Exercise performed immediately after fructose ingestion enhances fructose oxidation and suppresses fructose storage[1]

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ABSTRACT

Background: Exercise prevents the adverse effects of a high-fructose diet through mechanisms that remain unknown.

Objective: We assessed the hypothesis that exercise prevents fructose-induced increases in very-low-density lipoprotein (VLDL) triglycerides by decreasing the fructose conversion into glucose and VLDL-triglyceride and fructose carbon storage into hepatic glycogen and lipids.

Design: Eight healthy men were studied on 3 occasions after 4 d consuming a weight-maintenance, high-fructose diet. On the fifth day, the men ingested an oral ^{13}C-labeled fructose load (0.75 g/kg), and their total fructose oxidation ($^{13}CO_2$ production), fructose storage (fructose ingestion minus ^{13}C-fructose oxidation), fructose conversion into blood ^{13}C glucose (gluconeogenesis from fructose), blood VLDL-^{13}C palmitate (a marker of hepatic de novo lipogenesis), and lactate concentrations were monitored over 7 postprandial h. On one occasion, participants remained lying down throughout the experiment [fructose treatment alone with no exercise condition (NoEx)], and on the other 2 occasions, they performed a 60-min exercise either 75 min before fructose ingestion [exercise, then fructose condition (ExFru)] or 90 min after fructose ingestion [fructose, then exercise condition (FruEx)].

Results: Fructose oxidation was significantly ($P < 0.001$) higher in the FruEx ($80\% \pm 3\%$ of ingested fructose) than in the ExFru ($46\% \pm 1\%$) and NoEx ($49\% \pm 1\%$). Consequently, fructose storage was lower in the FruEx than in the other 2 conditions ($P < 0.001$). Fructose conversion into blood ^{13}C glucose, VLDL-^{13}C palmitate, and postprandial plasma lactate concentrations was not significantly different between conditions.

Conclusions: Compared with sedentary conditions, exercise performed immediately after fructose ingestion increases fructose oxidation and decreases fructose storage. In contrast, exercise performed before fructose ingestion does not significantly alter fructose oxidation and storage. In both conditions, exercise did not abolish fructose conversion into glucose or its incorporation into VLDL triglycerides. This trial was registered at clinicaltrials.gov as NCT01866215. *Am J Clin Nutr* 2016;103:348–55.

INTRODUCTION

It has been proposed that dietary fructose may play a causal role in the development of obesity, diabetes, and cardiovascular diseases. The consumption of a diet rich in fructose or fructose-containing caloric sweeteners causes hepatic insulin resistance (1, 2), stimulates hepatic de novo lipogenesis (3), increases blood triglyceride concentrations (4), and promotes liver-fat deposition (5) in humans. There is currently much concern that these alterations may contribute to the development of insulin resistance and nonalcoholic liver disease in the long term.

An increase in physical activity has been shown to efficiently improve metabolic homeostasis and reduce cardiovascular disease risk in patients with the metabolic syndrome as well as in healthy participants (6–8). Several studies have specifically reported that an increase in physical activity also prevented fructose-induced metabolic alterations (9, 10) possibly by altering the metabolic fate of fructose ingested before or while exercising.

The disposal of ingested fructose carbons is mainly partitioned into lactic acid production, fructose conversion into plasma glucose and hepatic glycogen, and lipid synthesis (11, 12). Because lactic acid production is more energy efficient than fructose conversion into glucose and glycogen is and much-more efficient than lipid synthesis is (13), we have proposed that fructose conversion into glucose and fat would occur mainly when lactic acid production is saturated (14). Therefore, in this study, we assessed the hypothesis that exercise may prevent a fructose-induced rise in VLDL triglycerides (VLDL-TGs)[6] by decreasing fructose conversion into glucose and VLDL-TGs and fructose carbon storage into hepatic glycogen and lipids.

To evaluate this hypothesis, we assessed how a single exercise session altered the metabolic fate of a pure fructose load in

a group of healthy nonobese subjects. Because exercise is expected to differentially affect endogenous glycogen and blood glucose use when performed in the fasting state or after ingestion of a meal (15, 16), we monitored the fate of an acute ^{13}C-fructose load ingested either immediately before or immediately after a standardized exercise (**Figure 1**). We specifically assessed the partitioning of fructose carbons into oxidation and storage by monitoring breath ^{13}CO$_2$ production and the conversion of fructose carbon into blood glucose by monitoring total ^{13}C glucose appearance with intravenous deuterated glucose. We also assessed the effects of exercise on blood lactate and VLDL-^{13}C palmitate concentrations as potential clues to exercise-induced alterations of hepatic lactate production from fructose and de novo lipogenesis, respectively.

METHODS

Subjects

Eight sedentary male volunteers [mean ± SD age: 22.5 ± 2.2 y; mean ± SD BMI (in kg/m^2): 22.8 ± 0.8] participated in the study. The subjects were in good health, nonsmokers, and not presently taking any medications and had no history of diabetes in their first-degree relatives. The study protocol was approved by the Human Research Ethics Committee of Canton de Vaud, and the participants provided informed written consent. The procedures were conducted in accordance with the Helsinki Declaration of 1975 as revised in 1983. This trial was registered at clinicaltrials.gov as NCT01866215.

Study design

Participants were studied on 3 occasions with fructose ingested on one occasion in sedentary conditions (i.e., without exercise [fructose treatment alone with no exercise condition (NoEx)]) or on the other occasions with an exercise bout performed immediately before fructose ingestion (exercise, then fructose condition (ExFru)] or after fructose ingestion [fructose, then exercise condition (FruEx)] in a randomized order. On each occasion, subjects received a controlled, high-fructose, weight-maintenance diet that contained 55% carbohydrate (30% fructose, 20% starch, and 5% nonfructose sugars), 30% lipids, and 15% proteins during the 4 d before each experiment. Dietary intake was calculated for each participant according to the Harris-Benedict equation (17) and a physical activity factor of 1.5. Diets were provided as prepacked food items and drinks. Fructose was provided as lemon-flavored drinks. Volunteers received instructions to consume all the foods and beverages that they received at specified times and to not consume any other foods or beverages except water. Subjects were also requested to restrain from physical activity during these 4 d.

On the fifth day, subjects underwent one of 3 experimental tests (**Figure 2**). For this purpose, they reported to the Clinical Research Center of Lausanne University Hospital at 0700 after a 12-h overnight fast. On arrival, subjects were weighed (Seca 708; Seca GmbH), and their body compositions were measured with the use of a bioelectrical impedance analysis (Imp DF50; ImpediMed). Participants were transferred to a bed, and a cannula was inserted into a vein of the right forearm for blood sampling.
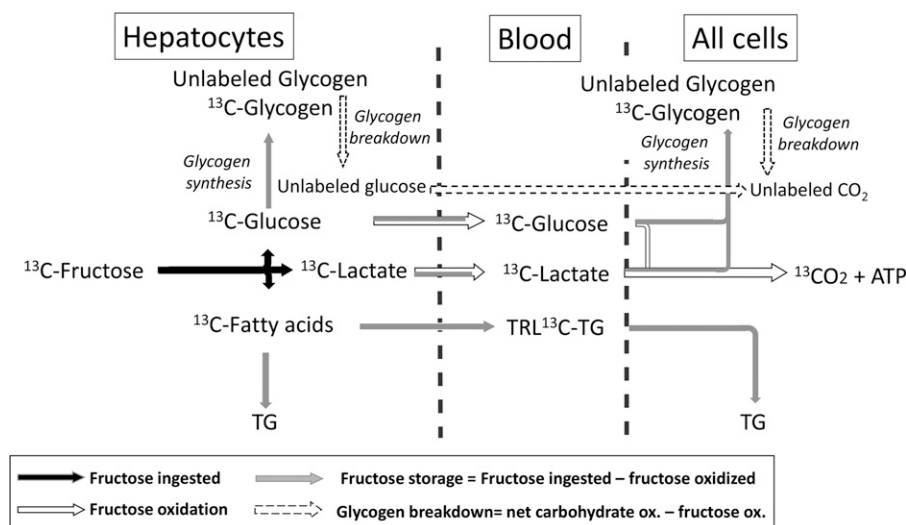
FIGURE 1 Overview of major pathways used for fructose metabolism. Fructose absorbed from the gut (solid black arrow) is mainly extracted by hepatic cells expressing fructokinase and aldolase B (i.e., hepatocytes, intestinal cells, and proximal renal tubule cells) where it is converted into lactate, glucose, and fatty acids. These metabolites are ubiquitous energy substrates that can be released into the bloodstream to be either oxidized or stored as glycogen or triglycerides in all cells of the organism. Because of the complexity of its metabolism, no single tracer method accurately assesses all pathways of fructose disposal. When a ^{13}C-labeled fructose load is administered, breath ^{13}CO$_2$ production provides an estimate of total fructose oxidation (direct oxidation of trioses-phosphate formed from fructose in the liver (not shown) and extrahepatic oxidation of lactate, glucose, and fatty acids synthesized from fructose). With the assumption that all ^{13}C-labeled fructose is completely absorbed during the experiment, (fructose ingested) – (fructose oxidized) represents fructose storage as intracellular triglycerides or glycogen. The measurement of net carbohydrate oxidation with the use of indirect calorimetry does not differentiate the oxidation of glycogen, glucose, lactate, or fructose because all these substrates have the same respiratory quotient, and it provides an estimate of the sum of their net oxidations. Note that standard indirect calorimetry equations compute the oxidation of glucose synthesized from amino acids as being part of net protein oxidation and not of net carbohydrate oxidation. The immediate oxidation of VLDL-TG synthesized from fructose can be assumed to be small, and thus was neglected. When a pure fructose load is ingested, [net carbohydrate oxidation (indirect calorimetry)] – [fructose oxidation (^{13}CO$_2$)] corresponds to the oxidation of endogenous glycogen (i.e., glycogen breakdown), and the rate of ^{13}C-glucose appearance in blood (measured with intravenous tracer infusion of deuterated glucose) corresponds to the release of fructose carbons as glucose in the systemic circulation. ox., oxidized; TG, triglycerides; TRL, triglyceride-rich lipoproteins.

A second cannula was inserted into a vein of the left forearm for tracer infusion. A primed, continuous infusion of $[6,6-^2H_2]$-glucose (Cambridge Isotope Laboratories) (bolus: 3 mg/kg body weight; continuous infusion: $30 \ \mu g \cdot kg^{-1} \cdot min^{-1}$) was administered throughout the test. Blood samples were collected at baseline ($T = 0$) and after 60, 120, 180, 240, 300, 360, 420, 480, and 540 min for the measurement of plasma substrate concentrations and isotopic enrichments. Energy expenditure and substrate oxidation were measured with the use of indirect calorimetry (Quark RMR; Cosmed Srl). An oral fructose load (0.75 g/kg body weight; D-Fructose, Fluka Analytic; Sigma Aldrich) that was enriched with 0.1% $[U-^{13}C_6]$-fructose (Cambridge Isotope Laboratories) was given at time 120 min. Breath samples were collected every 60 min to measure breath $^{13}CO_2$ enrichment. A timed urine collection was performed throughout the test to determine urinary nitrogen excretion.

Each volunteer was studied during 3 experimental conditions according to a randomized order. The sequence of conditions was determined with the use of computer-generated random numbers (R software, version 3.0.2; CRAN). A 4-wk washout period separated the following 3 experimental conditions.

> *1*) No exercise (NoEx): the participant remained installed in bed over the 9 h of the metabolic test and received a fructose load at time 120 min.
>
> *2*) Exercise before fructose ingestion (ExFru): the subject cycled during 1 h at 100 W starting at time 45 min and received a fructose load at time 120 min while remaining in bed the rest of the time. The cycling exercise was performed on an ergometer (Ergoline GmbH). During exercise, oxygen uptake and

carbon dioxide production were measured breath by breath (SensorMedicsVmax; Sensormedics Corp.) during 5 min every 20 min. Blood and breath samples were also collected 20, 40, and 60 min after the beginning of the exercise session.

> *3*) Exercise after fructose ingestion (FruEx): the participant received a fructose load at time 120 min and performed 1 h of cycling exercise at 100 W starting at time 210 min. To account for the exercise-induced increase in glucose fluxes, the $6,6-^2H_2$-glucose infusion rate was increased to $75 \ \mu g \cdot kg^{-1} \cdot min^{-1}$ at the beginning of exercise and was reduced to $30 \ \mu g \cdot kg^{-1} \cdot min^{-1}$ at the end of exercise.

Analytic procedures

Plasma glucose, lactate, nonesterified fatty acids (NEFAs), triglycerides, and urinary urea were measured with the use of enzymatic methods (Randox Laboratories). Insulin and glucagon were measured with the use of a radioimmunoassay (Millipore). The VLDL subfraction was separated by ultracentrifugation as previously described (18). VLDL concentrations were measured with the use of an enzymatic method (Randox Laboratories).

For the plasma glucose isotopic analysis, plasma was deproteinized with the use of barium hydroxide, neutral compounds were isolated by passing the supernatant over anion- and cation-exchange resins, and glucose penta-acetyl derivatives were obtained by adding acetic anhydride and pyridine. Plasma $6,6-^2H_2$ isotopic enrichment was measured with the use of gas chromatography–mass spectrometry (Agilent Technologies). Plasma ^{13}C glucose isotopic enrichment was measured with the use of gas chromatography–combustion–isotope ratio mass
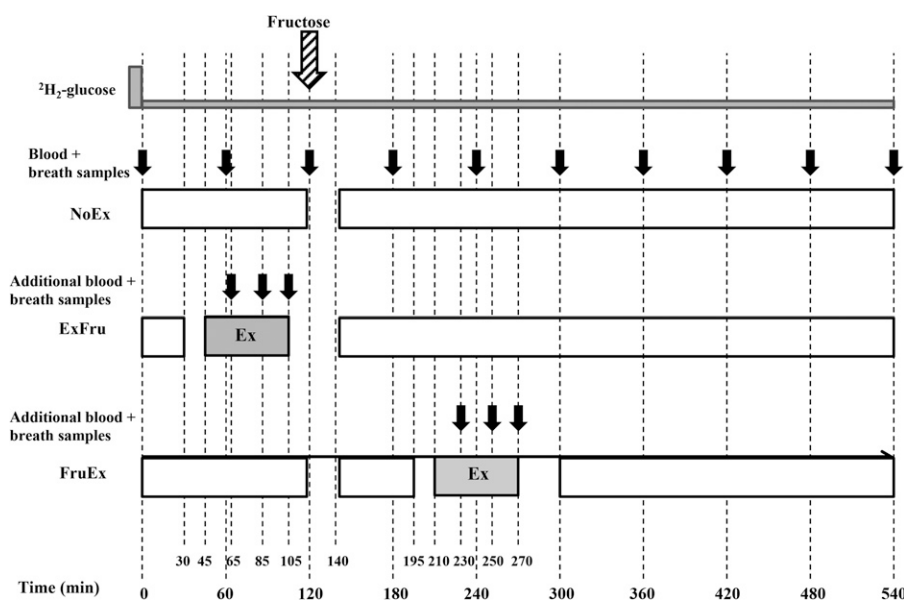
FIGURE 2 Experimental design of day 5 for each condition. In the NoEx, the participant remained in bed over the 9 h of the metabolic test and received a fructose load enriched with 0.1% $[U-^{13}C_6]$-fructose, which was given at time (T) = 120 min (striped arrow). Blood and breath samples (black arrows) were collected at baseline ($T = 0$) and after 60, 120, 180, 240, 300, 360, 420, 480, and 540 min for measurements of plasma substrate concentrations and isotopic enrichments as well as measurements of breath $^{13}CO_2$ enrichment. $[6,6-^2H_2]$-glucose (bolus: 3 mg/kg body weight; continuous infusion: $30 \ \mu g \cdot kg^{-1} \cdot min^{-1}$) was infused throughout the test. In the ExFru, the subject cycled during 1 h at 100 W starting at $T = 45$ min and received a fructose load at $T = 120$ min while remaining in bed the rest of the time. White bars represent the time when the volunteers were resting, and gray bars represent the exercise sessions. Blood and breath samples were also collected 20, 40, and 60 min after the beginning of the exercise session. The $[6,6-^2H_2]$-glucose infusion rate was increased to $75 \ \mu g \cdot kg^{-1} \cdot min^{-1}$ during exercise. In the FruEx, 1 h of cycling exercise at 100 W was performed starting at $T = 210$ min. The 3 experimental conditions were followed in a randomized order. Ex, 60-min exercise; ExFru: exercise, then fructose condition; FruEx, fructose, then exercise condition; NoEx, fructose treatment alone with no exercise condition.

TABLE 1
Anthropometric variables and fasting metabolic substrates and hormones[1]

	NoEx	ExFru	FruEx
Body mass, kg	71.9 ± 5.3	71.1 ± 4.8	72.3 ± 4.9
Fat mass, kg	13.4 ± 3.5	13.1 ± 4.0	13.7 ± 3.6
Systolic blood pressure, mm Hg	118 ± 6	120 ± 8	119 ± 6
Diastolic blood pressure, mm Hg	69 ± 4	70 ± 4	70 ± 5
Glucose, mmol/L	4.89 ± 0.38	4.92 ± 0.37	4.89 ± 0.41
Lactate, mmol/L	0.84 ± 0.36	0.80 ± 0.17	0.84 ± 0.32
Insulin, mU/mL	7.50 ± 2.12	8.00 ± 3.42	7.89 ± 3.04
Glucagon, pg/mL	67.5 ± 32.2	69.7 ± 38.4	69.0 ± 24.9
Triglycerides, mmol/L	1.01 ± 0.42	0.87 ± 0.25	1.07 ± 0.5
VLDL-TGs, mmol/L	0.33 ± 0.12	0.29 ± 0.09	0.34 ± 0.13
NEFAs, mmol/L	0.40 ± 0.11	0.41 ± 0.09	0.46 ± 0.15

[1]All values are means ± SDs (n = 8 participants in each analysis; analyzed by original assigned groups). The normality of the distribution was inspected visually and checked with the use of Shapiro-Wilk tests. Skewed distributions (lactate) were log transformed before the statistical analysis. Changes in anthropometric variables and fasting metabolic variables at T = 0 min of each condition were assessed by Student's paired t tests with Bonferroni corrections for multiple testing. $P < 0.05$ was considered significant. Participants showed no significant difference in anthropometric variables and fasting metabolic substrates and hormones between the 3 tests performed. All P values were NS. ExFru, exercise, then fructose condition; FruEx, fructose, then exercise condition; NEFA, nonesterified fatty acid; NoEx, fructose treatment alone with no exercise condition; VLDL-TG, VLDL triglyceride.

spectrometry (Isoprime; Isoprime Ltd.) at the Centre for Research in Human Nutrition Rhône-Alpes.

Breath $^{13}CO_2$ enrichment was measured by isotope-ratio mass spectrometry (Sercon Ltd.). ^{13}C-palmitate enrichment and concentrations in VLDL were measured after total lipid extractions from plasma and the preparation of fatty acid methyl esters from triglyceride fractions. The palmitate $^{13}C:^{12}C$ ratio in fatty acid methyl ester derivatives was determined with the use of Delta Plus XP GC-combustion isotope ratio mass spectrometry (Thermo Electron Corporation, Bremen, Germany). VLDL-TG palmitate content (percentage of weight) was measured by GC-isotope-ratio mass spectrometry (Thermo Electron, Bremen, Germany) as reported previously (9).

Calculations

Plasma concentrations of VLDL ^{13}C palmitate (nmol/L) were calculated as

$$[\text{VLDL-TG-}^{13}\text{C-palmitate isotopic enrichment (atom percent excess)}] \times \text{weight \% palmitate} \times [\text{VLDL-TG (nmol/L)}] \quad (1)$$

Fructose conversion into plasma glucose was calculated as

$$\text{EGP} \times \{(^{13}\text{CG}_1 + {}^{13}\text{CG}_2) \div 2 + \text{pV} \times [(G_1 + G_2) \div 2 \\ \times ({}^{13}G_2 - {}^{13}G_1) \div (t_2 - t_1)]\} \div {}^{13}\text{CF} \quad (2)$$

where EGP is the endogenous glucose production ($g \cdot kg^{-1} \cdot h^{-1}$) calculated with [6,6-$^2H_2$]-glucose and Steele's equations for non–steady state conditions (19), ^{13}CG is the isotopic enrichment of plasma glucose (atom percent excess), G is the glucose con-

centration (g/L), ^{13}CF is the isotopic enrichment of oral fructose, p is the pool fraction (set at 0.75), V is the glucose distribution space (set at 0.2 L/kg body weight), and t is the time (h).

Total fructose oxidation (g/min) was calculated as

$$\frac{\text{CO}_2 \text{ IE}}{\text{Fructose IE} \times 0.8} \times \frac{\dot{V}\text{CO}_2}{0.134} \times \frac{180}{10^6} \quad (3)$$

where CO_2 IE is the isotopic enrichment of breath carbon dioxide, and fructose IE is the isotopic enrichment of the oral fructose load, both of which are expressed as the atom percent excess, 0.8 is the recovery of ^{13}C from fructose in breath carbon dioxide, $\dot{V}CO_2$ is the total respiratory carbon dioxide (mL/min), 0.134 is the volume of carbon dioxide (mL) produced by the oxidation of 1 μmol fructose, 180 is the molar weight of fructose, and 10^6 allows for the conversion from micrograms to grams.

When cumulated over a 7-h postprandial period, the absorption of ingested fructose can be assumed to be essentially complete, although a few grams may be malabsorbed (20). In such conditions, the amount of fructose ingested minus fructose oxidation represents the sum of fructose carbons retained in the body as glycogen and fat (i.e., fructose storage).

The net carbohydrate oxidation calculated with the use of indirect calorimetry (21) includes the conversion of fructose (or glucose) into fat as long as the fat synthesis does not exceed fat oxidation (i.e., nonnet fat synthesis) (22). With the ingestion of pure fructose (i.e., with no exogenous carbohydrate other than fructose), the difference between the amount of fructose ingested and the cumulated net carbohydrate oxidation represents exclusively the net storage of hepatic and muscle glycogen. The net glycogen storage between times 120 and 540 was calculated as

$$\text{Fructose ingested (g)} - \text{net carbohydrate oxidation (g)} \quad (4)$$

Glycogen breakdown between times 120 and 540 (g) was calculated as

$$(\text{Net carbohydrate oxidation}) - ({}^{13}\text{C fructose oxidation}) \quad (5)$$

Statistical analysis

Anthropometric variables are expressed as means ± SDs, whereas all other values are expressed as means ± SEMs. The normality of the distribution was inspected visually and checked with the use of Shapiro-Wilk tests. When necessary, skewed distributions (baseline lactate) were log transformed before statistical analyses.

Changes in anthropometric variables and fasting metabolic variables at T = 0 min of each condition were assessed with the use of Student's paired t tests with Bonferroni corrections for multiple testing. Metabolic effects of the conditions (NoEx, ExFru, and FruEx) were evaluated by the calculation of incremental AUCs either over 9 or 7 h after fructose consumption and compared with the use of Student's paired t tests with Bonferroni correction for multiple testing. All statistical calculations were performed with R software (version 3.0.2). $P < 0.05$ was considered statistically significant.

RESULTS

All participants were recruited and completed the 3 experimental conditions between July 2012 and June 2013. One

participant who was initially included had to stop the exercise session (because of faintness) and was removed from the analysis and replaced to reach the number of 8 completed participants that were needed on the basis of the sample size calculation. No other harms or unintended effects were reported by participants or observed by investigators during the metabolic tests.

Anthropometric variables and fasting metabolic variables

Participants showed no significant differences in body weight, body fat mass, or blood pressure between the 3 experimental conditions (**Table 1**). Their fasting plasma metabolite and hormone concentrations were also not significantly different (Table 1).

Metabolic effects of fructose without exercise (NoEx)

Fructose ingestion in subjects remaining awake and lying in their bed (NoEx) induced a slight increase in blood glucose and insulin, a marked increase in blood lactate, and a marked suppression of NEFA (**Figure 3**). Estimated pathways used for oral fructose disposal, total energy expenditure, and net substrate oxidation, cumulated over 2-h basal and over 7 h postprandial are shown in **Table 2**. Postprandial oxidation of exogenous fructose corresponded to 49.3 ± 0.9%, total fructose storage to 50.7 ± 0.9%, net glycogen storage to 25.3 ± 5.6%, and fructose conversion into plasma glucose to 15.5 ± 0.7% of the ingested load (Table 2). Blood ^{13}C-palmitate enrichment and ^{13}C palmitate concentrations in VLDL increased progressively from

time 120 to reach a peak at time 300, and declined slowly thereafter (**Figure 4**).

Effects of exercise performed before fructose ingestion (ExFru)

In the ExFru, total energy expenditure and net carbohydrate oxidation ($P < 0.001$ compared with NoEx; Table 2) were significantly higher than in the NoEx during the preprandial 2-h period during which exercise was performed. Glycogen breakdown during this period amounted to 139.0 ± 6.0 g over 2 h (data not shown). Thereafter, the ingestion of the fructose load elicited plasma glucose and lactate responses that did not significantly differ from those in the NoEx (all P = NS compared with NoEx; Figure 3). In contrast, plasma insulin concentrations tended to be lower (P = 0.06 compared with NoEx; Figure 3), and plasma NEFA concentrations were significantly lower ($P <$ 0.01 compared with NoEx; Figure 3). Fructose oxidation (45.7 ± 1.2%), fructose storage (54.3 ± 1.2%), net glycogen storage (41.3 ± 4.5% of the fructose load), and glycogen breakdown that were cumulated over 7 h postprandial, did not show any significant difference compared with in the NoEx (all P = NS; Table 2).

Postprandial total plasma triglyceride concentrations were not significantly altered (P = NS compared with NoEx), but VLDL-TG concentrations were lower ($P < 0.05$ compared with NoEx). AUCs for VLDL-^{13}C palmitate enrichment and concentrations were not significantly different from those in the NoEx (Figure 4).
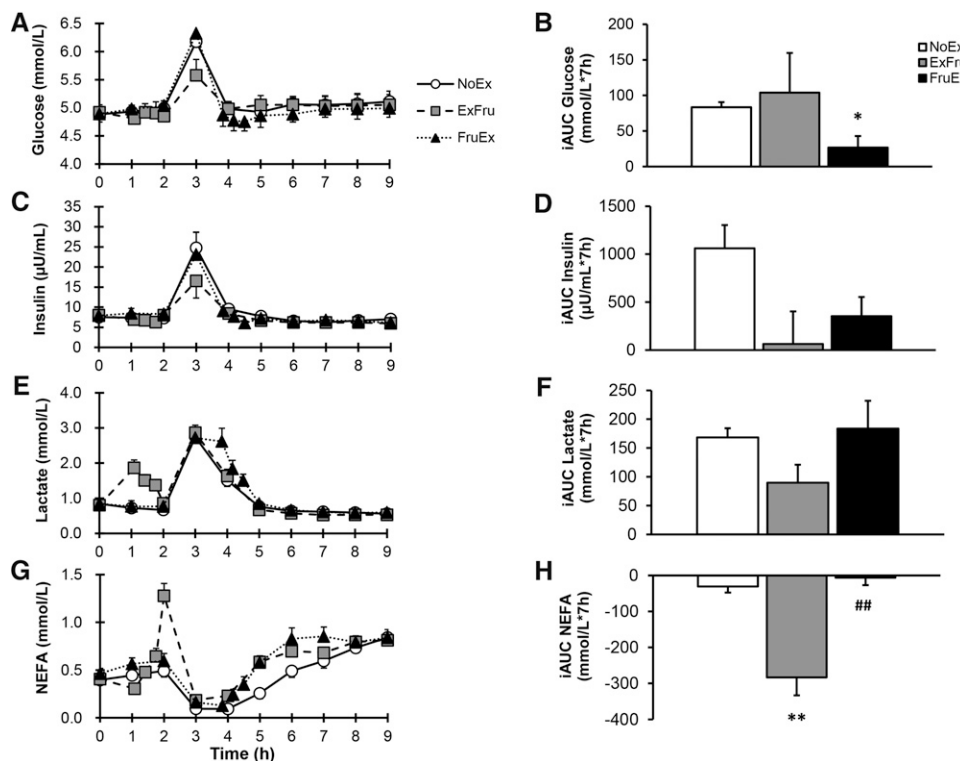

FIGURE 3 Mean ± SEM glucose, insulin, lactate, and NEFA concentrations and postprandial AUCs. n = 8 participants in each analysis; analyzed by original assigned groups. The normality of the distribution was inspected visually and checked with the use of Shapiro-Wilk tests. All data were normally distributed. Metabolic effects of the conditions (NoEx, FruEx, and ExFru) were evaluated by the calculation of the iAUC over 7 h after fructose consumption and compared with the use of Student's paired t test, with Bonferroni corrections for multiple testing. $P < 0.05$ was considered significant. In the ExFru, insulin tended to be lower (P = 0.06 compared with NoEx). *$P < 0.05$ compared with NoEx; **$P < 0.01$ compared with NoEx; ##$P < 0.01$ compared with ExFru. ExFru, exercise, then fructose condition; FruEx, fructose, then exercise condition; iAUC, incremental AUC; NEFA, nonesterified fatty acids; NoEx, fructose treatment alone with no exercise condition.

TABLE 2
Estimated pathways used for oral fructose disposal, total energy expenditure, and net substrate oxidation[1]

	Period AUC, min	NoEx	ExFru	FruEx	*P* NoEx vs. ExFru	NoEx vs. FruEx	ExFru vs. FruEx
Fructose oxidation, g over 7 h	120–540	26.6 ± 1.0	24.4 ± 1.0	43.5 ± 1.9	0.053	<0.001	<0.001
Fructose storage, g over 7 h	120–540	27.3 ± 0.6	28.9 ± 0.8	10.7 ± 1.4	0.25	<0.001	<0.001
Net glycogen storage, g over 7 h	120–540	13.5 ± 2.9	22.3 ± 2.7	−116.7 ± 5.8	0.24	<0.001	<0.001
Glycogen breakdown, g over 7 h	120–540	13.8 ± 3.0	6.6 ± 2.2	127.4 ± 5.1	0.32	<0.001	<0.001
Fructose conversion into plasma glucose, g over 7 h	120–540	8.4 ± 0.5	8.4 ± 0.4	8.6 ± 0.7	1.00	1.00	1.00
Energy expenditure, kcal over 2, 7, or 9 h	0–120	141 ± 5.2	635.3 ± 12.1	152.3 ± 6.9	<0.001	0.21	<0.001
	120–540	537.0 ± 16.2	528.6 ± 20.4	1034.8 ± 29.9	1.00	<0.001	<0.001
	0–540	678 ± 20.9	1163.9 ± 28.6	1187.1 ± 36.3	<0.001	<0.001	0.81
CHOX, g over 2, 7, or 9 h	0–120	9.3 ± 0.9	139 ± 6	7.7 ± 1.6	<0.001	0.81	<0.001
	120–540	40.4 ± 3.5	31 ± 1.9	170.9 ± 6.4	0.20	<0.001	<0.001
	0–540	49.8 ± 4.2	170 ± 6.0	178.6 ± 7.5	<0.001	<0.001	1.00

[1]All values are means ± SEMs (*n* = 8 participants in each analysis; analyzed by original assigned groups). The normality of the distribution was inspected visually and checked with the use of Shapiro-Wilk tests. All data were normally distributed. Metabolic effects of the conditions (NoEx, FruEx, and ExFru) were evaluated by the calculation of incremental AUCs over 120 min (prefructose load), 420 min (after fructose load), or 540 min (overall) and comparison with the use of Student's paired *t* tests with Bonferroni corrections for multiple testing. *P* < 0.05 was considered significant. CHOX, carbohydrate oxidation; ExFru, exercise, then fructose condition; FruEx, fructose, then exercise condition; NoEx, fructose treatment alone with no exercise condition.

Effects of exercise performed after fructose ingestion (FruEx)

In the FruEx, there were no significant differences in plasma insulin, lactate, and triglyceride concentrations compared with those in the NoEx and ExFru except for a lower plasma glucose concentration over the 7 h after fructose ingestion (*P* = 0.038 compared with NoEx' Figures 3 and 4). Exercise performed 90 min after fructose ingestion significantly increased the total energy expenditure and net carbohydrate oxidation (both *P* < 0.001 compared with NoEx; Table 2) to values similar to those observed in the ExFru, whereas lipid oxidation remained comparable to that in the NoEx and ExFru (data not shown). Postprandial fructose oxidation (80.2 ± 2.6% of the fructose load) was nearly doubled compared with the NoEx and ExFru (both *P* < 0.001), and glycogen breakdown was higher than in the NoEx and ExFru (*P* < 0.001). Fructose storage (19.8 ± 2.6% of the fructose load) and net glycogen storage were both lower than in the NoEx and ExFru (all *P* < 0.001; Table 2)

The AUC of VLDL-^{13}C palmitate enrichment was significantly reduced compared with in the NoEx (*P* = 0.039 compared with NoEx; Figure 4). In contrast, AUCs for VLDL-^{13}C-palmitate concentrations were not significantly different from those in the NoEx (Figure 4).

DISCUSSION

To our knowledge, this study allowed us to obtain novel original data regarding the effects of exercise on the fructose metabolic pathways used for its postprandial metabolism.

When fructose was administered in resting conditions, 49% of the exogenous load was oxidized over 7 h postprandial. The amount of newly synthesized, labeled glucose released in the systemic circulation during this time corresponded to 15% of the fructose load. Fifty-one percent of ingested fructose was stored, but the net glycogen storage corresponded only to 25% of ingested fructose. The remaining 26% may be explained by the loss of ^{13}C carbon labels that were due to *1*) whole-body glycogen turnover (i.e., storage of ^{13}C-labeled glycogen with the simultaneous breakdown and oxidation of unlabeled glycogen), *2*) storage of fructose as fat, or *3*) incomplete gut absorption of oral fructose. The appearance of ^{13}C carbons in VLDL palmitate unequivocally indicated that hepatic de novo lipogenesis from fructose occurred. However, a quantitative estimate of hepatic fat synthesis was not possible. Therefore, we conclude that hepatic de novo lipogenesis was active after fructose ingestion. However, the total amount of fat synthesis was likely to be small.

When exercise was performed before fructose ingestion, postprandial fructose oxidation tended to be lower, whereas fructose storage was not significantly different from that in sedentary conditions (i.e., without exercise). Cumulative energy expenditure was largely superior to the energy content of the fructose meal, resulting in an energy deficit of 950 kcal. As a consequence, glycogen utilization during exercise was not fully compensated by glycogen storage after fructose ingestion, which resulted in a global glycogen deficit of 146 g over the total 9 h. Labeled fructose carbons were nonetheless recovered as VLDL palmitate, which indicated that de novo lipogenesis remained active and, hence, that fructose carbons were not exclusively channeled toward glycogen storage.

In contrast, exercise performed immediately after fructose ingestion had substantial effects on postprandial fructose metabolism. It increased the total energy expenditure and net carbohydrate oxidation to the same extent as with the ExFru and resulted in the same total energy and glycogen deficits. However, it markedly enhanced fructose oxidation and decreased fructose and net glycogen storage. Despite this preferential fructose oxidation, the fructose conversion into blood glucose was unchanged, and fat synthesis was still active, as documented by the ongoing incorporation of labeled carbons in VLDL palmitate. Blood lactate, insulin, and triglyceride concentrations were hardly altered compared with in the other 2 conditions.

Our current data support our postulation that exercising after fructose ingestion increases fructose conversion into lactic acid followed by its oxidation in the working muscle. This process has previously been shown with the use of ^{13}C-labeled tracers in
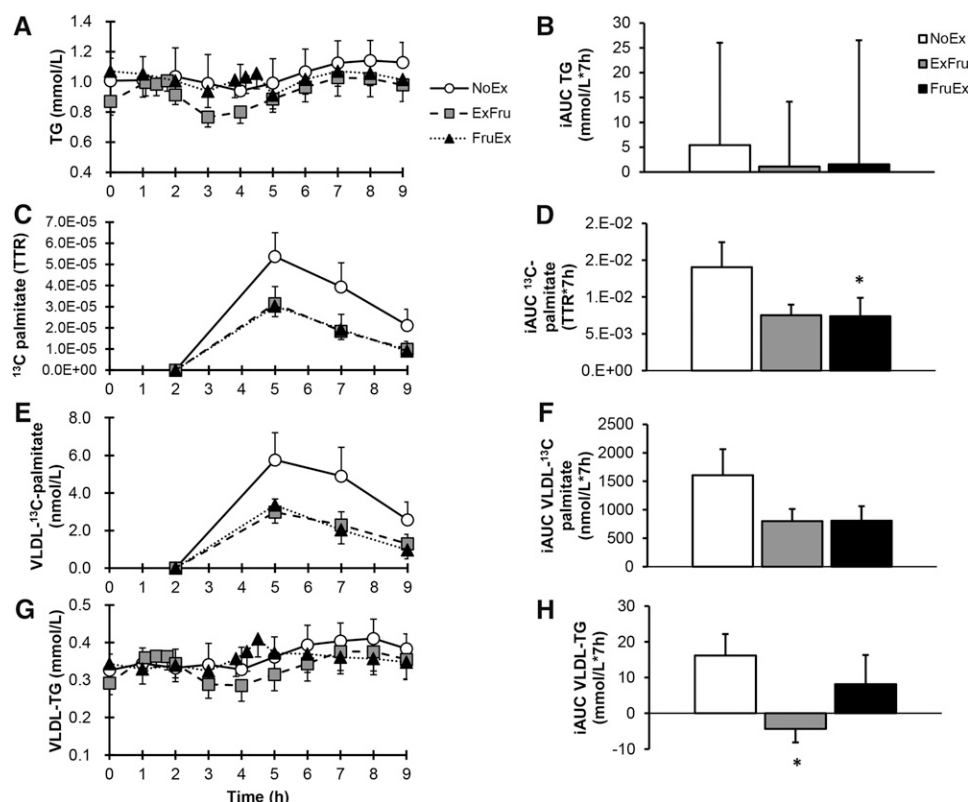
FIGURE 4 Mean ± SEM TG concentrations, ^{13}C-palmitate enrichment, ^{13}C palmitate concentrations in VLDL, VLDL-TG concentrations, and post-prandial AUCs. $n = 8$ participants in each analysis; analyzed by original assigned groups. The normality of the distribution was inspected visually and checked with the use of Shapiro-Wilk tests. All data were normally distributed. Metabolic effects of the conditions (NoEx, FruEx, and ExFru) were evaluated by the calculation of the iAUC over 7 h after fructose consumption and compared with the use of Student's paired t tests with Bonferroni corrections for multiple testing. $P < 0.05$ was considered significant. *$P < 0.05$. ExFru, exercise, then fructose condition; FruEx, fructose, then exercise condition; iAUC, incremental AUC; NEFA, nonesterified fatty acids; NoEx, fructose treatment alone with no exercise condition; TG, triglyceride; TTR, tracer-to-tracee ratio.

exercising subjects who were fed glucose-fructose mixtures (23). In contrast, the data do not confirm our hypothesis that exercising before fructose ingestion would massively divert fructose carbons into glycogen storage. Furthermore, our observations that exercise did not decrease fructose conversion into plasma glucose and did not abolish de novo lipogenesis were in contradiction with our starting hypothesis that de novo lipogenesis would become active only when other pathways were saturated.

It has been reported that exercise can revert hypertriglyceridemia induced by high-fructose or high-carbohydrate weight-maintenance diets (9, 24), and this effect has been proposed as one major mechanism by which exercise may protect against the development of cardiovascular diseases. However, in our current experiments, exercise had no significant effects on plasma triglyceride concentrations. This finding was not unexpected because the ingestion of pure fructose elicits no or only minimal increases in plasma triglyceride concentrations. The addition of fructose to a mixed meal is known to enhance postprandial triglyceride excursions, possibly by impairing extrahepatic triglyceride clearance (25). Such an effect could not be observed in our experiment because no exogenous lipids were administered together with fructose. Our current data provide targeted information regarding the effects of exercise on fructose disposal but provide little information on how exercise specifically prevents the development of hypertriglyceridemia. Pre-

viously, we have documented that hepatic de novo lipogenesis activity increased over time in subjects who were fed a weight-maintenance high-fructose diet. In addition, we have also reported that exercise prevented both this upregulation of hepatic de novo lipogenesis and the development of fasting and postprandial hypertriglyceridemia (9). The mechanisms that are responsible for these effects of exercise on short-term adaptations to dietary fructose remain unknown. We can only speculate that exercise, when performed after fructose ingestion, may contribute to this effect by decreasing the need to store fructose carbons within the organism.

Our current study had important limitations. First, the monitoring of breath ^{13}CO$_2$ release provided a reasonable estimate of total fructose oxidation but did not allow for the differentiation of ^{13}CO$_2$ produced from lactate, glucose, and fatty acids. Second, we did not monitor intestinal fructose absorption in this study. Nonetheless, we are confident that fructose malabsorption, if present, was quantitatively small because participants did not get diarrhea or bloating, which are side effects typically elicited by malabsorption of as little as 5–10 g carbohydrate (20). However, it remains possible that fructose malabsorption <5g passed undetected in some of the participants and, hence, that fructose storage was somewhat overestimated (26). Third, we obtained only qualitative estimates for de novo lipogenesis from fructose because the need to simultaneously assess ^{13}CO$_2$ production and the cost of ^{13}C fructose precluded the use of

highly enriched fructose and the calculation of intrahepatic acetyl-CoA enrichment by mass isotopomer analysis. Fourth, the amount of fructose disposed through systemic lactate was not measured by tracer methods because this would have required a duplication of all tests in each volunteer to separately monitor glucose and lactate kinetics. As a consequence, our data did not allow for a quantitative estimate of the amount of fructose ultimately stored as glycogen and fat or in which organs or tissues this action occurred. Nonetheless, the data provide reliable estimates for fructose oxidation, fructose conversion into plasma glucose, and net glycogen storage and do not support allegations that fructose is mainly converted into fat (27). Nonetheless, fat synthesis from fructose, even if quantitatively minor, may play an important role in the development of fructose-induced hypertriglyceridemia (5).

In conclusion, our results indicate that exercise performed after fructose ingestion markedly enhances fructose oxidation and decreases fructose storage, whereas a similar exercise performed before fructose ingestion does not alter postprandial fructose disposal in healthy individuals. Exercise does not inhibit fructose conversion into plasma glucose and does not abolish fructose conversion into fat. These results suggest that exercise improves sugar-induced hypertriglyceridemia by other mechanisms.

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