

# Postexercise repletion of muscle energy stores with fructose or glucose in mixed meals<sup>1,2</sup>

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## ABSTRACT

**Background:** Postexercise nutrition is paramount to the restoration of muscle energy stores by providing carbohydrate and fat as precursors of glycogen and intramyocellular lipid (IMCL) synthesis. Compared with glucose, fructose ingestion results in lower postprandial glucose and higher lactate and triglyceride concentrations. We hypothesized that these differences in substrate concentration would be associated with a different partition of energy stored as IMCLs or glycogen postexercise.

**Objective:** The purpose of this study was to compare the effect of isocaloric liquid mixed meals containing fat, protein, and either fructose or glucose on the repletion of muscle energy stores over 24 h after a strenuous exercise session.

**Design:** Eight male endurance athletes (mean  $\pm$  SEM age:  $29 \pm 2$  y; peak oxygen consumption:  $66.8 \pm 1.3$  mL  $\cdot$  kg<sup>-1</sup>  $\cdot$  min<sup>-1</sup>) were studied twice. On each occasion, muscle energy stores were first lowered by a combination of a 3-d controlled diet and prolonged exercise. After assessment of glycogen and IMCL concentrations in vastus muscles, subjects rested for 24 h and ingested mixed meals providing fat and protein together with 4.4 g/kg fructose (the fructose condition; FRU) or glucose (the glucose condition; GLU). Postprandial metabolism was assessed over 6 h, and glycogen and IMCL concentrations were measured again after 24 h. Finally, energy metabolism was evaluated during a subsequent exercise session.

**Results:** FRU and GLU resulted in similar IMCL [ $+2.4 \pm 0.4$  compared with  $+2.0 \pm 0.6$  mmol  $\cdot$  kg<sup>-1</sup> wet weight  $\cdot$  d<sup>-1</sup>; time  $\times$  condition (mixed-model analysis):  $P = 0.45$ ] and muscle glycogen ( $+10.9 \pm 0.9$  compared with  $+12.3 \pm 1.9$  mmol  $\cdot$  kg<sup>-1</sup> wet weight  $\cdot$  d<sup>-1</sup>; time  $\times$  condition:  $P = 0.45$ ) repletion. Fructose consumption in FRU increased postprandial net carbohydrate oxidation and decreased net carbohydrate storage (estimating total, muscle, and liver glycogen synthesis) compared with GLU ( $+117 \pm 9$  compared with  $+135 \pm 9$  g/6 h, respectively;  $P < 0.01$ ). Compared with GLU, FRU also resulted in lower plasma glucose concentrations and decreased exercise performance the next day.

**Conclusions:** Mixed meals containing fat, protein, and either fructose or glucose elicit similar repletion of IMCLs and muscle glycogen. Under such conditions, fructose lowers whole-body glycogen synthesis and impairs subsequent exercise performance, presumably because of lower hepatic glycogen stores. This trial was registered at clinicaltrials.gov as NCT01866215. *Am J Clin Nutr* 2017;105:609–17.

**Keywords:** athletes, intramyocellular lipids, muscle glycogen, recovery, hypertriglyceridemia, lactate shuttle

## INTRODUCTION

Adequate recovery of endogenous energy stores is critical for exercise performance and is largely dependent on nutrition (1, 2). The maintenance of muscle glycogen, in particular, has been considered a major challenge for endurance athletes because of its limited content, importance as a substrate source, and association with the development of fatigue during exercise (3, 4). Consequently, most nutritional guidelines recommend the increase of postexercise dietary carbohydrate content to favor muscle glycogen repletion between sessions (2).

Consumption of beverages or foods containing glucose or maltodextrins is generally advocated to improve postexercise muscle glycogen repletion because of the increase in plasma glucose and insulin concentrations they elicit. In contrast, fructose is considered not to be a prime energy source for muscle, and most (5–7) but not all (1) studies have found lower muscle glycogen repletion after fructose ingestion than after glucose ingestion. However, these observations were made with pure glucose and maltodextrin rather than with mixed meals. In addition, muscle glycogen synthesis was monitored during the hours immediately after exercise ( $<8$  h postexercise), which corresponds with a period in which muscle insulin sensitivity is enhanced. Unlike glucose, fructose is primarily metabolized in the liver, and pure fructose was shown to efficiently restore hepatic glycogen after exercise (8). Substantial amounts of fructose are also released as blood glucose and lactate from the liver (9, 10). In addition, fructose metabolism can be modulated by coingested nutrients such as glucose or amino acids (9, 10). For these reasons, the comparison of the presence of fructose and glucose in a mixed meal on muscle glycogen repletion still remains unclear. Fructose and glucose could also differentially affect muscle fat storage. Indeed, beside glycogen, intramyocellular

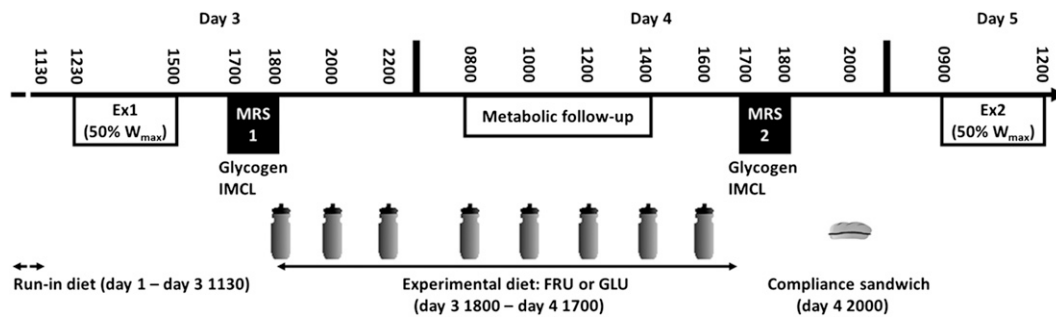
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**FIGURE 1** Setting of the 5-d experimental sequences. Subjects were studied on 2 occasions, each consisting of a 5-d sequence of dietary and physical activity control. Having first ingested a weight-maintenance controlled diet between day 1 and day 3, subjects were supervised while cycling for 2 h 30 min at 50  $W_{\max}$  (Ex1: 1230–1500 on day 3). Lowered IMCL and glycogen stores in vastus muscles were then measured by MRS (MRS1: 1700–1800 on day 3) before subjects started a 24-h period of dietary recovery during which they remained inactive and received the experimental diets. A total of 2.5 g fat/kg, 1.9 g protein/kg, and 5.6 g carbohydrate/kg, including 4.4 g/kg of either pure fructose or pure glucose, was administered in 8 liquid mixed meals provided at 1800, 2000, and 2200 (day 3), and 0800, 1000, 1200, 1400, and 1600 (day 4). The effect of 3 of these meals was assessed during a postprandial metabolic follow-up of 6 h (0800–1400 on day 4), and muscle energy stores were again measured after the end of the 24-h period of recovery (MRS2, day 4: 1700–1800). To improve compliance, subjects received a standardized small sandwich to ingest at the end of day 4. Finally, subjects, who fasted overnight, participated in a second endurance session to assess the effect of the previous diet on energy metabolism and subsequent exercise capacity (Ex2: 0900–1200 on day 5). Ex, exercise; FRU, fructose condition; GLU, glucose condition; IMCL, intramyocellular lipid; MRS, magnetic resonance spectroscopy;  $W_{\max}$ , maximal workload.

lipids (IMCLs)<sup>5</sup> represent an alternative energy source to muscle glycogen, which can be mobilized during exercise (11). As for muscle glycogen, IMCL stores are replenished in the postexercise period. However, the relative contributions of plasma free fatty acids (FFAs) (12) and lipoprotein-bound triglycerides from dietary fat (13) to postexercise IMCL replenishment remain controversial (14). High-fructose diets are consistently associated with increased fasting and postprandial plasma triglyceride concentrations (15). These effects are accounted for by both increased hepatic VLDL triglyceride secretion (16) and decreased adipose tissue triglyceride clearance (17). We therefore hypothesized that the consumption of fructose might stimulate IMCL replenishment more efficiently than might glucose consumption.

The primary aim of this study (NCT01866215) was therefore to compare the effectiveness of isocaloric mixed meals containing fat and protein with either pure fructose or pure glucose on IMCL and muscle glycogen replenishment over a 24-h period after a strenuous exercise session. We also wanted to assess whether these mixed meals containing fructose (the fructose condition; FRU) or glucose (the glucose condition; GLU) had different effects on whole-body metabolism during a subsequent standardized exercise session.

## METHODS

All variables are presented as means  $\pm$  SEMs. Eleven well-trained young men were recruited from local triathlon and cycling clubs. Two of them dropped out during the experiment because of gastrointestinal symptoms during the fructose condition. One subject completed the study but was excluded from analysis because of apparent failure to comply with dietary instructions (see Results section). The 8 remaining subjects [age:  $29 \pm 2$  y; weight:  $71.2 \pm 1.9$  kg; BMI (in  $\text{kg}/\text{m}^2$ ):  $22.6 \pm 0.4$ ; peak oxygen consumption:  $66.8 \pm 1.3$   $\text{mL} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ; maximal workload ( $W_{\max}$ ):  $368 \pm 12$  W] were nonsmokers and

weight-stable, took no medication, had no family history of diabetes and had  $\geq 3$  y of cycling training and racing experience. In addition, all of them were fully informed about the nature of and risks involved in the testing procedure and provided written consent before beginning the study. Except for magnetic resonance spectroscopy (MRS) measurements, which were done at the Department of Clinical Research and Radiology, University of Bern, the experiments were performed at the Clinical Research Center, University of Lausanne, Switzerland. The experimental protocol was approved by the Ethics Committee of the Canton de Vaud, Lausanne, Switzerland, and all procedures were performed in accordance with the 1983 revision of the Declaration of Helsinki.

## Study design

The subjects of this double-blind, crossover-controlled, randomized clinical trial were studied on 2 occasions separated by 2–4 wk. The experiments (Figure 1) started with a run-in period aimed at lowering the muscle energy stores by using a 2.5-d controlled diet (day 1 to 1130 on day 3) followed by a prolonged exercise session (exercise 1: 1230–1500 on day 3). IMCL and muscle glycogen concentrations were then measured (MRS1: 1700 on day 3). Thereafter, 2 experimental isocaloric mixed diets that contained either fructose or glucose were provided as 8 liquid meals ingested at 1800, 2000, and 2200 on day 3, and at 0800, 1000, 1200, 1400, and 1600 on day 4. To evaluate the effects of these diets, plasma metabolic markers and energy metabolism were monitored after an overnight fast (0800 on day 4) and over 6 h postprandial (0800–1400 on day 4). Thereafter, IMCLs and muscle glycogen were measured at 1700 (MRS2). The next day, all subjects reported to the Clinical Research Center after an overnight fast to have their metabolism monitored during 3 h of standardized exercise (exercise 2: 0900–1200 on day 5).

## Preliminary visit

Between 1 and 2 wk before the first experimental condition, subjects performed an incremental test to exhaustion on an electrically braked leg cycle ergometer (Excalibur; Lode). Peak

<sup>5</sup> Abbreviations used: FFA, free fatty acid; FRU, fructose condition; GLU, glucose condition; IMCL, intramyocellular lipid; MRS, magnetic resonance spectroscopy;  $W_{\max}$ , maximal workload; WW, wet weight.

oxygen consumption was determined by the continuous monitoring of respiratory gas exchange with the use of indirect calorimetry (Quark CPET; Cosmed) as previously described (18). Briefly, after a 5-min warm-up at 60 W, ergometer power was increased by 35 W every 3 min.  $W_{\max}$  was calculated by linear regression of the time completed and was used for setting subsequent exercise intensities.

#### **Experimental sequences: run-in period (day 1 to 1700 on day 3)**

The purpose of this period was to lower muscle energy stores at the beginning of the 2 experimental periods. Subjects were provided an outpatient weight-maintenance diet (day 1 to 1130 on day 3) providing 1.5 times calculated basal energy expenditure (19) and containing 55% carbohydrate, 30% fat, and 15% protein. Food items were weighed (P10; Mettler Toledo) and prepacked by the experimenters. Subjects were instructed to ingest all the food supplied and nothing else except water, and were asked to return emptied containers. To further standardize muscle energy stores, subjects were instructed to perform, under both conditions, 1 h of endurance exercise on day 1, remain sedentary on day 2, and perform a supervised constant-load cycling exercise session (exercise 1: 2.5 h at 50%  $W_{\max}$ ) between 1230 and 1500 on day 3. After this session, subjects traveled by public transportation to the MRI scanning facility for an initial assessment of muscle IMCL and glycogen concentrations by MRS (MRS1: 1700–1800 on day 3). Travel time was  $\sim 90$  min, with a walk of  $<5$  min. Subjects were escorted by an investigator to ensure that physical activity remained minimal and that no food was consumed. Altogether, a comparable protocol was previously used to standardize baseline IMCL and muscle glycogen concentrations (13).

#### **MRS measurements**

Noninvasive measurements of IMCLs, followed by glycogen concentrations, in the vastus muscles were performed with the use of a 3-Tesla whole-body scanner (Verio; Siemens) before (MRS1: 1700 on day 3) and after (MRS2: 1700 on day 4) the dietary interventions. To ensure reproducible anatomical positioning, the subjects' right legs were fixed in a specially designed cast and the first session positioning served as an intraindividual reference. Care was taken to minimize MRI-visible extramyocellular lipids in the voxel, and measurements were performed at the same time of day, by the same operator. A double-tuned, linearly polarized proton-MRS/ $^{13}\text{C}$ -MRS surface coil (Lammers Medical Technology) was used, and constant magnetic field was assessed by a shimming package (Siemens), followed by post hoc calculation of the shim currents. To determine vastus intermedius IMCL contents by proton MRS, a  $9 \times 9 \times 18 \text{ mm}^3$  voxel was used, and nonwater-suppressed spectra (1 acquisition) and water-suppressed spectra (96 averages) were acquired with the use of a point-resolved spectroscopy localization sequence (retention time: 3 s; echo time: 30 ms). Glycogen measures by  $^{13}\text{C}$ -MRS targeted a larger region in the vastus lateralis and medialis, and 3 consecutive spectra (retention time: 200 ms; 2048 averages each; 7 min/spectrum) were acquired and compared with a reference spectrum (retention time: 200 ms; 32 averages; chromium-doped acetone, keto-group singlet on center frequency). Relaxation effects were not corrected, and spectra were analyzed with the use of

jMRUI version 3.1 (20). Water and total creatine signals were used as internal standards to determine IMCL and glycogen concentrations, respectively [water reference: 39.8 mol/kg wet weight (WW); total creatine reference: 33.8 mmol/kg WW] (13, 21).

#### **Dietary intervention (1700 on day 3 to 1700 on day 4)**

The experimental diets were provided during the whole experimental period, and were consumed partly under investigators' supervision. Random assignment and meal preparation were performed by an experimenter who was not otherwise involved in the experiments, leaving subjects and investigators blinded. Both FRU and GLU contained the same total energy and macronutrient composition ( $3508 \pm 154$  kcal as 5.6 g carbohydrate/kg, 2.5 g fat/kg, and 1.9 g protein/kg), and were consumed as 8 equal liquid meals ( $308 \pm 13$  mL each) ingested at 1800, 2000, and 2200 (day 3, unsupervised), and 0800, 1000, 1200, 1400, and 1600 (day 4, supervised). This timing was selected to ensure continuous energy provision while limiting the effect of prior exercise. The choice of a liquid meal was made because of the convenience of preparing accurate, tailor-made liquid diets according to the energy needs of each subject. The total energy administered during the experimental period was calculated to match total energy expenditure. This was done by summing each participant's daily metabolic requirements [basal energy expenditure (19) with a 1.4 physical activity level to account for the fact that subjects remained in bed most of the day] and the energy expended during exercise 1 (assuming a muscle energy efficiency of 25%). Fat was provided with the use of a mixture of fresh full cream, soy cream, and soy milk, and protein was provided with the use of soy milk protein and whey proteins (Whey Protein 94; Sponser Sport Food). All of these products are available commercially. Carbohydrate was added as either 5.0 g glucose/kg in GLU, or 4.4 g fructose/kg with 0.6 g glucose/kg in FRU [D-(+)-Glucose and D-(–)-Fructose; Sigma-Aldrich]. Using small amounts of glucose in the fructose meals prevents intestinal fructose malabsorption (22). After MRS2, subjects were instructed to ingest a small sandwich ( $347 \pm 11$  kcal containing 0.6 g carbohydrate/kg (mainly starch), 0.1 g fat/kg, and 0.5 g protein/kg) under both conditions, and to continue fasting thereafter (except for water).

#### **Metabolic follow-up (0800–1400 on day 4)**

The metabolic effects of the experimental diets were directly assessed on day 4 (0800–1400). Fasting subjects reported to the Clinical Research Center at 0700 and were installed on a bed after a void. A venous cannula was inserted into a forearm vein to allow repeated blood sampling at 0800 (baseline), 0830, 0900, 1000, 1030, 1100, 1200, 1230, 1300, and 1400. Respiratory exchange measurements (indirect calorimetry) were performed continuously in the fasting state (0730–0800) and, after the provision of 3 experimental meals, at 0800, 1000, and 1200. Throughout the test, urine was collected in a container to determine urea nitrogen excretion and the exact time at which urine collection was ended. The exact time elapsed since the initial miction was used to calculate urinary urea excretion rate. At 1400, metabolic follow-up was discontinued, and the subjects were instructed to ingest the last 2 liquid meals (1400 and 1600)

while traveling to the MRS laboratory to repeat measurements (MRS2: 1700–1800 on day 4).

### Energy metabolism during standardized endurance exercise (0900–1200 on day 5)

The effect of the experimental diets on energy metabolism were evaluated on day 5 during a prolonged cycling exercise session performed in the fasting state. Upon arrival at the laboratory (0700), subjects were asked to void. They were then weighed, and a cannula was inserted into a forearm vein for repeated blood sampling (0800, 0830, 0900, 0920, 0940, 1000, 1020, 1040, 1100, 1120, 1140, and 1200). After an initial baseline resting period (0800–0900), subjects mounted the ergometer and started cycling at a constant workload for 3 h or until exhaustion (exercise 2: 50%  $W_{max}$ , 0900–1200). Subjects had free access to water, but otherwise remained in a fasting state. Their energy expenditure, respiratory exchange ratio, and substrate oxidation were measured by indirect calorimetry. As on the previous day, urine was collected throughout the test to determine protein oxidation.

### Analyses

Blood samples were collected in EDTA-coated tubes and centrifuged (10 min;  $2800 \times g$ ;  $4^{\circ}\text{C}$ ), and plasma aliquots were stored at  $-20^{\circ}\text{C}$  until analyzed. Plasma FFAs, triglycerides, glucose, lactate, and urinary nitrogen were measured by enzymatic methods (RX Monza; Randox Laboratories), and insulin and glucagon were determined by radioimmunoassay with the use of commercial kits (Merck Millipore).

### Calculations

Energy expenditure (kilocalories per minute) and net carbohydrate, lipid, and protein oxidations (grams per minute) were calculated from respiratory gas exchanges with the use of standard indirect calorimetry equations (23). Net rates of oxidation measured by indirect calorimetry are a reflection of each nutrient's balance, but it is not possible to specify the metabolic pathways used for each nutrient's oxidation. For example, net carbohydrate oxidation represents the sum of exogenous glucose, fructose, and endogenous glycogen oxidation, without including oxidation of glucose formed from amino acid precursors, which would be included in net protein oxidation (24). Net protein oxidation was calculated while assuming that urinary urea nitrogen represented 85% total nitrogen excretion, which may be overestimated during exercise because of sweat losses. During the postprandial follow-up, net lipid and carbohydrate oxidations were calculated postprandially over 6 h. Net carbohydrate storage was obtained by subtracting cumulated net carbohydrate oxidation over 6 h from total carbohydrate ingested over the same period, assuming complete carbohydrate absorption. This calculation does not include the eventual storage of newly synthesized fat because de novo lipogenesis from glucose or fructose is computed as carbohydrate oxidation by indirect calorimetry equations, and, hence, provides an estimate of net whole body glycogen storage (without, however, differentiating between hepatic and muscle glycogen synthesis) (24). Net IMCL and muscle glycogen synthesis over 24 h were calculated by subtracting MRS1 from MRS2 values.

### Statistics

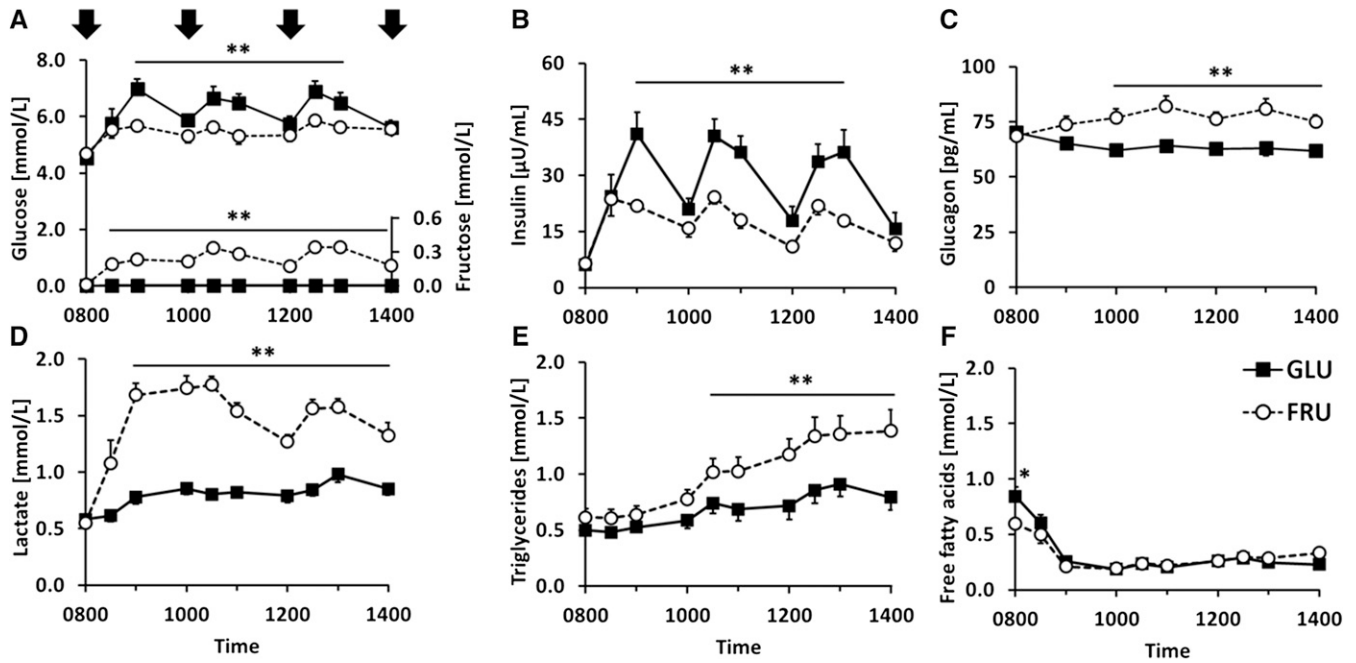
Treatment allocation was determined by random generation of blocks of 4 sequences until completion of the study. A sample size of 8 subjects was calculated ( $1-\beta$ : 80%;  $\alpha = 0.05$ ) to detect an  $\sim 35\%$  difference in IMCL recovery between fructose and glucose consumption. Outlier subjects were detected by a Grubb test. Distribution normality and homoscedasticity were verified with the use of Shapiro-Wilk and Bartlett tests and data were log-transformed when appropriate (postprandial test: glucagon, triglycerides, and FFAs; exercise test: lactate and glucagon). Unless otherwise specified, significance was determined with the use of a paired Student's *t* test. Significance of changes over time was determined with the use of mixed-models analysis, with fixed effects of time and condition, and random effects for subject-specific intercepts and slopes. Time and condition interaction and baseline values were included in the models whenever the goodness of fit was improved. Pearson's correlation test was used to determine linear associations. Subsequent exercise capacity was evaluated by plotting the cycling time of subjects as a cumulative survival curve, and both conditions were compared with the use of the log-rank test. Analyses were performed with the use of R, version 3.0.3, and the level of significance was set as 2-tailed  $P = 0.05$ .

### RESULTS

The experimental part of the study was completed between March 2013 and March 2014. All subjects reported that they strictly followed the outpatient dietary and physical activity instructions before both conditions. However, 1 subject had highly variable muscle glycogen (FRU: 68.2 mmol/kg WW, compared with GLU: 34.2 mmol/kg WW) and IMCL (FRU: 11.5 mmol/kg WW, compared with GLU: 7.8 mmol/kg WW) concentrations on day 4, and was identified as an outlier (Grubb's test:  $P < 0.05$ ). Furthermore, his muscle glycogen concentration did not increase between day 4 and day 5. Because dietary intake was not directly supervised before MRS1 (day 3), we speculated that this subject did not adhere to the run-in instructions, and he was therefore removed from all analyses. In the remaining 8 subjects, body weight and heart rate during exercise 1 were similar across conditions, as well as day-4 IMCL (FRU:  $6.5 \pm 1.0$  mmol/kg WW compared with GLU:  $6.0 \pm 0.9$  mmol/kg WW;  $P = 0.22$ ) and muscle glycogen (FRU:  $32.7 \pm 4.0$  mmol/kg WW compared with GLU:  $35.9 \pm 3.1$  mmol/kg WW;  $P = 0.44$ ) concentrations.

### Follow-up of the metabolic effects of fructose and glucose (day 4, 0800–1400)

Fasting glucose, fructose, insulin, glucagon, lactate, and triglyceride concentrations were not different between FRU and GLU (all  $P > 0.05$ ) (Figure 2A–E), but fasting FFA concentrations were lower in FRU than in GLU ( $0.6 \pm 0.0$  mmol/L compared with  $0.9 \pm 0.1$  mmol/L, respectively;  $P = 0.01$ ) (Figure 2F). Thereafter, postprandial glucose and insulin responses were lower, and glucagon, lactate, and triglyceride concentrations were higher in FRU than in GLU (all time  $\times$  condition:  $P < 0.01$ ) (Figure 2A–E). Postprandial FFA concentrations were almost completely suppressed under both conditions (Figure 2F).



**FIGURE 2** Plasma markers during postprandial follow-up (day 4, 0800–1400). Changes over time in plasma glucose (A), insulin (B), glucagon (C), lactate (D), triglyceride (E), and free fatty acid (F) concentrations after ingestion of the meals in FRU and GLU. Values are means  $\pm$  SEMs,  $n = 8$  subjects. Postprandial glucose and insulin responses were lower and glucagon, lactate, and triglyceride concentrations were higher in FRU than in GLU (all time  $\times$  condition:  $P < 0.01$ ). Distribution normality was visually inspected and checked with the use of a Shapiro-Wilk test; glucagon, triglyceride, and free fatty acid data were log-transformed for analyses. Metabolic effects of FRU and GLU were compared with the use of a mixed model with time and condition as fixed effects. Time and condition interaction and baseline values were included in the models whenever the goodness of fit was improved. Paired contrasts were used to determine differences between conditions at specific time points,  $*P < 0.05$  and  $**P < 0.01$ . Lines: FRU and GLU significantly different over all covered time points. Arrows: meal ingestion (0800, 1000, and 1200). FRU, fructose condition; GLU, glucose condition.

Fasting resting metabolic rate, respiratory exchange ratio, and substrate oxidation were similar in FRU and GLU (data not shown). Postprandial energy expenditure was higher in FRU than in GLU ( $567 \pm 21$  compared with  $542 \pm 21$  kcal/6 h, respectively;  $P = 0.03$ ), corresponding to a higher respiratory exchange ratio ( $0.81 \pm 0.01$  compared with  $0.78 \pm 0.01$ , respectively;  $P < 0.01$ ), and, hence, to an increased net carbohydrate oxidation (FRU:  $47 \pm 4$  g/6 h compared with GLU:  $29 \pm 3$  g/6 h;  $P < 0.01$ ). Because the same amount of carbohydrate was ingested under both conditions ( $165 \pm 7$  g/6 h), carbohydrate balance (accounting for whole-body glycogen storage) was lower in FRU than in GLU ( $+117 \pm 9$  compared with  $+135 \pm 9$  g/6 h, respectively;  $P < 0.01$ ). In contrast, lipid oxidation was lower in FRU than in GLU ( $31 \pm 2$  compared with  $35 \pm 3$  g/6 h,

respectively;  $P = 0.02$ ), resulting in a higher lipid balance (FRU:  $+36 \pm 2$  g/6 h compared with GLU:  $+32 \pm 2$  g/6 h;  $P = 0.02$ ) (**Table 1**). Protein oxidation and balance over 6 h were similar across both conditions (data not shown).

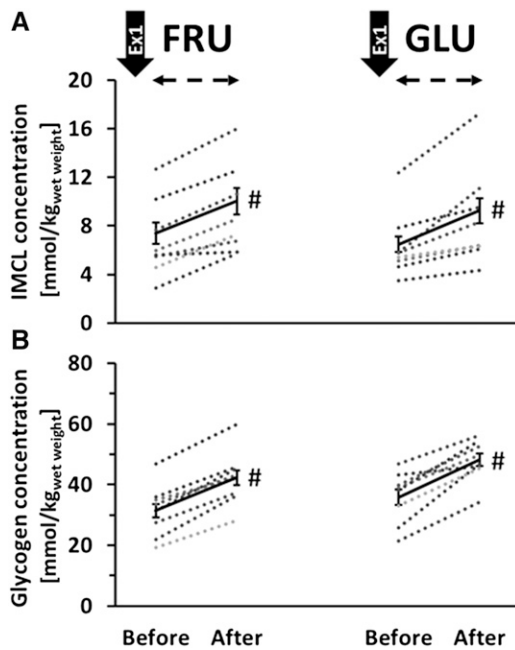
#### Recovery of muscle energy stores (day 3, 1700, to day 4, 1700)

Two MRS measurements were performed 24 h apart to determine the effects of diet on IMCL and muscle glycogen repletion (**Figure 3**). Both FRU and GLU were effective in raising IMCL concentrations from MRS1 to MRS2, but with no difference between treatments (FRU:  $+2.4 \pm 0.4$  mmol/kg WW compared with GLU:  $+2.0 \pm 0.6$  mmol/kg WW; time:  $P < 0.01$ ;

**TABLE 1**  
Whole-body substrate balance over the 6-h follow-up (day 4, 0800–1400)<sup>1</sup>

|                 | Intake      |             | Net oxidation |              | Storage       |               |
|-----------------|-------------|-------------|---------------|--------------|---------------|---------------|
|                 | FRU         | GLU         | FRU           | GLU          | FRU           | GLU           |
| Carbohydrate, g | 165 $\pm$ 7 | 165 $\pm$ 7 | 47 $\pm$ 4**  | 29 $\pm$ 3** | 117 $\pm$ 9** | 135 $\pm$ 9** |
| GLU             | 17 $\pm$ 1  | 134 $\pm$ 6 | —             | —            | —             | —             |
| FRU             | 117 $\pm$ 5 | —           | —             | —            | —             | —             |
| Other           | 31 $\pm$ 5  | 31 $\pm$ 5  | —             | —            | —             | —             |
| Fat, g          | 67 $\pm$ 3  | 67 $\pm$ 3  | 31 $\pm$ 2*   | 35 $\pm$ 3*  | 36 $\pm$ 2*   | 32 $\pm$ 2*   |

<sup>1</sup> Values are means  $\pm$  SEMs,  $n = 8$  subjects. Intake values correspond to the 3 experimental meals ingested during the postprandial metabolic follow-up. Substrate oxidation data were averaged over the entire 6-h period, and whole-body storage was calculated by difference from intake values. \*\*\*FRU and GLU significantly different as assessed by Student's paired  $t$  test,  $*P < 0.05$  and  $**P < 0.01$ . FRU, fructose condition; GLU, glucose condition.



**FIGURE 3** Effect of diet on energy storage in vastus muscles. Changes in IMCL (A) and muscle glycogen (B) concentrations before and after FRU and GLU diets. Values are means  $\pm$  SEMs,  $n = 8$  subjects. FRU and GLU were similarly effective in increasing IMCL (time:  $P < 0.01$ ; time  $\times$  condition:  $P = 0.45$ ) and muscle glycogen (time:  $P < 0.01$ ; time  $\times$  condition:  $P = 0.45$ ) concentrations. Distribution normality was visually inspected and checked with the use of a Shapiro-Wilk test; no transformations were necessary for analyses. Metabolic effects of FRU and GLU were compared with the use of a mixed model with time and condition as fixed effects. Models included time and condition interaction, and baseline values were included in the models whenever the goodness of fit was significantly improved. #Before and after dietary intervention significantly different,  $P < 0.01$ . Ex, exercise; FRU, fructose condition; GLU, glucose condition; IMCL, intramyocellular lipid.

time  $\times$  condition:  $P = 0.45$ ) (Figure 3A). Muscle glycogen contents were also effectively but similarly raised in FRU and GLU ( $+10.9 \pm 0.9$  mmol/kg WW compared with  $+12.3 \pm 1.9$  mmol/kg WW, respectively; time:  $P < 0.01$ ; time  $\times$  condition:  $P = 0.45$ ) (Figure 3B).

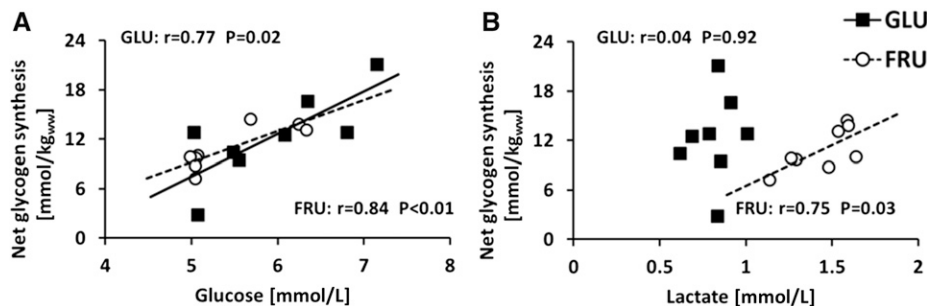
To obtain information regarding potent precursors of these stores, we determined linear associations between mean plasma concentrations of circulating substrates (metabolic follow-up) and muscle energy storage (Figure 4). Glycogen repletion was associated with plasma glucose under both conditions (GLU:  $r = 0.77$  and  $P = 0.02$ ; FRU:  $r = 0.84$  and  $P < 0.01$ ) (Figure 4A), and with

plasma lactate only in FRU ( $r = 0.75$  and  $P = 0.03$ ) (Figure 4B). No other relations were found.

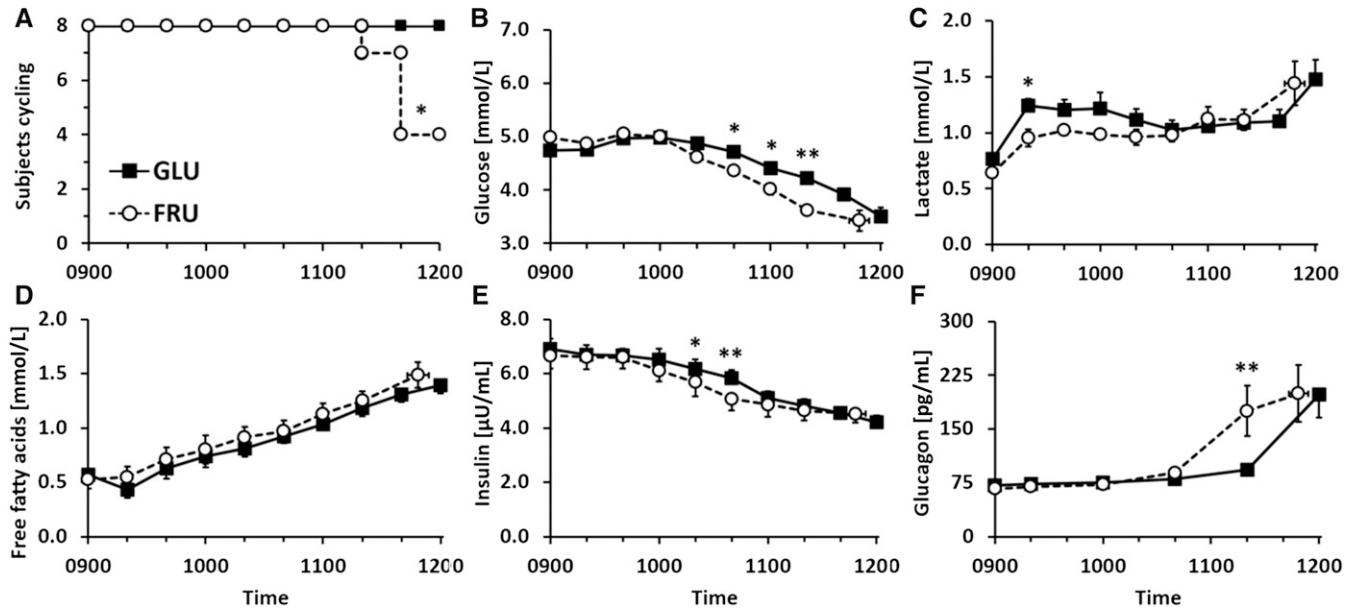
### Energy metabolism during subsequent exercise (day 5, 0900–1200)

On day 5, the subjects who fasted overnight, reported to the laboratory for an endurance exercise trial (3 h at 50%  $W_{\max}$ :  $180 \pm 10$  W). Pre-exercise body weight, glucose, lactate, FFA, triglyceride, insulin, and glucagon concentrations, as well as energy expenditure, respiratory exchange ratio, and substrate oxidation, were all similar in FRU and GLU (data not shown; all  $P > 0.05$ ). In contrast to GLU, in which all subjects cycled for 3 h, 4 of 8 subjects were unable to maintain the target power output after the diet provided in FRU (Figure 5), suggesting an impaired endurance exercise capacity (mean completion: 2 h 48 min  $\pm$  0 h 05 min; log-rank test:  $P = 0.03$ ) (Figure 5A). Plasma glucose concentrations increased transiently at the beginning of exercise, but then declined progressively under both conditions to reach significantly lower values in FRU than in GLU (time:  $P < 0.01$ ; time  $\times$  condition:  $P = 0.01$ ) (Figure 5B). Plasma lactate concentrations were initially lower, but increased more during exercise in FRU than in GLU (time  $\times$  condition:  $P = 0.03$ ) (Figure 5C), whereas FFA concentrations were similarly raised under both conditions (time  $\times$  condition:  $P = 0.80$ ) (Figure 5D). At the last point completed by all subjects (i.e., at 1120), the lower plasma glucose concentration in FRU than in GLU ( $3.6 \pm 0.3$  compared with  $4.2 \pm 0.1$  mmol/L;  $P < 0.01$ ) (Figure 5A) was associated with higher glucagon ( $183 \pm 3$  compared with  $89 \pm 6$  pg/mL, respectively;  $P < 0.01$ ) (Figure 5F) and similarly low insulin ( $4.6 \pm 0.4$  compared with  $4.8 \pm 0.3$   $\mu$ U/mL, respectively;  $P = 0.48$ ) (Figure 5E) concentrations, suggesting that endogenous glucose output was impaired in FRU.

During exercise, mean energy expenditure ( $14.1 \pm 0.6$  compared with  $14.1 \pm 0.4$  kcal/min, respectively;  $P = 0.98$ ), respiratory exchange ratio ( $0.83 \pm 0.01$  compared with  $0.84 \pm 0.01$  g/min, respectively;  $P = 0.18$ ), carbohydrate oxidation ( $1.62 \pm 0.12$  compared with  $1.70 \pm 0.12$  g/min, respectively;  $P = 0.63$ ) and lipid oxidation ( $0.70 \pm 0.10$  compared with  $0.67 \pm 0.10$  g/min, respectively;  $P = 0.29$ ) were not significantly different between FRU and GLU. Protein oxidation, however, was slightly higher in FRU than in GLU ( $0.06 \pm 0.02$  compared with  $0.05 \pm 0.01$  g/min, respectively;  $P = 0.03$ ).



**FIGURE 4** Associations between muscle glycogen synthesis and mean glucose (A) and lactate (B) concentrations after the ingestion of FRU or GLU meals. For each condition,  $n = 8$  subjects. Distribution normality was visually inspected and checked with the use of a Shapiro-Wilk test; no transformations were applied. Linear relation between covariates was determined with the use of a Pearson's test, and correlation coefficients with corresponding  $P$  values are indicated. FRU, fructose condition; GLU, glucose condition; WW, wet weight.



**FIGURE 5** Plasma substrates and hormones during subsequent exercise (day 5, 0900–1200). Cumulative curves of subjects still cycling during exercise 2, with survival AUCs compared with the use of the log-rank test; \*FRU and GLU significantly different,  $P < 0.05$  (A). Changes over time in plasma glucose (B), lactate (C), free fatty acid (D), insulin (E), and glucagon (F) concentrations in subjects who previously consumed FRU or GLU diets. Metabolic effects of FRU and GLU conditions were compared with the use of a mixed-model with time and condition as fixed effects. Time and condition interaction and baseline values were included in the models whenever the goodness of fit was improved. Paired contrasts were used to determine differences between conditions at specific time points; \* $P < 0.05$  and \*\* $P < 0.01$ . Because several subjects did not complete the exercise session in FRU, all values collected after 1120 are drawn as a single point for FRU ( $n = 8$  until 1120; then  $n = 4$  under FRU conditions). Values are means  $\pm$  SEMs,  $n = 8$  subjects. Plasma glucose concentrations increased transiently at the beginning of exercise, but then declined progressively under both conditions to reach significantly lower values in FRU than in GLU (time  $\times$  condition:  $P = 0.01$ ). However, plasma lactate concentrations increased more during exercise in FRU than in GLU (time  $\times$  condition:  $P = 0.03$ ), whereas free fatty acid concentrations were similarly raised under both conditions (time  $\times$  condition:  $P = 0.80$ ). This was also associated with a tendency toward higher glucagon response in FRU than in GLU (time  $\times$  condition:  $P = 0.09$ ) and similarly decreased insulin (time  $\times$  condition:  $P = 0.72$ ). Distribution normality was visually inspected and checked with the use of a Shapiro-Wilk test; lactate and glucagon data were log-transformed for analyses. FRU, fructose condition; GLU, glucose condition.

## DISCUSSION

The aim of this study was to determine how fructose or glucose ingested with mixed meals would contribute to postexercise muscle lipids and, more importantly for subsequent performance, glycogen repletion. We hypothesized that fructose would favor IMCL repletion and alter muscle glycogen synthesis (8, 25). To assess this hypothesis, we studied healthy volunteers after a single session of exercise that decreased IMCL and glycogen concentrations to values close to those reported in the literature under similar conditions (10, 12). Participants thereafter consumed liquid mixed meals that contained glucose or fructose, which elicited postprandial responses consistent with reports that compared fructose- and glucose-based meals in resting humans (15, 17).

Contrary to our hypothesis, fructose- and glucose-containing meals induced comparable IMCL repletion. This occurred despite 1.5-fold higher plasma triglyceride concentrations in FRU than in GLU, suggesting that muscle fatty acid uptake was not dependent on triglyceride-rich lipoprotein concentrations. One explanation could be a higher insulin-induced vasodilation in GLU than in FRU (26), thus compensating for lower triglyceride concentrations by greater muscle blood flow. Alternatively, this may reflect that IMCL synthesis is mainly dependent on whole-body lipolysis and plasma FFA concentrations (27, 28), and that increased concentrations of plasma triglyceride-rich lipoproteins contributed little to this process. Consistent with a prime role of FFAs in IMCL synthesis (11), postprandial FFA concentrations

were indeed not different between the 2 conditions. Further specifically designed experiments will be needed to identify the metabolic pathways used for IMCL repletion in FRU.

Both FRU and GLU resulted in similar muscle glycogen repletion. This was unexpected, because plasma glucose and insulin, which are prime determinants of muscle glycogen synthesis, were markedly higher in GLU than in FRU. FRU, but not GLU, increased postprandial fructose concentrations, but to values an order of magnitude lower than plasma glucose concentrations. This, and the lower affinity of muscle hexokinase for fructose than glucose, strongly suggests that plasma fructose was not a major precursor of muscle glycogen (9, 10). The possibility that the small amounts of glucose present in FRU were sufficient to reach a plateau in muscle glycogen repletion is also very unlikely, considering the dose-response relations between glucose loads, postprandial glycemia, and muscle glycogen synthesis (1). Rather, we postulate that the similar muscle glycogen repletion in FRU and GLU was mainly explained by conversion of fructose-derived circulating glucose and lactate. Such muscle glycogen synthesis from lactate has been described in animal models, with considerable between-species variations (29). Muscle glycogen synthesis has further been shown to be stimulated by increased lactate concentrations (30). No similar data are currently available in humans after oral fructose consumption, but it has been reported that lactate contributed to muscle glycogen repletion when fructose was administered intravenously after exercise (31).

Compared with glucose consumption, fructose consumption increased postprandial energy expenditure and net carbohydrate oxidation. As a consequence, whole-body carbohydrate storage, calculated as the difference between ingested and oxidized carbohydrate over the 6 h during which resting indirect calorimetry was performed, was 18 g lower in FRU than in GLU. This provides a reliable estimate of whole-body glycogen storage (24) when carbohydrate absorption is complete. For FRU, this may not hold true because of fructose malabsorption (32). Fructose malabsorption would, however, result in an overestimation of whole-body glycogen storage, and, hence, an underestimated difference in glycogen storage between FRU and GLU. Carbohydrate oxidation was monitored for 6 h under supervised resting conditions, but cannot be extrapolated to the whole 24 h because physical activity, although maintained as low as possible, was certainly higher during the rest of the day. One may nonetheless speculate that whole-body net carbohydrate oxidation remained higher, and, hence, that whole-body glycogen storage was lower in FRU than in GLU. Given that muscle glycogen repletion was similar with the consumption of both sugars, one may then suspect that hepatic glycogen synthesis was somewhat lower with fructose consumption than with that of glucose. This conclusion is at odds with several reports showing that fructose is a better hepatic glycogen precursor than glucose (8, 25, 33), and will need to be confirmed with direct assessment of hepatic glycogen in future studies.

Glucose and fructose also altered metabolic homeostasis differently during a subsequent standardized exercise session. This part of our experimental protocol was mainly exploratory and did not intend to specifically assess hepatic or muscle metabolism. Yet, plasma glucose concentrations decreased more sharply and were significantly lower in the second session of exercise, whereas glucagon increased earlier in FRU than in GLU. This would certainly be consistent with lower hepatic glycogen concentrations and lower hepatic glucose production in FRU, but we cannot discard the fact that other mechanisms, such as increased muscle glucose transport, may occur. Although it was not our aim to evaluate exercise performance, we also observed that one-half of the subjects had to stop exercising in FRU, whereas all subjects were able to complete the exercise in GLU. This strongly suggests that exercise performance may not be adequately maintained by a very high fructose diet administered during postexercise recovery. In this context, our observation of higher plasma glucagon and lactate concentrations in FRU may reflect counterregulatory responses to low endogenous glucose production. Failure to complete the exercise trial may then be related to lower glycemia, which may be associated with lower energy supply to both skeletal muscle and the brain, and, hence, to both impaired muscle performance and increased central fatigue (34). This remains, however, an academic rather than practical issue, because our experimental diets were designed to assess the effects of mixed meals comprising fructose or glucose on muscle energy repletion, but were not aimed at providing practical recommendations for athletes. This would require further studies focused on exercise performance, and including various ratios of fructose to glucose, rather than the individual sugars.

This study presents several limitations. First, we studied trained athletes because of their high turnover of glycogen and IMCLs. However, this population also exhibits high oxidative

capacity and insulin sensitivity, both of which could affect muscle energy storage. Second, we did not measure hepatic glycogen, nor did we measure substrate kinetics. Third, we designed liquid meals from commercially available foods and nutrients and administered them as split doses every 2 h for convenience and purposes of accuracy. This could have affected our results, which may not be extrapolated to other nutrient sources. The FRU diet also contained much larger ratios of fructose to glucose than usually consumed. Our aim was, however, not to develop a novel feeding procedure for athletes, but to assess whether fructose specifically affected the metabolic pathways used for muscle energy storage.

In conclusion, this study indicates that pure fructose or glucose ingested together with fat and protein in the 24 h period after a strenuous exercise session leads to similar energy storage in IMCLs and muscle glycogen. Muscle glycogen synthesis was likely fueled in FRU by glucose and lactate derived from fructose and released into the blood by splanchnic organs. Net whole-body glycogen storage was lower, and performance during subsequent exercise was decreased in FRU.

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The authors' responsibilities were as follows—RR, VL, LE, CB, RK, PS, and LT: designed the study; RR, VL, and JC: recruited subjects and performed metabolic tests; ASD and KZ: performed scanner measurements; RR: analyzed the data; RR and LT: drafted the manuscript; LT: had primary responsibility for the final content; and all authors: read and approved the final manuscript. LT previously received research support from Nestlé and Ajinomoto for research unrelated to this trial. None of the other authors reported a conflict of interest related to the study.

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