Influence of differing macronutrient intakes on muscle glycogen resynthesis after resistance exercise

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Roy, B. D., and M. A. Tarnopolsky. Influence of differing macronutrient intakes on muscle glycogen resynthesis after resistance exercise. J. Appl. Physiol. 84(3): 890–896, 1998.—The provision of additional protein (Pro) to a carbohydrate (CHO) supplement resulted in an enhanced rate of muscle glycogen resynthesis after endurance exercise (Zawadzki et al., J. Appl. Physiol. 72: 1854–1859, 1992). A comparison of isoenergetic CHO and CHO/Pro formula drinks on muscle glycogen resynthesis has not been examined after either endurance or resistance exercise. We studied the effect of isoenergetic CHO (1 g/kg) and CHO/Profat (66% CHO, 23% Pro, 11% fat) defined formula drinks and placebo (Pl) given immediately (t = 0 h) and 1 h (t = +1 h) after resistance exercise in 10 healthy young men. They performed a whole body workout (9 exercises/3 sets at 80% 1 repetition maximum) with unilateral knee extension exercise [exercise (Ex) and control (Con) leg]. The CHO/Profat and CHO trials resulted in significantly greater (P < 0.05) plasma insulin and glucose concentration compared with Pl. Muscle glycogen was significantly lower (P < 0.05) for the Ex vs. Con leg immediately postexercise for all three conditions. The rate of glycogen resynthesis was significantly greater (P < 0.05) for both CHO/Profat and CHO (23.0 ± 4.5 and 19.3 ± 6.1 mmol·kg dry muscle·h⁻¹, respectively) vs. Pl (Ex = 2.8 ± 2.3 and Con = 1.4 ± 3.6 mmol·kg dry muscle·h⁻¹). These results demonstrated that a bout of resistance exercise resulted in a significant decrease in muscle glycogen and that consumption of an isoenergetic CHO or CHO/Profat formula drink resulted in similar rates of muscle glycogen resynthesis after resistance exercise. This suggests that total energy content and CHO content are important in the resynthesis of muscle glycogen.

NUTRITIONAL SUPPLEMENTATION after endurance exercise is known to increase the rate of muscle glycogen resynthesis (6, 7). However, there has been very little investigation into the role of nutritional supplementation after resistance exercise (14). Similar to endurance exercise, resistance exercise results in significantly decreased muscle glycogen (10, 16, 20). However, the magnitude of the decrease is not as great as that observed with endurance exercise (19). The influence of carbohydrate (CHO) supplementation after resistance training has been investigated (14). It was found that CHO supplementation resulted in glycogen resynthesis rates similar to those observed after endurance exercise (14). However, others have observed greater rates of muscle glycogen resynthesis and higher plasma insulin levels with the consumption of a CHO/protein (Pro) supplement after endurance exercise (21). Nonisoenergetic supplements made the interpretation of these latter findings difficult; however, they suggested that Pro and CHO supplements were more efficacious in promoting glycogen resynthesis than was CHO alone.

The greater rate of glycogen synthesis in the study by Zawadzki et al. (21) was attributed to the greater plasma insulin release that occurred with the consumption of the CHO/Pro supplement. Insulin release is stimulated primarily by CHO; however, Pro has also been demonstrated to stimulate its release (13). There is evidence to suggest that consumption of CHO and Pro in combination could result in a greater plasma insulin response (18, 21).

A more rapid rate of muscle glycogen recovery may be of benefit to an individual performing multiple workouts per day. Furthermore, an enhanced postexercise insulin response would be of benefit to an athlete performing resistance exercise, since it may attenuate muscle protein degradation (17) and/or increase muscle protein synthesis.

Therefore, the purpose of this investigation was to determine the effects of macronutrient intakes on the insulin response and rate of muscle glycogen resynthesis after a resistance training session while 24-h energy intake is controlled.

METHODS

Subjects

Ten young (19–21 yr) males were recruited and screened to ensure that they were healthy and had been participating in a resistance training program for at least 2 yr before the investigation (~4 times/wk; Table 1). Before inclusion in the investigation, subjects’ training logs were evaluated to ensure that no recent significant gains in strength or alterations in training had occurred. Subjects were also required not to alter their training for the duration of the study. The experimental procedures, risks, and benefits were explained to each subject before written consent was obtained after approval from the McMaster University Human Ethics Committee.

Design

The subjects participated in a placebo-controlled, double-blind trial. All subjects completed each of the following conditions: CHO/Profat (~66% CHO—23% Pro—11% fat; commercially available sports drink, Mead-Johnson, Canada; CHO source: 56% sucrose-44% glucose polymer from corn syrup solids), CHO only (identical CHO source as CHO/Profat), and placebo (Pl; Sucralose, sucrose derivative not recognized as CHO by body) trials. All trials were separated by a minimum of 2 wk. The trial sequence was chosen randomly, but, in error, the technician who blinded the study had all subjects participating in each condition in the same sequence. However, both the subjects and the other investigators were blinded to the supplement allocation (double blinded).
Before the experimental trials, the subjects' one repetition maximum was determined for eight different exercises (see below), and body density was determined by hydrostatic weighing. In addition, subjects collected 4-day diet records that were analyzed by use of a nutritional analysis software package (Nutritionist IV, First Data Bank, San Bruno, CA). From this, individual diets were designed for each subject for the 3 days before each trial (diet checklist) and for the trial day itself (prepackaged diet). The diets were isoenergetic, isonitrogenous, and flesh free. The prepackaged diet composition varied according to the postexercise drink that was administered (Table 2). The total daily energy intake remained constant for the three trials (Table 3). Postexercise energy intake for the CHO/Pro/fat and CHO was the same, and only the macronutrient composition varied (Table 2). The energy and macronutrients supplied by the predefined formula drinks replaced part of the habitual intake of the subjects. In addition, the amount of energy and macronutrients supplied from predefined formula drinks was the same for each trial day. This was achieved by the administration of a drink with breakfast (Table 2).

The subjects refrained from any resistance exercise for 3 days and all exercise for 2 days before each trial. They consumed meals at days and all exercise for 2 days before each trial. They were allowed to drink water ad libitum but were required to consume exactly the same quantity for the ensuing two trials. On completion of the exercise (t = 0 h), a blood sample was collected, a muscle biopsy was obtained from the vastus lateralis of the Ex leg using the suction-modified Bergström needle biopsy technique, and a drink was given [CHO/Pro/fat (trial 1) isoenergetic to CHO (1 g/kg; trial 2) and placebo (trial 3; t = 0 h)]. Blood samples were then collected every 20 min for the next 2 h and 40 min. Additional blood samples were collected at 4, 4.25, and 4.5 h postexercise. A second drink was given at the 1-h postexercise point (identical to that at t = 0 h), and a second needle muscle biopsy was collected at 4-h postexercise time point.

All blood samples were collected into prechilled, heparinized tubes and centrifuged immediately. The plasma was stored at −50°C for subsequent analysis of lactate, glucose, and insulin (see below). All biopsy samples were dissected free of visible connective tissue, quenched in liquid nitrogen, and stored at −50°C for subsequent analysis of muscle glycogen (see below).

Analyses

Plasma. All plasma samples were assayed for insulin by radioimmunoassay (Diagnostic Products, Los Angeles, CA). The intra-assay coefficient of variation (CV) was 3.2%. In addition, glucose concentration was also determined for every sample (Sigma Diagnostics Kit 135, St. Louis, MO), with an intra-assay CV of 3.5%.

Plasma lactate concentration was determined for the baseline sample and for the immediately postexercise sample. Plasma lactate was determined in duplicate using a YSI 23L lactate analyzer (Yellow Springs Instruments, Yellow Springs, OH).

Muscle glycogen. Before analysis, the muscle samples were lyophilized and powdered, and any visible remaining blood or connective tissue was removed before weighing. Glycogen concentration was determined by a method adapted from that described by Bergmeyer (2). Briefly, 160 µL of NaOH were added to 2.0–4.0 mg of dry muscle tissue and mixed thoroughly. After incubation at 80°C for 10 min, 640 µL of a combined acid-buffer solution (HCl-citrate) were added to neutralize the sample. Amyloglucosidase (40 µL; Sigma Chemical, St. Louis, MO) was then added, and the sample was mixed and allowed to sit for 80 min. Then, 80 µL of a reagent solution [375 mM triethanolamine, 150 mM KOH, 112.5 mM

### Table 1. Subjects' descriptive data and habitual diet characteristics

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<tr>
<th>Subject No.</th>
<th>Age, yr</th>
<th>Mass, kg</th>
<th>Ht, cm</th>
<th>Body Fat, %</th>
<th>Years Trn</th>
<th>Energy, kcal</th>
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<th>%Pro</th>
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<td>58</td>
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Mean ± SE 19.6 ± 0.22 86.8 ± 3.10 182.3 ± 2.48 12.3 ± 0.004 4.4 ± 0.43 3,030 ± 320.80 50 ± 2.31 18 ± 1.12 32 ± 2.14


### Table 2. Distribution of nutritional supplements for each trial

<table>
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<th>Breakfast (0800)</th>
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<th>Snack (1400)</th>
<th>Post-Ex</th>
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<td>S</td>
<td>PI</td>
</tr>
<tr>
<td>CHO/Profat</td>
<td>L</td>
<td>S</td>
<td>CHO (1 g/kg)</td>
</tr>
<tr>
<td>PI + B</td>
<td>L</td>
<td>S</td>
<td>CHO/Profat</td>
</tr>
</tbody>
</table>

Post-Ex, supplement given at t = 0 and t = +1 h postexercise, PI, placebo; B, breakfast; L, lunch; S, snack.
expressed as means ± SE. Values are translated from rate calculations.

Calculations

The mean rate of muscle glycogen resynthesis was calculated from rate = (Gpost – Gpre)/t, where Gpre is the muscle glycogen concentration immediately postexercise, Gpost is the muscle glycogen concentration at the interval t postexercise, and t is the time interval between the two biopsies.

Statistics

Muscle and blood data were analyzed using repeated-measures analysis of variance (ANOVA; time × treatment; Statistica, version 5.0, StatSoft, Tulsa, OK). When a significant interaction occurred, Tukey’s post hoc analysis was used to locate the pairwise differences. Integrated area under the curve calculations were done using a custom-made software package. Area under the curve data were analyzed using a one-way repeated-measures ANOVA, where P < 0.05 was considered to be indicative of statistical significance. Values are expressed as means ± SE.

RESULTS

The exercise stimulus resulted in similar increases in plasma lactate for all three conditions (Fig. 2).

Significantly higher baseline plasma glucose concentrations were observed for both the CHO/Pro/fat and CHO trials (P < 0.05). In all three conditions, exercise resulted in slightly (nonsignificant) higher plasma glucose (Fig. 3A). Consumption of CHO/Pro/fat resulted in significantly greater glucose at 20, 40, 60, 100, 120, 140, and 160 min postexercise compared with the Pl condition (P < 0.01; Fig. 3A). The CHO trial resulted in a similar glucose response to the CHO/Pro/fat trial, with significant increases at 20, 40, 60, 100, 120, and 140 min postexercise vs. the Pl condition (P < 0.01; Fig. 3A). The area under the glucose curve was not significantly different between the CHO/Pro/fat and CHO conditions (CHO/Pro/fat = 5.87 ± 0.27 mg/dl and CHO = 5.59 ± 0.35 mg/dl), but these were greater compared with Pl (P < 0.01; Fig. 4A).

There were no differences in plasma insulin concentrations among trials at the beginning of exercise (t = 0 h), the end of exercise (t = 6 h), and the time of the second muscle biopsy (t = 4 h). Plasma insulin concentrations were significantly higher for the CHO/Pro/fat vs. Pl condition at the +20-, +80-, +100-, and +120-min postexercise time points (Fig. 3B; P < 0.01). Similarly, the CHO condition significantly elevated plasma insulin concentrations at +20, +40, +60, +80, +100, +120, and +140 min compared with Pl (Fig. 3B; P < 0.01). The area under the insulin curve was approximately three times greater for both CHO/Pro/fat and CHO vs. Pl (P < 0.01; CHO/Pro/fat = 28.9 ± 2.7 µU·h⁻¹·l⁻¹, CHO = 33.6 ± 4.6 µU·h⁻¹·l⁻¹, Pl = 10.1 ± 1.2 µU·h⁻¹·l⁻¹; Fig. 4B).

Immediately postexercise, muscle glycogen concentrations in all three conditions were significantly lower (but not different between trials) than in the unexercised Con leg (P < 0.05; CHO/Pro/fat = 220.3 ± 26.5 mmol/kg dry muscle, CHO = 235.1 ± 27.7 mmol/kg dry muscle, Pl = 247.6 ± 22.9 mmol/kg dry muscle, Con = 352.3 ± 32.04 mmol/kg dry muscle; Fig. 5A). This amount represented an average decrease of ~36% in muscle glycogen in the vastus lateralis (comparing postexercise glycogen on all 3 conditions vs. postexercise glycogen).
gen in Pl-Con (nonexercised leg). For both the CHO/Pro/fat and CHO trials, the muscle glycogen concentration was significantly higher after 4 h compared with the immediate postexercise time point ($P < 0.05$; Fig. 5A; CHO/Pro/fat = 220.3 ± 26.5 to 312.2 ± 23.0 mmol/kg dry muscle, CHO = 235.1 ± 27.7 to 312.2 ± 38.8 mmol/kg dry muscle). The Pl treatment, however, did not result in any increases in muscle glycogen concentration after 4 h in either the Ex or Con leg (Fig. 5A; Pl: Ex = 247.6 ± 22.9 to 255.7 ± 27.5 mmol/kg dry muscle, Con = 352.3 ± 32.0 to 367.5 ± 43.0 mmol/kg dry muscle). Both CHO/Pro/fat and CHO trials led to significantly greater mean rate of muscle glycogen resynthesis compared with the Pl condition (Fig. 5B; CHO/Pro/fat = 23.0 ± 4.5 mmol·kg dry muscle$^{-1}·$h$^{-1}$, CHO = 19.3 ± 6.1 mmol·kg dry muscle$^{-1}·$h$^{-1}$; Pl: Ex = 2.0 ± 2.3 mmol·kg dry muscle$^{-1}·$h$^{-1}$, Con = 3.8 ± 4.7 mmol·kg dry muscle$^{-1}·$h$^{-1}$).

**DISCUSSION**

The purpose of this investigation was to determine the effects of energy intake of differing macronutrient composition on muscle glycogen resynthesis after a bout of whole body resistance exercise. This is the first report that has compared differing macronutrient intakes after exercise while also controlling for supplement energy intake and total daily energy intake in the placebo condition. As previously demonstrated in resistance exercise, CHO supplements (1 mg/kg, given at 0 and +1 h postexercise) resulted in significantly increased plasma glucose and insulin (17). Similarly, the CHO/Pro/fat condition in this study led to similar increases in both plasma glucose and insulin as with CHO alone. This suggests that CHO/Pro/fat was a similar stimulus for insulin release as with CHO alone. The increases in plasma insulin and glucose observed in the present study were similar to those observed by others after both endurance (8, 9) and resistance (17) exercise.

Both the CHO/Pro/fat and CHO conditions resulted in similar rates of muscle glycogen resynthesis. These were considerably higher than those observed for the Pl condition (both Ex + Con legs). The rates of resynthesis in the present study were similar to those observed after endurance exercise (21). In the study by Zawadzki et al. (21), it was found that a combined CHO/Pro supplement resulted in a rate of muscle glycogen resynthesis greater than for either CHO or Pro alone. However, this combined CHO/Pro supplement was the individual CHO supplement added to the Pro supplement, thus resulting in a supplement with a 42% greater energy content. The finding that the isoenergetic conditions in the present study resulted in similar rates of muscle glycogen resynthesis suggests that total energy consumed postexercise is also an important factor in the resynthesis of glycogen. The attenuated rates of muscle glycogen resynthesis observed with the Pl condition demonstrated the importance of energy and macronutrient intake after resistance exercise.

There may have been the possibility of an order effect in the present study because of an inadvertent error in supplement allocation order by the research assistant who blinded the study. However, there are a number of factors that indicate that this was not the case and did not influence the results. First, because subjects were all highly trained, it was unlikely that any significant gains in strength were made between each trial, and there were no reasons for systematic changes in training in either direction. Second, each subject performed the same amount of work for each condition. Third, a similar increase in plasma lactate values was observed from preexercise to postexercise for the three conditions (Fig. 2). Fourth, muscle glycogen concentration in the exercised leg immediately postexercise was the...
same for all three conditions (Fig. 5A). Also, most importantly, both the investigators and the subjects were unaware of supplement allocation (double blind). All of the above support the unlikeliness of an order effect.

One unexpected finding in the present study was the significantly different baseline plasma glucose concentrations (lower for PI condition). It is unlikely that this difference was indicative of differences in CHO consumption within the 3 h before each trial caused by the almost identical plasma insulin concentrations and dietary controls. We have observed a similar trend in a similar study with endurance exercise, which may relate to a relative "rebound" hypoglycemia for the PI condition (greater CHO consumption with breakfast) and/or an increase in gluconeogenic supply for the CHO/Profat and CHO conditions because of the lower total energy intake earlier in the day.

Rates of muscle glycogen resynthesis were greater in the present study for CHO/Profat and CHO conditions after resistance exercise than those observed by others for CHO alone (14) but are similar to those rates observed with the consumption of CHO after high-intensity exercise (11). These differences were probably because of the differences in subject training status. In the present study, highly trained resistance athletes were studied, whereas the subjects in the study by Pascoe et al. (14) were untrained. The difference between trained and untrained individuals is probably related to insulin sensitivity and GLUT-4 content within the muscle (15). Athletes have been demonstrated to have enhanced insulin sensitivity compared with sedentary controls (5). This increased sensitivity appears to be related to the GLUT-4 content of the skeletal muscle (5). Short-term aerobic training has been demonstrated to increase the GLUT-4 content of the muscle (15). It is probable that resistance training also increases the expression of GLUT-4, thus increasing the transport capacity of glucose into the cell and increasing the insulin sensitivity of the cell. Further support for this explanation is the positive correlation that has been observed between GLUT-4 content and the rate of

Fig. 4. A: area under curve for glucose; B: area under curve for insulin. *Significant (P < 0.05) difference between conditions.

Fig. 5. A: muscle glycogen concentration for dietary conditions (CHO/Profat, CHO, and PI for exercised leg) and for control (rested leg) on PI trial; B: mean rate of muscle glycogen resynthesis over first 4 h after exercise. *Significant (P < 0.05) difference.
muscle glycogen resynthesis after endurance exercise (12).

Plasma insulin concentrations were similar for both the CHO/Pro/fat and CHO trials in the present study. This is not consistent with previous work in this area (21). The lack of difference in insulin concentration between the CHO/Pro/fat and the CHO trials of the present study is probably related to the isoenergetic nature of the conditions. It therefore appears that insulin release is not potentiated by CHO/Pro compared with CHO only after resistance exercise. However, the Pro content may be important to a resistance athlete in that hyperinsulinemia appears to be most effective in promoting muscle protein synthesis when hyperaminoacidemia is present (1).

There is evidence demonstrating that the coinestion of both fat and Pro may influence the metabolic response to CHO (4, 21); however, the insulin response in the present study did not suggest such an interaction. It should be noted that the fat content in the CHO/Pro/fat condition was minimal (~11% of total energy consumed). For example, an 80-kg individual would have consumed <4 g of fat with each postexercise beverage. Such a small quantity would not be likely to have much influence on CHO metabolism. There have been reports in the literature of even greater amounts of total fat consumption (~31% of total energy intake) over a 24-h period having no effect on the total amount of muscle glycogen resynthesis after exercise compared with CHO alone (3). We therefore feel that the fat component of the CHO/Pro/fat condition had little influence on muscle glycogen storage and the response to CHO feedings.

Significant decreases in muscle glycogen were demonstrated in the present study after resistance exercise. The three sets of three different knee extensor exercises in the present protocol resulted in an ~36% decrease in muscle glycogen. Others (10, 14, 16, 20) have also observed significant decreases in muscle glycogen after resistance exercise. The decrease in the present study was similar to that seen by others when both intensity and volume of the exercise are considered. At the 4-h time period after completion of the exercise, there was very little resynthesis of muscle glycogen in the Pl condition. This indicates that nutritional intake is important in the resynthesis of muscle glycogen after resistance exercise. It also demonstrates that over this time there was only a very small contribution to glycogen resynthesis from gluconeogenic precursor delivery to the liver and subsequent hepatic glucose release in the Pl condition. Hepatic gluconeogenesis may have contributed to glycogen resynthesis indirectly, via the glucose-alanine cycle for the CHO/Pro/fat trial. It is possible that both the protein and fat component contributed gluconeogenic precursors into the circulation, thereby increasing the available substrates for gluconeogenesis in the liver. We can only speculate as to this possibility, since no measurements of plasma amino acids or glycerol were made. In addition, the relative contribution from gluconeogenesis would probably have been minimized in the CHO/Pro/fat and CHO trials caused by the elevation in plasma insulin and an expected decrease in plasma glucagon.

The findings of the present study have direct importance for individuals or athletes who perform multiple bouts of exercise within a given day and/or individuals with very low energy intakes (i.e., body builders before competitions). For example, individuals who perform multiple bouts of exercise within a single day could ensure an adequate supply of muscle glycogen for subsequent bouts of exercise with the consumption of a supplement postexercise. In addition, postexercise consumption of supplements may have a positive influence on protein metabolism (17), which requires further investigation.

In summary, our results indicated that the consumption of a 1 g/kg CHO or CHO/Pro/fat of equal energy content immediately and 1 h after completion of a resistance training bout significantly increased the rate of muscle glycogen resynthesis over the first 4 h after the completion of the exercise compared with a placebo. This suggests that total energy content and CHO content are important in the resynthesis of muscle glycogen.

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