Van Hall, G., S. M. Shirreffs, and J. A. L. Calbet. Muscle glycogen resynthesis during recovery from cycle exercise: no effect of additional protein ingestion. J Appl Physiol 88: 1631–1636, 2000.—In the present study, we have investigated the effect of carbohydrate and protein hydrolysate ingestion on muscle glycogen resynthesis during 4 h of recovery from intense cycle exercise. Five volunteers were studied during recovery while they ingested, immediately after exercise, a 600-ml bolus and then every 15 min a 150-ml bolus containing 1) 1.67 g·kg body wt \(-1\) of sucrose and 0.5 g·kg body wt \(-1\) of a whey protein hydrolysate (CHO/protein), 2) 1.67 g·kg body wt \(-1\) of sucrose (CHO), and 3) water. CHO/protein and CHO ingestion caused an increased arterial glucose concentration compared with water ingestion during 4 h of recovery. With CHO ingestion, glucose concentration was 1–1.5 mmol/l higher during the first hour of recovery compared with CHO/protein ingestion. Leg glucose uptake was initially 0.7 mmol/min with water ingestion and decreased gradually with no measurable glucose uptake observed at 3 h of recovery. Leg glucose uptake was rather constant at 0.9 mmol/min with CHO/protein and CHO ingestion, and insulin levels were stable at 70, 45, and 5 mU/l for CHO/protein, CHO, and water ingestion, respectively. Glycogen resynthesis rates were \(52 \pm 7, 48 \pm 5, \text{ and } 18 \pm 6\) for the first 1.5 h of recovery and decreased to \(30 \pm 6, 36 \pm 3, \text{ and } 8 \pm 6\) mmol·kg body mass \(-1\)·h \(-1\) between 1.5 and 4 h of recovery (CHO/protein, CHO, and water ingestion, respectively). No differences could be observed between CHO/protein and CHO ingestion. It is concluded that coingestion of carbohydrate and protein, compared with ingestion of carbohydrate alone, did not increase leg glucose uptake or glycogen resynthesis rate further when carbohydrate was ingested in sufficient amounts every 15 min to induce an optimal rate of glycogen resynthesis.

METHODS

Subjects. Five healthy trained volunteers participated in this study. Their mean age, mass, height, and maximal oxygen uptake were \(26 \pm 2\) yr, \(74 \pm 2\) kg, \(1.78 \pm 0.03\) m, and \(61 \pm 2\) ml·kg body mass \(-1\)·min \(-1\), respectively. The subjects were informed about possible risks and discomfort involved before giving their voluntary consent to participate. The study was performed according to the Declaration of Helsinki and was approved by the Ethical Committee of Copenhagen-Fredriksberg.

Protocol. Each subject underwent three experimental trials separated by 1 mo. The three trials were identical except that during the recovery period the subjects ingested drinks that contained carbohydrate and a protein hydrolysate (CHO/protein), carbohydrate (CHO), or water (water). The CHO/protein and CHO trials were randomized, and the water trial was performed thereafter.

Before the experimental trials, maximal power output \(W_{\text{max}}\) and maximal oxygen uptake were determined during a graded exercise test on a cycle ergometer (model 818, Monark) as described previously (10). The protocol of the experimental day is shown in Fig. 1. The subjects reported to the laboratory at 8:00 AM. After the subjects changed clothes and their body mass was measured, intense cycle ergometer exercise was started to decrease the muscle glycogen content. The glycogen-depletion protocol was described previously by Kuipers et al. (10). In short, after a warm-up period of 10 min at 50% of their \(W_{\text{max}}\) the subjects had to cycle in blocks of 2-min duration at an alternating workload of 90 and 50% of their respective \(W_{\text{max}}\). This was continued until they were not able to complete

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the 2 min at 90% $W_{\text{max}}$. That moment was defined as the inability to maintain a pedal rate above 60 rpm. The high-intensity block was then reduced to 80% $W_{\text{max}}$. Again the subjects had to cycle until they were unable to complete the 2 min at 80% $W_{\text{max}}$. When the 80% $W_{\text{max}}$ could not be completed, subjects were allowed to stop. During the glycogen-depletion exercise, the subjects were cooled with standing floor fans and water was provided ad libitum.

After cessation of the glycogen-depletion exercise, the subject immediately took a shower. Then, catheters for blood sampling and blood flow measurements were placed under local anesthesia (Lidocaine, 20 mg/ml) in the femoral artery and vein in the inguinal region of the leg. The tip of the catheters was advanced to 5–6 cm proximal of the inguinal ligament. The arterial catheter was 20 gauge and 20 cm in length (Ohmeda, Swindon, UK), and the venous catheter was a radiopaque TFE catheter with side holes (Cook, Bjaeverskov, Denmark). A thermistor was inserted through the venous catheter for blood flow measurement by the constant infusion thermodilution technique (2). Placement of the catheters took ~20 min, after which the subjects remained in supine position for another 10–15 min before mounting the cycle ergometer for an incremental exercise bout. This second exercise bout started within 1 h of the end of the glycogen-depletion bout and was performed to abolish the effect on metabolism of the recovery period due to placement of the catheters so that immediate recovery could be followed. This exercise bout consisted of 14 min at 40% $W_{\text{max}}$ followed by 4 min at 60, 70, and 80% $W_{\text{max}}$. This exercise bout was to near exhaustion for the subjects, and one of the subjects was not able to complete the last 2 min at the highest workload in two of the three trials.

After the exercise, the subjects dismounted the ergometer and lay on the bed for the next 4 h. Immediately after they lay down, local anesthesia was applied to the skin (Lidocaine, 20 mg/ml), and an incision was made to obtain a muscle biopsy with the needle-biopsy technique (3) from the vastus lateralis muscle. The time between the end of the exercise bout and the muscle biopsy was on average 5 min. The first and second biopsies were taken through the same incision. However, to avoid sampling from a damaged site, the Bergstrom needle was directed 3–4 cm in a more proximal direction compared with the initial biopsy. The third biopsy was taken from a new incision ~4 cm distal from the first incision. The first blood sample and muscle biopsy were taken just before the first bolus of the drink was provided. The second and third biopsies were taken after 1.5 and 4 h of recovery. During the first hour of recovery, femoral arterial and venous blood samples were taken and blood flow was measured every 10 min; thereafter, these procedures were repeated every 30 min.

The drinks during recovery were provided as a bolus. The first bolus was 600 ml, and, thereafter, the subjects received 150 ml every 15 min for the next 4 h of recovery. The drinks contained 1) 1.67 g·kg body wt⁻¹·l⁻¹ of sucrose and 0.5 g·kg body wt⁻¹·l⁻¹ of a whey protein hydrolysate (MD Foods, Arhus, Denmark) with an average chain length of 3.7 (CHO/protein), 2) 1.67 g·kg body wt⁻¹·l⁻¹ sucrose (CHO), and 3) water (water).

**Muscle analysis.** Muscle specimens of ~50–70 mg wet weight were immediately frozen in liquid nitrogen, freeze-dried, and freed from adherent blood and connective tissue. Glycogen was measured in duplicate with 2–3 mg of dry muscle tissue. For the glycogen determination, glycogen was hydrolyzed with 1 M HCl for 3 h at 100°C and then neutralized with 0.12 M Tris-2.1 M KOH saturated with KCl. The supernatant was analyzed for glucose (Roche UniKit, Paris, France) on an automatic analyzer (Cobas Fara, Roche, Basel, Switzerland).

**Blood analysis.** Femoral arterial and venous blood was collected into EDTA-containing tubes, immediately centrifuged at 4°C to obtain plasma, and stored at ~40°C. Plasma glucose was analyzed on an automatic analyzer (Cobas Fara, Roche, France). Plasma insulin (Pharmacia, Uppsala, Sweden) and glucagon (Linco, St. Charles, MO) were determined with a radioimmunoassay kit.

Calculations. Glucose uptake across the leg was calculated as the difference in concentration between femoral arterial and venous plasma multiplied by the blood flow. Muscle glycogen resynthesis rate was calculated between 0 and 1.5 h ($t_1$) and 1.5 and 4 h ($t_2$) of recovery from the following equation (in which brackets denote concentration): glycogen resynthesis rate = (glycogen$_{t_2}$ - glycogen$_{t_1}$) / (t$_2$ - t$_1$). Net glucose uptake in millimoles for the first 1.5 h and between 1.5 and 4 h of recovery was calculated from the leg glucose uptake rates of the two consecutive time points, multiplied by the time span, and summed for the time period of 0–1.5 and 1.5–4 h. Glucose needed for glycogen resynthesis was estimated from the net increase in muscle glycogen concentration multiplied by 1.4 kg of dry weight muscle. If it is assumed that 6 kg of the ~8 kg of leg muscle (1.4 kg dry wt) was engaged in the cycle exercise (15) and depleted to the same extent as the vastus lateralis muscle.

Statistical analysis. All data are means ± SE from five subjects. Statistical analysis of the data was done by using the nonparametric Wilcoxon signed-rank test to determine differences between data obtained in the three different trials for each point in time. Statistical significance was set at $P < 0.05$.

**RESULTS**

Arterial glucose concentration and leg glucose uptake (Fig. 2). The highest arterial glucose concentration was reached 20 min after the first bolus of CHO/protein and CHO was ingested, with values of 8.7 ± 0.3 and 9.6 ± 0.2 mmol/l, respectively, after which glucose concentration decreased gradually to ~6.3 mmol/l for both drinks. In contrast, with water ingestion, glucose concentration was lower for the first 20 min but returned to a stable level of ~5.2 mmol/l after 1 h of recovery. The arterial glucose concentration was higher with CHO/protein and CHO ingestion compared with the concen-
Leg glucose uptake was highest (1.6 mmol/min) 10 and 20 min after the first bolus of the CHO/protein and CHO drinks was ingested. Leg glucose uptake then decreased, and, after 1 h, the leg glucose uptake was at a steady state of ~9 mmol/min. No differences in leg glucose uptake between the CHO/protein and CHO drink could be observed at any time during the recovery. With water ingestion, the leg glucose uptake was initially 0.7 mmol/min but decreased gradually, and, after 3 h of recovery, no measurable glucose uptake could be observed.

Arterial insulin and glucagon concentrations (Fig. 3). Arterial insulin increased considerably during recovery as a result of CHO ingestion. During the entire 4 h of recovery, insulin concentration was higher with CHO/protein ingestion (70–80 mU/l) than with CHO ingestion (40–50 mU/l), and it was only ~5 mU/l with water ingestion. Arterial glucagon decreased after ingestion of the first bolus for all three drinks. The arterial glucagon concentration was higher during recovery with CHO/protein (90 ng/l) compared with CHO ingestion (50 ng/l). With only water ingestion, the glucagon concentration was between that of the CHO/protein and CHO trial, but it failed to have a consistently significant difference from either drink.

Muscle glycogen content and resynthesis rate (Fig. 4). The glycogen depletion exercise and the 26 min of incremental exercise resulted in a muscle glycogen concentration that was the same for the three trials (CHO/protein, 69 ± 10; CHO, 90 ± 23; water, 78 ± 11 mmol·glycosyl units⁻¹·kg dry muscle⁻¹).

No differences in glycogen resynthesis rate were observed between the CHO/protein and CHO trial for either the first 1.5 h or between 1.5 and 4 h of recovery. Glycogen resynthesis rate during the first 1.5 h of recovery was ~50 mmol glycosyl units·kg dry...
muscle·h and decreased to a rate of 30 mmol glycosyl units·kg dry muscle·h between 1.5 and 4 h of recovery. With only water ingestion, the glycogen resynthesis rate during the first 1.5 h was 18 mmol glycosyl units·kg dry muscle·h and decreased to 8 mmol glycosyl units·kg dry muscle·h thereafter.

Leg glucose uptake compared with glucose incorporated in muscle glycogen (Fig. 5). If we assume that 6 kg of muscle in the leg (of a total of 8 kg) is depleted to the same extent as the vastus lateralis muscle during cycle exercise, the comparison between total leg glucose uptake and glucose needed for glycogen resynthesis can be made. In Fig. 5, the total leg glucose uptake during the 0–1.5 and 1.5–4 h of recovery is shown together with the estimated amount of glucose needed for the glycogen synthesized (Fig. 4). During the first 1.5 h of recovery, a nearly 100% match is found for the amount of glucose taken up by the leg and the amount of glucose needed for the observed increase in muscle glycogen content. Between 1.5 and 4 h of recovery it appears that more glucose is taken up by the leg than is incorporated into glycogen.

DISCUSSION

In this study we have investigated muscle glycogen resynthesis and leg glucose uptake during 4 h of recovery from intense cycle exercise leading to glycogen depletion. The major findings were that neither a high rate of muscle glycogen resynthesis with CHO intake nor leg glucose uptake was affected by additional protein ingestion.

Glucose uptake by the skeletal muscle is facilitated mainly via the GLUT-4 transporter. Stimulation of glucose transport by insulin is mediated by translocation of GLUT-4 from the intracellular sites to the plasma membrane (11). Skeletal muscle translocation of GLUT-4 to the plasma membrane appears also to be facilitated by muscle contraction independent of insulin. It is suggested that, during the initial phase of recovery from exercise, GLUT-4 still resides in the plasma membrane because of contraction-mediated GLUT-4 translocation during the exercise bout, and thus glucose can be transported independently of insulin (6, 8). After the relatively short period of insulin independent muscle glucose uptake, glucose uptake and GLUT-4 translocation to the plasma membrane become insulin dependent. However, during recovery from exercise, there is a marked increase in the sensitivity to insulin of muscle glucose uptake and glycogen synthesis (14, 16, 18). Recently, evidence has been presented suggesting that the rate of muscle glucose uptake may control the rate of glycogen resynthesis after exercise. This proposed mechanism seems to fit nicely with our data from when the subjects ingested water. Glucose uptake was highest during the initial recovery period, and leg glucose uptake decreased with the longer time of recovery. GLUT-4 transporters that remained in the plasma membrane from the exercise bout may have caused the higher glucose uptake at the initial stage. As recovery proceeds, the muscle becomes more and more dependent on insulin to maintain
GLUT-4 transporters in the plasma membrane and/or to translocate GLUT-4 to the plasma membrane. Because the insulin level in the water trial was low, the number of GLUT-4 transporters in the plasma membrane declined and, therefore, glucose uptake ceased. During the first hour of recovery, leg glucose uptake was the same for CHO/protein and CHO ingestion despite a substantially lower arterial glucose concentration with CHO/protein ingestion. This indicates that during the first 0.5 h of recovery, arterial glucose concentrations, which are considerably above basal concentrations, do not affect skeletal muscle glucose uptake. With CHO/protein and CHO ingestion, leg glucose uptake was higher during the initial 20–30 min of recovery. This might have been caused by a greater amount of plasma membrane-bound GLUT-4 as a result of the previous contraction. However, glucose uptake then decreased to a constant level despite a high insulin concentration. Furthermore, the higher insulin levels with CHO/protein compared with CHO ingestion did not increase leg glucose uptake in the CHO/protein trial and potentially did not affect insulin-mediated GLUT-4 translocation to the plasma membrane. Because for several hours after exercise the muscle may be more sensitive to insulin (5, 14, 16), a relative low insulin concentration may already have elicited the maximal glucose uptake and GLUT-4 translocation to the plasma membrane. Also, with CHO/protein and CHO ingestion, arterial glucose concentration did not have an effect on leg glucose uptake because glucose concentration declined with recovery time, but leg glucose uptake remained constant after 1 h of recovery. Muscle glycogen was measured after 1.5 and 4 h of recovery, and, therefore, we cannot say whether the glycogen resynthesis rate is declining over that time period.

During the first hour of recovery, arterial glucose concentration was significantly lower with CHO/protein ingestion compared with CHO alone. This lower glucose concentration might have been caused by a reduced glucose appearance from the gastrointestinal tract or a larger clearance by tissues other than the leg. Gastric emptying rate is suggested to depend on energy density of the ingested solution (17, 19), and because the energy density of the CHO/protein drink was higher than that of the CHO drink, gastric emptying may have been slowed down, thus slowing glucose appearance. This study clearly shows that changes in arterial or venous glucose concentrations do not give an indication of muscle glucose uptake because glucose uptake was not different with CHO/protein intake despite lower glucose concentrations.

Skeletal muscle glycogen resynthesis after exercise has been shown to be biphasic (12, 14); there is the initial insulin-independent phase of 30–60 min with a high glycogen resynthesis rate followed by an insulin-dependent phase with a slower glycogen resynthesis rate. When Price and colleagues (14) studied glycogen resynthesis in insulin-resistant subjects and controls they did not observe a difference in glycogen resynthesis rate during the insulin-independent phase. However, during the insulin-dependent phase, glycogen resynthesis rate was lower in the insulin-resistant subjects (13). These findings clearly indicate that from 1 h of recovery onward insulin might play an important role in the regulation of muscle glycogen resynthesis. Zawadzki and colleagues (20) reported a higher glycogen resynthesis rate during 4 h of recovery from exercise when carbohydrates were coingested with protein compared with when carbohydrates were ingested alone. They suggested that this was likely due to the higher insulin level with protein coingestion, potentially via stimulation of glycogen synthase and/or glucose uptake by the muscle. Despite a substantial insulin difference between the CHO/protein and CHO trials, we were not able to find a higher glycogen resynthesis rate. There are several possible reasons to explain the apparent difference. First, the glycogen resynthesis rate of ~41 µmol·kg dry muscle⁻¹·h⁻¹, as observed by Zawadzki and colleagues with CHO/protein intake, is nearly identical to the glycogen resynthesis rate in this study, both for CHO/protein and CHO ingestion if the average glycogen resynthesis rate from 0 to 4 h of recovery is considered (39.8 µmol · kg dry muscle⁻¹ · h⁻¹). This glycogen resynthesis rate may be the maximal achievable after prolonged dynamic exercise with sufficient and regular carbohydrate loading despite higher insulin levels. Second, the higher insulin concentration in the CHO/protein trial might not have been effective. Therefore, a relative low insulin level might have elicited the maximal positive achievable effect on glycogen resynthesis during recovery from intense exercise. Third, although not directly linked to insulin levels, is the much lower muscle glycogen content immediately after exercise in our study, with an average of 79 vs. ~183 mmol/kg dry muscle (213 µmol/g protein), that could have had an effect on the time course of the fast and slow glycogen recovery phase. It is suggested that glycogen resynthesis rate is affected by initial concentration when muscle glycogen content is <120 mmol/kg dry muscle (14).

If we assume that 6 kg of muscle in the leg (of a total of ~8 kg) is depleted to the same extent as the vastus lateralis muscle during cycle exercise, the comparison between total leg glucose uptake and glucose needed for glycogen resynthesis can be made. In Fig 5 the total leg glucose uptake during the 0- to 1.5-h and the 1.5- to 4-h recovery period is shown together with the estimated amount of glucose needed for glycogen resynthesis. During the first 1.5 h of recovery, a nearly 100% match is found for the amount of glucose taken up by the leg and the amount of glucose needed for the observed increase in muscle glycogen content. Between 1.5 and 4 h of recovery, it appears that more glucose is taken up by the leg than incorporated into glycogen. These results indicate that during the initial phase of recovery, glucose is mainly used for glycogen resynthesis and potentially less for oxidation. Furthermore, during the early stages of recovery, glucose uptake might be the rate-limiting factor for glycogen resynthesis (see discussion above). On the other hand, between 1.5 and 4 h of recovery glucose uptake appears not to be rate limiting.
for glycogen resynthesis because more glucose is taken up than is stored in muscle glycogen. With water ingestion, glucose uptake is lower than the amount of glucose needed for glycogen resynthesis. This may have been due to the fact that the differences in both glucose arterial venous differences and muscle glycogen content are small and will increase variably. Another possibility is that <6 kg of muscle in the leg is depleted to the same extent as in the quadriceps and that we overestimate glucose needed for glycogen resynthesis.

In conclusion, coingestion of carbohydrate and protein compared with ingestion of carbohydrate alone did not increase leg glucose uptake or the glycogen resynthesis rate after intense cycle exercise. It seems that protein ingestion per se or via stimulation of insulin secretion cannot increase muscle glycogen resynthesis rate in humans when an optimal carbohydrate supplementation regime is followed during recovery from exercise.

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