Dietary carbohydrate and postexercise synthesis of proglycogen and macroglycogen in human skeletal muscle

K. B. ADAMO, M. A. TARNOPOLSKY, AND T. E. GRAHAM
Department of Human Biology and Nutritional Sciences, University of Guelph, Guelph, Ontario N1G 2W1; and Department of Medicine and Kinesiology, McMaster University, Hamilton, Ontario, Canada L8N 3Z5

Adamo, K. B., M. A. Tarnopolsky, and T. E. Graham. Dietary carbohydrate and postexercise synthesis of proglycogen and macroglycogen in human skeletal muscle. Am. J. Physiol. 275 (Endocrinol. Metab. 38): E229–E234, 1998.—This study examined the role of carbohydrate (CHO) ingestion on the resynthesis of two pools of glycogen, proglycogen (PG) and macroglycogen (MG), in human skeletal muscle. Nine males completed an exhaustive glycogen depletion exercise bout at 70% maximal O2 consumption on two occasions. Subsequent 48-h dietary interventions consisted of either high (HC, 75% of energy intake) or low (LC, 32% of energy intake) CHO diets. Muscle biopsies were taken at exhaustion (EXH) and 4, 24, and 48 h later. The total muscle glycogen (Gt) at EXH for the HC and LC conditions was not significantly different, and the MG represented ~12% of the Gt. From EXH to 4 h, there was an increase in the PG only for HC and no change in MG in either diet (P < 0.05). From 4 to 24 h, the concentration of PG increased in both conditions (P < 0.05). Between 24 and 48 h, in HC the majority of the increase in Gt was due to the MG pool (P < 0.05). The MG and PG concentrations for HC were significantly greater than for LC at 24 and 48 h (P < 0.05). At 48 h the MG represented 40% of the Gt for the HC diet and only 21% for the LC diet. There was no change in the net rates of synthesis of PG or MG over 48 h for LC (P < 0.05). The net rate of PG synthesis from 0 to 4 h for HC was 16 ± 1.68 mmol glucosyl units·kg dry wt·1·h1, which was threefold greater than for LC (P < 0.05). The net rate of PG synthesis decreased significantly from 4 to 24 h for HC, whereas the net rate of MG synthesis was not different over 48 h but was significantly greater than in LC (P < 0.05). The two pools are synthesized at very different rates; both are significantly different, and the MG represented ~12% of the Gt. From EXH to 4 h, there was an increase in the PG only for HC and no change in MG in either diet (P < 0.05). From 4 to 24 h, the concentration of PG increased in both conditions (P < 0.05). Between 24 and 48 h, in HC the majority of the increase in Gt was due to the MG pool (P < 0.05). The MG and PG concentrations for HC were significantly greater than for LC at 24 and 48 h (P < 0.05). At 48 h the MG represented 40% of the Gt for the HC diet and only 21% for the LC diet. There was no change in the net rates of synthesis of PG or MG over 48 h for LC (P < 0.05). The net rate of PG synthesis from 0 to 4 h for HC was 16 ± 1.68 mmol glucosyl units·kg dry wt·1·h1, which was threefold greater than for LC (P < 0.05). The net rate of PG synthesis decreased significantly from 4 to 24 h for HC, whereas the net rate of MG synthesis was not different over 48 h but was significantly greater than in LC (P < 0.05). The two pools are synthesized at very different rates; both are sensitive to CHO, and the supercompensation associated with HC is due to a greater synthesis in the MG pool.

IT HAS BEEN CLEARLY DEMONSTRATED that muscle glycogen stores can be increased to above normal concentration, i.e., supercompensation (5). This has been shown to be beneficial to athletes who are involved in multiple daily training bouts or compete in long-term intense exercise, such as marathon running (5). The most important prerequisites for achieving glycogen supercompensation by the classic method are having the subjects deplete glycogen in the involved muscle groups and then consume a high carbohydrate (CHO) diet (11, 26). The glycogen repletion process is biphasic, exhibiting a rapid early phase (<24 h) and a slow phase lasting for several days (11, 25). It has been shown under these conditions that it takes ~24 h for muscle glycogen to return to normal levels after exhaustive exercise and that, if a high CHO diet is continued, the supercompensated level can be achieved in the following days. It has been proposed that glycogen synthase (GS) activity plays a key role in determining the rates of glucose uptake and glycogen synthesis in muscle (3, 6, 7). However, the increased GS activity observed immediately after exercise has returned to normal before this supercompensation has occurred (6, 17, 28).

Recently it has been found that the primer for glycogen synthesis is an autoglucosylating protein referred to as glycogenin. This protein is able to catalyze the addition of glucosyl units to its Tyr-194 binding site, and this glucosylated protein is required as a prerequisite to glycogen synthesis (18–20). While identifying and studying rodent muscle glycogenin, Lomako and colleagues (18–20) discovered that there were two forms of glycogen. These glycogen pools were shown to differ in size and protein content, with macroglycogen (MG) being the larger at 107 Da and proglycogen (PG) the smaller at 400 kDa. Both molecular forms contain only a single glycogenin but different amounts of CHO; thus, they are separable via solubility in perchloric acid (PCA) because MG is soluble and PG precipitates.

We recently demonstrated that human muscle glycogen is composed of MG and PG (1). In muscle with a normal total glycogen concentration (300–350 mmol glucosyl units/kg dry wt), MG was ~20–25% of the total, and in samples having high concentrations of glycogen, the MG form represented a greater proportion compared with lower glycogen states. Similarly, Jansson (16) demonstrated that there was an increasing percentage of acid-soluble glycogen as the total concentration of muscle glycogen increased. She found that PCA-soluble glycogen constituted 25% of the total when glycogen concentrations were ~350 mmol/kg dry wt and that increases in total glycogen above that concentration were mainly due to the soluble form (MG).

On the basis of our recent findings (1) and the observations of Jansson (16), we proposed that supercompensation is due to increased synthesis of MG. We hypothesized that the smaller PG form will dominate the initial resynthesis phase and will be the predominant form until the concentration reaches the normal resting range of 300–350 mmol glucosyl units/kg dry wt. To address these hypotheses, we examined the resynthesis of MG and PG for 48 h after glycogen-
Table 1. Dietary summary

<table>
<thead>
<tr>
<th>Energy, kcal/day</th>
<th>CHO, g</th>
<th>Protein, g</th>
<th>Fat, g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Habitual</td>
<td>3,109.7 ± 183.0</td>
<td>404.5 ± 33.9</td>
<td>120.1 ± 15.1</td>
</tr>
<tr>
<td>Low CHO</td>
<td>3,211.8 ± 64.7</td>
<td>261.6 ± 8.3</td>
<td>155.9 ± 4.8</td>
</tr>
<tr>
<td>High CHO</td>
<td>3,263 ± 95.9</td>
<td>640.2 ± 18.5</td>
<td>77.6 ± 3.0</td>
</tr>
</tbody>
</table>

Values are means ± SE. CHO, carbohydrate.

depleting exercise and employed high and low CHO diets to manipulate the repletion rates.

METHODS

The experimental protocol was approved by the University of Guelph’s Human Subjects Committee, and nine recreationally active male subjects volunteered and gave their written consent to participate in this study. Subject characteristics [range and (mean ± SE)] were for age 19–28 yr (23.1 ± 1.2 yr), for height 170–191 cm (180.6 ± 1.9 cm), for weight 68–89 kg (77.6 ± 2.2 kg), and for maximum \( \dot{V}O_2 \) consumption (\( \dot{V}O_2_{\text{max}} \)) 53–68 ml·kg\(^{-1}\)·min\(^{-1}\) (58.7 ± 1.7 ml·kg\(^{-1}\)·min\(^{-1}\)).

Experimental Protocol

The entire experimental protocol is illustrated in Fig. 1. Subjects arrived in the morning at the testing center and completed an exhaustive glycogen depletion ride at 70% of \( \dot{V}O_2_{\text{max}} \). Exhaustion was determined as the point at which subjects could no longer maintain the pedaling frequency at a constant resistance.

At exhaustion, a muscle biopsy (EXH) was taken from the vastus lateralis muscle by use of the percutaneous needle biopsy technique. A catheter was placed percutaneously into a medial antecubital vein, from which a blood sample was taken and a saline (0.9%; 308 mosmol/l) drip was started. After the first sample, blood was drawn every 30 min for 4 h. During the 4-h period the subjects on the LC diet were allowed water only ad libitum; however, those who were on the HC diet were given 500 ml of a 70-g maltodextrin solution every hour for the first 3 h starting immediately after the EXH biopsy.

A second biopsy (4B) was taken 4 h postrecovery. The subjects returned the next day at about 24 h (24B) after the EXH biopsy for a third sample and again about 48 h after the EXH biopsy for a final biopsy sample (48B). The trial was repeated 2 wk later with the same subjects ingesting the opposite diets.

Analyses

Blood. Blood samples were immediately separated into two aliquots; 3 ml were transferred to a nonheparinized tube and allowed to clot, and serum was stored for subsequent insulin measurements. The other aliquot was transferred into a sodium heparinized tube, and a 200-µl sample was added to 1 ml of 0.6 M PCA and centrifuged with the supernatant collected for whole blood glucose analysis. All samples were stored at -80°C. The whole blood extracts were analyzed in duplicate (4) for glucose with a Perkin-Elmer LS-50 fluorometer. Serum insulin concentration was measured quantitatively using a 125I radioimmunoassay kit (Coat-A-Count; DPC, Los Angeles, CA).

Muscle. Muscle biopsies were stored at -80°C until they were freeze-dried and dissected free of visible blood, connective tissue, and other nonmuscle elements. A 1.5- to 3-mg portion of freeze-dried muscle was extracted following the MG and PG isolation method (1) followed by enzymatic measurement of glucosyl units (4), which was reported as millimole glucosyl units per kilogram dry weight.

Statistical Analysis

Total glycogen (\( G_t \)) was reported as the sum of MG + PG. The percentage of MG (and PG) was determined by dividing

after a prolonged exhaustive exercise (to be described below), they were provided with two boxes of prepackaged meals, formulated in the same manner as the control diet, for the ensuing 48 h. Each box contained a menu including three meals and snacks for that day, and the subjects were instructed to eat solely the food supplied. This was confirmed by direct interview. Each subject received an HC diet (75% of energy from CHO) in one trial and in the other trial an LC diet (32% of energy from CHO) (Table 1).
the concentration of each pool by the $G_t$ and multiplying by 100 (MG/$G_t \times 100$). The net synthesis rates were calculated by taking the difference between the concentrations at two time points and dividing by the time interval in hours [e.g., $(4B - EXH)/4h$. Two-way (time and treatment) ANOVA for repeated measures was used to compare the blood glucose, insulin, and MG, PG, and $G_t$ concentration data from the two trials. When these analyses revealed significant differences, a Tukey post hoc test was used to locate the pair-wise differences. Differences were considered to be significant if $P < 0.05$. All muscle glycogen results are presented as means ± SE for 9 HC and 8 LC subjects; no data were collected for one subject who did not perform this part of the study because of illness. Blood glucose concentrations are also reported as means ± SE. There is one less subject in these data than in the glycogen data because of the inability to attain blood samples from one of the subjects.

RESULTS

Muscle MG and PG Concentrations

The $G_t$ at EXH was not significant between trials and measured 79 ± 15.6 and 113 ± 20.1 mmol glucosyl units/kg dry wt for the HC and LC conditions, respectively (Fig. 2, A and B). At exhaustion, the MG concentration represented 13% of the $G_t$ in the HC condition and 11% in the LC condition.

During the first 4 h after exercise, there was a significant increase in PG of 68 mmol glucosyl units/kg dry wt for the HC trial, with no significant change in PG for the LC diet or in MG for either diet ($P < 0.05$). From 4 to 24 h, the concentration of PG increased by 152 and 65 mmol glucosyl units/kg dry wt for HC and LC, respectively, whereas the MG pool rose only for HC ($P < 0.05$).

Between 24 and 48 h, the significant elevation of $G_t$ in HC was due to the MG concentration, which increased by 79 mmol glucosyl units, whereas the PG concentration did not change significantly ($P < 0.05$; Fig. 2A). For the LC condition, there was no significant change in the PG or MG concentration from 24 to 48 h (Fig. 2B). Both the MG and PG concentrations were much greater for HC than for LC and at 24 and 48 h ($P < 0.05$). At 48 h the MG represented 40% of the $G_t$ for the HC diet but only 21% for the LC (Fig. 2, A and B). The four subjects with the highest $G_t$ concentrations had MG concentrations representing ~50% of the total.

Net Glycogen Synthesis Rates

PG synthesis. During the first 4 h after the exhaustive exercise, there was a threefold difference ($P < 0.05$) in the net rate of PG synthesis between the HC and LC conditions. The rates of PG synthesis were 15.9 ± 1.7 and 5.4 ± 1.8 mmol glucosyl units·kg dry wt $^{-1}$·h$^{-1}$ for HC and LC, respectively (Fig. 3A). There was a significant decrease in the net rate of PG synthesis from 4 to 24 h for HC to 6.3 ± 1.1 mmol glucosyl units·kg dry wt $^{-1}$·h$^{-1}$ ($P < 0.05$).

There was no net change in the net synthesis rate of PG over the 48-h LC trial period ($P < 0.05$). The average net PG synthesis rate over the course of the LC trial was 3.3 ± 1.1 mmol glucosyl units·kg dry wt $^{-1}$·h$^{-1}$ (Fig. 3A).

MG synthesis. The net rate of MG synthesis did not change significantly over 48 h within each trial; however, between trials, the net rate of MG synthesis was significantly greater during the HC trial. The average of the three values of net synthesis rate over the HC trial was $3.7 ± 0.4$ mmol glucosyl units·kg dry wt $^{-1}$·h$^{-1}$, whereas for the LC condition, the rate was only $0.7 ± 0.5$ mmol glucosyl units·kg dry wt $^{-1}$·h$^{-1}$ (Fig. 3B). During the HC 24- to 48-h period, the net MG synthesis rate of 3.5 ± 0.8 mmol glucosyl units·kg dry wt $^{-1}$·h$^{-1}$ was eightfold greater than that of PG, corresponding with the significantly greater synthesis of MG during this time.

Blood Glucose and Insulin

Blood glucose was not significantly different at EXH between the two trials. The blood glucose increased significantly within 30 min in the HC trial after ingestion of the CHO drink and remained elevated throughout the first 4-h measurement period (Table 2). Simi-
Traditionally, the resting concentration trained subjects. The subjects exercised strenuously, resting glycogen levels, as have been shown in well- did not expect any of them to have higher than normal we did not take resting biopsies. Our subjects were nutritional intake. To minimize the number of biopsies, in terms of magnitude, and both are sensitive to and PG are metabolized differently both in timing and different metabolic pools under physiological condi- tion remained unchanged in the LC trial (Table 2).

The average net PG resynthesis rate of 16 mmol glucosyl units·kg dry wt·1·h−1 for the first 4 h, when an HC diet is provided, is much higher than any other synthesis data in this study, but the net synthesis rate is probably even higher in the first 1–2 h after glycogen depletion. The net PG synthesis rate is much slower when CHO are restricted. It is also worth pointing out that, even when there was no dietary provision of nutri-

FIG. 3. Net rate of PG synthesis (A) and MG synthesis (B) for HC and LC over 48 h of recovery. Values with same letter are not different within a trial; *P < 0.05 vs. LC.

Discussion

The purpose of this study was to determine whether the two structural forms of muscle glycogen function as different metabolic pools under physiological conditions. The major findings of this study were that MG and PG are metabolized differently both in timing and in terms of magnitude, and both are sensitive to nutritional intake. To minimize the number of biopsies, we did not take resting biopsies. Our subjects were recreationally active males and not athletes; hence we did not expect any of them to have higher than normal resting glycogen levels, as have been shown in well-trained subjects. The subjects exercised strenuously, and then we controlled diet and exercise for the 2 days before the test. Traditionally, the resting concentration has been reported as 350–400 mmol glucosyl units/kg dry wt, and we have considered an increase beyond this level to represent supercompensation.

At exhaustion, MG represented only 11–13% of the Gt, and during the first 4 h of recovery, the synthesis was predominantly in the PG pool during the HC trial. As well, for the first 24 h of the HC treatment, the synthesis was predominantly PG, with a modest increase in MG. In contrast, during the second 24 h, the synthesis of MG was significantly greater than that of PG. The PG synthesis was the more dynamic, varying ~40-fold. Similar responses occurred with LC, but the changes were more modest, particularly in the synthesis of MG.

Our data show that the PG pool is especially sensitive to dietary CHO availability. It appears that the PG pool is the major contributor during the early recovery phase, with elevated blood glucose and insulin concentrations. The synthesis of MG is slower and more constant; MG contributes up to 25% of the Gt after 24 h when an HC diet is provided. The increase in Gt beyond this time point is predominantly in the MG pool, and it represents 40–50% of the Gt after 48 h.

The purpose of this study was to determine whether...
gents, as in the LC diet, the PG synthesis rate still tended to be higher in the first 4 h than later in recovery when CHO and other nutrients were ingested. In other words, the ingestion of moderate CHO after 4 h failed to generate a high PG synthesis rate. The net MG synthesis rates are much slower after exercise and stay relatively constant. This net synthesis rate also was affected by dietary CHO, because it was greater for the HC than for the LC condition. Over the first 4 h in the HC condition, the net PG synthesis rate was fivefold greater than that seen in the MG pool. Hence, glycogen resynthesis is first generated in PG, and the later increase and ultimately the supercompensation in Glc occur predominantly in MG (Fig. 2A).

At all times, any net synthesis of MG that occurs without a corresponding decrease in PG reflects a synthesis of PG as well. However, because our data were derived from changes in concentrations, we restricted ourselves to our net changes. This is a greater factor early in recovery. For example, in HC and LC from EXH to 4 h, the "corrected" PG rates would be 19.2 ± 3.7 and 6.5 ± 0.7 mmol glucosyl units·kg dry wt−1·h−1, respectively. Thus our reported differences are conservative. This "discrepancy" becomes much less in the 24- to 48-h period because the rates are much lower; however, it does suggest that there is some PG synthesis still occurring. The relationship between PG and MG is summarized in Fig. 4. PG appears to increase to 250–300 mmol glucosyl units/kg dry wt, and subsequently MG continues to increase while the PG concentration remains relatively constant. Because the MG is derived from the PG precursor, and the concentration of the PG is not significantly increasing, it seems that the PG is being synthesized at the same rate as the MG is being formed. This transition from PG to MG occurs at a Glc concentration of 300–350 mmol glucosyl units/kg dry wt. This agrees with the finding of Jansson (16), where glycogen synthesis above the 350 mmol concentration was due to the acid-soluble (MG) form. This apparent threshold of 350 mmol glucosyl units/kg dry wt corresponds to the normal resting concentration of muscle glycogen (12, 14). We had previously observed a similar pattern while developing the analytic method for separating the two glycogen pools (1). However, the data in the upper concentration range from that study were limited, and samples were obtained from various protocols. Hence further investigation was required to elucidate this relationship.

These human data correlate with work by Lomako et al. (20) and Huang et al. (13), who used labeled glucose to study glycogen synthesis in resting rodent muscle. These investigators demonstrated that the given amount of label first appeared in the PG pool and then later in the MG pool, with its coincidental disappearance from the PG pool, suggesting that PG is the precursor for MG.

The relationships illustrated in Fig. 3, A and B, are consistent with the proposal by Lomako et al. (20) that there are two forms of GS. They speculated that there is one form, proglycogen synthase (PGS), controlling the synthesis of PG and a second form, macroglycogen synthase (MGS), controlling the synthesis of MG. We are aware of no evidence that there are two isoforms in humans, but our data do demonstrate that, functionally, they could very well exist. Previously it has been shown that GS is highly active immediately after exercise, when inactive glucose 6-phosphate-dependent glycogen synthase (GSd) has been converted to active glucose 6-phosphate-independent GS (GSI) (6, 7, 10, 15, 28). GS activity has also been shown to be inversely correlated with glycogen levels. The postexercise increase in GSd is reversed after 24 h when a normal resting Glc has been reached, yet the slow rise in muscle glycogen (supercompensation) still occurs (8, 27). Our data demonstrate that the apparent GS activity associated with PGS has a huge range; early in recovery the synthase is five times more active than that associated with MGS. It is dramatically downregulated during the last 24 h, and its activity is only one-eighth the rate of MGS. Whether they are structurally different enzymes or not, the GS forms associated with PG and MG are obviously regulated very differently.

It appears that PG is synthesized rapidly, especially when glucose and insulin are high, and then declines over the next 48 h, whereas MG synthesis remains lower and steady. The synthesis rate of MG is apparently unaffected by the insulin concentration, because its net synthesis rate does not change over the 48 h of either trial. The concentration of MG continues to increase when PG synthesis has declined between 24 and 48 h (Fig. 4). Thus not only does the PGS appear to be insulin sensitive, but also, later in recovery, it declines to a very low rate, whereas MGS does not change. Whether these are truly two structurally different forms of synthase or the same form under different regulation is unknown.

It is believed that the glycogenin molecule represents the "saturable factor" or the limiting factor in this relationship. GS depends on the autocatalytic ability of glycogenin to self-glucosylate, providing the primer molecule and acting as the substrate for GS. It is imperative that each glycogen molecule, whether it is
PG or MG, contain one molecule of glycogenin; hence, the amount of glycogenin available could play a significant role in directing the repletion of the glycogen stores. It is possible to build only as many PG molecules as there are glycogenin proteins; hence, this could also limit the amount of MG to be made.

To our knowledge this is the only study of human muscle that identifies the relationship between MG and PG during recovery, and it is the first to use exercise and diet manipulation to alter the stores to and PG during recovery, and it is the first to use

We express appreciation to Mitch Kanter from the Gatorade Sports Science Institute for contributions to the experimental design and for supplying the Gatorade used as the carbohydrate supplement. Preminda Sathsasivam and Kim Robertson deserve special thanks for time and technical assistance during this study. The help of Cyndy McLean, Farah Thong, and Praseedha J anakiram during the exercise trials was also greatly appreciated.

This study was supported by the Natural Sciences and Engineering Research Council of Canada (NSERC). K. B. Adamo received an NSERC studentship in collaboration with the Gatorade Sports Science Institute.

Address for reprint requests: T. Graham, Human Biology and Nutritional Sciences, Univ of Guelph, Guelph, ON, Canada N1G 2W1.

Received 27 J January 1998; accepted in final form 21 April 1998.

REFERENCES


