Metabolic effects of a prolonged, very-high-dose dietary fructose challenge in healthy subjects

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ABSTRACT

Background: Increased fructose intake has been associated with metabolic consequences such as impaired hepatic lipid metabolism and development of nonalcoholic fatty liver disease (NAFLD).

Objectives: The aim of this study was to investigate the role of fructose in glucose and lipid metabolism in the liver, heart, skeletal muscle, and adipose tissue.

Methods: Ten healthy subjects (age: 28 ± 19 y; BMI: 22.2 ± 0.7 kg/m^2) underwent comprehensive metabolic phenotyping prior to and 8 wk following a high-fructose diet (150 g daily). Eleven patients with NAFLD (age: 39.4 ± 3.95 y; BMI: 28.4 ± 1.25) were characterized as "positive controls." Insulin sensitivity was analyzed by a 2-step hyperinsulinemic euglycemic clamp, and postprandial interorgan crosstalk of lipid and glucose metabolism was evaluated, by determining postprandial hepatic and intramyocellular lipid and glycogen accumulation, employing magnetic resonance spectroscopy (MRS) at 7 T. Myocardial lipid content and myocardial function were assessed by ^{1}H MRS imaging and MRI at 3 T.

Results: High fructose intake resulted in lower intake of other dietary sugars and did not increase total daily energy intake. Ectopic lipid deposition and postprandial glycogen storage in the liver and skeletal muscle were not altered. Postprandial changes in hepatic lipids were measured [Δhepatocellular lipid (HCL)_healthy_baseline: −15.9 ± 10.7 compared with ± ΔHCL_healthy_follow-up: −6.9 ± 4.6; $P = 0.17$] and hepatic glycogen (Δglycogen_baseline: 64.4 ± 14.1 compared with Δglycogen_follow-up: 51.1 ± 9.8; $P = 0.42$). Myocardial function and myocardial mass remained stable. As expected, impaired hepatic glycogen storage and increased ectopic lipid storage in the liver and skeletal muscle were observed in insulin-resistant patients with NAFLD.

Conclusions: Ingestion of a high dose of fructose for 8 wk was not associated with relevant metabolic consequences in the presence of a stable energy intake, slightly lower body weight, and potentially incomplete absorption of the orally administered fructose load. This indicated that young, metabolically healthy subjects can at least temporarily compensate for increased fructose intake. This trial was registered at www.clinicaltrials.gov as NCT02075164. *Am J Clin Nutr* 2019;00:1–9.

Keywords: fructose, glucose metabolism, nonalcoholic fatty liver disease, insulin resistance, glycogen, ectopic fat

Introduction

Excessive calories are generally delivered through the consumption of food with high fat content, and sugar-sweetened or alcoholic beverages (1). Sucrose (consisting of fructose and glucose) and fructose are commonly used industrial sweeteners with similar biochemical structures, but different metabolism (1). Postprandial insulin release in response to glucose is much higher compared with fructose (2). Faster fructose metabolism compared with glucose can possibly explain hepatic ATP depletion after phosphorylation of fructose to fructose 1-phosphate (1). Fructose has been shown to promote hepatic de novo lipogenesis (DNL) in humans by: *1*) ATP depletion; *2*) suppression of

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Supplemental Figure 1 and Supplemental Tables 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/.

Data described in the article, code book, and analytic code will be publicly and freely available without restriction at www.pubmed.gov.

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Abbreviations used: CO, cardiac output; DNL, de novo lipogenesis; ECG, electrocardiography; EF, ejection fraction; EGP, endogenous glucose production; HCL, hepatocellular lipid; IMCL, intramyocellular lipid; IPAQ, International Physical Activity Questionnaire; MET, metabolic equivalent; MRS, magnetic resonance spectroscopy; MYCL, myocardial lipid; NAFLD, nonalcoholic fatty liver disease; OGIS, oral glucose insulin sensitivity index; OGTT, oral-glucose-tolerance test; SV, stroke volume.

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mitochondrial fatty acid oxidation in the liver; and *3*) stimulating DNL (3). Fructose has been identified as a potential major player in the pathogenesis and progression of nonalcoholic fatty liver disease (NAFLD), because fructose consumption is closely associated with nonalcoholic steatohepatitis and advanced fibrosis (4, 5), although the underlying mechanisms remain poorly understood.

The impact of fructose on alterations in glucose and lipid metabolism, as well as on ectopic lipid accumulation, has been studied over the past decades, revealing inconsistent results (3, 6–10). Currently it is unclear whether the potentially adverse metabolic effects of fructose are specific and independent of energy intake. Importantly, the effects of increased dietary intake of fructose on metabolic interorgan crosstalk have not been clarified.

We hypothesized the first step in the impairment of metabolic interorgan crosstalk to be the liver hit by fructose, resulting in increased DNL and VLDL production. Moreover, fructose can impair insulin sensitivity in skeletal muscle, where excess carbohydrates will be shifted from skeletal muscle to the liver, thereby also increasing DNL and VLDL secretion. Fructose-stimulated hepatic DNL and VLDL secretion, combined with insulin resistance, might lead to increased ectopic fat deposition in peripheral tissue, including skeletal muscle and the heart, contributing to a vicious circle that interconnects lipotoxicity and metabolic syndrome.

To our knowledge, the present study is the first to investigate the effect of prolonged, very-high-dose fructose ingestion on the metabolic crosstalk between insulin-sensitive tissues in young healthy subjects.

Methods

Study cohort

Ten young, healthy subjects, without regular medication intake and without a history of regular alcohol consumption, completed this interventional dietary trial. As a positive control group for method validation, we included 11 nondiabetic patients with NAFLD with normal glucose tolerance. The diagnosis of NAFLD was confirmed by biopsy in 5 patients, or by ultrasound and FibroScan (Echosens) in the remaining 6 patients. Prior to their inclusion, all participants provided informed consent. The study was approved by the ethics committee of the Medical University of Vienna (No. 1022/2013), and was conducted in accordance with the Declaration of Helsinki. All participants were screened by physical examination, history, and oral-glucose-tolerance test (OGTT), to exclude impairments of carbohydrate metabolism, and comprehensive blood tests were conducted to exclude other diseases. Additionally, contraindications for magnetic resonance spectroscopy (MRS) were assessed prior to study inclusion. Prior to the fructose challenge, healthy participants underwent an H_2 breath test to exclude fructose intolerance.

From a total of 16 included subjects, 10 completed the study. Six healthy volunteers dropped out during the "active phase" of the study, but prior to dietary intervention (**Supplemental Figure 1**).

Study design

All healthy participants and 5 patients with NAFLD (serving as positive controls for baseline metabolic characterization) underwent baseline metabolic phenotyping consisting of: *1*) an OGTT; *2*) hyperinsulinemic euglycemic clamp with stable glucose isotope tracers for measurement of whole-body and hepatic insulin sensitivity; *3*) mixed meal test in combination with ^{1}H and ^{13}C MRS at 7 T, to monitor postprandial substrate distribution between skeletal muscle and liver; and *4*) ^{1}H MRS and MRI at 3 T for determination of myocardial lipids (MYCLs) and myocardial function. Six additional patients with NAFLD underwent the same procedure, except for the hyperinsulinemic euglycemic clamp. Insulin sensitivity in this subgroup was determined by the OGTT-based oral glucose insulin sensitivity index (OGIS) (11), and compared with healthy subjects. In healthy participants, metabolic phenotyping was performed at baseline and 8 wk following the dietary high-fructose challenge (150 g fructose/d over 8 wk in addition to their ordinary diet). The fructose was provided by the researchers, and the participants were allowed to select the rest of their diet. They were told to retain and document their dietary habits, including their regular carbohydrate ingestion, as well as their usual physical activity.

All metabolic tests were performed after an overnight fast. After inclusion, all healthy participants were initially provided with 28 portions of fructose (150 g each) for daily ingestion. After 4 wk on a high-fructose diet, a follow-up visit was made for measurement of liver function and other metabolic parameters, and an additional 28 portions of sugar for the following 4 wk were provided. All participants were instructed to document their diet and the daily ingestion of fructose in a diary, and to return potentially unused portions of sugar.

The maximum number of returned portions at the end of the study was 7 (1 female participant); 2 other participants returned 5 portions of fructose each. All other participants ingested $\geq$53 of the provided portions. Thus 95.6% of provided portions were ingested. One subject failed to record dietary protocols in the first 4 wk of the study and was excluded from the analysis of dietary protocols only.

Dietary assessments using a prospective method (Freiburger Ernährungsprotokoll, Justus-Liebig-University Giessen) were conducted 7 d in a row, prior to the intervention and during the 8 wk of dietary intervention. The Freiburger dietary protocols (http://www.ernaehrung.de/static/pdf/freiburger-ernaehrungsprotokoll.pdf) are ready-made protocols that allow participants to document meals using common kitchen sizes. It was not necessary to accurately weigh the consumed food. Participants recorded their dietary intake on a daily basis.

Calories of the macronutrients were calculated based on their caloric equivalent, in reference to Elmadfa and Leitzmann (12). For fructose, a caloric equivalent of 4.1 kcal/g fructose was used. Additionally, the International Physical Activity Questionnaire —Short Form (IPAQ-SF) was recorded over the intervention period and metabolic equivalent (MET) minutes per week were calculated, as described in IPAQ guidelines (13).

An OGTT was performed in fasting conditions. A polyethylene catheter was set in an antecubital vein, and blood for the measurement of plasma glucose, insulin, and C-peptide was collected at baseline, as well as 30, 60, 90, and 120 min after

ingestion of a solution comprising 300 mL water and 75 g glucose. The OGIS was used to estimate glucose clearance (11).

Hyperinsulinemic euglycemic clamp

Following an overnight fast, 2 catheters (Vasofix; Braun) were inserted in the left and right antecubital vein for blood sampling and infusions, respectively. A primed continuous infusion (bolus: 4 mg × kg lean body weight; remaining period: 0.04 mg/min × kg lean body weight) of D-[6,6-$^2H_2$]-glucose (98% enriched; Cambridge Isotope Laboratories) was started at −120 min for measurement of endogenous glucose production (EGP). Blood samples for determination of EGP were drawn at −120, −5, 0, 80, 100, 120, 200, 220, and 240 min of applying the clamp. The clamp goal was determined from the mean of 3 plasma glucose measurements, prior to using the clamp. Blood sugar values of 80 mg/dL and 100 mg/dL were taken as the clamp goal where the calculated clamp goal was outside this range. The hyperinsulinemic-euglycemic clamp test was started with a primed continuous insulin (Actrapid; Novo Nordisk) infusion of 15 mU/min/m^2 of body surface area, during 0 to 120 min; thereafter, insulin infusion was increased to 40 mU/min/m^2 to achieve standardized postprandial hyperinsulinemia (during 120 to 240 min). Plasma glucose was measured every 5 min and maintained at the clamp goal by infusing variable amounts of D-glucose, enriched with D-[6,6-$^2H_2$]-glucose. For calculation of M values, which are a measure of insulin sensitivity, blood was collected every 20 min (14).

Mixed meal test

Participants arrived at our study center at 07:00 following an overnight fast. The meal challenge was performed in combination with ^{1}H and ^{13}C MRS at 7 T, to measure changes in lipid and glycogen content in the liver and skeletal muscle (15–17). Two high-calorie liquid meals containing 100% of required daily energy (Fortimel Energy; Nutricia: 150 kcal/100 mL; 18.4 g carbohydrates/100 mL; 5.8 g lipids/100 mL; 5.9 g protein/100 mL), and additionally, 25% of required daily energy in the form of table sugar, were mixed together and served at 10:30 and at 13:30.

At 17:00, ^{1}H and ^{13}C MRS at 7 T was repeated for assessment of postprandial lipid and glycogen content in the liver and skeletal muscle. Additionally, blood for the measurement of plasma glucose, insulin, C-peptide, and triglycerides was collected at hourly intervals.

MYCL assessment was performed using ^{1}H MRS at 3 T (Tim Trio; Siemens Healthcare) in overnight fasting conditions, as previously described (18, 19). The volume of interest (∼6–8 cm^3) was positioned in the interventricular septum to avoid signal alterations by epicardial fat. The magnetic resonance signal acquisition was performed during multiple breath holds and triggered by an electrocardiography (ECG) signal. MYCL was calculated as the ratio of summed areas of lipid-methylene and lipid-methyl signals to that of water signal.

Assessment of left ventricular function

ECG-triggered cine true fast imaging in ARGUS software allowed us to illustrate myocardial function. Endocardial and epicardial borders were manually identified in end-systolic and end-diastolic phases. Mean muscle volume in end-diastolic and end-systolic phases multiplied, with a density of 1.05 g/cm^3 representing myocardial mass. All data were normalized to body surface area using the Dubois formula (19).

Ejection fraction (EF), stroke volume (SV), and cardiac output (CO) were the main parameters used to describe left ventricular systolic function.

Laboratory analyses and calculations

All standard laboratory parameter concentrations of glucose, insulin, and C-peptide were analyzed at the Core Lab of the Medical University of Vienna. For details, see www.kimcl.at.

Glucose enrichment was measured as tracer-to-trace ratio (20), and EGP was calculated as previously described (21, 22).

Statistical analyses

All data are given as mean ± SEM. Statistical analyses were performed using SPSS version 24 (IBM). Statistical comparison was performed employing paired and unpaired Student *t* tests, as appropriate. The level of statistical significance was set at $P \leq 0.05$.

Results

Baseline characteristics, diet, energy intake, and physical activity

In this study, we included 10 young, healthy, lean participants with normal serum liver enzymes and normal serum lipids. Despite daily ingestion of 150 g fructose (equivalent to 618 kcal) over 8 wk, BMI slightly decreased in healthy subjects (**Table 1**). Notably, no significant changes in total energy intake were observed during the study (**Figure 1**). Mean intake of dietary sugar excluding fructose was significantly lower during the study than at baseline ($P < 0.05$); before challenge: 381 kcal/d; week 4: 267 kcal/d; week 8: 269 kcal/d. Recorded mean MET (minutes per week) did not change during the study. The physical activity of the 10 subjects during the intervention was classified as either "moderate" or "high," referring to IPAQ guidelines 2005 (3936.7 ± 792.1 MET-min). No increases in serum liver enzymes and lipids were observed.

OGTT

Glucose clearance during the OGTT calculated by OGIS did not change after fructose consumption (healthy_baseline: 509 ± 16 mL/min/m^2, compared with healthy_follow-up: 488.9 ± 12.7 mL/min/m^2; $P = 0.24$).

Hyperinsulinemic euglycemic clamp

Eight weeks of high-caloric fructose consumption did not affect insulin-stimulated glucose metabolism in the presence of standardized moderate ($P = 0.19$) and postprandial hyperinsulinemia ($P = 0.15$) in healthy subjects (**Figure 2**). Insulin-mediated suppression of EGP was observed during application of the clamp, without a significant effect of fructose consumption (EGP after 240 min of clamp, healthy_baseline:

TABLE 1 Anthropometric and laboratory data of patients with NAFLD and of healthy subjects[1]

	NAFLD	Healthy_baseline	*P* value[2] NAFLD vs. healthy_baseline	Healthy_follow-up	*P* value[3] healthy_baseline vs. healthy_follow-up
n	11	10		10	
Female	3	5		5	
Male	8	5		5	
Age, y	39.4 ± 3.95	28 ± 1.9	0.02*	—	
BMI, kg/m^2	28.4 ± 1.25	22.2 ± 0.7	0.001*	21.8 ± 0.7	0.026*
AST, U/L	36.9 ± 6.4	23.8 ± 1.7	0.07	22 ± 2.4	0.343
ALT, U/L	65.7 ± 17.2	19.5 ± 1.8	0.02*	16.8 ± 2.7	0.223
GGT U/L	63.09 ± 12.8	14 ± 1.8	0.003*	11.8 ± 1.3	0.01*
ALP, U/L	71.8 ± 7.88	52.4 ± 4.6	0.049*	50.5 ± 4.0	0.1
Triglyceride, mg/dL	153.3 ± 18.7	71.6 ± 7.9	0.001*	89.1 ± 14.9	0.066
LDL, mg/dL	122.63 ± 9.6	86.9 ± 9.3	0.015*	80.4 ± 6.3	0.436
HDL, mg/dL	47.7 ± 4.07	65.6 ± 5.6	0.019*	61.3 ± 4.7	0.12
HbA1C, %	5.15 ± 0.08	4.9 ± 0.09	0.1	4.97 ± 0.09	0.71
OGIS, mL/min/m^2	395.3 ± 30.9	509 ± 16	0.006*	488.9 ± 12.7	0.24

[1]All values are given as mean ± SEM. *$P < 0.05$. ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, γ-glutamyltransferase; HbA1C, glycated hemoglobin; NAFLD, nonalcoholic fatty liver disease; OGIS, oral glucose insulin sensitivity index.
[2]Unpaired Student *t* test was used to compare the data between NAFLD and healthy subjects.
[3]Paired Student *t* test was used to compare the data between healthy_baseline and healthy_follow-up.

0.1 ± 0.003 mg/kg/min, compared with EGP healthy_follow-up: 0.006 ± 0.003 mg/kg/min; $P = 0.34$).

Mixed meal test

Impact of the fructose challenge on plasma glucose, insulin, and C-peptide concentrations.

Plasma glucose, insulin, and C-peptide concentrations during the mixed meal test were not impaired following the fructose challenge in healthy subjects. Additionally, triglyceride concentrations were comparable (**Table 2**).

Impact of fructose challenge on hepatic and skeletal muscle lipid and glycogen content.

Lipid and glycogen content in the liver and skeletal muscle assessed by ^{1}H and ^{13}C MRS remained unchanged after 8 wk of dietary intervention with fructose. The fructose challenge also did not affect postprandial lipid and glycogen accumulation in the liver and skeletal muscle

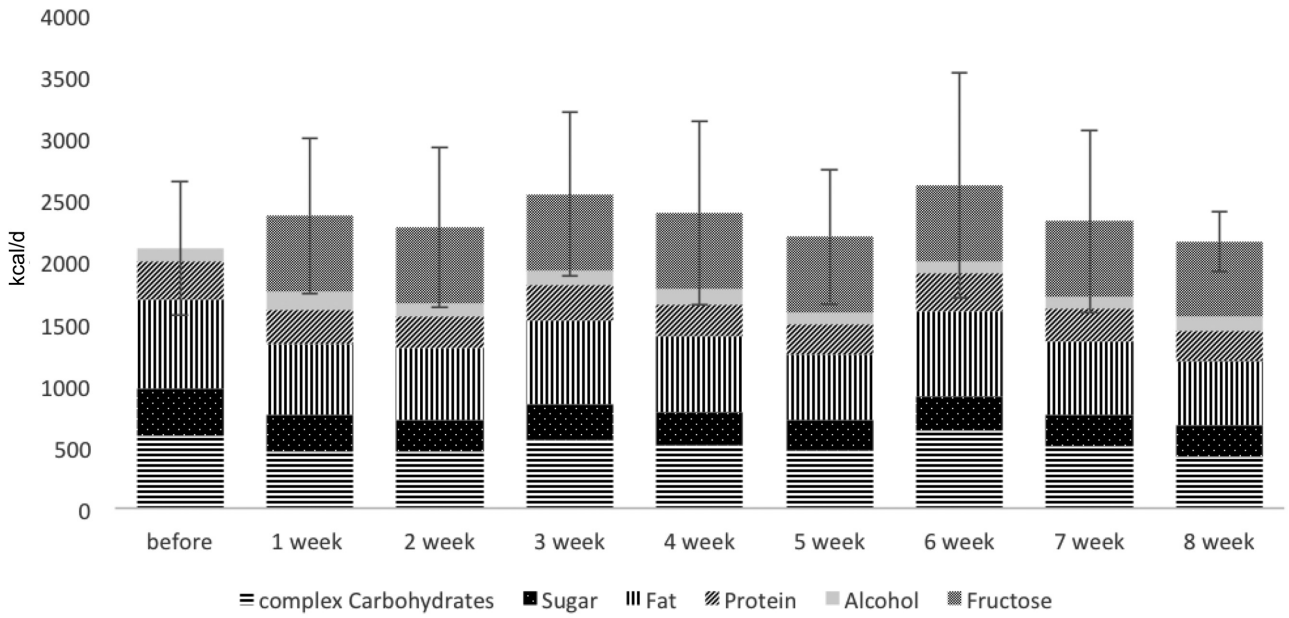

FIGURE 1 The time course in total energy and nutrient intake during the study in healthy subjects. $n = 10$ for both healthy_baseline and healthy_follow-up. Error bars represent the data variability.

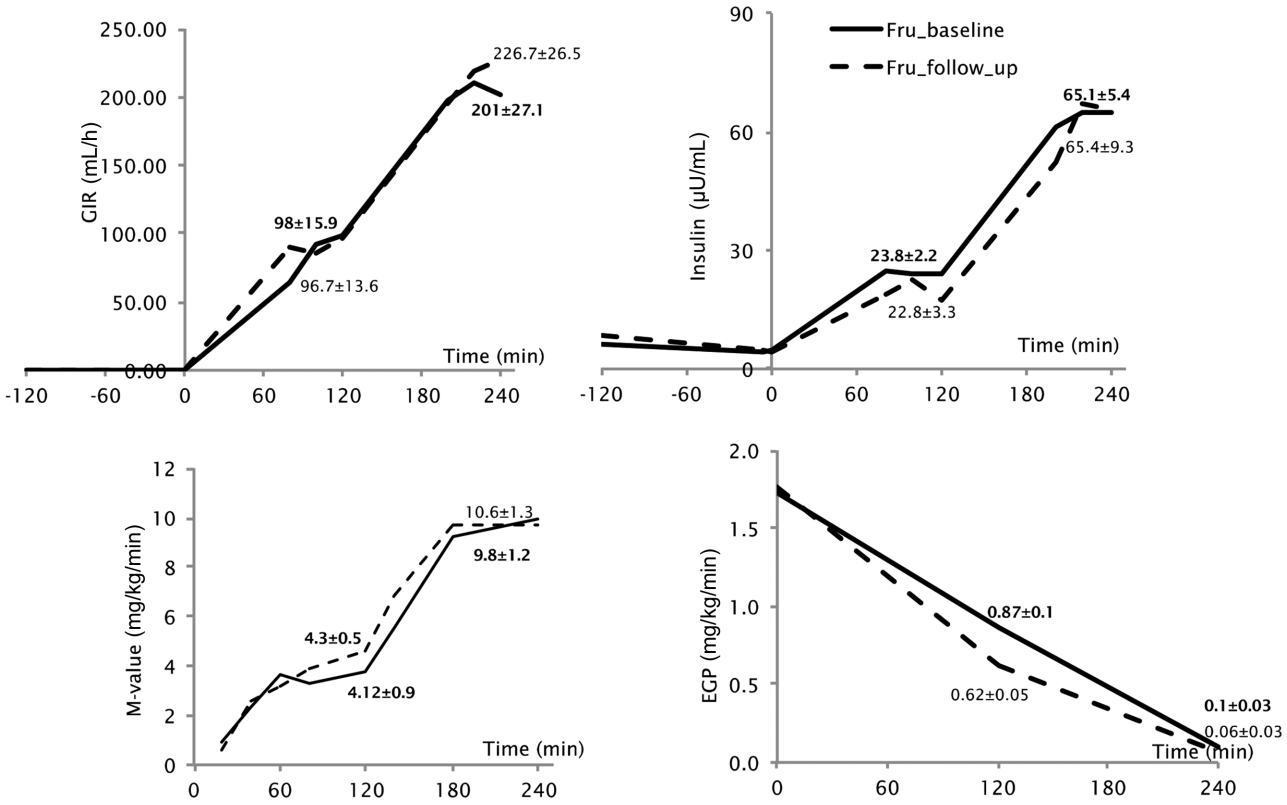

FIGURE 2 The solid line shows GIR, serum insulin concentrations, M value (measure of insulin sensitivity), and EGP during clamp tests in healthy subjects at baseline; the dashed line indicates the parameters after the 8-wk fructose challenge. Eight weeks of fructose ingestion did not impair whole-body or hepatic insulin sensitivity. All values are given as mean ± SEM. Paired Student *t* test was used to compare the data at defined time points before and during steady-state conditions during low-dose (100–120 min) and high-dose (220–240 min) insulin infusion between healthy_baseline ($n = 10$) and healthy_follow-up ($n = 10$). EGP, endogenous glucose production; Fru, fructose; GIR, glucose infusion rate.

after mixed meal challenges (see **Table 3**) [Δhepatocellular lipid (HCL)_healthy_baseline: −15.9 ± 10.7% compared with ΔHCL_healthy_follow-up: −6.9 ± 4.6%; $P = 0.17$] and (Δglycogen_liver_healthy_baseline: 64.4 ± 14.1% compared with Δglycogen_liver_follow-up: 51.1 ± 9.8%; $P = 0.42$).

Myocardial data

Increased DNL in the liver could be responsible for increased ectopic lipid deposition in organs with high uptake of free fatty acids, such as myocardium, and could play an important role in the development of cardiac lipotoxicity and cardiomyopathy. To address potential changes in the heart as a critical part of the interorgan crosstalk during the fructose challenge, we also explored the effects of high fructose intake on MYCL accumulation and changes in myocardial function.

Notably, a high fructose diet did not affect systolic myocardial function (EF healthy_baseline: 56.3 ± 3.2% compared with EF healthy_follow-up: 57.6 ± 1.9%; $P = 0.69$), MYCLs (MYCL healthy_baseline: 1.4 ± 0.15% compared with MYCL healthy_follow-up: 1.3 ± 0.14%; $P = 0.25$), or myocardial mass (myocardial mass healthy_baseline: 48.4 ± 3.7 g/m^2 compared

TABLE 2 Plasma glucose, insulin, and C-peptide concentrations during MMT at baseline, and following the fructose challenge in patients with NAFLD and in healthy subjects[1]

	NAFLD	Healthy_baseline	*P* value[2] NAFLD vs. healthy_baseline	Healthy_follow-up	*P* value[3] healthy_baseline vs. healthy_follow-up
n	11	10		10	
AUC_glucose, mg/dL × min	48,264 ± 3022	45,834 ± 1973.5	0.5	47,352 ± 1954.5	0.07
AUC_insulin, μU/mL × min	33,942 ± 7844	17,746 ± 2630.4	0.07	17,404 ± 3944.7	0.94
AUC_C-peptide,ng/mL × min	5269 ± 720	3602 ± 230.6	0.04*	3658 ± 233	0.86
AUC_MMT_triglyceride, mg/dL × min	118,677 ± 17,353	43,761 ± 5131.9	0.001*	45,780 ± 3840	0.64

[1] All values are given as mean ± SEM. *$P < 0.05$. MMT, mixed meal test; NAFLD, nonalcoholic fatty liver disease.
[2] Unpaired Student *t* test used to compare the data between NAFLD and healthy subjects.
[3] Paired Student *t* test used to compare the data between healthy_baseline and healthy_follow-up.

TABLE 3 Interorgan crosstalk analyzed during mixed meal test in patients with NAFLD and in healthy subjects shows the impact of fructose on baseline and postprandial lipid and glycogen storage in the liver and skeletal muscle[1]

	NAFLD	Healthy_baseline	*P* value[2] NAFLD vs. healthy_baseline	Healthy_follow-up	*P* value[3] healthy_baseline vs. healthy_follow-up
n	11	10		10	
Δglycogen_muscle, a.u.	15.4 ± 5.6	22.12 ± 6.3	0.44	11.2 ± 5.8	0.13
IMCL_basal, a.u.	1.7 ± 0.18	1.4 ± 0.15	0.14	1.3 ± 0.14	0.35
ΔIMCL, % of baseline	23.1 ± 7.9	− 1.5 ± 8.8	0.055*	8.7 ± 8.9	0.24
Δglycogen_liver, a.u.	25.2 ± 5.2	64.4 ± 14.1	0.017*	51.1 ± 9.8	0.42
HCL_basal, a.u.	15.6 ± 4.2	1.11 ± 0.2	0.007	1.6 ± 0.5	0.1
ΔHCL, % of baseline	16.1 ± 8.6	− 15.9 ± 10.7	0.035*	− 6.9 ± 4.6	0.17

[1]All values are given as mean ± SEM. *$P < 0.05$. a.u., arbitrary unit; HCL, hepatocellular lipid; IMCL, intramyocellular lipid; NAFLD, nonalcoholic fatty liver disease.
[2]Unpaired Student *t* test used to compare the data between NAFLD and healthy subjects.
[3]Paired Student *t* test used to compare the data between healthy_baseline and healthy_follow-up.

with myocardial mass healthy_follow-up: 50.3 ± 3.5 g/m^2; $P = 0.7$), as assessed by ^{1}H MRS and MRI at 3 T (**Table 4**).

Gender differences

The analysis of a possible gender effect did not reveal any significant difference regarding fructose-induced metabolic changes and is presented in **Supplemental Table 1**.

Discussion

We have shown that an 8-wk high-fructose dietary challenge was associated with stable caloric intake and no increase in body weight, as confirmed by dietary records. Under these conditions we could not observe any changes in hepatic and skeletal muscle lipid or glycogen content. Also skeletal muscle insulin sensitivity and postprandial excursions of plasma glucose, insulin, C-peptide, and triglycerides were not affected by fructose.

We did not observe any effects of fructose on postprandial metabolic interorgan crosstalk with respect to postprandial lipid and glycogen storage in the liver and skeletal muscle. Left ventricular myocardial function, and MYCL content and morphology were not altered by high dietary fructose consumption.

Patients with NAFLD were characterized by increased hepatic lipid storage, blunted hepatic glycogen synthesis, and increased myocardial mass.

The volunteers lost a small amount of weight and reduced their intake of "dietary sugars," which consisted chiefly of sucrose (50% fructose) and fructose. Thus, the net increase of absorbed fructose during the dietary challenge was reduced by changes in dietary fructose intake secondary to the observed changes in sugar intake. Furthermore, fructose could have partly escaped absorption, because it was not taken with glucose, and any fructose that was not absorbed would have been processed by the microbiome to noncarbohydrate moieties. Thus, the "net fructose" intake might have been less than the 150 g/d provided.

We performed comprehensive metabolic phenotyping by employing sensitive gold standard methods to investigate the effects of prolonged, high-dose fructose consumption in healthy volunteers.

The primary outcome parameters in this study were insulin sensitivity and HCLs. Secondary outcome parameters were intramyocellular lipid (IMCL), MYCL, as well as glycogen content in the liver and skeletal muscle of healthy participants. Recent studies have revealed inconsistent conclusions regarding the effects of dietary fructose. Insulin sensitivity decreased after

TABLE 4 Data of left ventricular function in patients with NAFLD and in healthy subjects at baseline and after fructose consumption[1]

	NAFLD	Healthy_baseline	*P* value[2] NAFLD vs. healthy_baseline	Healthy_follow-up	*P* value[3] healthy_baseline vs. healthy_follow-up
n	11	10		10	
EF, %	61.2 ± 1.9	56.3 ± 3.2	0.14	57.6 ± 1.9	0.69
EDV, mL/m^2	57.4 ± 4.5	66.3 ± 5.7	0.049*	58.6 ± 3.4	0.06
ESV, mL/m^2	22 ± 1.9	27.9 ± 2.2	0.17	24.6 ± 3.4	0.11
SV, mL/m^2	36.3 ± 2.9	36.5 ± 3.2	0.85	34.0 ± 2.7	0.33
CO mL/min	3.4 ± 0.48	2.2 ± 0.49	0.045*	2.1 ± 0.15	0.51
Myocardial mass, g/m^2	74.6 ± 6.3	48.4 ± 3.7	0.005*	50.3 ± 3.5	0.7
MYCL, %	1.8 ± 0.18	1.4 ± 0.15	0.14	1.3 ± 0.14	0.25

[1]Values are given as mean ± SEM. *$P < 0.05$. CO, cardiac output; EDV, end-diastolic volume; EF, ejection fraction; ESV, end-systolic volume; MYCL, intramyocardial lipid; NAFLD, nonalcoholic fatty liver disease; SV, stroke volume.
[2]Unpaired Student *t* test used to compare the data between NAFLD and healthy subjects.
[3]Paired Student *t* test used to compare the data between healthy_baseline and healthy_follow-up.

fructose-rich meals, but not after glucose-rich meals in 50-y-old male subjects and in postmenopausal women (8), but remained unchanged in healthy male subjects after consumption of 1.5 g fructose/kg body weight, in combination with a high-fat diet, after 4 wk (6).

Unaffected intrahepatocellular lipid content was observed after 4 wk of fructose supplementation in healthy, lean subjects (6). However, fructose ingestion increased DNL, as measured using stable isotope tracers during application of a hyperinsulinemic euglycemic clamp in normal-weight subjects, after 9 d of a high-fructose diet (7), and after 10 and 12 wk of a high-fructose diet in overweight subjects (3, 8). An overview of important recent studies is summarized in **Supplemental Table 2**, which presents inconsistent data on the potential metabolic effects of fructose.

The impact of fructose on metabolism has been shown to be dose dependent (23), and the daily consumption of >100 g fructose is regarded as very high (9). In the present study, large amounts of fructose, >3-fold the average daily intake reported in the United States (24), over a prolonged period of 8 wk, were administered. However, we did not observe any relevant effects of high-dose fructose ingestion in the presence of stable energy intake and body weight, which enabled us to conclude that healthy subjects can compensate for increased fructose intake.

Dietary records (Figure 1) indicate a spontaneous reduction in the intake of other dietary sugars during fructose intervention. Stable intake of energy (Figure 1) in the presence of unchanged physical activity is the most likely explanation for the observed lack of increase in body weight.

Stable body weight, despite high fructose consumption, has previously been reported in healthy subjects (6, 9). Only minor increases in body weight were observed in abdominally obese subjects after consumption of 75 g fructose over 12 wk (3). This indicates that metabolically healthy subjects adjust the sugar and energy intake to maintain a stable energy intake. Therefore, it was possible to analyze potential adverse metabolic effects of fructose per se, independently from increases in body weight observed in a selection of previous studies (Supplemental Table 2). Lack of compliance as a reason for a lack of weight gain should also be considered. We are aware that dietary intervention in an outpatient setting cannot be completely controlled. However, the participants exhibited significant effort and motivation, by documenting dietary intake and physical activity on a daily basis, and returned unused fructose portions, which indicated that >95% of issued fructose had been ingested. Thus a lack of compliance is an unlikely reason for the lack of weight gain.

Furthermore, as noted above, incomplete intestinal absorption of fructose cannot be excluded. All study participants underwent breath testing to exclude fructose intolerance during the screening phase, prior to dietary intervention. The fructose was provided by the researchers, and the participants were allowed to select the rest of their diet. Importantly, they were told to maintain their dietary habits, including their regular carbohydrate ingestion, as well as their usual physical activity. Despite all these precautions, the high dose of fructose administered in the present study might have occasionally exceeded the intestinal absorption capacity, which could have contributed to a less than expected energy intake. Recent data indicate that low doses of fructose can be cleared by the intestine via conversion of fructose to glucose, lactate, and glycerate, indicating that the small intestine shields the liver from otherwise potentially toxic fructose exposure. However, high doses of fructose overwhelm intestinal fructose absorption and clearance, resulting in fructose reaching both the liver and colonic microbiota (25). Thus, high-dose fructose ingestion is potentially necessary to overcome these protective mechanisms.

The results of previous studies are in line with the results of the present research, suggesting that the metabolic effects of fructose or glucose ingestion can be comparable, and likely related to changes in body weight/energy intake (6, 7, 9) (Supplemental Table 2).

It was demonstrated that young, lean, insulin-sensitive subjects stored excess energy in muscle and the liver in the form of glycogen. In insulin-resistant subjects, however, glycogen synthesis is impaired, and ingested substrates are shifted to hepatic DNL (26). These defects in skeletal muscle glycogen synthesis in insulin-resistant subjects can result in increased plasma concentrations of triglycerides, lower HDL cholesterol, and increased triglyceride synthesis in the liver (26). Thus, skeletal muscle insulin resistance can be closely related to hepatic insulin resistance, and contribute to increased fat accumulation in the liver and other insulin-sensitive tissues, for example, the myocardium. To date, the effects of an increased dietary intake of fructose on metabolic interorgan crosstalk have not been elucidated.

To our knowledge, this is the first study that investigated the long-term effects of a high-dose fructose diet on postprandial glycogen storage in the liver and skeletal muscle. It should be noted that the final portion of fructose was ingested on the day prior to final metabolic phenotyping. Thus, this study was not designed to evaluate potential acute effects of fructose, which have previously been reported (10, 27, 28).

More specifically, Rosset et al. (10) demonstrated decreased net whole-body glycogen content in endurance athletes following ingestion of a liquid meal containing a combination of fat, protein, and 4.4 g/kg/body weight fructose, with 0.6 g glucose/kg to prevent intestinal fructose malabsorption, but not after a liquid meal containing the same amount of fat and protein, with 5 g glucose/kg. Petersen et al. (29) confirmed an acute stimulatory effect of low-dose fructose administration on hepatic glycogen synthesis in healthy subjects.

Our study is in line with another, earlier study indicating that ectopic lipid deposition in 7 young healthy males did not increase following a high-fructose diet (1.5 g/kg body weight/d). In contrast to this finding, HCL increased after a 7-d hypercaloric fructose diet (3.5 g/kg fat-free mass/d) in healthy males (partly offspring of type 2 diabetes patients) (30, 31), or when combined with saturated fat (32). This indicates that not fructose per se, but fructose combined with factors that include genetic predisposition and hypercaloric diet, can increase ectopic lipid deposition. In mice, a high-fat diet and high-sucrose/high-fat diet were accompanied by weight gain, decreased insulin sensitivity, and increased adipocyte size, whereas a high-fructose diet induced a less pronounced increase of adipocytes, and did not change body weight or insulin sensitivity (33). IMCLs have been shown to remain stable after high fructose consumption over 4 wk (1.5 g/kg body weight/d) (6), or to increase in healthy subjects with a family history of diabetes, following the consumption of 35% of energy requirements in the form of fructose, in addition to their ordinary diet (30).

In line with published data, multiple features of impaired lipid and glucose metabolism were observed in patients with NAFLD, including impaired whole-body and hepatic insulin sensitivity, increased hepatic and IMCL content, and impaired postprandial glycogen storage.

In our study, patients with NAFLD showed comparable end-diastolic volume and end-systolic volume, as well as similar EF and SV. Myocardial mass and CO were significantly higher in patients with NAFLD. Impairment of left ventricular systolic function or an increase of myocardial mass were not observed following a medium-term high-fructose diet. Overall, these data are in line with the published literature, and confirm the validity and sensitivity of the applied study methodology (34).

Unfortunately, the net amount of fructose absorbed was not directly measured. As noted above, the "net fructose" intake might have been less than the 150 g/d provided, due to a reduced intake of other dietary sugars, and possibly incomplete fructose absorption.

Further limitations of the current study were that it was not a randomized controlled trial, the study's sample size was small, and the duration of the fructose challenge was limited. Thus, a type II error cannot absolutely be excluded. However, the methods employed for metabolic phenotyping, including dynamic tests and advanced MRS, have all been previously validated, and their specificity for distinguishing different metabolic phenotypes proven (17, 26, 35, 36). Based on the present data, we estimated that subtle differences and trends could become statistically significant following inclusion of a substantially higher number of subjects (n = 35–40). However, the physiological relevance of such a small effect remains questionable.

In summary, our data suggest that in healthy subjects, a prolonged high-dose intake of fructose does not impair basal and postprandial whole-body or tissue-specific glucose and lipid metabolism in the presence of a stable caloric intake and potentially incomplete absorption of the orally administered fructose load.

The authors' responsibilities were as follows—MG, LP: performed magnetic resonance tomography measurements and calculations; S Traussnigg, CK, EH, TL: recruited study participants; TR-H, AS: analyzed dietary protocols; HB, AG, CB: were responsible for the acquisition of data; PW, TL, AH: performed experiments; PW, AL, S Trattnig, M Krššák, M Krebs, MT: provided writing assistance; AK-W, M Krebs, M Krššák, MT: designed the project; M Krebs, M Krššák, MT: provided final support; and all authors: read and approved the final manuscript. MT is speaker for BMS, Falk Foundation, Gilead, and MSD, and is a member of advisory boards for Albireo, Falk Pharma GmbH, Genfit, Gilead, Intercept, MSD, Novartis, Phenex, and Regulus. He has additionally received travel grants from Abbvie, Falk, Gilead, and Intercept, and unrestricted research grants from Albireo, Cymabay, Falk, Gilead, Intercept, MSD, and Takeda. He is also coinventor of patents for the medical use of norUDCA, filed by the Medical University of Graz. M Krebs has received research support from Sanofi, AstraZeneca, and Ipsen, as well as speaker and consulting fees from AstraZeneca, Novartis, Novo Nordisc, Lilly, Merck, Böhringer, Roche, and Sanofi. All other authors report no conflicts of interest.

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