate even at a basal state, possibly reflecting the persistence of increased lipogenic gene expression. This "metabolic switch" in hepatocytes may enable them to quickly respond to recurrent fructose loads with an increased lipogenic capacity, but may also enhance lipogenesis fed by short-chain FAs produced by bacterial fermentation *i.e.* acetate.[14] Contrasting with our hypothesis, fructose and sucrose likewise increase the FSR. This may result from the facilitating effects of glucose ingestion, which is important for the induction of lipogenic gene expression. Firstly, glucose strongly enhances intestinal fructose uptake and secondly, insulin is required for the maximal induction of SREBP1c and lipogenic gene expression.[33,34] Moreover, fructose stimulates hepatic glucose uptake through glucokinase activation, possibly enhancing glucose flux towards the liver and increasing the abundance of glycolytic intermediates and lipogenic substrates.[35,36] This is in line with the notion that the monosaccharide composition determines the extent of "monosaccharide flooding" of the liver and thus is a key determinant of lipogenic gene expression and therefore hepatic lipogenic activity.

Enhanced lipogenesis after both fructose and sucrose ingestion is seemingly contrary to our previous observation of an increased relative abundance of plasma palmitate only after daily consumption of SSBs containing fructose but not sucrose.[7] However, the MIDA approach used in this study assesses basal *de novo* FA synthesis, whereas measurement of plasma palmitate reflects hepatic FA synthesis in both the postprandial and fasting states. The reported increased ratio of palmitic to linoleic acid after prolonged daily fructose consumption may therefore reflect the importance of fructose as a lipogenic *substrate*. VLDL-TG secretion was not increased at the basal state in this study, consistent with unchanged/normal fasting TG levels after the dietary intervention. Nevertheless, a fructose-induced enhanced lipogenic activity may increase *postprandial* hepatic FA/TG production and fat content[37] and contribute to postprandial hypertriglyceridemia after consumption of high-fructose loads (*e.g.* SSBs). This may not be primarily due to the accumulation of newly synthesized FAs, but rather due to concomitant down-regulation of FA oxidation.[36] Thus, preformed and newly synthesized FAs, as well as copious glycerol from fructolysis, may promote re-esterification and VLDL production.[32,38] The effect of fructose consumption on hepatic fat content was not examined in this study. A recent study by Smajis *et al.* in healthy men demonstrated that the daily consumption of 150 g of fructose over 8 weeks did not result in net fat retention in the liver.[39] However, the authors did not specify whether fructose was consumed in liquid form or solid food, rendering it difficult to compare the 2 studies. Thus, it remains an open question whether fructose *in the form of SSB* with fast fructose absorption and significant overflow to the liver increases hepatic fat content in the long term when possible compensatory mechanisms such as increased VLDL-TG secretion may be exhausted beyond their limits. Nevertheless, our data demonstrates that fructose consumed in SSBs is a potent stimulator of *de novo* lipogenesis which is recognized *per se* as a risk factor for NAFLD and diabetes.[40,41] Increased hepatic lipogenic activity and concurrently increased intestinal fructose absorption and hepatic clearance capacity may increase the susceptibility to liver-related pathologies.[42] Moreover, a recent study demonstrated that acetate generated by microbial fermentation of fructose also feeds hepatic lipogenesis, pointing to possible interactions between fructose and dietary sources of acetate such as ethanol and fermentable fibers.[14]

VLDL-TG synthesis and secretion is also determined by the FA flux towards the liver.[32] Accordingly, we measured rates of peripheral lipolysis during the measurement of FA synthesis, when substrate for FA synthesis was provided by a glucose infusion, which induced an insulin response. Rates of peripheral lipolysis did not differ between the groups, indicating that SSB consumption over several weeks does not induce adipose tissue insulin resistance. This is in contrast to a study reporting impaired insulin-induced suppression of adipose tissue lipolysis after only 6 days of high-fructose overfeeding (3 g/kg of body weight fructose provided as 20% fructose solutions).[23] Notably, our study investigated the metabolic effects of SSB consumption close to a real-life setting, instead of sugar overfeeding.

Impaired FA utilization may play a role in the etiology of skeletal muscle and hepatic insulin resistance.[43] We measured plasma FFA oxidation to assess whether regular SSB consumption is a primary factor that decreases basal FA oxidation. Plasma FFA oxidation was not impaired by moderate SSB consumption. Replacement of lipid energy substrate in the skeletal muscle by metabolites generated from fructose, *i.e.* lactate or glucose, may spare lipids from oxidation and increase intramuscular fat content, which is supposed to decrease muscular FFA uptake and oxidation.[43,44] Decreased FFA utilization by the skeletal muscle is supposed to increase FFA flux to the liver, which could – in combination with impaired hepatic FA oxidation due to regular fructose consumption – promote hepatic fat deposition and insulin resistance.[16,43]

Dietary composition impacts whole-body fuel selection. Lipolysis and the proportion of released FA that is oxidized inversely correlate with CHO intake.[45] Five days of high-CHO overfeeding (type of CHO not specified) impacts whole-body fuel selection even at an overnight fasting state in healthy men. It induces an insulin-resistant state with increased hepatic glucose production and oxidation despite increased serum insulin concentrations.[45] To test whether daily SSB consumption increases CHO oxidation in the fasting state we measured CHO and total fat oxidation rates by indirect calorimetry. Unlike individuals overfed with CHOs for 5 days, individuals with

prolonged moderate intakes of SSBs containing fructose, sucrose or glucose for several weeks did not show increased fasting CHO oxidation.[45]

Not only fat deposition *per se* but also fat distribution, independently of obesity, is of particular importance for the development of type 2 diabetes.[46] However, determination of subcutaneous, intramuscular or hepatic fat deposition was beyond the scope of this study. Overall SSB interventions tended to increase body weight and fat. It might be hypothesized that the significant increase of % body fat and fasting leptin concentrations after the glucose intervention were caused by an increase of mainly subcutaneous adipose tissue, which was observed to produce higher leptin amounts than visceral fat.[47]

This study confirmed that consumption of fructose-containing SSBs changes LDL composition as described previously.[48] In the intervention group with added sucrose, there was a significant change of the LDL particle distribution towards smaller, more atherogenic particles associated with cardiovascular disease.[49]

To our knowledge, this is the first study to apply tracer-based methodology to quantify metabolic changes induced by interventional SSBs (with moderate fructose, sucrose or glucose content) alongside the habitual diet. Thus, this study provides findings that are highly relevant to everyday life. The finding that regular consumption of fructose-containing beverages increases hepatic basal lipogenic activity is in accordance with mechanistic animal studies that showed that fructose and sucrose are more potent inducers of lipogenic gene expression than glucose.[10]

This study bears some limitations. Inherent problems of this type of study remain i) little control for compliance to the protocol of individual individuals and ii) unknown intestinal capacities (fructose tolerability) of the individuals to take up fructose. Accordingly, inter-subject variability may reflect individual compliance and differences in intestinal fructose uptake. Though a valuable tool for tracing *in vivo* kinetics of human metabolism, tracer-based methodology provides only estimations of kinetics as it is based on various assumptions and mathematical models. Thus, in the present study the use of 13C-acetate as a tracer and MIDA may have led to an underestimation of *de novo* fatty acid synthesis.[50] We measured the synthesis and secretion of VLDL-TG formed from plasma glycerol which represents a fraction of total VLDL-TG. The contribution of VLDL-TG with glycerol originating from the glyceroneogenic or glycolytic pathway has not been assessed in the study.[51]

Conclusions

In summary, our study provides evidence that daily consumed fructose-containing beverages induce profound alterations in hepatic lipid metabolism, manifested as an increased basal lipogenic capacity (increased FSR of newly synthesized FA). Very interestingly, pure fructose (80 g fructose/day) and sucrose (40 g fructose plus 40 g glucose/day) increased basal hepatic FA synthesis comparably. Other features of the metabolic syndrome, *i.e.* fasting hypertriglyceridemia, hyperglycemia, hyperinsulinemia, peripheral/adipose tissue insulin resistance were not observed in this study of 7 weeks. This indicates that increased basal hepatic FA synthesis is probably the first metabolic change induced by regular fructose-containing SSB consumption. We hypothesize that this metabolic switch towards a higher lipogenic activity in the liver may pave the way for further changes affecting metabolic health.

Abbreviations

BMI, body mass index; CHO, carbohydrate; FA, fatty acid; FFA, free fatty acid; FSR, fractional secretion rate; HFCS, high-fructose corn syrup; MIDA, mass isotopomer distribution analysis; NAFLD, non-alcoholic fatty liver disease; NPRQ, non-protein respiratory quotient; OGTT, oral glucose tolerance test; REE, resting energy expenditure; sdLDL, small dense lipoprotein; SSB, sugar-sweetened beverage; TG, triglyceride; VLDL, very low density lipoprotein.

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Conflict of interest

The authors have declared that no conflicts of interest exist with regard to this study.

Please refer to the accompanying ICMJE disclosure forms for further details.

Authors' contributions

M.H., B.G., and K.B. designed the research; B.G., A.N., M.H., A.E., H.K., L.T., and P.A.G. conducted the research; B.G., N.D. and P.A.G., analyzed the data; B.G. and P.A.G wrote the manuscript, and A.N., M.H., A.E., H.K., L.T. and G.A.S. revised the manuscript; B.G. and P.A.G. had access to all data and had primary responsibility for the final content.

Data availability statement

All data, materials and methods in this study are available upon request from the authors.

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Supplementary data

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Author names in bold designate shared co-first authorship

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