Different acute effects of fructose and glucose administration on hepatic fat content

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ABSTRACT

Background: Diets rich in fat and added sugars (especially fructose) play an important role in the pathogenesis of nonalcoholic liver disease (NAFLD), but there is only limited information on the acute effects of these nutrients on hepatic fat content (HFC).

Objectives: We therefore explored how the administration of high-fat load, glucose, fructose, and combinations thereof affects HFC measured in vivo using proton magnetic resonance spectroscopy (^{1}H-MRS) in healthy subjects.

Methods: Ten healthy nonsteatotic male volunteers (age 38.5 ± 9.6 y, body mass index [BMI, kg/m^2] 26.9 ± 2.7) underwent, in random order, 6 experiments, each lasting 8 h, that included: *1*) fasting; *2*) a high-fat load (150 g of fat [dairy cream] at time 0); *3*) glucose (3 doses of 50 g at 0, 2, and 4 h); *4*) a high-fat load with glucose; *5*) fructose (3 doses of 50 g at 0, 2, and 4 h); and *6*) a high-fat load with fructose. HFC was measured using ^{1}H-MRS prior to test meal administration (before time 0) and at 3 and 6 h. Plasma concentrations of triglycerides, nonesterified fatty acids, glucose, and insulin were monitored throughout each experiment.

Results: HFC increased to 119 ± 19% ($P < 0.05$) and 117 ± 17% ($P < 0.01$) of baseline when subjects consumed a high-fat load alone or a high-fat load with fructose, respectively, but was not affected when glucose was coadministered with a high-fat load. HFC was not affected when subjects had fasted or had consumed repeated doses of fructose. When subjects were administered 3 doses of glucose, HFC dropped to 85 ± 13% ($P < 0.05$) of baseline.

Conclusions: Our results demonstrate that fructose and glucose have a different immediate impact on HFC in humans in vivo. **Clinical trial registry:** The study was registered at clinicaltrials.gov and obtained clinicaltrials.gov identifier: NCT03680248. *Am J Clin Nutr* 2019;109:1519–1526.

Keywords: hepatic fat content, glucose, fructose, insulin, nonesterified fatty acids, dietary fat, proton magnetic resonance spectroscopy, healthy volunteers

Introduction

The epidemic of nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis is becoming a major challenge for health systems worldwide (1). Importantly, the first stage of the disease is simple steatosis caused by excessive fat accumulation in the liver (in the absence of significant alcohol consumption). Hepatic fat comes from 3 major sources—nonesterified fatty acids (NEFAs) released from adipose tissue, dietary fat, and hepatic de novo lipogenesis (DNL) (2). However, hepatic triglycerides (TGs) are subsequently metabolized or, more importantly, quickly secreted from the liver in VLDLs to provide fuel for extrahepatic tissues, which explains why TGs should ideally not accumulate in the liver. Steatosis develops when the delicate balance between these processes is disrupted and hepatic TG production outweighs TG export to other tissues.

In healthy nonsteatotic subjects, the daily turnover of hepatic TGs might even exceed hepatic fat content (HFC) (3). It is estimated that ≤20% of dietary fat is delivered to the liver both as TGs of chylomicron remnants and spillover fatty acids released from dietary TGs by lipoprotein lipase in the circulation and not captured by extrahepatic tissues. Therefore, a sufficient

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Supplemental Table 1 and Supplemental Figures 1 and 2 are available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents at https://academic.oup.com/ajcn/.

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Abbreviations used: ALT, alanine aminotransferase; AST, aspartate aminotransferase; AUIC, area under increment curve; CRP, C-reactive protein; DNL, de novo lipogenesis; FGF, fibroblast growth factor; HFC, hepatic fat content; NAFLD, nonalcoholic liver disease; NEFA, nonesterified fatty acid; PRESS, point resolved spectroscopy sequence; TG, triglyceride; VOI, volume of interest; ^{1}H-MRS, proton magnetic resonance spectroscopy.

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TABLE 1 Characteristics of male volunteers included in the study[1]

Parameter	Mean ± SD
Number	10
Age at study entry (y)	38.5 ± 9.6
BMI (kg/m^2)	26.9 ± 2.7
Waist circumference (cm)	93.1 ± 8.5
Total body fat (kg)	20.1 ± 5.4
Hepatic fat content (%)	1.9 ± 1.0
TG (mmol/L)	1.4 ± 1.0
Cholesterol (mmol/L)	4.6 ± 0.7
NEFA (after overnight fast) (mmol/L)	0.33 ± 0.07
Fasting glucose (mmol/L)	5.5 ± 0.3
Fasting insulin (mIU/L)	6.0 ± 2.4
HOMA-IR	1.5 ± 0.7

[1]The values are an average of 6 measurements obtained throughout the study (except for age). NEFA, nonesterified fatty acids; TG, triglycerides.

amount of dietary fat provided to healthy nonsteatotic subjects should induce changes in HFC that are detectable using proton magnetic resonance spectroscopy (^{1}H-MRS) within hours (4). However, liver fat accumulation can be strongly affected by the coadministration of other nutrients, especially carbohydrates, which induce regulatory responses diverting metabolism to carbohydrate oxidation and fat storage and possibly also affecting HFC.

The current epidemic of NAFLD seems to be tightly linked to excessive fructose consumption (5, 6). However, it has not yet been clarified whether fructose is just a marker of increased caloric intake leading to insulin resistance and ectopic fat accumulation or the culprit directly involved in fat accumulation in the liver. It has been documented that metabolism of fructose, contrary to that of glucose, can indeed induce metabolic changes possibly promoting hepatic fat accumulation (7, 8) but up to now there is no direct proof that fructose consumption induces immediate fat accumulation in the liver.

In this study we therefore aimed to determine, using ^{1}H-MRS, the effects of fructose and/or glucose administration on HFC. The sugars were administered alone or in combination with a high-fat load (150 g fat [dairy cream]). HFC was measured using ^{1}H-MRS in the morning after an overnight fast before a fat load, and 3 and 6 h later after the start of dietary intervention.

Methods

Subjects

Ten healthy male volunteers were enrolled into the study (**Table 1** and **Supplemental Figure 1**). Participants were recruited from May 2016 to July 2017. The inclusion criteria were: normal response to the oral glucose tolerance test; normal values of glycated hemoglobin, alanine aminotransferase (ALT), and aspartate aminotransferase (AST); and HFC <5%, as determined by ^{1}H-MRS. Exclusion criteria included: body mass index (BMI, in kg/m^2) >30; use of pharmacological agents affecting insulin sensitivity and lipid metabolism; and inability to undergo ^{1}H-MRS examination.

Experimental design

Each of the 10 volunteers underwent 6 experiments, each lasting 8 h, that differed in the diet consumed during the experiment. After an overnight fast all experiments started in the morning with ^{1}H-MRS examination for HFC determination. Next, a cannula for blood draws was inserted into the antecubital vein and the first blood sample taken; the volunteers then consumed the experimental breakfast (time 0). HFC was again determined by ^{1}H-MRS 3 and 6 h after the experimental breakfast (**Figure 1**). The blood for determination of TGs, NEFAs, glucose, insulin, and glucagon was subsequently drawn at 0.5, 1, 2, 2.5, 3, 4, 4.5, 5, and 6 h in each experiment. The dietary interventions were as follows: *1*) fasting (Fasting experiment); *2*) a high-fat load alone (460 mL dairy cream containing 150 g fat, 19.5 g carbohydrate, 13.5 g protein) administered at time 0 h (Fat experiment); *3*) 50 g glucose dissolved in fruit tea administered at 0, 2, and 4 h (Glucose experiment); *4*) a high-fat load at time 0 + 50 g glucose at 0, 2, and 4 h (Fat + Glucose experiment); *5*) 50 g fructose dissolved in fruit tea administered at 0, 2, and 4 h (Fructose experiment); and *6*) a high-fat load at time 0 + 50 g fructose at 0, 2, and 4 h (Fat + Fructose experiment) (Figure 1). The order of the experiments was randomized and the interval between individual experiments was ≥14 d (14–595 d, median interval 34 d). The volunteers were asked not to change their lifestyle and eating habits throughout the study. In each of the experiments, information on the volunteers' physical activity and food on the day before the experiment and their changes between experiments was obtained. In each experiment, weight and waist and hip circumferences were also measured, and the body fat content and body composition were determined by bioelectrical impedance analysis (BODYSTAT 1500, Bodystat Ltd) to verify that there were no significant changes in the lifestyle between experiments. Moreover, cholesterol, AST, ALT, and C-reactive protein (CRP) were determined in baseline fasting samples (time 0) to check the health status of the subjects.

Magnetic resonance measurements

HFC was measured by in vivo ^{1}H-MRS. Examinations were performed using a 3T whole body scanner (3T Trio, Siemens) equipped with an 8-channel surface body coil. Automatic and manual shimming were combined to reach a linewidth <50 Hz. Standard PRESS (point-resolved spectroscopy sequence) single-voxel spectroscopy was used (echo time 30 ms and repetition time 4500 ms) to measure HFC. The voxel size of our volume of interest (VOI) was set at 40 × 30 × 25 mm, and the VOI position in liver segments V/VIII was carefully checked during all consecutive examinations. One spectrum acquisition during each breath-hold was obtained with the measurement repeated 3 times. Relaxation times were measured using the same sequence (PRESS) with echoes of 30, 50, 68, 135, 180, and 270 ms.

Calculations of T2 were done using MATLAB software (Mathworks). T2 relaxation times of water and CH_2 groups were found in the range 30 ± 3 ms and 53 ± 11 ms without any extreme values, and these T2 times were in agreement with published data. T1 values were not calculated as only 1 acquisition spectrum was measured.

Spectra were evaluated using LCModel version 6.2 (http://s-provencher.com/lcmodel.shtml). Three lipid signals of aliphatic protons in the range 0.0−3.5 ppm (–CH_2 1.2−1.4 ppm; –CH_3 0.8−0.9 ppm; –CH_2CH_2CH=CH 1.9−2.1 ppm) and water and olefinic proton signals (4.7 and 5.3 ppm) were fitted as described

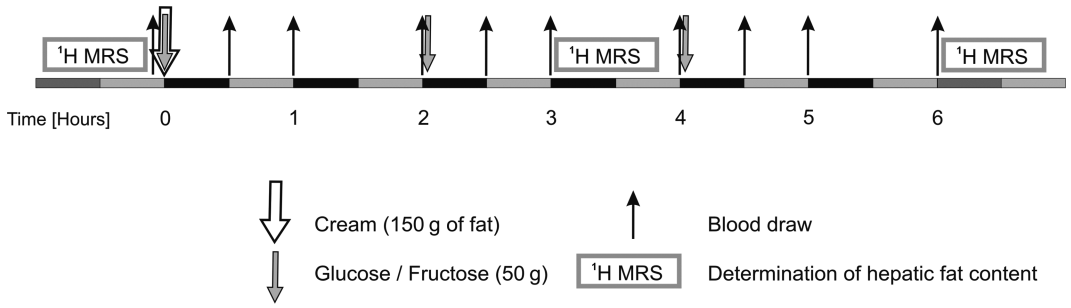

FIGURE 1 Study design. Ten healthy male adults underwent the 6 experiments. Hepatic fat content was determined 3 times during each experiment using proton magnetic resonance spectroscopy (^{1}H-MRS) and 10 blood samples were taken. The experiments differed in the type of food administered. In each experiment, subjects received one of the following: fasting; high-fat load (150 g fat at 0 h); 3 × 50 g glucose at 0, 2, and 4 h; high-fat load (150 g fat at 0 h) and 3 × 50 g glucose at 0, 2, and 4 h; 3 × 50 g fructose at 0, 2, and 4 h; high-fat load (150 g fat at 0 h) and 3 × 50 g fructose at 0, 2, and 4 h.

in an earlier article (9). The percentage of HFC was calculated according to Longo et al. (10).

Biochemical analysis

Blood was collected into vacutainers with EDTA and immediately chilled on ice. The aliquots of plasma were subsequently stored at −80°C until analysis. The concentration of TGs was determined using enzymatic kits manufactured by Roche Diagnostics, glucose concentration was determined using a PLIVA Lachema Diagnostika kit, and NEFAs were measured using a kit manufactured by Wako Chemicals GmbH. Insulin was measured using an immunoradiometric assay kit (Beckman Coulter) and glucagon using a radioimmunoassay kit (EMD Millipore Corporation).

Statistics

The data are presented as means ± SD. The area under increment curves (AUICs) for biochemical parameters in the plasma were calculated using the trapezoid rule after correction to baseline values. The statistical analyses were carried out using GraphPad Prism 5 (GraphPad Software). The primary end point of our study was to determine whether HFC changes over time after particular dietary interventions. The changes in TGs, NEFAs, glucose, insulin, and glucagon were evaluated as secondary end points. The percentage changes in HFC were evaluated using ANOVA for repeated measures and Dunnett's multiple comparison posttest. Changes in biochemical parameters over time were evaluated using the same tests. AUIC values were compared using repeated measures ANOVA and Tukey's multiple comparison posttest. One-sample *t*-test was used to determine the AUIC difference from zero. A P value <0.05 was considered statistically significant.

Study approval

All participants gave written informed consent prior to inclusion in the study, which had been approved by the Joint Ethics Committee of the Institute for Clinical and Experimental Medicine and Thomayer Hospital in Prague, Czech Republic. All work was conducted in compliance with the principles of the Declaration of Helsinki.

Results

Each of the 10 volunteers underwent all 6 experiments. Importantly, the baseline values of parameters reflecting the health and metabolic status of subjects did not differ between experiments (**Supplemental Table 1**), suggesting that there were no pronounced changes in the lifestyle of subjects. This allowed us to study the effect of particular dietary interventions.

Fasting has no effect on HFC

In a control experiment with no food administered to subjects (Fasting experiment), TG and glucose concentrations did not change during the experiment, whereas the NEFA concentration steadily rose from 0.34 ± 0.16 to 0.59 ± 0.10 mmol/L ($P < 0.001$) at the end of the experiment (**Figure 2**). In line with such a finding was the observation that the NEFA AUIC was higher than zero (0.87 ± 0.76 mmol∗h/L, $P = 0.005$) (**Figure 3**). The insulin concentration declined during the experiment to approximately one-half of baseline and, concordantly, the insulin AUIC reached a negative value. Glucagon concentration was unaffected and because there were no differences in the glucagon AUICs between all experiments, glucagon data (**Supplemental Figure 2**) were not further considered. HFC did not change throughout this experiment as measured after an overnight fast and 3 and 6 h later (**Figure 4**).

High-fat load (150 g) induces an increase in HFC

Administration of 150 g of fat (Fat experiment) increased triglyceridemia, and TG concentrations did not return to baseline until the end of the experiment (Figure 2), with the TG AUIC being 5.40 ± 2.22 mmol∗h/L. The NEFA concentration rose from 0.34 ± 0.12 to 0.76 ± 0.22 mmol/L ($P < 0.001$) at the end of the experiment, and the NEFA AUIC reached a positive value of 1.08 ± 0.68 mmol∗h/L ($P < 0.001$) and was nonsignificantly different from the NEFA AUIC in the Fasting experiment. Glucose concentration marginally decreased throughout the experiment as documented by a negative AUIC value of −1.51 ± 1.77 mmol∗h/L ($P < 0.025$). Insulinemia slightly increased during the experiment, with the insulin AUIC reaching 18.5 ± 19.4 mIU∗h/L ($P = 0.015$). HFC rose by 19% ($P < 0.05$), from 1.99 ± 1.28% to 2.25 ± 1.34% after 6 h (Figure 4).

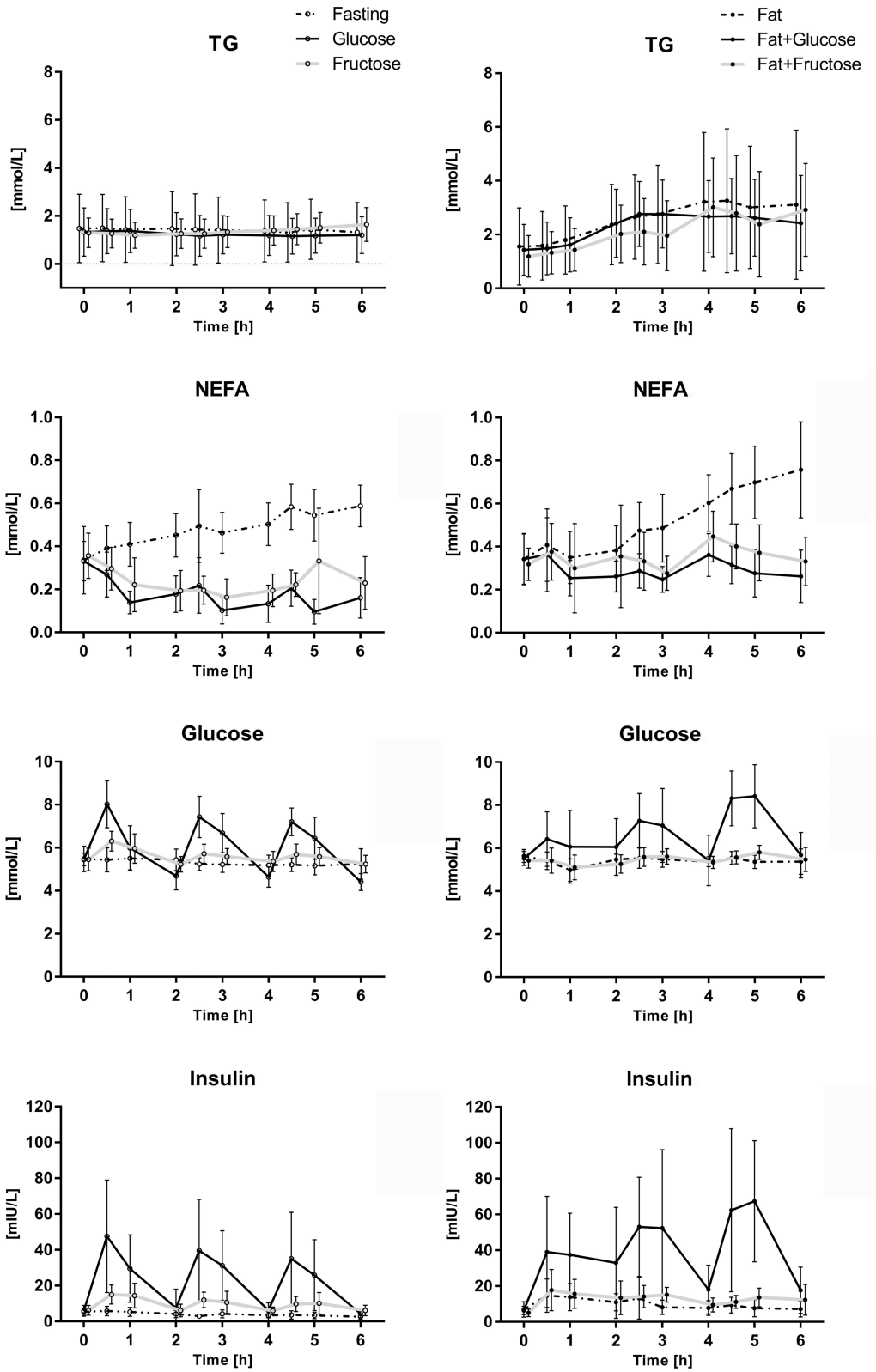

FIGURE 2 Dynamics of plasma TG, NEFA, glucose, and insulin during the 6 experiments: Fasting; Fat (150 g fat in dairy cream at 0 h); Glucose (3 × 50 g glucose at 0, 2, and 4 h); Fat + Glucose (150 g fat at 0 h and 3 × 50 g glucose at 0, 2, and 4 h); Fructose (3 × 50 g fructose at 0, 2, and 4 h); Fat + Fructose (150 g fat at 0 h and 3 × 50 g fructose at 0, 2, and 4 h). Experiments without fat load are presented in the lefthand panels, experiments with fat load in the righthand panels. All experiments were carried out in 10 healthy male volunteers. Data are means ± SD. NEFA, nonesterified fatty acids; TG, triglycerides.

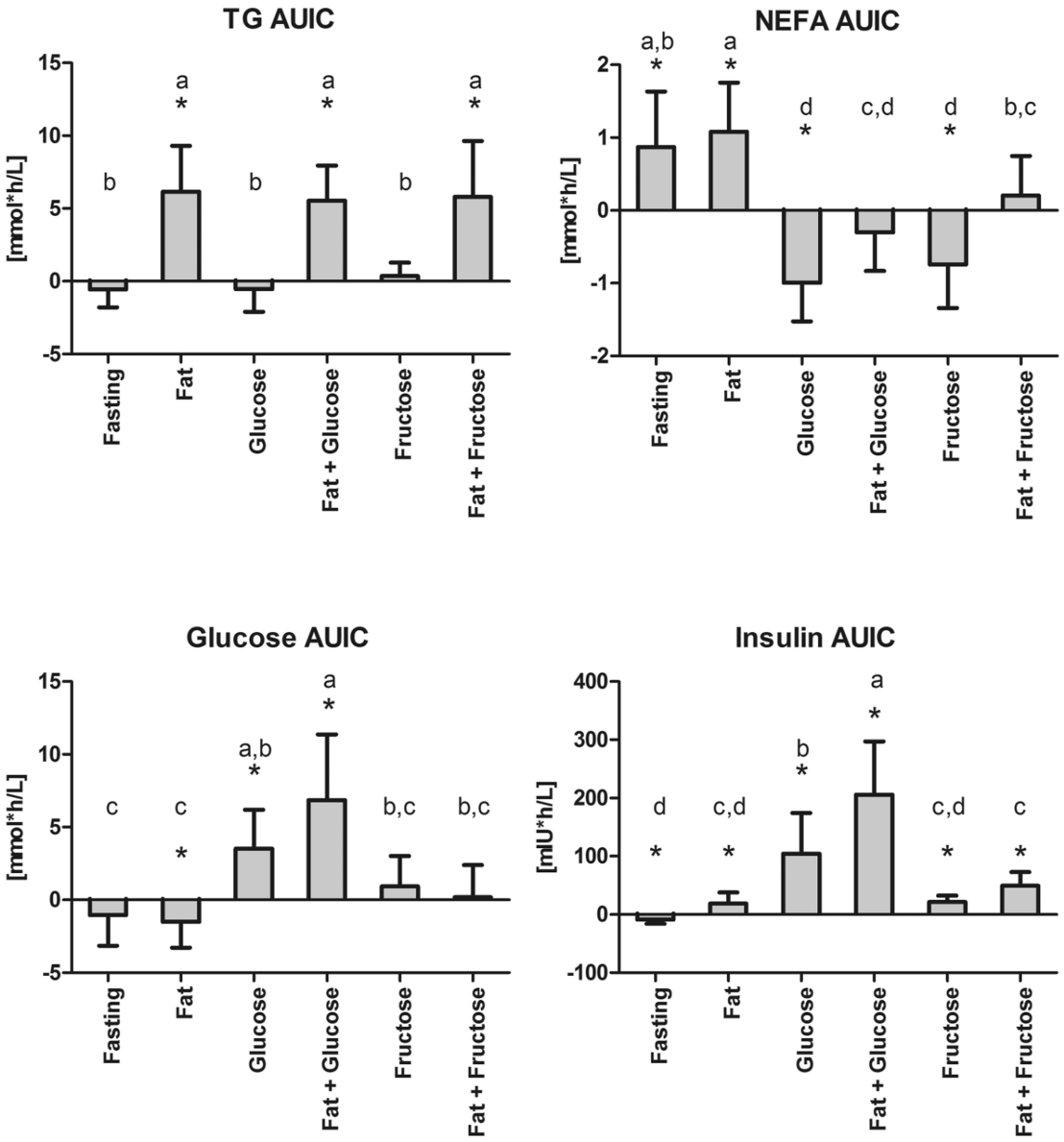

FIGURE 3 Area under the 6-h increment curves (AUICs) of TG, NEFA, glucose, and insulin during the 6 experiments: Fasting; Fat (150 g fat in dairy cream at 0 h); Glucose (3 × 50 g glucose at 0, 2, and 4 h); Fat + Glucose (150 g fat at 0 h and 3 × 50 g glucose at 0, 2, and 4 h); Fructose (3 × 50 g fructose at 0, 2, and 4 h); Fat + Fructose (150 g fat at 0 h and 3 × 50 g fructose at 0, 2, and 4 h). All experiments were carried out in 10 healthy male volunteers. Data are means ± SD. $^{*}P < 0.05$: significantly different from zero by the 1-sample *t*-test. The same letter indicates values that do not differ using repeated measures ANOVA and Tukey's multiple comparison posttest. NEFA, nonesterified fatty acids; TG, triglycerides.

Repeated loads of glucose (3 × 50 g) induce a decrease in HFC

When subjects received 3 doses of 50 g glucose at 0, 2, and 4 h (Glucose experiment), 3 corresponding peaks of glucose and insulin concentrations at 0.5, 2.5, and 4.5 h were observed. Correspondingly, pronounced increases in the AUICs for both glucose and insulin were observed, being 3.52 ± 2.68 mmol∗h/L ($P = 0.003$) and 104 ± 70 mIU∗h/L ($P = 0.001$), respectively. Glucose administration had no statistically significant effect on triglyceridemia, with the TG AUIC not differing from zero. However, the NEFA concentration declined significantly throughout the experiment—the NEFA AUIC equaled −0.99 ± 0.53 mmol∗h/L ($P < 0.001$). HFC decreased by 15% ($P < 0.05$), from 1.73 ± 0.83% to 1.47 ± 0.75%, 6 h after fat administration (Figure 4).

Coadministration of glucose (3 × 50 g) with a high-fat load does not affect HFC

When giving 50 g glucose to subjects together with a fat load and then again after 2 and 4 h (Fat + Glucose experiment), corresponding increases in plasma glucose concentration were noted; the glucose AUIC of 6.85 ± 4.53 mmol∗h/L did not differ significantly from that in the Glucose experiment (Figure 3). Insulin concentrations responded similarly to glucose, with a tendency to a more pronounced response with time, that is,

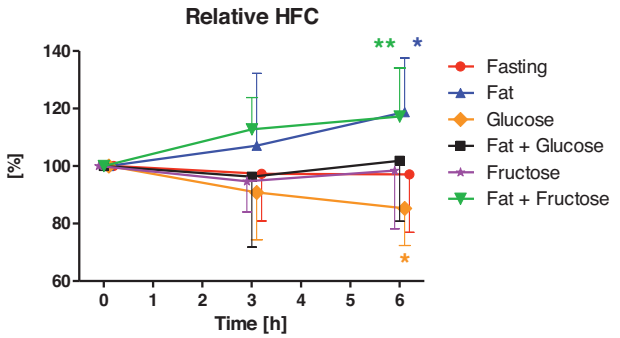

FIGURE 4 Dynamics of hepatic fat content (HFC) during the 6 experiments. Fasting; Fat (150 g fat in dairy cream at 0 h); Glucose (3 × 50 g glucose at 0, 2, and 4 h); Fat + Glucose (150 g fat at 0 h and 3 × 50 g glucose at 0, 2, and 4 h); Fructose (3 × 50 g fructose at 0, 2, and 4 h); Fat + Fructose (150 g fat at 0 h and 3 × 50 g fructose at 0, 2, and 4 h). Proton magnetic resonance spectroscopy for determination of HFC was carried out before ingestion of cream (time 0) and 3 and 6 h later. All experiments were carried out in 10 healthy male volunteers. Data expressed as a percentage of baseline morning values are means ± SD. *,**Significantly different from baseline (ANOVA for repeated measures and Dunnett's multiple comparison posttest): $^{*}P < 0.05$, $^{**}P < 0.01$.

the insulin AUIC was doubled compared with that in the Glucose experiment (206 ± 92 mIU∗h/L, $P < 0.0001$). Triglyceridemia rose during the experiment, with the TG AUIC reaching 5.22 ± 2.34 mmol∗h/L. NEFA concentrations did not change significantly throughout the experiment, with the NEFA AUIC of −0.30 ± 0.53 mmol∗h/L not differing from zero and being lower than the NEFA AUIC in the Fat experiment ($P < 0.001$). Finally, HFC was not affected in this experiment (Figure 4).

Repeated loads of fructose (3 × 50 g) have no effect on HFC

When subjects received only 3 doses of 50 g fructose in another experiment at 0, 2, and 4 h (Fructose experiment), glucose concentrations rose slightly from 5.45 ± 0.52 to 6.30 ± 0.46 mmol/L at 0.5 h ($P < 0.001$) but did not differ from baseline at a later stage of the experiment. The glucose AUIC of 0.93 ± 2.09 mmol∗h/L did not differ from zero or from the glucose AUIC in both the Fasting and Glucose experiments. Insulin responded to all loads of fructose by a mild increase in its concentration 30 min later; however, the insulin AUIC of 21.3 ± 11.4 mIU∗h/L was one-fifth of that in the Glucose experiment ($P < 0.001$) and did not differ from the insulin AUIC in the Fasting experiment. The TG concentration did not change throughout the experiment except for a small but a statistically significant increase in the last 1.5 h of the experiment. However, the TG AUIC of 0.52 ± 0.80 mmol∗h/L did not differ from zero. The NEFA concentration decreased during the experiment as documented by a negative NEFA AUIC of −0.74 ± 0.59 mmol∗h/L. Such a decrease in the AUIC was comparable to that after glucose administration and significantly lower than the NEFA AUIC in the Fasting experiment ($P < 0.001$). No changes in HFC were noted throughout this experiment (Figure 4).

Coadministration of fructose (3 × 50 g) with a high-fat load induces an increase in HFC

When 50 g fructose was given to subjects together with a fat load and then again after 2 and 4 h (Fat + Fructose experiment), the response of the TG concentration was not significantly different from that in the other experiments with a high-fat load; there were no statistically significant differences in the TG AUIC between the Fat, Fat + Glucose, and Fat + Fructose experiments. The NEFA concentration did not change throughout the experiment, with the NEFA AUIC of 0.20 ± 0.54 mmol∗h/L not differing from zero nor from the NEFA AUIC in the Fat + Glucose experiment. However, it was higher than the NEFA AUIC in the Fructose experiment ($P < 0.05$) and lower than that in the Fat experiment ($P < 0.05$). The glucose concentration did not change throughout the experiment, with the glucose AUIC of 0.19 ± 2.21 mmol∗h/L being less than one-tenth of the same parameter in the Fat + Glucose experiment ($P < 0.001$). Insulinemia slightly increased during the experiment; however, the insulin AUIC of 49.2 ± 23.9 mIU∗h/L was one-fourth of that in the Fat + Glucose experiment. Finally, HFC rose by 17% ($P < 0.01$), from 1.70 ± 0.86% to 1.92 ± 0.92%, after 6 h (Figure 4); the magnitude of such an increase did not differ from that in the experiment with a high-fat load alone.

Discussion

In this study using ^{1}H-MRS for determination of HFC in healthy nonobese male volunteers, we found that administration of three 50-g doses of fructose within 4 h has an entirely different acute impact on liver fat accumulation than administration of the same amount of glucose. Fructose administration has no effect on HFC whereas glucose administration induces a decrease in HFC after 6 h. When coadministering both sugars with a high-fat load, fructose administration induces an HFC increase after 6 h whereas glucose administration has no such effect. To the best of our knowledge, this is the first study furnishing such evidence of different immediate effects of both simple sugars on liver fat in humans in vivo.

Hepatic fat comes from 3 major sources—NEFAs (released from adipose tissue), dietary TGs, and de novo lipogenesis (DNL). The availability of these sources for TG synthesis and storage in the liver is highly variable during the day and is under strict metabolic control. Most plasma NEFAs are released from adipose tissue after intracellular TG lipolysis that can be suppressed by insulin and stimulated by catecholamines (11). Dietary TG enters the liver in remnants of chylomicrons and as spillover fatty acids, that is, fatty acids released from chylomicrons by lipoprotein lipase and not taken up by extrahepatic tissues (12). De novo TG lipogenesis from nonlipid precursors is believed to take place postprandially and be under insulin control (13).

Importantly, when subjects fasted during the Fasting experiment, no change in HFC was observed. Our results suggest that under these conditions the loss of TGs from the liver due to their oxidation and export in VLDLs is fully compensated by an increased flux of NEFAs from adipose tissue to the liver. When subjects received 150 g fat in dairy cream in the Fat experiment, HFC increased by 19% 6 h later. Dairy cream

administration induced an expected increase in dietary fat influx and the same increase in NEFA concentration as in the Fasting experiment. Therefore, a combination of both increased NEFA influx and entry of dietary fat was likely to contribute to hepatic fat accumulation.

The fact that a pure fat load can induce liver fat accumulation was noted in a recent study in which administration of ~1.2 g of pure fat/kg body weight resulted in an increase in HFC as high as 35% (4). The HFC increment of only 19% observed in our study even after a higher fat load could be due to the presence of small amounts of carbohydrate (19.5 g) and protein (13.5 g) in dairy cream inducing a small yet significant insulin response. The insulin could partially suppress the adipose tissue lipolysis and the contribution of NEFAs of adipose origin to hepatic fat accumulation. The fact that the NEFA AUIC does not differ between the Fasting and the Fat experiments, even when NEFAs of adipose origin might be reduced by insulin in response to dairy cream administration, could then be explained by the contribution of spillover fatty acids to the NEFA pool (14).

Administration of three 50-g doses of glucose alone induced 3 corresponding peaks in glucose and insulin concentrations. Plasma NEFA concentration was efficiently suppressed throughout the experiment and HFC also decreased. Such an observation strongly suggests that insulin secretion induced by glucose administration efficiently inhibits NEFA release from adipose tissue. It also shows that NEFA supply is a major and critical source of liver fat even in healthy nonsteatotic subjects; a decrease in NEFA concentration apparently overrides the known effects of insulin on DNL and VLDL secretion (15–17). These findings are further corroborated by the observation that glucose coadministered with a high-fat load prevents the increase in HFC observed in the Fat experiment. Again, such an observation can be explained by insulin-induced inhibition of adipose tissue lipolysis leading to suppression of NEFA concentration. However, HFC does not decline because the limited supply of NEFAs to the liver in the Fat + Glucose experiment is compensated by dietary fat present both in chylomicron remnants and spillover fatty acids. Similar findings were reported in a study where glucose supplementation during exercise protected against an increase in HFC during the recovery phase (18).

These data together indicate that a contribution of both NEFAs and dietary TGs might be required to increase HFC.

In light of these findings, it is striking that administration of fructose and glucose differently affects HFC despite their identical effects on NEFA and TG concentrations. Both sugars suppress NEFA concentration, although insulin secretion in response to fructose seems to be too low to sufficiently affect NEFA release from adipose tissue. It cannot be excluded that lipolysis in adipose tissue is inhibited through the action of fibroblast growth factor (FGF21) as fructose has been demonstrated to be a much stronger inducer of FGF21 secretion from the liver than glucose (19), and there are data showing that FGF21 suppresses lipolysis in adipocytes (20). However, regardless of the mechanism, the relative increase in HFC following fructose administration compared with that following glucose administration cannot be then explained by increased NEFA availability from the plasma or increased supply of dietary TG. The implication is that another major source of liver fat—DNL—must be involved.

It has already been demonstrated that fructose—but not glucose—administration stimulates an immediate increase in DNL from [^{13}C]acetate (21). Unlike glucose, which is extensively metabolized by peripheral tissues, most of the fructose is taken up and metabolized directly in the liver. The fructolysis is faster than glycolysis because it is not subject to any feedback control, and the trioses produced during fructolysis can directly enter lipogenesis (7, 22). More importantly, fructose switches the metabolic pathways in the hepatocyte through carbohydrate-responsive element-binding protein (ChREBP) and sterol regulatory element-binding protein 1c signaling (SREBP1c) pathways to DNL more efficiently than glucose (8).

Two other studies analyzing the acute effects of nutrients on acute changes in HFC used a mixed meal with a high fat content and produced ambiguous results. The first study (23), involving 10 subjects, did not find any effect of a meal containing 50 g fat on HFC after 4 h. It seems likely that the 50-g load is not sufficient to induce significant changes in HFC. The other study (24) used a higher fat load and observed significant changes in HFC in 9 subjects after 3 and 5 h. These results seem to contrast with those of our study because the meal used induced significant insulin secretion. However, the carbohydrates were provided only as part of the experimental meal and NEFA concentration was suppressed only within the first 2 h, rapidly increasing thereafter, suggesting that NEFAs of adipose tissue origin can contribute to hepatic fat accumulation. The unique design of our study, with repeated doses of glucose and fructose, enabled us to efficiently suppress lipolysis in adipose tissue not only at the beginning but also throughout the experiment. Therefore, for studies of acute changes in HFC the use of repeated loads of nutrients under study could be important because of the relatively short-lived effect of hormonal regulation. Moreover, such an experimental arrangement is much closer to the way in which nutrients are taken in real life.

The results of our study suggest that, in healthy insulin-sensitive subjects, the detrimental immediate effects of a fat load on HFC can be prevented by inducing sufficient insulin secretion by the glucose present in the food. Likewise, the results suggest that a marked reduction of fructose consumption and/or its replacement by glucose could be an effective strategy to favorably affect HFC. It remains to be clarified whether the same mechanisms operate in obese and/or insulin-resistant subjects.

In conclusion, this is the first study to demonstrate, using ^{1}H-MRS, in humans in vivo that fructose consumption, unlike glucose consumption, has an immediate adverse effect on HFC even if the effects of both sugars on the availability of NEFAs and dietary fat for liver fat do not differ.

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The authors' contributions were as follows—JK, RP, and MH: designed research; TD, JK, and KZ: conducted experiments; TD, MDr, and PŠ: conducted ^{1}H-MRS examinations; TD, JK, MDr, PŠ, and MDe: processed and analyzed data; TD, JK, RP, and MH: wrote the paper; TD and JK had primary responsibility for final content. All authors read and approved the final manuscript.

The authors have declared that no conflict of interest exists.

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