Postexercise Muscle Glycogen Synthesis with Combined Glucose and Fructose Ingestion

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ABSTRACT


**Purpose:** To evaluate the efficacy of using combined glucose and fructose (GF) ingestion as a means to stimulate short-term (4 h) postexercise muscle glycogen synthesis compared to glucose only (G). **Methods:** On two separate occasions, six endurance-trained men performed an exhaustive glycogen-depleting exercise bout followed by a 4-h recovery period. Muscle biopsy samples were obtained from the vastus lateralis muscle at 0, 1, and 4 h after exercise. Subjects ingested carbohydrate solutions containing G (90 g h\(^{-1}\)) or GF (G = 60 g h\(^{-1}\); F = 30 g h\(^{-1}\)) commencing immediately after exercise and every 30 min thereafter. **Results:** Immediate postexercise muscle glycogen concentrations were similar in both trials (G = 128 ± 25 mmol kg\(^{-1}\) dry muscle [dm] vs GF = 112 ± 16 mmol kg\(^{-1}\) dm; \(P > 0.05\)). Total glycogen storage during the 4-h recovery period was 176 ± 33 and 155 ± 31 mmol kg\(^{-1}\) dm for G and GF, respectively (G vs GF, \(P > 0.05\)). Hence, mean muscle glycogen synthesis rates during the 4-h recovery period did not differ between the two conditions (G = 44 ± 8 mmol kg\(^{-1}\) dm h\(^{-1}\) vs GF = 39 ± 8 mmol kg\(^{-1}\) dm h\(^{-1}\), \(P > 0.05\)). Plasma glucose and serum insulin responses during the recovery period were similar in both conditions, although plasma lactate concentrations were significantly elevated during GF compared to G (by ~0.8 mmol L\(^{-1}\), \(P < 0.05\)). **Conclusions:** Glucose and glucose/fructose (2:1 ratio) solutions, ingested at a rate of 90 g h\(^{-1}\), are equally effective at restoring muscle glycogen in exercised muscles during the recovery from exhaustive exercise.

**Key Words:** EXERCISE RECOVERY, CARBOHYDRATE SUPPLEMENTATION, ABSORPTION, LACTATE

Fatigue during prolonged strenuous exercise is associated with glycogen depletion in working skeletal muscles, and the restoration of muscle glycogen is an important component in the recovery of exercise capacity (3). Glucose ingestion immediately and at regular intervals after exercise increases glycogenic substrate availability (i.e., glucose) and optimizes exercise- and insulin-induced stimulation of muscle glucose utilization, facilitating rapid short-term (<8 h) muscle glycogen storage (13). However, when compared to glucose, short-term postexercise muscle glycogen synthesis rates are ~45% lower when fructose is ingested compared with an equivalent quantity of glucose only. The enhanced carbohydrate delivery observed at rest and during exercise is attributed to increased total intestinal carbohydrate absorption (20). For example, Shi et al. (29,30) demonstrated increased total intestinal carbohydrate absorption at rest when glucose and fructose are ingested simultaneously compared with glucose only. Moreover, we (15–18,21) and others (1) reported improved maximal ingested carbohydrate delivery and oxidation during exercise with combined glucose and fructose ingestion compared with the ingestion of an equivalent quantity of glucose only. The enhanced carbohydrate delivery observed at rest and during exercise is attributed to increased total intestinal carbohydrate absorption through the stimulation of multiple and distinct intestinal transporters (glucose and fructose absorption is facilitated by sodium-dependent glucose transporter 1 [SGLT1] and glucose transporter 5 [GLUT5], respectively), leading to increased systemic ingested carbohydrate availability (20).

Interestingly, the systemic availability of ingested carbohydrate has been proposed as the main rate-limiting step in muscle glycogen synthesis in the early postexercise period (14,19,32,33). A logical extension of the results from
studies performed at rest and during exercise is that combined ingestion of glucose and fructose could also increase total systemic carbohydrate availability after exercise. Moreover, glucose and fructose can both independently promote muscle glycogen synthesis albeit at different rates, vide supra, and thus simultaneous ingestion of glucose and fructose could be a viable strategy to facilitate muscle glycogen synthesis after exercise. Therefore, the purpose of the present study was to test the hypothesis that combined glucose and fructose ingestion will stimulate short-term (4 h) postexercise muscle glycogen synthesis to a greater extent than the ingestion of an iso-energetic quantity of glucose only.

METHODS

Subjects. Six healthy, endurance-trained men participated in this study. All subjects were engaged in training for athletic events that included endurance cycling (i.e., road cycling, duathlon, or triathlon). The subjects’ training had specifically included cycling for 3.4 ± 1.1 yr before participation in the study, and before that, each subject reported a long history of training for endurance-type sports (e.g., soccer/rugby/field hockey, long-distance running, and swimming). Their mean age, body mass, height, maximal oxygen uptake (VO2max), and maximal cycle ergometer power output (Wmax) were 26 ± 2 yr, 75 ± 3 kg, 1.81 ± 0.02 m, 5.1 ± 0.2 L·min⁻¹ (67 ± 3 mL·kg⁻¹·min⁻¹), and 336 ± 11 W (4.6 ± 0.2 W·kg⁻¹), respectively. The number of subjects was equivalent to that required to detect mean differences in total muscle glycogen storage between the experimental beverages (calculated a priori on the basis of data from Jentjens et al. (18)), with a power of >0.80 and a two-tailed alpha level of 0.05 (35). All subjects were informed of the purpose, practical details, and risks associated with the procedures before giving their written informed consent to participate. The study was approved by the local research ethics committee.

Experimental design. After preliminary testing, each subject completed two exercise trials consisting of a glycogen-depleting exercise bout followed by a 4-h recovery period during which participants ingested an 18% glucose solution (G; Meritose 200; Amylum Europe NV, Belgium) or an iso-energetic glucose plus fructose solution (GF; Fruitone S; Fiske Foods Ingredients, UK) (the ingested G/F ratio was 2:1). In the G trial, glucose was ingested at a rate of 90 g·h⁻¹, and in the GF trial, glucose and fructose were ingested at 60 and 30 g·h⁻¹, respectively (~1.2 g·kg⁻¹·h⁻¹). The order of the trials was randomly assigned and separated by at least 7 d. In an attempt to minimize the differences in resting muscle glycogen concentration, subjects completed a diet recall log in which they recorded diet patterns 24 h before the first trial. Subjects were instructed to follow the same patterns before the second trial and were further asked to avoid exercise 1 d in advance of both trials.

Preliminary testing. Within 2 wk of the commencement of the main experimental trials, VO2max and Wmax were determined during an incremental exercise test performed on a cycle ergometer (Excalibur Sport; Lode, Groningen, Netherlands) as described elsewhere (34). Gas exchange measurements during this test were made using an automated online gas analysis system (Oxycon Pro; Jaeger, Wuerzberg, Germany). Wmax values were used to determine appropriate exercise intensities for the subsequently described experimental trials.

Experimental protocol. On the day of the experimental trials, subjects reported to the laboratory in the morning after an overnight fast (~10 h). After the body mass was measured, participants mounted a cycle ergometer (Excalibur Sport) and performed a high-intensity intermittent exercise protocol. Briefly, after a warm-up period consisting of 10-min cycling at 50% of their predetermined Wmax, subjects were instructed to cycle 2-min block periods at alternating workloads between 90% and 50% Wmax. When subjects were unable to complete a 2-min block at 90% Wmax (unable to maintain cadence of 60 rpm despite strong verbal encouragement), the high-intensity block was reduced to 80% Wmax. Again, subjects continued cycling until they were unable to complete a 2-min block at 80% Wmax, after which the high-intensity block was reduced to 70% Wmax. When the 70% Wmax blocks could not be completed, subjects were allowed to stop. The average absolute workloads eliciting 90%, 80%, and 70% of Wmax in the present study were 303 ± 10, 269 ± 9, and 235 ± 7 W, respectively. The exercise protocol used has been described previously by Kuipers et al. (23) and has been used extensively to induce muscle glycogen depletion in trained athletes (e.g., (19,32,33)). During the exercise tests, subjects were cooled with standing floor fans, and water was provided ad libitum.

After cessation of the exercise bout, subjects dismounted the ergometer and lay supine on a bed, and a venous blood sample was drawn from an indwelling catheter placed in an antecubital arm vein. This was followed immediately by the collection of a muscle biopsy obtained from the vastus lateralis muscle through a skin incision under local anesthesia (2% xylocaine), using the suction-modified Bergstrom needle biopsy technique (11). One of the test carbohydrate solutions was provided immediately after the initial muscle biopsy (time (t) = 0 min). Subjects remained supine for the duration of the study. A second muscle biopsy was collected from the same leg 60 min into the recovery period. For this biopsy, a small amount of local anesthesia was reapplied to the muscle fascia through the initial skin incision before the muscle biopsy was taken but with the Bergstrom needle directed ~3 cm in a more proximal direction compared with the initial biopsy site. A final muscle biopsy was obtained from the contralateral leg after 240 min of recovery. The precautions of directing the needle angle proximal to the initial biopsy site for the second biopsy and performing the third biopsy on the
The total exercise duration was 1% of the participants’ maximal duration of the recovery period. The final exercise duration was 240 min. The drinks ingested during the recovery period were provided as an initial bolus of 460 mL (~83 g of carbohydrate), with a further 220 mL (~40 g of carbohydrate) provided every 30 min (after the metabolic measurements were taken) for the duration of the recovery period.

**Blood and muscle analyses.** Venous blood samples (~4 mL) were separated into prechilled ethylenediaminetetraacetate-acid-containing tubes and were centrifuged immediately at 4°C, with the remainder (~4 mL) placed in plain tubes and allowed to clot before centrifugation. Thereafter, aliquots of plasma or serum were frozen in liquid nitrogen and stored at −25°C until further analysis. Plasma samples were assayed for glucose and lactate concentration on a semiautomatic analyzer (Cobas Mira S-Plus; ABX Diagnostics, UK) using commercially available kits (Glucose HK and Lactic Acid; ABX Diagnostics). Serum insulin concentrations were determined by enzymelinked immunosassay (Insulin ELISA; IDS, UK).

On collection, muscle biopsy samples (between 50 and 100 mg wet weight (w.w.)) were blotted, dissected free of visible fat and connective tissue, immediately frozen in liquid nitrogen, and stored at −70°C until further analysis for muscle glycogen content. For the determination of muscle glycogen concentration, ~25–50 mg w.w. were freeze-dried, and muscle samples were subsequently further dissected free of connective tissue, visible fat, and blood using a light microscope. Thereafter, muscle samples were powdered, hydrolyzed by adding 500 μL of 1 mol L⁻¹ HCl to 2–3 mg of dry muscle (dm) tissue, and incubated for 3 h at 100°C. After cooling to room temperature, samples were neutralized with 267 μL of 0.12 mol L⁻¹ Tris/2.1 mol L⁻¹ KOH saturated with KCl. Samples were then centrifuged, and the supernatant was analyzed in duplicate for glucose concentration on a semiautomatic analyzer (Cobas Mira S-Plus; ABX Diagnostics) using a commercially available kit (Glucose HK; ABX Diagnostics). Where tissue size permitted (>55% of determinations), muscle glycogen content was determined in duplicate. The intrasay coefficient of variation for muscle glycogen determination was <5%.

**Calculations.** Glucose and insulin area under the curve (AUC) was determined using the trapezoid method (25). Muscle glycogen concentrations are expressed as millimoles per kilogram of dm (mmol·kg⁻¹·dm), and glycogen synthesis rates as millimoles per kilogram of dm per hour (mmol·kg⁻¹·dm·h⁻¹) calculated from the following equation: muscle glycogen synthesis rate = \((G_{IB} \cdot G_{IA}/Δt)\), where \(G_{IB} - G_{IA}\) is the muscle glycogen concentrations at \(B (1 or 4)\) and \(A (0 and 1)\) hours after drink consumption and \(Δt\) is the time between the two biopsies.

**Statistical analysis.** All data are expressed as means ± SE from six subjects. Differences in muscle glycogen concentration and synthesis rate between trials and over time within each trial were compared using paired \(t\)-tests with the Bonferroni procedure for multiple pairwise comparisons applied. Two-way ANOVA with repeated measures (trial × time) was used to compare differences in plasma glucose, lactate, and serum insulin, with significant interaction effects followed by post hoc comparisons (Tukey HSD). All other measures (exercise variables and insulin/glucose AUC) were compared using paired \(t\)-tests. For all statistical analyses, significance was accepted at \(P < 0.05\).

**RESULTS**

**Exercise protocol.** The total exercise duration was similar in both experimental trials (138 ± 8 vs 129 ± 9 min for G and GF, respectively). In addition, the number of high-intensity exercise blocks completed was also similar between trials (90% \(W_{max} = 18 ± 4\) vs \(19 ± 3\); 80% \(W_{max} = 6 ± 1\) vs \(4 ± 1\); 70% \(W_{max} = 6 ± 2\) vs \(5 ± 2\); for G and GF, respectively). Thus, the average heart rate was also similar between trials (158 ± 2 vs 159 ± 2 for G and GF, respectively), corresponding to 85 ± 1% of the participants’ maximal heart rate attained during preliminary exercise testing.

**Muscle glycogen.** Mean muscle glycogen concentrations during the recovery period are depicted in Figure 1. The immediate postexercise muscle glycogen concentration was similar between the trials (128 ± 25 and 112 ± 16 mmol·kg⁻¹·dm for G and GF, respectively). Sixty minutes after carbohydrate ingestion commenced, mean glycogen content increased nonsignificantly (ns) to 156 ± 27 and 169 ± 36 mmol·kg⁻¹·dm in G and GF, respectively. The final glycogen content (303 ± 46 and 267 ± 38 mmol·kg⁻¹·dm in G and GF, respectively) was significantly (\(P < 0.05\)) higher than that measured immediately postexercise and that measured 60 min after carbohydrate ingestion commenced. However, there was no significant difference in the final muscle glycogen content between G and GF. Accordingly, by the end of the 4-h recovery period, muscle glycogen concentration had increased by a similar amount in both

![FIGURE 1—Muscle glycogen concentrations during the 4-h recovery period. Values are means ± SE; n = 6. G indicates glucose; GF, glucose and fructose. * Significant difference from \(t = 0\); † significant difference from \(t = 60\).](image-url)
Plasma glucose, lactate, and serum insulin. Plasma glucose, lactate, and serum insulin concentrations for both trials during the 4-h recovery period are shown in Figure 2.

Plasma glucose concentrations were similar for both trials at the first postexercise sample point (t = 0, 3.55 ± 0.31 vs 3.53 ± 0.19 mmol·L⁻¹ for G and GF, respectively; Fig. 2A). The ingestion of carbohydrate resulted in an increase in plasma glucose concentration measured 30 min after ingestion (7.26 ± 0.39 and 7.81 ± 0.24 mmol·L⁻¹ for G and GF, respectively, P < 0.05 vs t = 0). Thereafter, plasma glucose concentration declined but remained significantly higher than the immediate postexercise value (P < 0.05) for the duration of the recovery period in both trials (average of 5.68–5.88 mmol·L⁻¹ for the 60- to 240-min period). There was no significant difference in plasma glucose concentration between the two trials at any time during the recovery period. Therefore, the total glucose AUC was not statistically different between the trials (1433 ± 56 vs 1505 ± 36 mmol·L⁻¹ for G and GF, respectively, P = 0.2).

Plasma lactate concentrations at t = 0 were similar in G and GF (2.51 ± 0.33 and 2.16 ± 0.26 mmol·L⁻¹, respectively; Fig. 2B). In the G trial, plasma lactate concentrations declined significantly by 30 min (to 1.45 ± 0.09 mmol·L⁻¹, P < 0.05 vs t = 0) and remained stable for the duration of the recovery period (1.49–1.56 mmol·L⁻¹, P < 0.05 vs t = 0). In contrast, plasma lactate concentration in the GF trial did not change significantly from t = 0, remaining between 1.86 ± 0.19 and 2.43 ± 0.11 mmol·L⁻¹ for the duration of the recovery period. Accordingly, plasma lactate concentration was significantly higher (P < 0.05, ~0.8 mmol·L⁻¹) in the GF trial compared with G between 60 and 240 min.

Serum insulin concentrations were similar at t = 0 in both trials (5 ± 1 vs 5 ± 0 μU·mL⁻¹ for G and GF, respectively; Fig. 2C). Ingestion of carbohydrate increased serum insulin concentration to 38 ± 10 and 36 ± 6 μU·mL⁻¹ for G and GF, respectively, by 30 min (P < 0.05 vs t = 0). Serum insulin remained elevated (P < 0.05 vs t = 0) for the duration of the recovery bout, ranging between 53–64 and 48–65 μU·mL⁻¹ for G and GF, respectively. There was no significant difference in serum insulin concentration between the two trials at any time during the recovery period. Hence, the total insulin AUC was not statistically different between the trials (12,860 ± 3424 vs 12,593 ± 2527 μU·mL⁻¹ for G and GF, respectively, P = 0.9).

DISCUSSION

In this study, we investigated the effect of ingesting a combination of glucose and free fructose on short-term (4 h) postexercise muscle glycogen synthesis. We confirm that ingestion of large quantities (90 g·h⁻¹) of carbohydrate at regular intervals promotes rapid muscle glycogen storage in recovery from exhaustive exercise, and importantly, we provide the first evidence that this can be achieved with the ingestion of glucose alone or as a combination of free glucose and free fructose. Our data showing a high but similar rate of postexercise glycogen synthesis in both experimental conditions are somewhat contradictory to our hypothesis that glycogen synthesis would be augmented above that observed with glucose when glucose and fructose were ingested. Nonetheless, our data are novel, and the findings have practical significance for the development of nutritional strategies to optimize short-term postexercise glycogen synthesis.
The main finding of the present study was that G and GF ingestion elicited similar rates of postexercise muscle glycogen synthesis. As stated above, this similarity in glycogen synthesis was contrary to our expectation. Although systemic carbohydrate delivery or availability was not directly assessed (i.e., by measuring systemic carbohydrate appearance rates), we hypothesized that the feeding regimen adopted would elevate total carbohydrate delivery in GF compared to G, and this would enhance postexercise muscle glycogen synthesis. This notion was justified as previous work demonstrates increased absorption of ingested carbohydrate at rest and oxidation during exercise when carbohydrate types that use multiple intestinal transport mechanisms are ingested (17,29,30). In fact, the specific GF dose used in the present study was previously reported to increase exogenous carbohydrate oxidation rates during exercise by up to 36% (18,21). However, despite the possibility of increased carbohydrate delivery with GF ingestion, a key consideration is the fate of fructose and subsequent glycogenic substrate availability.

At rest, ~50% of ingested fructose appears in the systemic circulation as glucose, with the remainder stored as liver glycogen, which is converted to lactate, used in triacylglycerol synthesis, or directly oxidized in the liver (9). In addition, ingested or infused fructose can be used for liver and skeletal muscle glycogen synthesis after exercise (4,5,7,26), the latter presumably occurring after conversion to glucose because direct incorporation of fructose into glycogen is a minor pathway for fructose disposal in human skeletal muscle (36). Thus, fructose alone can provide substrate for glycogenesis, albeit most likely by indirect pathways. The metabolic fate of fructose when coingested with glucose is less established. Recently, despite strong indirect evidence (from exogenous carbohydrate oxidation rates) that total carbohydrate delivery was augmented with ingestion of GF compared with G during exercise, we unexpectedly observed similar systemic glucose appearance rates with G or GF ingestion (21). The implication of this finding is that splanchnic glucose output was not increased by GF compared with G, at least during exercise (21). This recent finding can be integrated with our present results and interpreted to mean that the similar rates of muscle glycogen synthesis in G and GF observed could be attributed to the failure of GF to increase systemic glucose availability because glucose is the major precursor for postexercise skeletal muscle glycogen synthesis (2). A similar plasma glucose (and serum insulin) response in G and GF indirectly supports this assertion (10).

The recent finding of similar systemic glucose availability with G or GF ingestion during exercise (21) also reveals a potential fate for fructose in the GF trial of the present study. For example, in that study (21), exogenous carbohydrate oxidation was significantly higher in GF compared with G despite similar blood glucose turnover during exercise. Thus, utilization of alternative carbohydrate fuels could explain the elevated exogenous carbohydrate oxidation during exercise with GF ingestion. One suggestion is enhanced lactate production and oxidation in GF compared with G (17,21). Analogous to when GF is ingested during exercise, plasma lactate concentrations were significantly elevated (by ~0.8 mmol·L⁻¹; Fig. 2B) throughout the entire 4-h recovery in GF compared with G in the present investigation. Thus, a significant portion of the ingested fructose could have been directed toward lactate production, the site of which could include the intestines, the liver, the kidneys, or the skeletal muscle (24,36). The elevated plasma lactate concentration when GF was ingested during the postexercise period could further explain the absence of augmented glycogen synthesis in GF because although lactate can be used for glycogen synthesis in skeletal muscle, oxidation is its primary fate during exercise recovery (6).

Alternatively, changes in splanchnic metabolism as a result of physical exercise could explain the present findings. Our hypothesis was in part on the basis of evidence suggesting that intestinal glucose transport (via SGLT1) is limited to ~1 g·min⁻¹ (22,27), thus providing that carbohydrate in excess of this rate would not increase carbohydrate delivery unless additional intestinal transport mechanisms were stimulated (e.g., GLUT5 via fructose ingestion). Indeed, several studies performed in the last few years have indirectly supported this notion, at least with respect to carbohydrate delivery/availability during exercise (15–18,21,34). However, splanchnic glucose output in response to a glucose load has been reported to be enhanced by prior exercise (12,28), and in exercised dogs, this effect has been attributed to increased intestinal glucose absorption (12). Thus, increased splanchnic carbohydrate output during the postexercise period may have concealed potential differences in carbohydrate availability and, consequently, muscle glycogen storage between trials in the present study. The absence of glucose turnover data precludes this conclusion from being firm, but again, the similar plasma glucose response in both trials could be interpreted to mean that immediate glycogenic (glucose) substrate availability was similar among conditions.

Finally, although our results did not show augmented glycogen synthesis with GF above that observed with G, they do have important practical significance for the development of strategies to maximize muscle glycogen synthesis in the short-term postexercise recovery period. Notably, the rates of muscle glycogen synthesis we observed over the 4-h postexercise recovery period in the present study were 44 ± 8 and 39 ± 8 mmol·kg⁻¹·dm⁻¹·h⁻¹ when G or GF were ingested, respectively. These are comparable with the highest rates (40–50 mmol·kg⁻¹·dm⁻¹·h⁻¹) observed in the literature on the basis of studies that have provided oral carbohydrate (glucose, glucose polymers, or sucrose) in large doses (~1.2 g·kg⁻¹·h⁻¹) during postexercise recovery (14,19,32,33). As mentioned in the introduction, previous studies would indicate that fructose is a relatively poor nutritional precursor for
glycogen synthesis compared with glucose. However, our results demonstrate that fructose is not detrimental to postexercise muscle glycogen synthesis when coingested with glucose using the strategy adopted herein. Therefore, on the basis of the present data and results from previous studies (14), consuming large amounts (90 g·h⁻¹ at ~1.2 g·kg⁻¹·h⁻¹) of glucose, glucose polymers, sucrose, or a combination of free glucose and free fructose (2:1 ratio) immediately and at regular intervals (approximately every 15–30 min) represents an effective nutritional strategy to promote rapid muscle glycogen storage during the initial 4 h of recovery from exhaustive exercise.

This study was supported by a research grant from GlaxoSmithKline Consumer Healthcare, United Kingdom. The results of the present study do not constitute endorsement by ACSM.

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