Effect of carbohydrate ingestion on glycogen resynthesis in human liver and skeletal muscle, measured by $^{13}$C MRS

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This study investigated the effect of carbohydrate (CHO) ingestion on postexercise glycogen resynthesis, measured simultaneously in liver and muscle (n = 6) by $^{13}$C magnetic resonance spectroscopy, and subsequent exercise capacity (n = 10). Subjects cycled at 70% maximal oxygen uptake for 83 ± 8 min on six separate occasions. At the end of exercise, subjects ingested 1 g/kg body mass (BM) glucose, sucrose, or placebo (control). Resynthesis of glycogen over a 4-h period after treatment ingestion was measured on the first three occasions, and subsequent exercise capacity was measured on occasions four through six. No glycogen was resynthesized during the control trial. Liver glycogen resynthesis was evident after glucose (13 ± 8 g) and sucrose (25 ± 5 g) ingestion, both of which were different from control (P < 0.01). No significant differences in muscle glycogen resynthesis were found among trials. A relationship between the CHO load (g) and change in liver glycogen content (g) was evident after 30, 90, 150, and 210 min of recovery (r = 0.59–0.79, P < 0.05). Furthermore, a modest relationship existed between change in liver glycogen content (g) and subsequent exercise capacity (r = 0.53, P < 0.05). However, no significant difference in mean exercise time was found (control: 35 ± 6 min, glucose: 40 ± 5, and sucrose: 46 ± 6 min). Therefore, 1 g/kg BM glucose or sucrose is sufficient to initiate postexercise liver glycogen resynthesis, which contributes to subsequent exercise capacity, but not muscle glycogen resynthesis.

$^{13}$C magnetic resonance spectroscopy; magnetic resonance imaging; submaximal exercise

Postexercise glycogen resynthesis in rodent liver and skeletal muscle is well documented (8, 14, 16). Likewise, changes in human skeletal muscle glycogen concentration during prolonged exercise and recovery are relatively well documented, both in whole muscle (2, 12) and in muscle fiber subtypes (10), due largely to widespread use of needle biopsy procedures. In the absence of exogenous carbohydrate (CHO), there is little change in human muscle glycogen concentration after exercise-induced glycogen depletion (20). After CHO ingestion, restoration of human muscle glycogen concentration is typically complete within 24 h (2, 10), but the optimal type of CHO, in terms of both glycogen resynthesis and restoration of exercise capacity, remains unclear.

Previous studies have shown that ingestion of CHO with a high glycemic index (GI) is more effective in promoting muscle glycogen resynthesis than ingestion of lower GI CHO (9, 29, 45). Although relatively high, sucrose has a lower GI than glucose, and glucose ingestion might therefore be expected to promote greater muscle glycogen resynthesis than sucrose. However, due to its fructose content, sucrose may preferentially restore liver, rather than muscle glycogen stores after exercise, as predicted from the data of Nilsson and Hultman (36) obtained in resting subjects. This may alter the extent to which exercise capacity is restored, by mediating blood glucose availability. Previous studies have demonstrated that fatigue during prolonged exercise at 70% maximal oxygen uptake (V$\text{O}_{2\text{max}}$) is preceded by a decline in the rate of CHO oxidation and that fatigue can be delayed by maintaining a high rate of CHO oxidation via oral glucose ingestion (13). The authors demonstrated that highly trained subjects administered glucose were able to exercise for longer than when fasting, without any further reduction in muscle glycogen. They suggested that toward the end of exercise, oxidation of CHO was maintained as a result of glucose ingestion, which was sufficient to offset the reduction in muscle glycogen oxidation that occurred as a result of glycogen depletion, enabling exercise to continue. The lowering of blood glucose, and subsequent reduction in muscle glycogen uptake and oxidation, seen during the final stages of exercise without CHO ingestion, was suggested to be a major cause of fatigue. Recent tracer studies have confirmed this suggestion that CHO oxidation is maintained by an
increase in blood glucose oxidation and have shown total glucose oxidation to be greater under hyperglycemic (49) compared with euglycemic (48) conditions. Together these studies suggest that any differences between glucose and sucrose in terms of postexercise liver glycogen resynthesis, and therefore blood glucose availability, may have important consequences for subsequent exercise capacity. To date, no studies have investigated the relative merits of glucose and sucrose administration, during recovery from exercise, in terms of subsequent endurance exercise capacity.

Relatively little data are available describing in vivo human liver glycogen homeostasis, because liver biopsies are not considered appropriate for research purposes (18) and are generally excluded in normal subjects. Over the last 15 years, information about human liver glycogen homeostasis has been provided almost exclusively by magnetic resonance spectroscopy (MRS). However, even with the advent of MRS, very little data have been gathered from normal subjects. The majority of information has been obtained from patient populations, primarily to study the pathophysiology of glucose homeostasis (11, 24, 33, 46). Existing data from normal subjects has focused predominantly on responses to feeding and fasting in the resting state (37–39, 43), and the nature of liver glycogen homeostasis during exercise and recovery remains unclear.

To date, no studies have used interleaved 13C liver and muscle spectra to quantify changes in human liver and muscle glycogen content with exercise and during recovery. No studies have related postexercise liver and muscle glycogen resynthesis to subsequent endurance exercise capacity. Furthermore, the relative merits of glucose and sucrose, in terms of the effect of liver and muscle glycogen resynthesis on subsequent exercise capacity, are unknown.

We hypothesize that postexercise administration of glucose and sucrose will have a differential effect on liver glycogen resynthesis, which may have important consequences for the restoration of exercise capacity. Therefore, the first aim of this study was to quantify changes in human liver and muscle glycogen content during 1) exercise and 2) the recovery period after CHO administration. A second aim was to compare the effects of glucose and sucrose ingestion on 1) postexercise liver and muscle glycogen resynthesis and 2) subsequent endurance exercise capacity.

**METHODS**

Subjects. Ten healthy males gave their written consent to take part in the present study, which was approved by the University of Nottingham Medical School Ethical Committee. All subjects were well trained (5–7 days/wk on a regular basis), and all competed in various sports at a good club or international level. Trained subjects were chosen to avoid a training effect from repeated exercise trials. Age, weight, body mass (BM) index, VO2max and normal daily energy intake were 25 ± 2 yr, 76.1 ± 1.8 kg, 23.7 ± 0.6 kg/m², 4.01 ± 0.171/min, and 12.6 ± 0.8 M/L/day, respectively (means ± SE). Before beginning the study, all subjects participated in a routine medical examination.

Experimental protocol. Preliminary measurements made on all subjects during the first five visits established their VO2max, normal dietary composition, and endurance exercise capacity, as detailed below. The next series of trials (visits 6–8) used endurance exercise to deplete tissue glycogen levels and examined the effect of CHO ingestion on postexercise liver and muscle glycogen resynthesis. The final series of trials (visits 9–11) depleted glycogen levels and administered CHO in a similar fashion. On these occasions, measurements of glycogen resynthesis were omitted; however, endurance exercise capacity was measured at the end of the recovery period. In addition, during visits 9–11, blood and expired air samples were collected throughout both exercise periods and recovery. It is not feasible to combine protocols for measurement of liver and muscle glycogen with those for blood and gas sampling for instrumentation reasons (4).

On the first visit to the laboratory, subjects were thoroughly familiarized with all procedures involved in the study. On the second visit, VO2max was determined for all subjects with a discontinuous, incremental exercise test to exhaustion on an electrically braked cycle ergometer (Lode N.V. Instrumenten, Groningen, Holland). This was verified on a third visit, separated from the second by a minimum of 3 days.

To promote similar liver and muscle glycogen concentrations before each of the subsequent trials, both diet and training were controlled for 3 days beforehand. Initially subjects weighed and recorded their normal diet for 3 days. CHO, fat, and protein intake, represented as a percentage of total daily energy intake, were 48 ± 2, 36 ± 2, and 16 ± 1%, respectively (means ± SE). An identical (weighed) diet was prescribed for 3 days before all subsequent trials, ensuring the same CHO and total energy intake on each occasion for an individual subject. In addition, subjects were instructed to maintain the same pattern of training throughout each of these 3-day periods and to exclude any strenuous training for 24 h before each trial.

Subjects reported back to the laboratory after an overnight fast on eight further occasions, having been instructed, in addition to dietary and training requirements, to abstain from alcohol intake for 24 h before each trial and from caffeine intake on the day of each trial. On the fourth visit, subjects cycled to volitional exhaustion at 70% VO2max. Exercise time-to-exhaustion was used as a measurement of endurance exercise capacity and was verified by the repetition of the protocol on a separate occasion.

On the following six occasions (visits 6–11), subjects cycled at 70% VO2max for a fixed period (exercise time-to-exhaustion – 10 min; bout 1), to deplete tissue glycogen levels by a similar amount on each occasion. Subjects were administered 3 ml/kg BM of plain water every 15 min during exercise, split into two drinks, and any deficits in nude BM after exercise were immediately restored with plain water. Immediately after exercise, subjects were administered a single bolus of 1 g/kg BM CHO in the form of 18.5% (wt/vol) glucose, 18.5% (wt/vol) sucrose, or an equivalent volume of placebo (control). CHO ingestion was followed by a 4-h recovery period. A crossover design was adopted, with subjects receiving all three treatments on separate occasions, in a double-blind fashion. These trials were separated by a minimum of 2 wk.

On visits 6–8, liver and muscle glycogen was measured in six subjects before and after exercise and during the 4-h recovery period after CHO ingestion. Glycogen measurements (mmol/l) were made by natural abundance 13C MRS. Liver and muscle volume (liters) was measured immediately after each liver and muscle spectrum acquisition, respectively, with multislice magnetic resonance imaging (MRI).
enabled calculation of total glycogen content in millimoles and therefore in grams. Liver spectra and volume measurements were obtained within the first 30 min after CHO ingestion and hourly thereafter. A typical sequence of spectra is shown in Fig. 1. Muscle spectra and volume measurements were obtained from 30–60 min and hourly thereafter. During visits 9–11, 10 subjects completed an identical pattern of exercise and CHO ingestion. On these occasions, arterialized-venous blood samples were obtained at rest in the fasting state, every 15 min during exercise, and at hourly intervals during the 4-h recovery period. Expired gas samples were obtained at rest, every 15 min during exercise, and at 30-min intervals during the recovery period. For the last 25 min of recovery, subjects were allowed to be mobile and to perform gentle stretching exercises in preparation for the second bout of exercise. At the end of the recovery period, endurance capacity was measured in 10 subjects. Subjects cycled at 70% \(V_{\text{O}_2}\text{max}\) to volitional exhaustion (bout 2), and the exercise time was recorded. Water was given during exercise as before. As before, trials were separated by a minimum of 2 wk.

All treatments were supplied by SmithKline Beecham. The solutions were supplied in identical containers, labeled with a code, which was released by SmithKline Beecham after completion of the study. All solutions were identical in appearance. The placebo consisted of a sugar-free solution bearing as close a resemblance as possible to the taste of the glucose and sucrose solutions. This allowed double-blind trials to be undertaken. Glucose and sucrose were chosen because they are the constituents of most commercially available preparations. Ingestion of 1 g/kg BM CHO was chosen to approximate the CHO intake associated with a maximal exercise at 70% maximal oxygen uptake (\(V_{\text{O}_2}\text{max}\)), within the first 30 min after carbohydrate (CHO) ingestion (see below). When subjects were positioned in the 3.0-T spectrometer, data were acquired with a simple pulse-acquire (decouple) sequence, with the excitation pulse flip angle determined by Ernst angle considerations. Repetition times were 171 and 114 ms for liver and muscle acquisitions, respectively. The flip angle at the \(^{13}\text{C}\) coil centers was 220°, which minimized spectral contamination from superficial adipose tissue and, for the liver spectra, from abdominal muscle. Broad-band proton decoupling was applied for the duration of each 27-ms sampling period. Without decoupling, the \(^{13}\text{C}\) resonance is sensitive to the spin orientations of attached protons and the signal is split into a doublet with a relatively low signal-to-noise ratio. A Waltz-8 decoupling sequence was used, resulting in a peak transmitted power of 30 W; the acquisition period and repetition times for liver and muscle ensured mean deposition remained within specific absorption rate limits of 8 and 12 W/kg, respectively. Proton decoupling had the effect of doubling the signal-to-noise ratio of the \(^{13}\text{C}\) glycogen signal. In addition, proton-decoupled \(^{13}\text{C}\) spectra were summed over 15 min to further improve the signal-to-noise ratio. Each spectrum consisted of 5,200 and 7,800 acquisitions for the liver and muscle, respectively. No adjustments to the spectrometer were made between measurements, except for retuning the \(^{13}\text{C}\) surface coil and reshimming the static magnetic field immediately before data acquisition. The signal of interest arises from the C-1 position of glycogen at 100.5 particles/min (relative to tetramethylsilane = 0). Resonances from other glycogen carbons (C-2 to C-6) are obscured by the lipid peaks. The spectra were line broadened with a filter matched to the C-1 glycogen line width, baseline flattened, and integrated (NMR 1; New Methods Research, New York, NY). The coefficient of variation, calculated for repeated analysis of a single spectrum, was 3.80% (\(n = 15\)). The lipid peak at 30 particles/min (\(-\text{CH}_2\)) was used as an internal standard, which did not vary by more than 6% over the course of the

Fig. 1. Typical sequence of proton decoupled, natural abundance \(^{13}\text{C}\) spectra obtained by magnetic resonance spectroscopy (MRS) at 3.0 T (5,200 scans). Resonance arises from the C-1 position of glycogen at 100.5 particles/minute (ppm) (relative to tetramethylsilane = 0). Sequence shows time course of human liver glycogen resynthesis during exercise and recovery. Spectra were obtained before and after prolonged submaximal exercise at 70% maximal oxygen uptake (\(V_{\text{O}_2}\text{max}\)), within the first 30 min after carbohydrate (CHO) ingestion (1 g/kg body mass) and then hourly during the remainder of recovery. Coefficient of variation, calculated for repeated analysis of a single spectrum, was 3.80% (\(n = 15\)).
study. The C3 signal was quantified by comparison with spectra from a glycogen phantom (200 mmol/l oyster glycogen and 150 mmol/l KCl; total volume 2 liters).

Initial positioning of the surface coils and measurements of liver and muscle volume were accomplished with multislice echo-planar imaging. This technique provides detailed anatomical images based on proton density, by noninvasive and nonradioactive means (34). Echo-planar imaging was performed on an in-house spectrometer, with a 1-m bore, 0.5-T whole body magnet (Oxford Magnet Technology). The optimal positions for the carbon-proton coils over the liver and quadriceps femoris were established with water-filled Eppendorf tubes as coil markers. Measurements of total liver and quadriceps femoris volume were coupled to each of the liver and quadriceps femoris spectroscopy measurements, respectively. Each image was acquired in 130 ms, with the MBEST (Modulus Blipped, Echo-Planar, single-pulse technique) imaging sequence with a readout gradient switching rate of 0.5 kHz. An ultra-fast multislice sequence was employed, taking ~3 s to acquire 10 transverse slices. Slice width was 10 mm, with an in-plane resolution of 3 mm. Usually, six sequential sets of 10 slices were required to define muscle volume from the bladder to the knee. Analysis of muscle volume began at the urethra and ended before the quadriceps femoris tapered to the insertion points. Three sequential sets of 10 slices were usually required to define liver volume from the superior to the inferior aspect. When liver images were obtained, subjects were instructed to hold their breath at the same point during the outward phase of the breathing cycle during each 3-s acquisition period. Together with the short acquisition time, this removes the motion artefact arising from respiration associated with conventional MRI (6). The total number of slices used for analysis differed between subjects due to anatomical differences but numbered ~20 slices for the liver and 25 slices for the muscle. The same number of slices was analyzed on each occasion for a given subject. Liver and muscle volumes were calculated with the stereology method within the ANALYZE image analysis program (Biomedical Imaging Resource, Mayo Foundation, Rochester, Minnesota). This method reduced the subjective element of manual tracing. The coefficient of variation, calculated for repeated analysis of a single image, was 1.9% (n = 15) for both liver and muscle.

Blood sampling and analysis. Blood was collected into fluoride oxalate for immediate determination of whole blood glucose concentration (Yellow Springs Instruments, Farnborough, UK). A second sample was collected into a plain tube, from which a 100-µl sample was immediately deproteinized in 1 ml 10% perchloric acid, centrifuged, and stored at -20°C for lactate analysis (30) at a later date. A further sample was collected into lithium heparin, mixed, and centrifuged. The plasma was divided between a tube containing EGTA-glutathione, which was stored at -80°C for future plasma catecholamine analysis (31), and a plain tube, which was snap-frozen in liquid nitrogen and stored at -80°C for plasma ammonia analysis (15) within 24 h.

Statistical analysis. All MRS data in the glucose and sucrose trials refer to six subjects. One subject was unable to complete all three visits; hence, MRS data in the control trial refers to 5 subjects. Changes in liver and muscle glycogen content pre- and postexercise were examined with Students t-test for paired data. Differences in glycogen resynthesis during recovery were examined with two-way repeated-measures ANOVA (SuperANOVA) with condition (control, glucose, and sucrose) and time (sampling points) as independent variables.

Measurements of endurance capacity, blood metabolites, and CHO oxidation refer to all 10 subjects. Reporting of blood metabolite data and CHO oxidation (g/min) data during the second bout of exercise is restricted to the first 15 min of exercise to ensure equal subject numbers, because exercise time-to-exhaustion differed between subjects. Differences in resting, pretreatment, and peak blood metabolite concentrations were examined with one-way ANOVA, with condition as the independent variable. Differences between resting and peak blood metabolite concentrations were examined with Student’s t-test for paired data. Differences in total CHO oxidation (g) during exercise and recovery, and differences in exercise time-to-exhaustion, blood metabolite accumulation, and CHO oxidation rate (g/min) during the initial 15 min of exercise bout 2, were examined by one-way ANOVA, with condition as the independent variable. Differences in blood glucose, serum insulin, and blood lactate concentration, and respiratory exchange rate values during the recovery period were examined with two-way repeated-measures ANOVA, with condition and time as independent variables.

The Duncan New Multiple Range test for comparing mean values was used as a post hoc test when significant interactions were detected. Relationships between 1) the CHO load and change in (∆) tissue glycogen and 2) tissue glycogen and subsequent exercise duration were examined by computing a correlation coefficient (r) with a linear fit and a second order polynomial fit, respectively (Statview). Statistical significance was accepted at the 5% level (P < 0.05). Values are presented in the text and in Tables 1–3 and Figures 1–5 as means ± SE.

RESULTS

Performance. During bout 1, subjects cycled for 83 ± 8 min on each occasion to deplete liver and muscle glycogen stores. After the recovery period, exercise time-to-volitional fatigue during bout 2 appeared to be longer after CHO administration, but no significant differences among trials were found (control: 35 ± 5, glucose: 40 ± 5, and sucrose: 46 ± 6 min).

CHO oxidation. As expected, total CHO oxidation during exercise bout 1 did not vary among trials (control: 152.5 ± 15.5; glucose: 155.1 ± 18.4; and sucrose: 160.9 ± 22.5 g, nonsignificant (NS)). Treatments were administered after exercise. During the recovery period after treatment ingestion, total CHO oxidation was significantly elevated above control levels (2.8 ± 2.0 g) in both the glucose (16.4 ± 4.1 g, P < 0.05) and sucrose (18.4 ± 5.7 g, P < 0.05) trials. Respiratory exchange ratio values during recovery are given in Table 1. After 15 min of exercise in bout 2, CHO oxidation had risen to 2.1 ± 0.2 and 2.1 ± 0.3 g/min in the glucose and sucrose trials, respectively, both of which were significantly different from control (1.2 ± 0.3 g/min; P < 0.05). Total CHO oxidation in bout 2 (which also reflects exercise duration) was higher in the sucrose trial (102.3 ± 18.7 g; P < 0.01) but not significantly higher in the glucose trial (67.0 ± 7.8 g), when compared with control (38.8 ± 7.5 g). No significant differences in CHO oxidation were found between the glucose and sucrose trials.

Blood metabolites. Fasting blood glucose was 4.6 ± 0.1, 4.4 ± 0.1, and 4.4 ± 0.1 mmol/l in the control, glucose, and sucrose trials, respectively (NS). At rest
of the drinks, blood glucose was 4.1 

<table>
<thead>
<tr>
<th>Blood glucose</th>
<th>PE</th>
<th>R1</th>
<th>R2</th>
<th>R3</th>
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<tr>
<td>Control</td>
<td>4.1 ± 0.1</td>
<td>4.6 ± 0.5</td>
<td>4.2 ± 0.1</td>
<td>3.9 ± 0.1</td>
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<tr>
<td>Glucose</td>
<td>3.8 ± 0.1</td>
<td>6.6 ± 0.6*</td>
<td>4.7 ± 0.2</td>
<td>3.7 ± 0.2</td>
</tr>
<tr>
<td>Sucrose</td>
<td>3.7 ± 0.2</td>
<td>5.6 ± 0.4</td>
<td>4.1 ± 0.1†</td>
<td>3.7 ± 0.1</td>
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Values are means ± SE.  

A significant difference in peak plasma ammonia concentration was found during exercise bout 1 (156 ± 16, 143 ± 9, and 164 ± 16 

μmol/l; NS in the control, glucose, and sucrose trials, respectively) or exercise bout 2 (183 ± 20, 159 ± 17, and 189 ± 20 

μmol/l; NS in the control, glucose, and sucrose trials, respectively). There was a tendency for the change in plasma ammonia concentration during the initial 15 min of exercise bout 2 to be lower after CHO administration, but the difference among means did not reach significance (102 ± 14, 65 ± 8, and 85 ± 11 

μmol/l in the control, glucose, and sucrose trials, respectively; P = 0.069).

There was no difference in the blood lactate or plasma catecholamine response to exercise and recovery among trials. To give an indication of the response, 

blood lactate concentration increased from a resting level of 1.3 ± 0.2 mmol/l (all trials, n = 30), to a peak of 5.9 ± 0.5 mmol/l (n = 30, P < 0.001) within 15 min during exercise bout 1. Values during recovery are given in Table 1. Lactate concentrations before and after 15 min of exercise bout 2 were no different among trials (control: 1.2 ± 0.2 and 3.5 ± 0.7, glucose: 1.5 ± 0.3 and 3.4 ± 0.7, sucrose: 1.4 ± 0.4 and 3.4 ± 0.5 mmol/l, respectively; NS). Resting epinephrine and norepinephrine were 0.29 ± 0.04 and 1.19 ± 0.13 mmol/l (n = 30), rising to 5.09 ± 1.25 mmol/l (n = 30, P = 0.001) and 14.06 ± 1.16 mmol/l (n = 30, P < 0.001) during exercise bout 1, respectively. Before and after 15 min of exercise bout 2, there was no difference among trials in epinephrine (control: 0.59 ± 0.21 and 2.42 ± 0.63, glucose: 0.92 ± 0.41 and 4.63 ± 1.42, sucrose: 0.34 ± 0.12 and 3.29 ± 1.73 mmol/l, respectively; NS) or norepinephrine (control: 1.06 ± 0.23 and 13.96 ± 2.40, glucose: 3.67 ± 1.82 and 12.96 ± 4.01, sucrose: 1.01 ± 0.21 and 10.37 ± 1.63 mmol/l, respectively; NS) concentration.

Liver volume and liver glycogen. Liver volume (liters) and liver glycogen concentration (mmol/l) at rest, after exercise, and during recovery are shown in Table 2. Rates of liver glycogen resynthesis are shown as concentration per unit time in Table 3.

Liver volume was lower during the recovery period after exercise compared with resting values and appeared to fall further in the absence of exogenous CHO (control; Table 2). This may be partly a consequence of a fall in liver glycogen concentration, which followed a similar pattern. CHO administration appeared to arrest the decline in both liver glycogen concentration and liver volume (Table 2). However, a significant correlation between liver glycogen concentration and liver volume was not found in either the control, glucose, or sucrose trials.

Liver glycogen content (g) declined during exercise by 54 ± 4 (P < 0.01), 56 ± 6 (P < 0.05), and 58 ± 6 (P < 0.05) in the control, glucose, and sucrose trials, respectively. The decline was of the same magnitude in all trials (NS). The time course of liver glycogen depletion and resynthesis is shown in Fig. 2, top. ANOVA demonstrated a significant difference among trials in Δliver glycogen content during recovery (P < 0.05; Fig. 3). It is evident from Fig. 3 that there was no glycogen resynthesis in the control trial after 30 min (−19 ± 12 g), 90 min (−11 ± 14 g), 150 min (−19 ± 12 g), or 210 min (−15 ± 16 g) of recovery. However, glycogen resynthesis was evident in both the glucose and sucrose trials (Fig. 3). The change in liver glycogen from postexercise values after glucose ingestion was significantly different from control after 30 min (12 ± 8 g, P < 0.01), 90 min (9 ± 5 g, P = 0.01), 150 min (17 ± 7 g, P < 0.01), and 210 min (13 ± 8 g, P < 0.01) of recovery. Similarly, the change in liver glycogen after sucrose ingestion was significantly different from control after 30 min (6 ± 6 g, P < 0.01), 90 min (10 ± 8 g, P = 0.01), 150 min (18 ± 4 g, P < 0.01), and 210 min (25 ± 5 g, P < 0.01) of recovery. No differences in liver glycogen resynthesis were found between the glucose and sucrose trials.
Table 2. Liver volume, liver glycogen concentration, muscle volume, and muscle glycogen concentration

<table>
<thead>
<tr>
<th></th>
<th>Rest</th>
<th>PE</th>
<th>R1</th>
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<td>Liver</td>
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<tr>
<td>C volume</td>
<td>1.87 ± 0.10</td>
<td>1.80 ± 0.09</td>
<td>1.75 ± 0.10</td>
<td>1.72 ± 0.12da</td>
<td>1.69 ± 0.14</td>
<td>1.63 ± 0.15a</td>
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<tr>
<td>C glycogen</td>
<td>375 ± 61</td>
<td>191 ± 52b</td>
<td>130 ± 12a</td>
<td>155 ± 11a</td>
<td>135 ± 16a</td>
<td>151 ± 18b</td>
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<td>G volume</td>
<td>1.89 ± 0.14</td>
<td>1.82 ± 0.14</td>
<td>1.76 ± 0.14b</td>
<td>1.79 ± 0.10</td>
<td>1.74 ± 0.11b</td>
<td>1.77 ± 0.13b</td>
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<td>G glycogen</td>
<td>397 ± 84</td>
<td>177 ± 43a</td>
<td>213 ± 44a</td>
<td>211 ± 49abc</td>
<td>242 ± 51c</td>
<td>228 ± 49</td>
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<td>S volume</td>
<td>1.85 ± 0.17</td>
<td>1.71 ± 0.13a</td>
<td>1.65 ± 0.16b</td>
<td>1.68 ± 0.19b</td>
<td>1.67 ± 0.14a</td>
<td>1.67 ± 0.13a</td>
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<td>S glycogen</td>
<td>387 ± 194</td>
<td>141 ± 64</td>
<td>161 ± 55</td>
<td>178 ± 50</td>
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<td>233 ± 77da</td>
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Muscle

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<tr>
<td>C volume</td>
<td>4.86 ± 0.27</td>
<td>5.17 ± 0.43</td>
<td>5.05 ± 0.40</td>
<td>5.10 ± 0.45</td>
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<td>C glycogen</td>
<td>166 ± 35</td>
<td>70 ±18a</td>
<td>58 ± 7a</td>
<td>69 ± 10aP</td>
<td>64 ± 3b</td>
<td>68 ± 8b</td>
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<td>G volume</td>
<td>5.06 ± 0.26</td>
<td>5.07 ± 0.25</td>
<td>5.03 ± 0.27</td>
<td>4.99 ± 0.24</td>
<td>4.95 ± 0.28</td>
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<td>G glycogen</td>
<td>153 ± 39</td>
<td>60 ± 12a</td>
<td>100 ± 46b</td>
<td>85 ± 36b</td>
<td>93 ± 38b</td>
<td>92 ± 38b</td>
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<td>S volume</td>
<td>4.83 ± 0.23</td>
<td>5.01 ± 0.21</td>
<td>5.03 ± 0.25</td>
<td>4.92 ± 0.23</td>
<td>4.99 ± 0.17</td>
<td>5.05 ± 0.20</td>
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<tr>
<td>S glycogen</td>
<td>159 ± 31</td>
<td>55 ± 13b</td>
<td>60 ± 8a</td>
<td>72 ± 13bd</td>
<td>72 ± 8b</td>
<td>80 ± 10a</td>
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</table>

Values are means ± SE. Liver volume (liters), liver glycogen concentration (mmol/l), muscle volume (liters), and muscle glycogen concentration (mmol/l) measured pre- (rest), postexercise at 70% \( \dot{V}O_{2\text{max}} \) and hourly for 4 h (R1–R4) after subsequent ingestion of 1 g/kg body mass glucose (G; \( n = 6 \)), sucrose (S; \( n = 6 \)), or an equivalent volume of placebo (control (C); \( n = 5 \)). Mean values were significantly different from rest: * \( P < 0.05 \), ** \( P < 0.01 \), and *** \( P < 0.001 \); PE: \( P < 0.05 \), d \( P < 0.01 \) of recovery. A weak but statistically significant relationship was evident between \( \Delta \) liver glycogen content (g) after 240 min of recovery and time-to-exhaustion during exercise bout 2 (\( n = 15 \); Fig. 4).

A relationship was found between the CHO load (g) and \( \Delta \) liver glycogen content (g) after 30 min (\( r = 0.63 \); \( P < 0.05 \)), 90 min (\( r = 0.59 \); \( P < 0.05 \)), 150 min (\( r = 0.79 \); \( P < 0.01 \)), and 210 min (\( r = 0.70 \); \( P < 0.01 \)) of recovery. A weak but statistically significant relationship was evident between \( \Delta \) liver glycogen content (g) after 240 min of recovery and time-to-exhaustion during exercise bout 2 (\( r = 0.44 \); NS). However, a relationship was found between the CHO load (g) and the sum of \( \Delta \) liver and muscle glycogen resynthesis (g) after 60 min (\( r = 0.64 \); \( P < 0.05 \)), 180 min (\( r = 0.65 \); \( P < 0.01 \)), and 240 min (\( r = 0.62 \); \( P < 0.05 \)) of recovery. A weak but statistically significant relationship was evident between the sum of \( \Delta \) liver and \( \Delta \) muscle glycogen content (g) after 240 min of recovery and time-to-exhaustion during exercise bout 2 (\( n = 15 \); Fig. 5).

The mean CHO load was 76 g in both glucose and sucrose trials. Assuming equal glycogen resynthesis in both legs, the calculated increase in total CHO disposal during the 4-h recovery period after glucose and sucrose ingestion ([CHO oxidation – control CHO oxidation] + [\( \Delta \) liver glycogen resynthesis 0–210 min – \( \Delta \) control liver glycogen resynthesis 0–210 min] + [\( \Delta \) muscle glycogen resynthesis 0–240 min – \( \Delta \) control muscle glycogen resynthesis 0–240 min, \( \times 2 \)]) was 64 and 82 g, respectively (NS between trials).

Table 3. Rates of liver and muscle glycogen resynthesis

<table>
<thead>
<tr>
<th></th>
<th>R1</th>
<th>R2</th>
<th>R3</th>
<th>R4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>–61 ± 42</td>
<td>25 ± 9</td>
<td>–20 ± 14</td>
<td>16 ± 18</td>
</tr>
<tr>
<td>Glucose</td>
<td>36 ± 20</td>
<td>–2 ± 25</td>
<td>31 ± 24</td>
<td>–14 ± 8</td>
</tr>
<tr>
<td>Sucrose</td>
<td>20 ± 13a</td>
<td>17 ± 10</td>
<td>28 ± 34</td>
<td>27 ± 17</td>
</tr>
<tr>
<td>Muscle</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>–12 ± 21</td>
<td>10 ± 15</td>
<td>–5 ± 9</td>
<td>4 ± 11</td>
</tr>
<tr>
<td>Glucose</td>
<td>40 ± 39</td>
<td>–15 ± 11</td>
<td>8 ± 6</td>
<td>–1 ± 4</td>
</tr>
<tr>
<td>Sucrose</td>
<td>5 ± 9</td>
<td>11 ± 7</td>
<td>0 ± 8</td>
<td>8 ± 9</td>
</tr>
</tbody>
</table>

Values are means ± SE. Rates of liver and muscle glycogen resynthesis (mmol·l⁻¹·h⁻¹) measured hourly for 4 h (R1–R4) after 83 min of exercise at 70% \( \dot{V}O_{2\text{max}} \) and subsequent ingestion of 1 g/kg body mass glucose (\( n = 6 \)), sucrose (\( n = 6 \)), or an equivalent volume of placebo (control, \( n = 5 \)). Mean values were significantly different from control: * \( P < 0.05 \).

DISCUSSION

This is the first investigation of simultaneous changes in human liver and skeletal muscle glycogen content, corrected for tissue volume, during exercise and recovery. It is also the first occasion postexercise resynthesis of liver and muscle glycogen has been related to subsequent endurance exercise capacity.

MRS allows direct, noninvasive, and repeated measurement of liver and muscle glycogen stores (18, 35, 37). Despite its large molecular size, glycogen is 100% visible with MRS (18, 40, 41). The first natural abun-
a mixed meal after a 30-h fast (human liver and muscle glycogen to study responses to made. Liver glycogen or restoration of exercise capacity were signals were not quantified, and no measurements of However, postexercise refeeding was not controlled, 13C in muscle glycogen during exercise and recovery (1). A higher field strength, successfully monitored changes physiological changes. Subsequent pilot investigations, with measurements lacked the sensitivity to monitor physi- muscle were obtained in the mid-1980s (26), but these observations of a decline in both liver and muscle glycogen after prolonged exhaustive exercise in humans (2, 22). Liver glycogen content increased during the recovery period after CHO ingestion, and these changes were significantly greater throughout recovery when compared with the control trial (Fig. 3). In the absence of exogenous CHO, hepatic glycogenolysis is the principal means of maintaining euglycemia during exercise, and the rate of glycogenolysis is directly related to the hepatic glycogen content (47). Figure 3 demonstrates that both glucose and sucrose ingestion increased liver glycogen stores, thereby increasing the potential for substrate delivery to skeletal muscle during a subsequent bout of exercise.

It has been suggested that the ability to perform prolonged submaximal exercise becomes increasingly dependent on blood glucose availability as exercise progresses, particularly in well-trained subjects (13). The authors demonstrated that as muscle glycogen stores were reduced, oxidation of CHO from alternative sources was increased to meet the energy demands of contraction. It was also shown that fatigue could be delayed by maintaining a high rate of CHO oxidation via oral glucose ingestion. Interestingly, later studies have indicated that the magnitude of the increase in contribution of blood glucose to total energy production during exercise is primarily a function of glucose availability (48, 49). Together these studies suggest that blood glucose availability, whether supplied from exogenous CHO or liver glycogenolysis, is important to endurance exercise capacity. It also suggests that differences between glucose and sucrose ingestion are evident in terms of postexercise liver glycogen resynthesis could have important consequences for subsequent exercise capacity.

The results of the present study provide support for the importance of blood glucose availability to postexercise glycogen resynthesis, and, in some degree, to subsequent exercise capacity. After the initial bout of glycogen-depleting exercise in the present study, no evidence of liver or muscle glycogen resynthesis was found in the control trial, in which no CHO was given. Liver glycogen resynthesis was evident during the recovery interval after CHO ingestion. Given that the Michaelis-Menten constant ($K_m$) of glucokinase is in the order of 8 mmol/l and blood glucose concentration was still ~6 mmol/l 1 h after CHO ingestion, it is likely that, as expected, the direct pathway probably contributed to a significant proportion of glycogen resynthesis. The importance of glucose availability is illustrated by the significant relationship found between the CHO load and liver glycogen content throughout recovery ($r = 0.59–0.79, P < 0.05$). Of course, this represents only net glycogen synthesis, and no account has been taken of liver glycogen turnover, which has been shown to proceed under conditions of net glycogen synthesis in

Fig. 2. Human liver (top) and skeletal muscle (bottom) glycogen content (g) before and after 83-min cycling exercise at 70% $V_{\text{O}_2\text{max}}$ and during recovery after ingestion of 1 g/kg body mass glucose or sucrose or an equivalent volume of placebo (control; n = 6). Glycogen measurements (mmol/l) were made by natural abundance $^{13}$C MRS. C-1 signal was quantified by comparison to spectra from a glycogen phantom (200 mmol/l oyster glycogen and 150 mmol/l KCl; total vol 2 liters). Lipid peak at 30 ppm (CH$_2$) was used as an internal standard. Liver and muscle volume (liters) was measured immediately after acquisition of each liver and muscle spectrum, respectively. This enabled calculation of total glycogen content in millimoles and therefore in grams. Liver spectra and volume measurements were obtained within the first 30 min after CHO ingestion and then hourly. Muscle spectra and volume measurements were obtained from 30–60 min and then hourly.
Furthermore, a modest but significant relationship was evident between δliver glycogen content at the end of the recovery period and subsequent exercise time-to-exhaustion (r = 0.53, P < 0.05). Based on animal studies, Terjung et al. (44) postulated that when both liver and muscle glycogen stores are depleted during exercise, repletion of liver glycogen might be the rate-limiting factor in the capacity for prolonged strenuous exercise. The present results indicate that, after a small CHO load in humans, subsequent endurance capacity may be dependent to some degree on a liver-mediated increase in muscle glucose delivery.

No differences in liver or muscle glycogen resynthesis or subsequent endurance exercise capacity were found when comparing responses to 1 g/kg BM glucose or sucrose ingestion. This is somewhat surprising given sucrose contains equimolar fractions of glucose and fructose, which are metabolized predominantly by muscle and liver, respectively (3, 50). Although the GLUT-5 transporter isoform is expressed in human skeletal muscle (23), the affinity of hexokinase for fructose is very low, and, in the absence of the enzyme fructokinase, phosphorylation of fructose will be very slow in comparison with glucose. It appears that skeletal muscle can only utilize significant quantities of fructose after conversion to glucose in the liver. Fructokinase is present in liver, where it has a low Kₘ, and, consequently, a high affinity for fructose. On the basis of this, sucrose might have been expected to preferentially restore liver, rather than muscle, glycogen stores after exercise. The finding that it did not may be related to the small quantity of CHO given, an increase in insulin sensitivity as a result of the exercise, or an increase in liver glycogen turnover in the sucrose trial.
In a previous study from this laboratory, Moriarty et al. (35) investigated the effects of glucose and sucrose ingestion on postexercise liver and muscle glycogen resynthesis alone. Tissue glycogen was represented as a percentage of the lipid peak. Muscle glycogen levels were shown to fall during exercise and to increase during recovery, but no changes in liver glycogen levels were found. Furthermore, no differences in liver or muscle glycogen resynthesis were found after glucose or sucrose ingestion. However, nutrient intake was not controlled before exercise, positioning of the 13C liver and muscle probes did not have the benefit of imaging techniques, and, perhaps as a consequence of this, inter- and intraindividual resting muscle and liver glycogen levels were highly variable. Proton decoupling was not used, resulting in a signal-to-noise ratio that was approximately one-half of that obtained in the present study. In addition, no attempt was made to quantify the glycogen signal. On the basis of the results of the present study, it appears methodological problems may account for failure of the authors to demonstrate a decline in liver glycogen during exercise or resynthesis of liver glycogen during recovery. It is possible that administration of a relatively large CHO bolus (177 g) by Moriarty et al. was sufficient to mask a difference between glucose and sucrose uptake and disposal by liver and muscle. The present study demonstrates, however, that a differential response does not exist after a smaller CHO load. This is supported, in the case of muscle glycogen resynthesis, by Blom et al. (5), who compared feedings of 0.7 g/kg BM glucose and sucrose given at 2-h intervals during a 6-h recovery period after exercise-induced glycogen depletion. With this relatively small intake, no difference in the average rate of muscle glycogen resynthesis was found between the two conditions (5.8 ± 1.0 and 6.2 ± 0.5 mmol·kg wet wt·h−1, respectively). Van den Bergh et al. (45) found a significantly higher rate of postexercise muscle glycogen resynthesis when repeated ingestion of 80 g of glucose (total 320 g) was compared with a similar regimen of fructose ingestion over an 8-h period. Furthermore, Burke et al. (9) measured muscle glycogen concentration 24 h after exercise, during which time subjects consumed 10 g/kg BM CHO (690 g) of either high or low GI foods. They demonstrated a clear advantage of high GI CHO sources compared with low GI sources in terms of short-term muscle glycogen resynthesis. Differences between these studies may have been a function of the type or quantity of the CHO load and/or recovery time, but they do suggest a high CHO load does not preclude the ability to measure differences between glucose- and sucrose-mediated glycogen resynthesis. Neither Burke et al. nor Blom et al. (5) performed measurements of liver glycogen resynthesis or assessed the effects of these different types of CHO on restoration of exercise capacity.

Significant changes in muscle glycogen content after CHO ingestion were not demonstrated. This is surprising given previous studies have demonstrated that short-term skeletal muscle glycogen resynthesis after exercise-induced glycogen depletion is linearly related to the exogenous CHO load; albeit, CHO ingestion was relatively high (12 g/kg BM; Ref. 42). Consequently, this finding may be due to administration of a relatively small CHO load, a proportion of which was extracted by the liver (~30%) before being made available to the relatively large mass of skeletal muscle. The relationship between CHO load and muscle glycogen resynthesis and the relationship of muscle glycogen availability to endurance exercise performance is well established in humans (20, 28). A possible dilution effect in the present study is supported by the absence of a relationship between the CHO load and muscle glycogen content during recovery or between muscle glycogen content and subsequent exercise performance. A relationship between the sum of glycogen synthesis in the liver and muscle and these variables would suggest a pattern of resynthesis, which, although undetectable in muscle alone, was similar to that in the liver. Accordingly, the CHO load was significantly correlated with the sum of Δliver and Δmuscle glycogen content during recovery (r = 0.62–0.65, P < 0.05). A modest but significant relationship was evident between the sum of Δliver and Δmuscle glycogen content at the end of the recovery period and subsequent exercise time-to-exhaustion (r = 0.55, P < 0.05).

The absence of a significant change in muscle glycogen, together with the inability to detect differences between glucose- and sucrose-mediated liver or muscle glycogen resynthesis, also appears to be due to the large interindividual variation in glycogen measurements. The relatively small interindividual variation in the control trial suggests that this represented a physiological variation in rates of CHO disposal rather than variation resulting from instrumentation. A number of factors may account for this variation. One factor may be interindividual variations in muscle composition. The rate of postexercise glycogen resynthesis, determined with biochemical analysis, has been shown to be 25% higher in human type I muscle fibers when compared with type II fibers during the initial 3 h of recovery (10). This is probably related to fiber-type variations in the rate of muscle glucose uptake. In this respect, it has been shown that muscles composed predominantly of type I fibers have a greater plasma membrane glucose transporter number (17), and more specifically, the highest content of the GLUT-4 glucose transporter isoform (25). A second factor may be the glucose load. As discussed earlier, glucose availability to the muscle will be higher after glucose than fructose ingestion. Variation in glycogen resynthesis, arising from fiber-type differences in glucose uptake and disposal, might therefore be expected to be greater after glucose ingestion.

Plasma ammonia accumulation during the initial period of exercise bout 2 tended to be lower after CHO administration, suggesting improved maintenance of the ATP-to-ADP ratio. This would be expected to translate into reduced rates of fatigue, but differences in exercise time-to-exhaustion did not reach significance.
From a methodological point of view, this study demonstrates for the first time that significant changes in liver volume occur with this experimental paradigm. These changes in volume may confound analysis of changes in liver glycogen concentration during prolonged exercise and recovery. Accordingly, coupling of tissue glycogen measurements to tissue volume measurements, enabling calculation of liver glycogen content (g), provides a more accurate means to investigate changes in liver glycogen under these conditions.

In conclusion, this study has demonstrated that 1 g/kg BM glucose or sucrose is sufficient to initiate postexercise liver glycogen resynthesis. A weak but statistically significant correlation between postexercise liver glycogen resynthesis and subsequent exercise capacity was demonstrated, but no difference in mean exercise time was found after placebo, glucose, or sucrose ingestion. The role of liver glycogen homeostasis during exercise and recovery has been previously underestimated. It is not possible to detect significant changes in postexercise muscle glycogen content after administration of relatively small amounts of CHO.

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