Mechanisms for the acute effect of fructose on postprandial lipemia[1–3]

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ABSTRACT

Background: A high fructose intake can lead to postprandial hypertriacylglycerolemia. The underlying mechanism is unclear.

Objective: The objective of the study was to investigate the mechanisms involved in fructose-induced hypertriacylglycerolemia and the contribution of de novo lipogenesis in an acute setting.

Design: In a randomized, crossover study, 14 subjects were given a fructose or glucose test meal after an overnight fast. [$^2H_2$]Palmitate and [U^{13}C]D-fructose or [U^{13}C]D-glucose were added to trace the handling of dietary fats and the fate of dietary sugars in the body. Blood samples were taken before and after the meal. Respiratory exchange ratio was measured by using indirect calorimetry, and breath samples were collected.

Results: Plasma triacylglycerol and VLDL-triacylglycerol concentrations were significantly higher ($P = 0.001$ for both), whereas the concentrations of insulin and [$^2H_2$]palmitate in nonesterified fatty acids were significantly lower after fructose than after glucose ($P = 0.002$ and 0.03, respectively). The respiratory exchange ratio was higher after fructose ($P = 0.04$); significantly ($P = 0.003$) more carbon from sugars was recovered in breath carbon dioxide over 6 h after fructose (30.5%) than after glucose (24.5%). At 240 min, newly synthesized fatty acids from fructose made up ≈0.4% of circulating VLDL-triacylglycerol, whereas newly synthesized triacylglycerol-glycerol made up 38%. Newly synthesized fatty acids and triacylglycerol-glycerol from glucose contributed almost none of VLDL-triacylglycerol ($P = 0.002$ and 0.007 for glucose and fructose, respectively).

Conclusions: The lower insulin excursion after fructose may result in less activation of adipose tissue lipoprotein lipase, which led to impaired triacylglycerol clearance. The contribution of de novo lipogenesis to fructose-induced hypertriacylglycerolemia is small, but its effect on altering the partitioning of fatty acids toward esterification may be considerable. *Am J Clin Nutr* 2007;85:1511–20.

KEY WORDS Fructose, hypertriglyceridemia, de novo lipogenesis, mechanisms, high-carbohydrate diets, stable isotopes, lipoprotein lipase, carbohydrate oxidation

INTRODUCTION

In small doses, dietary fructose appears to be beneficial in enhancing glucose tolerance (1, 2). However, when consumed in large amounts, fructose has been shown to lead to hypertriacylglycerolemia (3). This fact is well established in animal models (4), but findings from past human studies are conflicting. The discrepancies between these findings are likely to be due to differences in the doses of fructose given (4) and in the duration of the diet intervention (5), to sex differences (6, 7), and to the use of different sugars—eg, sucrose (a fructose-glucose disaccharide) (8–11) or glucose—as controls. When compared with glucose, diets containing >15% of energy as fructose are consistently associated with increases in both fasting and postprandial triacylglycerol concentrations in humans (6, 7, 12, 13).

Postprandial lipemia is a known risk marker for coronary heart disease (14). Despite the recognition of the effect of high fructose consumption on postprandial hypertriacylglycerolemia, the mechanism underlying this relation is unclear. An understanding of the mechanisms involved in fructose's induction of hypertriacylglycerolemia in humans may help ascertain whether high fructose consumption contributes to cardiovascular disease risk. However, research on these mechanisms comes mainly from rat studies, whereas conclusions from human studies are usually inferred from research on high-carbohydrate diets, in which the carbohydrate content is not specifically fructose.

Increased plasma triacylglycerol concentrations during high-carbohydrate feeding in humans can be the result of an impairment of VLDL catabolism, enhanced VLDL synthesis, or both (15). An impairment of removal was shown to be the cause of fructose-induced hypertriacylglycerolemia, when rats had a lower VLDL-triacylglycerol (VLDL-TG) fractional catabolic rate after being fed a fructose solution (10% carbohydrate) acutely (16 h) and chronically (14 d) than did rats fed a glucose solution on the same schedule (16). A change in lipoprotein lipase (LPL) activity resulting in a decreased clearance of VLDL particles has been implicated, but evidence from animal studies is conflicting (17). Another possibility, which has not been investigated, is that structural changes in chylomicrons after fructose may affect their susceptibility to lipolysis by LPL and thus contribute to reduced clearance of triacylglycerol.

Another route by which fructose may increase plasma triacylglycerol concentrations is by alteration of the rate of hepatic fatty acid synthesis (18). Fructose feeding, both acute and chronic, has been shown to stimulate hepatic de novo lipogenesis (DNL) in rats (19) and in humans (20–22). However, the extent to which DNL contributes to the hypertriacylglycerolemic effects of fructose in an acute setting is unclear.

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[2] Supported by a grant from Heart UK. Fruisana (UK) and Aarhus United UK Ltd donated the fructose and the oils, respectively, used in the study.

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TABLE 1
Characteristics of the study subjects[1]

	Value
Age (y)	43 ± 15.3
BMI (kg/m^2)	25.3 ± 3.2
Waist-hip ratio	0.88 ± 0.05
Systolic blood pressure (mm Hg)	118 ± 9.2
Diastolic blood pressure (mm Hg)	77 ± 10.7
Percentage body fat (%)	27.3 ± 12.4
Plasma glucose (mmol/L)	5.0 ± 0.3
Plasma triacyglycerol (mmol/L)	1.24 ± 0.6
Plasma cholesterol (mmol/L)	5.0 ± 1.0
HDL cholesterol (mmol/L)	1.3 ± 0.3
Plasma insulin (pmol/L)	58.2 ± 15.6

[1] All values are $\bar{x}$ ± SD, n = 14. Value used for each subject was the mean from the 2 visits. Values did not differ significantly between visits.

In the present study, we sought to investigate the mechanisms for the acute effect of fructose on postprandial lipemia by comparing fructose to glucose in a group of healthy men and postmenopausal women. Stable isotopes were used to trace the handling of dietary fats and the fate of the dietary sugars in the body. We added [$^2H_2$]palmitate to the test meals to trace the fate of dietary fats. The susceptibility of chylomicrons to LPL hydrolysis was also investigated by exposing them to lipolysis by LPL in vitro. D-[U^{13}C]Fructose or D-[U^{13}C]glucose was added to the test meals to measure the incorporation of ^{13}C into the fatty acid and glycerol components of newly synthesized (de novo) plasma triacylglycerol formed from the sugars. This step was taken to assess the potential for fructose and glucose to act as substrates for DNL. Oxidation rates of these sugar substrates were estimated from $^{13}CO_2$ enrichment in breath.

SUBJECTS AND METHODS

Subjects

Fourteen healthy subjects (8 men) were recruited by advertisement. The subjects were in good health; had no history of gastrointestinal, endocrine, or cardiovascular disease; and were currently not taking any prescribed medication (including hormone replacement therapy). The subjects ranged in age from 21 to 64 y and had a body mass index (in kg/m^2) of 22 to 31. Further characteristics of the subjects are shown in **Table 1**.

All subjects gave written informed consent. The study was approved by the Oxfordshire Clinical Research Ethics Committee.

Experimental design

This was a single-blind, randomized, crossover acute study in which the subjects visited the metabolic unit for 2 experimental studies, which were separated by a 6-wk washout period. Subjects were given instructions to avoid vigorous exercise and alcohol and to have a low-fat evening meal the day before each study.

After an overnight fast and before the subject was given a test meal, 2 baseline blood samples were taken through an antecubital venous cannula. Each test meal consisted of an emulsified chocolate drink and a lemon-flavored sugar drink, which together contained 0.75 g sugar (fructose or glucose)/kg body wt and 0.5 g oil (85% palm oil and 15% safflower oil)/kg body wt. In addition, 250 mg D-[U^{13}C]fructose or D-[U^{13}C]glucose (isotope purity 99%; CK Gas Products Ltd, Winchester, United Kingdom) was added to the fructose or glucose test meal, respectively, to trace the fate of dietary sugars. We also added 500 mg [$^2H_2$]palmitate (isotope purity 98%; CK Gas Products Ltd) to each test meal to trace the fate of dietary fats. Further blood samples were then taken at intervals for 6 h after the meal. During the study, respiratory exchange was measured throughout the second half of every hour by using a ventilated-hood indirect calorimeter (Deltatrac; Datex, Helsinki, Finland), and samples of breath were collected into Exetainer tubes (Labco Ltd, High Wycombe, United Kingdom) for measurement of $^{13}CO_2$ enrichment.

Biochemical and mass spectrometric analyses

Analysis of metabolites and hormones

Blood samples were collected into heparinized syringes. A portion was added to 7% (wt:vol) perchloric acid, and the rest was centrifuged at 4 °C to separate plasma. 3-Hydroxybutyrate (3-OHB), glycerol, and lactate concentrations in the perchloric extracts of blood were measured enzymatically as described previously (23) with the use of a centrifugal analyzer (IL Monarch; Instrumentation Laboratory Ltd, Warrington, United Kingdom). Plasma glucose, cholesterol, triacylglycerol, nonesterified fatty acid (NEFA), and insulin concentrations were measured as described previously (24). Plasma fructose was analyzed by using a colorimetric assay modified from Roe (25) and Truswell et al (26).

Isolation of lipoprotein fractions

The following procedures were done for 12 subjects. Separations of Svedberg flotation rate (S_f) > 400 lipoprotein fraction (containing chylomicrons) and S_f 20–400 lipoprotein fraction (containing VLDL) were made by sequential flotation in an ultracentrifuge, and their concentrations were measured as previously described in detail (24).

In vitro lipolysis

In vitro lipolysis of the S_f > 400 lipoprotein fractions was measured by using a method adapted from Fisher et al (27). Briefly, 20 nmol S_f > 400 fraction lipoprotein-triacylglycerol (lipoprotein-TG) was brought to a volume of 140 μL with 0.9% NaCl. To this, 60 μL of 4% bovine serum albumin solution (essentially NEFA-free; Sigma Aldrich, St Louis, MO) was added for a final concentration of 1.2% albumin. The samples were incubated, with shaking, for 15 min at 37 °C with 1.02 units of bovine milk LPL (Sigma Aldrich) in a buffer made of $(NH_4)_2$ SO_4 (at a concentration of 3.2 mol/L) containing 150 IU heparin/L. The reaction was stopped at 0, 2.5, 5, 7.5, and 10 min by adding 10 μL tetrahydrolipstatin solution (3 mg/mL ethanol), mixed by vortex, and placed on ice. The NEFAs released were then measured enzymatically.

Lipid extraction and fatty acid analysis

To determine the specific fatty acid composition and isotopic enrichment, total lipids were extracted from plasma, and fatty acid methyl esters (FAMEs) were prepared from NEFA and triacylglycerol fractions as described previously (28). Similar procedures were followed for the other lipoprotein-TG. Fatty acid compositions (g/100 g total fatty acids) in these fractions were determined by gas chromatography (GC) (28).

Analysis of [$^2H_2$]palmitate and ^{13}C enrichments

[$^2H_2$]Palmitate enrichments in the FAME derivatives were determined by GC-mass spectrometry (GC-MS) (24). The ratio

of ^{13}C to ^{12}C (^{13}C:^{12}C) in the FAME derivatives was ascertained by using a Delta Plus XP GC-combustion-isotope ratio MS (Thermo Electron Corporation, Bremen, Germany). Tricosanoic acid methyl ester was used as an isotopic enrichment standard (28). A quality-control sample (a certified standard of eicosanoic acid FAME; Department of Geological Sciences, Indiana University, Bloomington, IN) was run with each set of samples. The retention times of individual fatty acids were verified by using a reference material (AOCS#6, RM-6; Thames Restek UK Ltd, Buckingham, United Kingdom) containing known proportions of the fatty acids of interest.

In the breath samples, carbon dioxide was separated from the presence of other gases by using a capillary column (CP-PoraPLOTQ; Varian Ltd, Oxford, United Kingdom) with dimensions of 27.5 m × 0.32 mm × 10 μm on the GC-combustion-isotope ratio MS. Splitless injection mode, an injection volume of 40 μL, and an injector temperature of 110 °C were used. The oven temperature was kept constant at 35 °C, and the total run time was 10 min. The column flow was held constant at 1.2 mL/min. The results were not accepted if background enrichment was not within the range of natural enrichment.

The ^{13}C enrichment results (from FAME derivatives and breath samples) expressed as $\delta^{13}C\ ^{0}/_{00}$ were converted to the tracer-to-tracee ratio (TTR) by using the following equation:

$$\text{TTR } (^{13}C{:}^{12}C) = [(\delta^{13}C‰/1000) + 1] \times 0.0112372 \quad (1)$$

The TTR of a baseline measurement (before administration of the stable-isotope tracer) was subtracted from each sample TTR to account for natural abundance. The concentration of ^{13}C incorporated into the fatty acids of S_f 20–400 lipoprotein-TG was calculated by using the following equation:

$$^{13}C \text{ in palmitate of } S_f \text{ 20–400 lipoprotein-TG } (\mu mol/L) = \text{TTR} \times \text{concentration of palmitate moiety in } S_f \text{ 20–400 lipoprotein-TG } (\mu mol/L) \times 3 \times 16 \times 17/16 \quad (2)$$

where [^{13}C]palmitate is used as an example and the multiplications by 3, 16, and 17/16 are used to account for the number of fatty acids per triacylglycerol, the number of carbon atoms per fatty acid, and the methyl group added during derivatization, respectively.

The rate of expiration of $^{13}CO_2$ in breath at each timepoint was calculated by multiplying the carbon dioxide production ($\dot{V}CO_2$; in mmol/min) by the TTR of $^{13}CO_2$:$^{12}CO_2$. The total amount of $^{13}CO_2$ (in mmol) produced in 6 h was calculated from the area under the curve (AUC). The glycerol component of the S_f 20–400 lipoprotein-TG (from 10 subjects) was obtained from the lipid extraction procedure and isolated by using ion-exchange chromatography as described previously (29). These [^{13}C]triacylglycerol-glycerol (TG-glycerol) samples were measured by using GC-MS (29). The concentration of ^{13}C incorporated into the TG-glycerol of S_f 20–400 lipoprotein-TG was calculated by using the following equation:

$$^{13}C \text{ in TG-glycerol of } S_f \text{ 20–400 lipoprotein-TG } (\mu mol/L) = \text{TTR} \times \text{concentration of TG-glycerol in } S_f 20–400 \text{ lipoprotein-TG } (\mu mol/L) \times 3 \quad (3)$$

where multiplication by 3 is used to account for the number of carbon atoms in TG-glycerol.

Statistical analysis and calculations

Data were analyzed by using SPSS for WINDOWS software (version 14; SPSS UK, Chertsey, United Kingdom). Statistical significance was set at $P < 0.05$. All data are presented as means ± SEMs unless otherwise stated. As shown in the figures, changes in concentrations with time were assessed by using repeated-measures analysis of variance (ANOVA) with time and treatment (test meal) as within-subject factors (except for plasma fructose concentrations). Values obtained for blood 3-OHB, S_f 20–400 lipoprotein-TG, and $S_f > 400$ lipoprotein-TG were log transformed for analysis.

Data were also analyzed as AUCs. These were calculated for the basal period (−30–0 min) and from the start of eating (0 min) to 360 min later (postprandial area). Incremental AUCs (iAUCs) were calculated by subtracting from the postprandial area the mean baseline value extrapolated over a period of 360 min, which reflected the changes occurring after the meal. Differences between the 2 test meals (fructose and glucose) were tested by using Wilcoxon's signed-rank test on iAUC values. Unless otherwise stated, P values given in the text were obtained by using these values.

The degree of difference in the concentrations of the various metabolites after the 2 test meals was calculated by dividing the mean iAUC value for all subjects obtained after fructose by the mean iAUC value for all subjects obtained after glucose. Respiratory exchange ratio and net substrate oxidation rates were calculated as described previously (30).

To trace the fate of dietary sugar, the percentages of labeled sugar that were oxidized to produce $^{13}CO_2$ in the breath or to form de novo triacylglycerol (fatty acids and glycerol components) were estimated. To estimate the proportion of labeled sugar that was oxidized to produce $^{13}CO_2$ in the breath, the total amount of $^{13}CO_2$ (mmol) produced in 6 h was first divided by 6 (number of ^{13}C atoms in a uniformly labeled [^{13}C]sugar) to give an equivalent amount (mmol) of uniformly labeled [^{13}C]sugar. This was then divided by the amount (mmol) of uniformly labeled [^{13}C]sugar added to the test meal.

We explored the fate of ingested fructose and glucose carbons at 240 min (the timepoint at which the concentration of [^{13}C]fatty acids peaked). The mean percentage of labeled sugar that formed [^{13}C]fatty acids in S_f 20–400 lipoprotein-TG at 240 min was calculated by using the following equation:

$$\text{Mean percentage of labeled sugar that formed } [^{13}C] \text{ fatty acids in } S_f \text{ 20–400 lipoprotein-TG at 240 min} = [\text{mean concentration of } ^{13}C \text{ in fatty acids } (\mu mol/L) \times 3 \text{ (L)/amount of uniformly labeled } [^{13}C] \text{ sugar } (\mu mol) \times 6] \times 100\% \quad (4)$$

where the multiplications by 3 and 6 are used to account for the volume of plasma in the body (in L) and the number of carbon atoms in the labeled sugar, respectively.

By the same principle, the mean percentage of labeled sugar that formed [^{13}C]TG-glycerol in S_f 20–400 lipoprotein-TG at 240 min was calculated by using the following equation:

Mean percentage of labeled sugar that formed [^{13}C] TG-glycerol in S_f 20–400 lipoprotein-TG at 240 min = [mean concentration of ^{13}C in TG-glycerol (μmol/L) × 3 (L)/amount of uniformly labeled [^{13}C] sugar (μmol) × 6] × 100% (*5*)

where the multiplications by 3 and 6 are used to account for the volume of plasma in the body (in L) and the number of carbon atoms in the labeled sugar, respectively.

The plasma volume of 3 L is typical (31). As explained below, figures obtained from the above calculations are estimates only.

To illustrate the lipogenic effects of the sugars on hypertriacylglycerolemia, the percentages of circulating S_f 20–400 lipoprotein-TG contributed by newly synthesized fatty acids and TG-glycerol were calculated for each subject at 240 min. These are referred to as percentages of de novo fatty acids and de novo TG-glycerol, respectively, as shown in the following equations:

Percentage of de novo fatty acids = [[^{13}C] fatty acids in S_f 20–400 lipoprotein-TG (μmol/L) × amount of sugar given (g)/S_f 20–400 lipoprotein-TG concentration (μmol/L) × 0.25 (g)] × 100% (*6*)

and

Percentage of de novo TG-glycerol = [[^{13}C] TG-glycerol in S_f 20–400 lipoprotein-TG (μmol/L) × amount of sugar given (g)/S_f 20–400 lipoprotein-TG concentration (μmol/L) × 0.25 (g)] × 100% (*7*)

in both of which equations, multiplication by 0.25 was used to account for the amount of [^{13}C]sugar (in g) in the test meal.

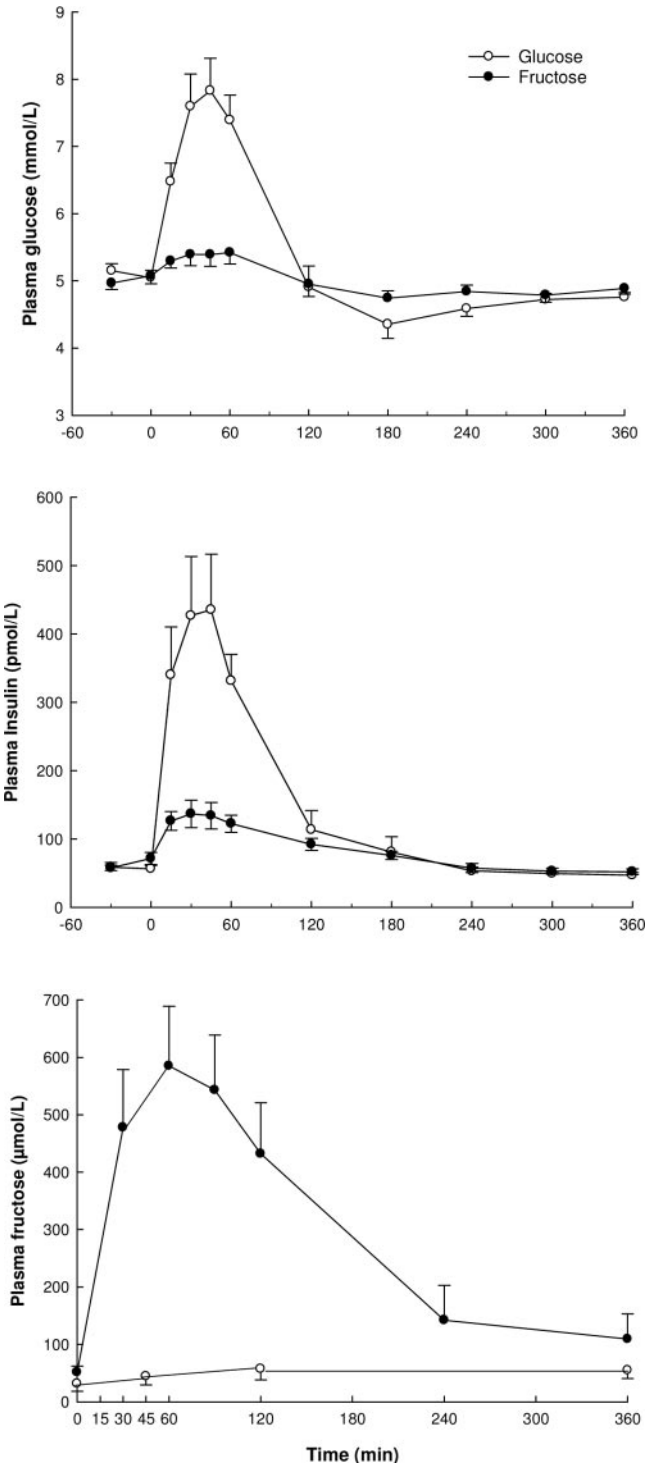

FIGURE 1. Mean (±SEM) concentrations of metabolites after test meals containing fructose or glucose. The test meal was taken at time 0. There were significant meal × time interactions for plasma glucose ($n = 12$) and plasma insulin ($n = 12$) ($P < 0.001$ for both) by repeated-measures ANOVA. Plasma fructose ($n = 12$) also was significantly ($P = 0.003$) higher after fructose than after glucose by incremental area under the curve.

RESULTS

Metabolites and [$^2H_2$]palmitate enrichments

Plasma glucose, insulin, and fructose

For both meals, plasma glucose concentrations peaked between 30 and 60 min and returned to baseline after 120 min. As expected, the increase in plasma glucose concentration was significantly ($P = 0.04$) higher after the glucose meal than after the fructose meal (**Figure 1**). The plasma insulin response followed

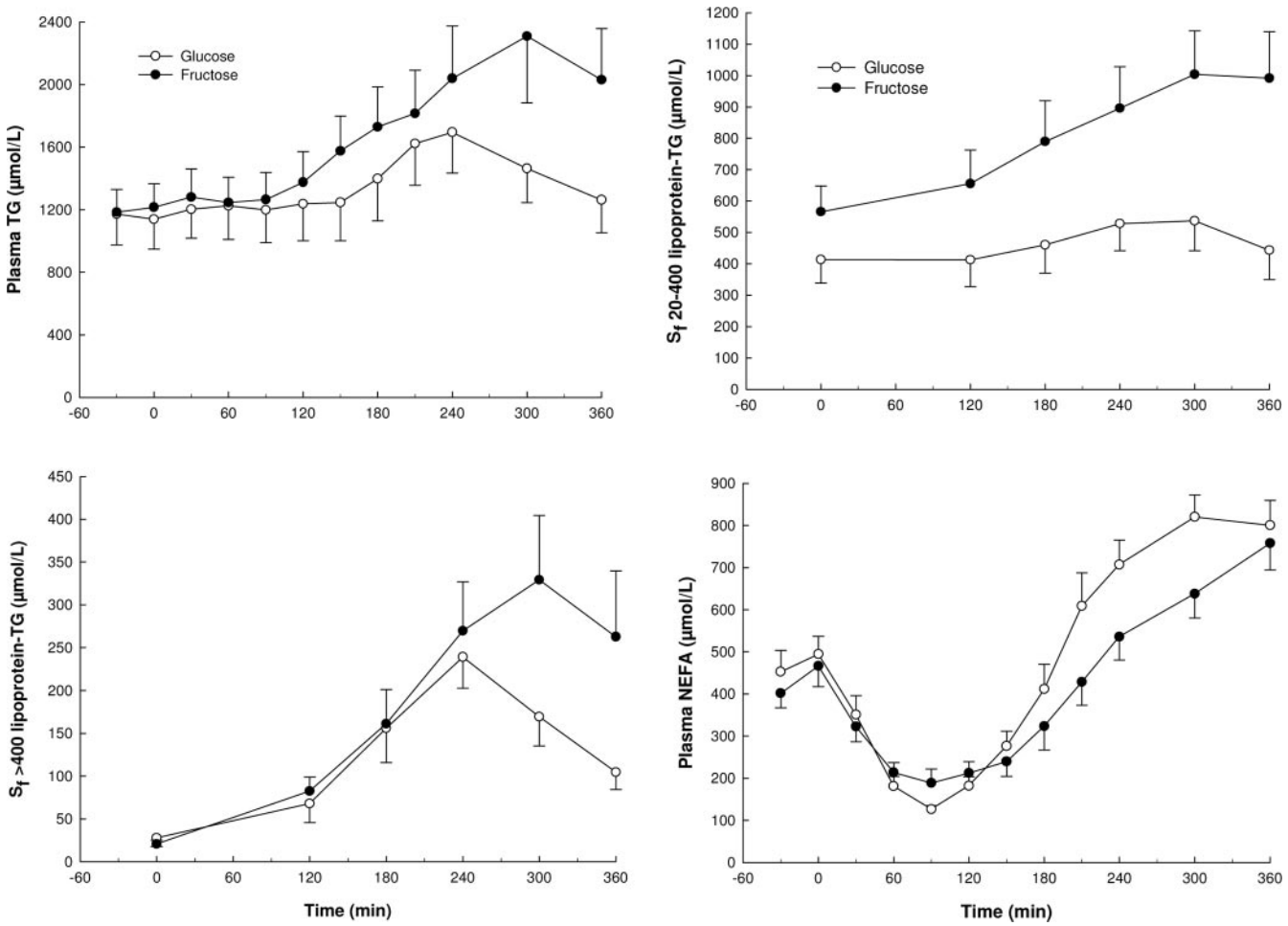

FIGURE 2. Mean (±SEM) concentrations of metabolites after test meals containing fructose or glucose. The test meal was taken at time 0. There were significant meal × time interactions for plasma triacyglycerol (TG; $n = 14$) ($P = 0.005$), Svedberg flotation rate (S_f) 20–400 lipoprotein-TG ($n = 12$) ($P = 0.03$), $S_f > 400$ lipoprotein-TG at postprandial period (240–360 min) ($n = 12$) ($P = 0.03$), and plasma nonesterified fatty acids (NEFA) ($n = 14$) ($P = 0.02$) by repeated-measures ANOVA.

a similar pattern, with a significantly ($P = 0.002$) higher concentration after glucose than after fructose (Figure 1).

After the fructose meal, the mean plasma fructose concentration rose significantly ($P = 0.003$), reaching a mean peak of 600 μmol/L at 60 min; it returned to baseline after 240 min (Figure 1). The maximum peak concentration reached in one of the subjects was 1118 μmol/L. After the glucose meal, mean plasma fructose concentrations stayed relatively constant at baseline (<50 μmol/L) throughout the study.

Plasma triacylglycerol, triacylglycerol-rich lipoproteins, and nonesterified fatty acids

After the meals, the mean plasma triacylglycerol concentration rose earlier after fructose (120 min) than after glucose (180 min). However, mean plasma triacylglycerol concentrations peaked later after fructose (300 min) than after glucose (240 min) and did not return to baseline within 360 min. Overall, the mean plasma triacylglycerol concentration was significantly ($P = 0.001$) higher after fructose than after glucose (**Figure 2**). The S_f 20–400 lipoprotein-TG concentration after fructose was 4.5 times that after glucose ($P = 0.001$); both concentrations achieved peak values at 300 min, and only the values in the glucose arm returned to baseline at 360 min (Figure 2). The $S_f >$ 400 lipoprotein-TG concentration also was significantly ($P = 0.05$) higher after the fructose than after the glucose. A peak delay was seen in the late postprandial period after fructose (Figure 2).

The concentration of plasma NEFAs fell after both meals, reaching a nadir at 90 min. The nadir was lower after glucose than after fructose, but the NEFA concentrations then rose quickly, and, beyond 180 min, they were consistently and significantly higher after glucose (Figure 2).

[$^2H_2$]Palmitate enrichment in plasma triacylglycerol, triacylglycerol-rich lipoproteins, and nonesterified fatty acids

[$^2H_2$]Palmitate was added to the test meal to trace the handling of dietary fats. Parallel to their unlabeled counterparts, the concentrations of [$^2H_2$]palmitate in plasma triacylglycerol ($P = 0.03$) and S_f 20–400 lipoprotein-TG ($P = 0.02$) (**Figure 3**) were significantly greater after fructose than after glucose. The concentration of [$^2H_2$]palmitate in $S_f > 400$ lipoprotein-TG was significantly greater in the later postprandial period (240–360 min) after fructose ($P = 0.05$ for meal × time interaction, ANOVA) than after glucose. [$^2H_2$]Palmitate appeared in NEFAs 90 min after the meals. The concentrations of [$^2H_2$]palmitate in NEFAs after both meals rose similarly until the 150-min timepoint, after which the concentration of [$^2H_2$]palmitate in NEFA

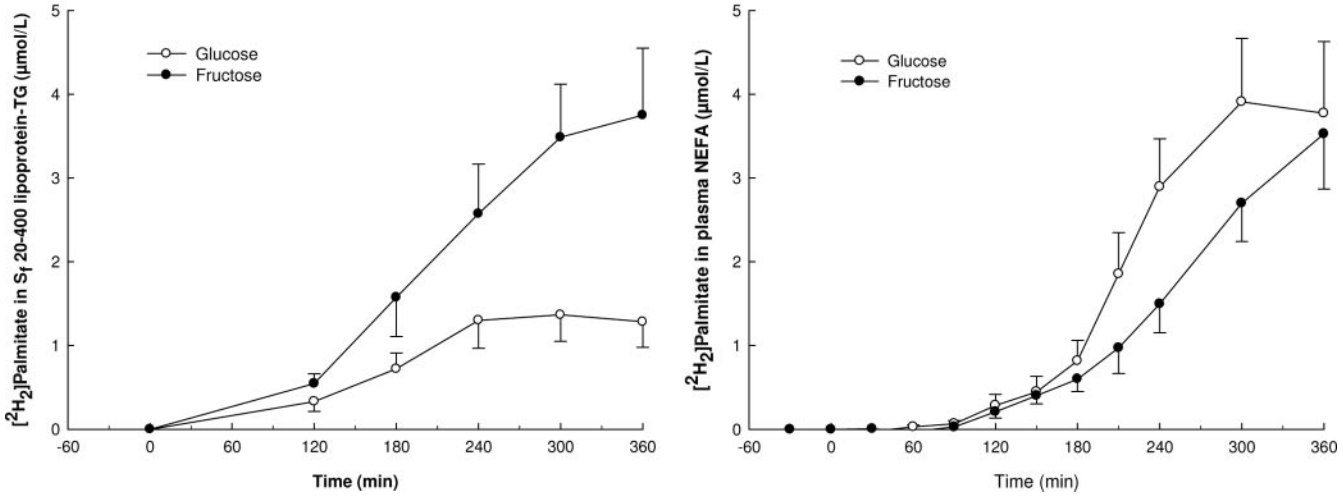

FIGURE 3. Mean (±SEM) concentrations of [$^2H_2$]palmitate in plasma fractions after test meals containing [$^2H_2$]palmitate and fructose or glucose. The test meal was taken at time 0. There were significant meal × time interactions for [$^2H_2$]palmitate in plasma Svedberg flotation rate (S_f) 20–400 lipoprotein-triacylglycerol (TG; $n = 11$) ($P = 0.006$) and [$^2H_2$]palmitate in plasma nonesterified fatty acids (NEFA; $n = 11$) ($P = 0.04$) by repeated-measures ANOVA.

rose significantly more rapidly after glucose than after fructose for the remaining period of the study ($P = 0.03$) (Figure 3).

In vitro lipolysis of chylomicrons

There was no difference in the amount of fatty acids released when $S_f > 400$ lipoprotein-TG after both meals were subjected to LPL in vitro ($P = 0.6$), which indicated that there was no difference in the rate of lipolysis. This suggested that the chylomicrons generated after the 2 meals did not differ in their susceptibility to LPL hydrolysis.

Oxidation and de novo lipogenesis

Blood 3-hydroxybutrate, glycerol, and lactate

Blood 3-OHB concentrations were suppressed immediately after both meals; after 120 min, they rose for the duration of the study. Mean blood 3-OHB concentrations rose significantly less after fructose than after glucose (**Figure 4**). There was no significant difference between the changes in concentrations of blood glycerol after the 2 meals ($P = 0.8$). In contrast, the blood lactate concentration after fructose was twice that after glucose (mean peak concentration: 2300 and 1100 μmol/L, respectively; $P = 0.002$).

Substrate oxidation rates

The production of $^{13}CO_2$ was higher after fructose than glucose, which indicates that significantly ($P = 0.003$) more fructose than glucose was oxidized (Figure 4). This finding concurred with the higher respiratory exchange ratio ($P = 0.04$ for meal × time interaction, ANOVA; $P = 0.937$ for iAUC), higher net carbohydrate oxidation rates ($P = 0.002$ for meal × time interaction, ANOVA; $P = 0.6$ for iAUC), and lower net fat oxidation rates ($P = 0.055$ for meal × time interaction, ANOVA;

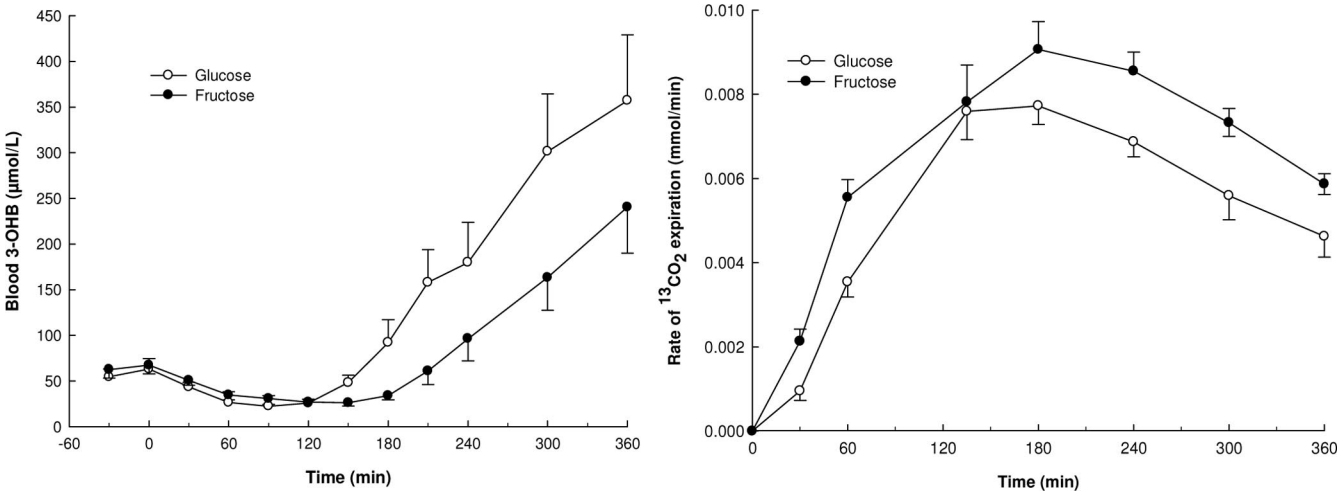

FIGURE 4. Mean (±SEM) concentrations of blood 3-hydroxybutyrate (3-OHB) and rates of expiration of $^{13}CO_2$ in breath samples after test meals containing [U^{13}C]fructose or [U^{13}C]glucose. The test meal was taken at time 0. The meal × time interaction was significant for blood 3-OHB ($n = 12$) ($P = 0.003$), and the meal effect was significant for breath $^{13}CO_2$ ($n = 12$) ($P = 0.002$) by repeated-measures ANOVA.

$P = 0.8$ for iAUC) observed after the fructose meal. The mean percentage of the labeled sugar recovered in breath carbon dioxide over 6 h was 30.5% and 24.5% after fructose and glucose, respectively ($P = 0.003$).

[^{13}C]Fatty acids of plasma Svedberg flotation rate 20–400 lipoprotein-triacylglycerol

D-[U^{13}C]Fructose or D-[U^{13}C]glucose was added to the test meals to trace the conversion of sugars to de novo fatty acids in plasma S_f 20–400 lipoprotein-TG. In general, ^{13}C enrichment in the fatty acids rose above baseline after fructose, whereas enrichments in the fatty acids remained close to baseline after glucose. The concentrations of [^{13}C]palmitate (16:0) ($P = 0.002$), [^{13}C]myristate (14:0) ($P = 0.01$) (**Figure 5**), and [^{13}C]stearate (18:0) ($P = 0.007$) in S_f 20–400 lipoprotein-TG were significantly higher after fructose than after glucose. The peak concentration of [^{13}C]palmitate was ≈10 times the peak concentrations of [^{13}C]myristate and [^{13}C]stearate.

The concentrations of [^{13}C]palmitoleate (16:1n−7) and [^{13}C]oleate (18:1n−9 plus n−7) were higher after fructose than after glucose, but the differences were not significant ($P = 0.07$ and 0.06, respectively). The concentration of [^{13}C]linoleate (18:2n−6), an essential fatty acid, was taken as a control. No appearance of [^{13}C]linoleate was seen after either meal. The percentage of labeled sugar that formed de novo [^{13}C]fatty acids in S_f 20–400 lipoprotein-TG at 240 min was ≈0.05% after fructose and ≈0% after glucose ($P = 0.007$ for glucose versus fructose).

[^{13}C]Triacylglycerol-glycerol of plasma Svedberg flotation rate 20–400 lipoprotein-triacylglycerol

The concentration of [^{13}C]TG-glycerol in S_f 20–400 lipoprotein-TG was significantly higher after fructose than glucose ($P = 0.007$) (Figure 5). The percentage of labeled sugar that formed [^{13}C]TG-glycerol in S_f 20–400 lipoprotein-TG at 240 min was ≈0.15% after fructose and ≈0% after glucose ($P = 0.007$).

Percentage of de novo fatty acids and de novo triacylglycerol-glycerol

There were wide between-subject variations in the percentage de novo fatty acids and percentage de novo TG-glycerol. At 240 min, the estimated mean values calculated for percentage de novo fatty acids were 0.4 ± 0.14% (range: −0.01% to 1.5%) after fructose and −0.0003 ± 0.0002% after glucose (−0.0005% to 0.0009%) ($P = 0.002$). For percentage de novo TG-glycerol, the estimated mean value was 38 ± 10% (−14% to 95%) after fructose, and values were significantly ($P = 0.007$) greater than zero. After glucose, the mean percent de novo TG-glycerol was −10 ± 10% (−92% to 19%), and values were not significantly greater than zero ($P = 0.4$).

DISCUSSION

As expected, fructose produced a significantly smaller glycemic excursion and induced a significantly smaller increase in insulin concentration than did glucose. Our findings on plasma fructose concurred with Holdsworth and Dawson (32), who recorded a maximum fructose concentration of 1 mmol/L in peripheral blood in humans after a fructose meal.

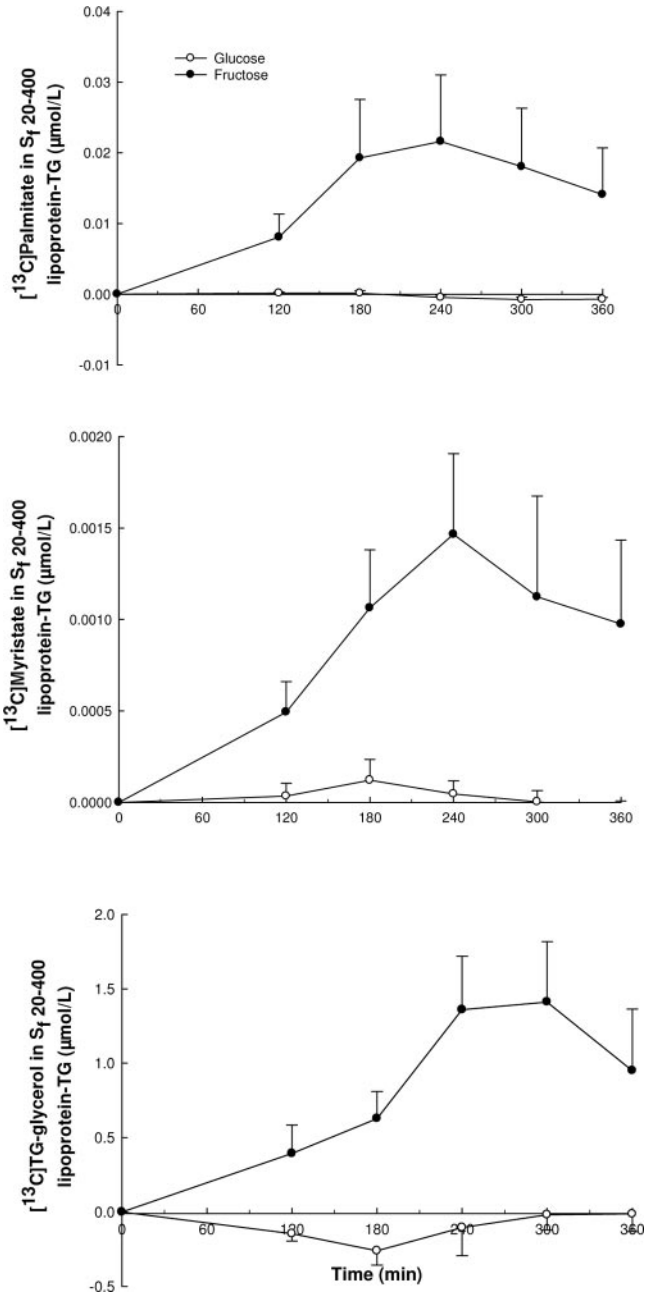

FIGURE 5. Mean (±SEM) concentrations of [^{13}C]fatty acids and triacylglyerol (TG)-glycerol in Svedberg flotation rate (S_f) 20–400 lipoprotein-TG after test meals containing [U^{13}C]fructose or [U^{13}C]glucose. The test meal was taken at time 0. There were significant meal × time interactions for [^{13}C]palmitate ($n = 12$) ($P = 0.045$), [^{13}C]myristate ($n = 10$; 2 samples did not have detectable myristate) ($P = 0.059$), and [^{13}C]TG-glycerol ($n = 10$) ($P = 0.023$) by repeated-measures ANOVA.

Concurring with the results of 2 other studies (33, 34), the results of the present study showed that fructose potentiates postprandial lipemia in the acute setting. We also found a significantly higher rise in VLDL-TG (S_f 20–400 lipoprotein-TG) concentration and a later peak in chylomicron-TG ($S_f > 400$ lipoprotein-TG) concentration after fructose, findings that were consistent with a delay in clearance or absorption.

In the adipose tissue, some fatty acids released by LPL are directed into fat storage and others are released into the circulation (which is known as the "spillover effect") (35). Adipose

tissue LPL activity is known to be up-regulated by insulin (36). In the present study, the concentration of isotopically labeled NEFAs was lower after fructose than after glucose. Yet, in concurrence with findings by Vrana et al (37), no difference was observed between the changes in blood glycerol concentrations after fructose and after glucose, which may indicate a similar amount of lipolysis from adipose tissue depots. It is thus likely that the lower NEFA concentration in the plasma in the late postprandial period after fructose than after glucose comes mainly from the lesser spillover of LPL-derived fatty acids from the chylomicron-TG than with glucose. In parallel with the lower NEFA concentration after fructose is the smaller increase in blood 3-OHB concentrations, which indicated that less NEFAs were subsequently oxidized in the liver after fructose than after glucose.

Because chylomicrons produced after the 2 meals were shown not to differ in their susceptibility to LPL hydrolysis, the results together suggest that the lower insulin excursion after fructose than after glucose could have reduced the activation of adipose tissue LPL. Because both chylomicrons and VLDL particles compete for hydrolysis by LPL, when the activity of LPL is limited, the higher affinity of LPL for chylomicrons than for VLDL (38) leads to less clearance of VLDL-TG. However, we cannot rule out decreased hepatic removal of VLDL-TG, as shown in fructose-fed rats (39).

Using an oral fructose load (diluted in water) of 0.5 or 1 g/kg, Delarue et al (40) reported that 56% or 59% of the fructose load, respectively, was oxidized over a 6-h study. In contrast, the present study used an oral fructose load of 0.75 g/kg and also 0.5 g oil/kg, and we found that only 35% of the fructose load was oxidized over 6 h. The oxidation of a lesser amount of fructose in the present study could be due to the presence of the dietary lipid, which is known to reduce hepatic glucose uptake (41, 42). However, it is also important to note that the rate of breath $^{13}CO_2$ expiration did not return to baseline at the end of the study, which suggests that the postprandial metabolism of sugars can extend beyond 6 h. More fructose (≈30% more, by extrapolation of Figure 4 to baseline) would have been oxidized if the study were extended for a longer time.

Even then, the lipogenic potential of fructose appears to be small. The results showed that only 0.05% and 0.15% of fructose were converted to de novo fatty acids and TG-glycerol, respectively, at 240 min. We did not have sufficient data from which to formally model the incorporation of fructose carbons into VLDL-TG. However, the turnover time for plasma VLDL-TG is ≈2 h (43), which would suggest that, integrated over the whole period of the experiment, <1% of the ingested sugar would have been converted to VLDL-TG. Thus, the remainder (≈40%) of the fructose is unaccounted for. A greater proportion of the remaining fructose than of the remaining glucose would have been diverted toward lactate formation—as is evident from the results of the present study and other studies (44, 45)—and converted to liver glycogen (46). The rest must have been used in the formation of the other end-products of fructose metabolism and indirectly oxidized by extrasplanchnic tissues (47).

In the present study, it was estimated that de novo fatty acids (formed from the labeled sugar) made up 0.4% of circulating VLDL-TG 240 min after a fructose meal. This percentage is different from that in the study by Schwarz et al (48), in which nearly 30% of circulating TG-palmitate was from hepatic DNL after fructose ingestion. The differences in fructose dosage, mode of administration, and methods used in quantifying DNL (12-h ^{13}C-acetate infusion) are likely to account for this. In addition, because we were able to estimate DNL only from one timepoint of the study and were unable to take into account the true isotopic enrichment of the fatty acid precursor pool, the value we obtained would be an underestimation of the actual values. Nevertheless, the value we obtained illustrates the argument that the contribution of fructose carbons to VLDL-TG fatty acids during DNL is not a quantitative explanation for acute fructose-induced hypertriacylglycerolemia. It is much more likely that the activation of hepatic DNL increased VLDL-TG secretion by altering the partitioning of fatty acids in the liver. Hepatic fatty acid oxidation is decreased by the increased concentrations of malonly-CoA (an intermediate in the pathway of DNL), which is an inhibitor of carnitine-palmitoyl transferase-1 (the enzyme involved in the transport of long-chain fatty acids into the mitochondria) (49), and fatty acids are channeled toward esterification. Results from the substrate oxidation rates support the above suggestion. Net fat oxidation was decreased and net carbohydrate oxidation was increased to a greater extent after fructose than after glucose. Thus, despite the minor contribution of de novo fatty acids to the overall increase of VLDL-TG by fructose, the effect of fructose on lipid metabolism may be considerable.

In contrast, de novo TG-glycerol (formed from the labeled sugar) made up 38% of circulating VLDL-TG-glycerol 240 min after fructose. It appears that fructose's contribution of glycerol to the fructose-induced hypertriacylglycerolemia is more significant than its contribution of fatty acids. It is thus likely that the production of glycerol 3-phosphate in the liver from the ingested fructose further increased fatty acid esterification at the expense of oxidation. Our study concurred with that of Maruhama (50) and confirmed that fructose has greater lipogenic potential than does glucose.

In agreement with Aarsland and Wolfe (51), we found that palmitate was the predominant product of carbohydrate-mediated lipogenesis, and that the pathway of further elongation and subsequent desaturation of de novo synthesized palmitate to generate stearate and oleate was quantitatively minor in this acute situation. More striking was the appearance of labeled myristate in plasma, which, to our knowledge, has not previously been reported in humans. In addition, as did those who conducted other high-carbohydrate feeding studies (52, 53), we noted the wide between-subject variations of percentage de novo fatty acids and percentage de novo TG-glycerol in the subjects after fructose feeding in the present study.

To summarize, our results suggest that a decrease in plasma-TG removal via a lower activation of adipose tissue LPL contributes largely to acute fructose-induced lipemia. Although the contribution of DNL to fructose-induced lipemia is small, the effect of DNL on the selective partitioning of fatty acids toward esterification instead of oxidation may be significant. More important, although fructose is a better substrate for DNL than is glucose, the lipogenic potential of fructose is probably better judged by its greater contribution to the glycerol component, rather than the fatty acid component of de novo triacylglycerol.

The per capita disappearance data for fructose in the United States indicate that the combined consumption of sucrose and high-fructose corn syrup has increased from 64 g/d in 1970 to 81 g/d in 1997 (3). These figures are close to or higher than the dose of fructose used in the present study. Thus, the mechanisms of

acute fructose induction of hypertriacylglycerolemia identified in the present study may be very applicable to current fructose consumption habits in humans. The similarity of fructose-induced hypertriacylglycerolemia to certain forms of endogenous hypertriacylglycerolemia is also suggestive of potential cardiovascular disease risks. This study of the mechanisms of fructose-induced lipemia in the acute setting would help provide a platform for further mechanistic research on long-term effects of fructose on plasma lipids.

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The authors' responsibilities were as follows—MF-FC (a PhD student): conduct of the entire study under the supervision of BAF and KNF; MF-FC, BAF, and KNF: designed the study; MF-FC: the draft of the manuscript; and BAF and KNF: assistance with laboratory methods, data interpretation, and writing of the manuscript. None of the authors had any financial or personal conflict of interest.

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