Muscle glycogen accumulation after a marathon: roles of fiber type and pro- and macroglycogen

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1Copenhagen Muscle Research Centre, August Krogh Institute, University of Copenhagen, DK-2100 Copenhagen, Denmark; and 2Human Biology and Nutritional Sciences, University of Guelph, Guelph, Ontario, Canada N1G 2W1

Asp, Sven, Jens R. Daugaard, Thomas Rohde, Kristi Adamo, and Terry Graham. Muscle glycogen accumulation after a marathon: roles of fiber type and pro- and macroglycogen. J. Appl. Physiol. 86(2): 474–478, 1999.—Muscle glycogen remains subnormal several days after muscle damaging exercise. The aims of this study were to investigate how muscle acid-soluble macroglycogen (MG) and acid-insoluble proglycogen (PG) pools are restored after a competitive marathon and also to determine whether glycogen accumulates differently in the various muscle fiber types. Six well-trained marathon runners participated in the study, and muscle biopsies were obtained from the vastus lateralis of the quadriceps muscle before, immediately after, and 1, 2, and 7 days (days 1, 2, and 7, respectively) after the marathon. During the race, 56 ± 3.8% of muscle glycogen was utilized, and a greater fraction of MG (72 ± 3.7%) was utilized compared with PG (34 ± 6.5%). On day 2, muscle glycogen and MG values remained lower than prerace values, despite a carbohydrate-rich diet, but they had both returned to prerace levels on day 7. The PG concentration was lower on day 1 compared with before the race, whereas there were no significant differences between the prerace PG concentration and the concentrations on days 2 and 7. On day 2 the glycogen concentration was particularly low in the type I fibers, indicating that local processes are important for the accumulation pattern. We conclude that a greater fraction of human muscle MG than of PG is utilized during a marathon and that accumulation of MG is particularly delayed after the pro and macroglycogen (PG) pools are restored after a competitive marathon. Furthermore, it has previously been reported that type I and IIA muscle fibers are more glycogen depleted than are type IIB muscle fibers immediately after a marathon (21), and, if a similar pattern is found in the days after a race, it might suggest that local changes are important for the glycogen accumulation pattern. To investigate this matter, we observed glycogen staining intensity in the various muscle fiber types before and 2 days after a race.

METHODS

Subjects. Six healthy, well-trained male runners participating in the Copenhagen Marathon 1996 (May 19, 1996), aged 28–35 yr, with no relevant medical records or history of cardiovascular disease, clotting disorders, diabetes, or other endocrine diseases, served as subjects. Subjects were recruited by advertisement in the local club for distance runners and were fully informed of any risks and discomfort associated with these experiments before giving their informed consent to participate. All were paid a small honorarium for the time and discomfort involved. Their mean weight and height were 73.0 kg (range 60.0–79.5 kg) and 182.4 cm (range 175.5–186.3 cm), respectively, and the average maximal O2 consumption, determined on a treadmill 2 wk before the marathon, was 58.2 ml·kg−1·min−1 (range 51.5–64.7 ml·kg−1·min−1). The mean running time for the included subjects was 3 h 7 min (range 2 h 54 min–3 h 23 min). Six more healthy well-trained male runners, aged 24–37 yr, participating in the Copenhagen Marathon 1997 (May 18, 1997), were included to determine the glycogen accumulation pattern in the various muscle fiber types. Their mean weight and height were 80.2 kg (range 75–91 kg) and 183 cm (range 175–190 cm), respectively, and the average maximal O2 consumption per kilogram body weight determined on a treadmill 2 wk before the marathon was 60.4 ml·kg−1·min−1 (range 54.9–64.5 ml·kg−1·min−1). The mean running time for these subjects was 3 h 27 min. (range 3 h 3 min–3 h 50 min). The study was approved by the Copenhagen Ethics Committee and conforms to the Code of Ethics of the World Medical Association.

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Running a marathon is one of the most strenuous physical events in sports and is followed by morphological signs of muscle damage (14, 23) and delayed muscle glycogen accumulation (21). Previous studies addressing the activities of glycogen-synthesizing enzymes (21) and the total content of the insulin-exercise-regulatable glucose transporters (GLUT-4) (5, 22) after a race revealed no obvious explanation for the impaired glycogen accumulation pattern. Glycogen exists in two forms in rodent and human muscle: (1, 15, 16): one form is acid soluble and termed macroglycogen (MG), and the other is acid insoluble and termed proglycogen (PG). In humans, the MG fraction increases when high glycogen concentrations are reached via diet manipulations (1). A recent human study (2) revealed that the two pools accumulate at very different rates after an exhaustive glycogen-depleting bicycle ride, that the PG pool is most sensitive to carbohydrate availability, and that glycogen supercompensation appears to be due to an increase in MG. Thus MG and PG concentrations are affected differently by various metabolic changes, and the purpose of this study was to investigate how the two glycogen fractions accumulate after prolonged, muscle-damaging exercise. For this purpose, muscle biopsies were obtained from the vastus lateralis of the quadriceps before, immediately after, and 1, 2, and 7 days after (days 1, 2, and 7, respectively) a competitive marathon. Furthermore, it has previously been reported that type I and IIA muscle fibers are more glycogen depleted than are type IIB muscle fibers immediately after a marathon (21), and, if a similar pattern is found in the days after a race, it might suggest that local changes are important for the glycogen accumulation pattern. To investigate this matter, we observed glycogen staining intensity in the various muscle fiber types before and 2 days after a race.
Medical Association (Declaration of Helsinki). Subjects were covered by state medical insurance and, in addition, by the same insurance as that of hospitalized patients in cases of complications.

Diet. Two days before the marathon, subjects commenced a standard weight-maintaining diet, containing at least 7 g carbohydrate/kg body wt, and kept a constant activity level for which light walking and bicycling were allowed, but the subjects abstained from any other forms of exercise. Subjects were instructed about the carbohydrate-rich diet, which was consumed daily for 7 days after the race. During this period, no alcohol, smoking, or drugs were allowed. During the race, the subjects were allowed to drink and eat ad libitum, but no foods or fluids were consumed during the last 5 km. Except for the postrace biopsy, muscle samples were taken at least 3 h after the subjects consumed a light breakfast similar to the meal on the marathon day.

Biopsies. Muscle biopsies were obtained from the vastus lateralis of the quadriceps muscle under local anesthesia from the nondominant and dominant leg. The ipsilateral muscle was chosen from the subject 15 min after the subjects crossed the finish line. The subjects' diet and activity routines were the same as for the day before the marathon, we avoided taking muscle biopsies just before the marathon, we took prerase muscle samples 9, 10, or 11 days earlier, when the subjects' activity routines were the same as for the 2 days before the race. The postrace biopsy was obtained within 15 min after the subjects crossed the finish line. The initial, prerase samples were obtained in a random manner from the nondonorant and dominant leg. The ipsilateral muscle was also sampled on days 1 and 2, whereas the contralateral muscle was used immediately after the race and on day 7. Ipsilateral biopsies were obtained at least 4 cm apart, to avoid the influence of muscle trauma associated with previous muscle biopsies (8). On days 3, 4, and 5 after the race, the subjects were encouraged to run 5–10 km slowly, whereas on the days before muscle samples were taken (days 1 and 6), the runners kept to a constant low-activity level, abstaining from exercise. A similar scheme was followed by the included subjects participating in the 1997 Copenhagen Marathon, from whom biopsy samples were taken for histochemical determination of fiber-type composition and glycogen content before the race and on day 2. These biopsy samples were embedded in TissueTek and frozen in isopentane cooled in liquid nitrogen and subsequently kept at −80°C.

Analytic procedures. Plasma creatine kinase (CK) was measured at 37°C by using a commercially available kit (Boehringer Mannheim). Muscle biopsies were freeze-dried and dissected free of blood and connective tissue before analysis. A 2- to 4-mg portion of freeze-dried muscle was extracted after the MG and PG isolation method (1), followed by enzymatic measurement of glucosyl units (17), reported in units of millimoles of glucosyl per kilogram dry weight (dw). Glycogen was measured on 2–4 mg of freeze-dried muscle by a hexokinase method after acid hydrolysis (17), by using an independent portion of the same biopsy sample.

For histochemical determination of glycogen and muscle fiber type, serial transverse sections were cut in a cryotome at −20°C from the embedded muscle sample. The sections (10 μm) were mounted on coverslips and stained for myofibrillar ATPase at pH 9.4 after both acid (pH 4.3 and 4.6) and alkaline (pH 10.3) preincubations (7) to identify type I, IIC, IIA, and IIB fibers. Because the transcript from the fastest human isoform (IIB) is homologous to rat IIX isoform (9) and thus may correspond to rat IIX fibers (10), IIX was chosen to represent the fastest human isoform in the present study. Sections (20 μm) were stained for glycogen by using the periodic acid-Schiff (PAS) reaction (18), and the relative glycogen contents in the individual muscle fibers were estimated from the PAS staining intensity (12), which was quantified by using a COMFAS image scanner (Sbsonys-COMFAS, Scan Beam, Hadsund, Denmark). The average number of fibers counted from each sample was 166 ± 15 fibers/sample before the race and 124 ± 13 fibers/sample on day 2.

Statistics and calculations. To compare mean glycogen (glycogen, MG and PG) values in muscle, a one-way analysis of variance for repeated measures was used. Student-Newman-Keuls test was used as the post hoc test. Linear regression was used to test correlations between glycogen and the sum of MG and PG and between glycogen and fractions of MG and PG. Student's paired t-test was used to compare the net PG synthesis (see RESULTS) and the MG synthesis from immediately after the race to day 1 and from day 1 to day 2, and to compare the glycogen accumulation in each fiber type before the race and on day 2, by using the Bonferroni correction if multiple comparisons were made. Because the CK data were not distributed normally, Wilcoxon's test was used for these data. The level of significance during all tests was set at P < 0.05. Figures 1–4 display means ± 2SE and n = 5–6 observations. All MG presumably comes from PG, and the net rate of PG is calculated as the sum of the change in the PG and MG concentrations.

RESULTS

The plasma CK concentration peaked 1 day (day 1) after the race at 2,655 U/l (range 1,568–4,420 U/l) and remained significantly (P < 0.05) elevated at 1,341 U/l (range 724–2,086 U/l) on day 2, whereas no difference was found between the prerace value at 241 U/l (range 91–458 U/l) and the level on day 7 at 236 U/l (range 125–453 U/l).

The muscle glycogen concentration before the race was 573 ± 38 mmol/kg dw, and the concentration had significantly decreased to 256 ± 33 mmol/kg dw immediately after the race (P < 0.05). Glycogen remained lower than the prerace value on days 1 and 2 at 335 ± 32 and 417 ± 22 mmol/kg dw, respectively (P < 0.05). On day 7, the concentration had returned to the prerace level (526 ± 30 mmol/kg dw).

The MG concentration before the race was 195 ± 35 mmol/kg dw, and the concentration had decreased to 56 ± 10 mmol/kg dw immediately after the race (P < 0.05; Fig. 1). MG remained lower than the prerace value on days 1 and 2 at 80 ± 12 and 119 ± 13 mmol/kg dw, respectively (P < 0.05). On day 7, the MG had returned to the prerace level (185 ± 15 mmol/kg dw). The muscle PG concentration before the race was 317 ± 23 mmol/kg dw, and it was reduced to 208 ± 23 mmol/kg dw immediately after the race (P < 0.05). PG remained lower than the prerace value at 249 ± 16 mmol/kg dw on day 1 (P < 0.05). On days 2 and 7, PG had returned to the prerace level (305 ± 15 and 350 ± 15 mmol/kg dw, respectively; Fig. 1). The net PG synthesis was higher than the MG synthesis from day 1 to day 2 (94 ± 23 vs. 39 ± 13 mmol/kg dw, P < 0.05) and was not significantly higher (0.05 < P < 0.1) from immediately after the race to day 1 (66 ± 20 vs. 25 ± 9.3 mmol/kg dw).

There was a highly significant correlation between muscle glycogen and the sum of PG and MG [sum = 41.4 + (0.895 x glycogen); R = 0.967, P < 0.05; Fig. 2]. The MG fraction correlated positively with the glycogen...
whereas the PG fraction correlated negatively with the glycogen content [%PG = 92.6 – (0.05 × glycogen); R = 0.894, P < 0.05; Fig. 3]. No difference in fiber-type composition was observed when the prerace and day 2 biopsies were compared, and, similarly, there were no differences in areas occupied by each fiber type on the two occasions (data not shown).

The glycogen accumulation patterns in the various muscle fiber types before the race and on day 2 are shown in Fig. 4. The PAS reaction showed that a significantly lower fraction (P < 0.05) of the type I (37 ± 12 vs. 77 ± 9%) and type IIA (70 ± 10 vs. 98 ± 1%) fibers was dark (glycogen filled) on day 2 compared with before the race. In contrast, a similar fraction of the...
type IIIX fibers stained dark on day 2 and before the race (88 ± 6 vs. 92 ± 5%). Before the race, the fractions of darkly staining fibers were similar in the various types. On day 2, significantly (P < 0.05) more type IIIX fibers (88 ± 6%) were dark compared with type IIA fibers (70 ± 10%) and significantly (P < 0.05) more type IIA fibers were dark compared with type I fibers (37 ± 12%). Because type IIC fibers were only present in one-half of the biopsies and the relative occurrence of this fiber type was low (0–5%), no statistics were applied to this group of fibers.

DISCUSSION

The principal findings in this study are that a greater fraction of muscle MG than of PG is utilized during a marathon and that changes in the MG content after the race can account for the muscle glycogen accumulation pattern, i.e., the accumulation of MG is particularly delayed. Also our data reveal that the glycogen accumulation is particularly delayed in the type I fibers, indicating that local changes are important for the pattern after a race.

Muscle glycogen restores within 2 days after glycogen-depleting concentric exercise, when an abundance of carbohydrates are consumed (2, 6, 19), whereas this accumulation process is delayed after different types of muscle-damaging exercise, of which a competitive marathon is an example (14, 21, 23). Previous studies addressing the activities of glycogen synthesizing enzymes (21) and the total content of the insulin/exercise regulatable glucose transporters (GLUT-4) (5) after a marathon revealed no changes that could account for the glycogen accumulation pattern, and underlying mechanisms remain obscure. In the present study, muscle glycogen concentrations remained 41 and 27% lower than prerace values 1 and 2 days, respectively, after the marathon, despite a carbohydrate-rich diet, and this accumulation pattern is in accordance with previous findings (5).

Muscle glycogen can be separated into two forms on the basis of differences in relative protein content and, hence, acid solubility (1, 15, 16). One form, termed MG, has low relative protein content and is acid soluble, whereas the other form, termed PG, has a higher relative protein content and is acid insoluble. Both forms contain a single molecule of glycogenin (37.5 kDa) but differ in the number of glycosyl units. MG has a molecular mass of 10^4 kDa, whereas PG has a molecular mass of 400 kDa, and the relative protein content is, therefore, much lower in MG than in PG. Glycogen begins with glycogenin functioning as both the protein “core” or “backbone” and also as the enzyme promoting the initial glucosylations (3). This leads to the formation of a PG molecule, and, as more glucosyl units are added, the PG expands to the MG form. It is not known whether the two forms are catabolized at different rates during exercise; however, in the present study, both forms had been catabolized, and ample amounts of both forms appeared to remain immediately after the race. The proportion of MG and PG varies widely in different tissues (3), and a human study clearly demonstrated that the proportion of muscle MG and PG can change via diet manipulations (2). It was found that, after an exhaustive glycogen-depleting bicycle ride, the two pools accumulate at very different rates, that both pools are sensitive to carbohydrate availability, and that supercompensation was due to an increase in MG. Thus it is evident that MG and PG concentrations are affected differently by diet manipulations and by nondamaging glycogen-depleting exercise. Adamo et al. (2) reported that the PG had been resynthesized to the normal resting levels 24 h after glycogen-depleting bicycle exercise, whereas, in the present study, the PG accumulation was delayed and restored only by day 2, even though the PG concentration postrace in the present study was much higher than that in the former study. MG accumulation was even more delayed and, on day 2, remained 39% below the prerace concentration. Thus MG accumulation was impaired even though the precursor, PG, was present in normal concentrations and the subjects were ingesting an abundance of carbohydrates. Concentrations of muscle metabolites, including MG and PG, are dependent on synthesis and degradation, and it is plausible that the nonuniform accumulation of MG and PG is caused by different enzymatic regulation. MG and glycogen were restored concomitantly, suggesting that delayed MG accumulation is the primary reason for the glycogen accumulation pattern. As mentioned above, the molecular mass of MG is ~10^4 kDa, and the molecular mass of PG is ~400 kDa; because they both contain one glycogenin molecule, it means that MG under normal circumstances contains 25 times more glycosyl units per glycogenin molecule than does PG. The rapid PG accumulation suggests that changes in the glycogenin protein are of minor importance for the muscle glycogen pattern.

The sum of PG and MG correlated highly with the glycogen concentration (R = 0.967, P < 0.05), revealing that both assay procedures extract the same number of glycosyl units and that PG and MG constitute additive fractions of the glycogen. There was a close linear correlation between the glycogen content and fractions of MG and PG (R = 0.894, P < 0.05), and the higher the muscle glycogen content the higher the fraction of MG and the lower the fraction of PG. These results agree with previous human studies (1, 2), in which it was found that the PG fraction was always in excess of MG and the MG fraction increased to 40% when the glycogen content was high, and with recent rat studies from our laboratory (W. Derave, personal communication). Our results are in accordance with the notion that glycosyl units are primarily directed toward MG, when glycogen is abundant, but, as mentioned above, the nonuniform time courses of the MG and PG storages might suggest different enzymatic regulation of the two glycogen fractions.

It has previously been reported that type I and IIA muscle fibers are more glycogen depleted after a marathon than are type IIB muscle fibers (21), and our histochemical glycogen staining revealed that a similar pattern is found 2 days after a race. The PAS staining
showed that the number of “filled” type I and II A fibers was low 2 days after the race compared with before the race, whereas no difference was observed on the two occasions in the type II X fibers (Fig. 4). The pronounced difference between the various fiber types, although these are anatomically located adjacent to each other, suggests that local factors are important for the glycogen accumulation pattern. It could be speculated that the primary reliance and metabolic demand on type I and II A fibers during the race results in Ca\(^{2+}\) accumulation within these muscle cells during and after the race. High-cyttoplasmic concentrations of Ca\(^{2+}\) are known to activate the less active “b” form of glycogen phosphorylase (13) and, hence, could increase the glycogen catabolism. Alternatively, local inflammatory responses (e.g., accumulating inflammatory cells; cytokine or prosta
glandin production) might be particularly pronounced in these fiber types, and the inflammatory cells might use a substantial fraction of the available glucose (11), or some factor might increase muscle glycogenolysis (20). It deserves to be mentioned that the muscle glycogen concentration in the type II muscle fibers is particularly affected after maximal eccentric contractions (4), and the difference in muscle-fiber selectivity after maximal eccentric contractions and a marathon might reflect differences in the way muscle damage is initiated.

We conclude that a greater fraction of MG is utilized during a marathon compared with PG. After the race, PG returns more rapidly back to the prerace concentration than does MG, and changes in the MG content appear to be the primary reason for the glycogen accumulation pattern. The fraction of MG increases with the glycogen concentrations. Finally, glycogen accumulation is particularly delayed in the type I fibers, indicating that local processes induced by the race are important for the accumulation pattern.

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