Impaired muscle glycogen resynthesis after a marathon is not caused by decreased muscle GLUT-4 content

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Asp, Sven, Thomas Rohde, and Erik A. Richter. Impaired muscle glycogen resynthesis after a marathon is not caused by decreased muscle GLUT-4 content. J. Appl. Physiol. 83(5): 1482-1485, 1997.—Our purpose was to investigate whether the slow rate of muscle glycogen resynthesis after a competitive marathon is associated with a decrease in the total muscle content of the muscle glucose transporter (GLUT-4). Seven well-trained marathon runners participated in the study, and muscle biopsies were obtained from the lateral head of the gastrocnemius muscle before, immediately after, and 1, 2, and 7 days after the marathon, as well as venous blood samples. Muscle GLUT-4 content was unaltered over the experimental period. Muscle glycogen concentration was 758 ± 53 mmol/kg dry weight before the marathon and decreased to 148 ± 39 mmol/kg dry weight immediately afterward. Despite a carbohydrate-rich diet (containing at least 7 g carbohydrate·kg body mass⁻¹·day⁻¹), the muscle glycogen concentration remained 30% lower than before-race values 2 days after the race, whereas it had returned to before-race levels 7 days after the race. We conclude that the total GLUT-4 protein content is unaltered in the lateral gastrocnemius after a competitive marathon and that the slow recovery of muscle glycogen after the race apparently involves factors other than changes in the total content of this protein.

A MARATHON is one of the most strenuous physical events in sports, and the muscles are under substantial strain during the race, leading to muscle damage (19, 30), systemic insulin resistance (29), and delayed muscle glycogen resynthesis (27). Unaccustomed eccentric exercise (involving forced lengthening of active muscle) induces changes in the postexercise period resembling the features after a marathon, including muscle damage (24), insulin resistance (4, 20), and sustained decreased muscle glycogen concentration (5, 6, 9, 12, 14, 25, 31). Furthermore, the muscle glucose transporter (GLUT-4) content is particularly affected by this type of unaccustomed muscle activity, and it has been suggested that decreased GLUT-4 content may be an important factor for the delayed glycogen resynthesis pattern after eccentric contractions (5, 6). To investigate whether a competitive marathon is associated with changes in the total muscle GLUT-4 content and to study whether such changes might coincide with delayed postrace muscle glycogen resynthesis, muscle biopsies were obtained from the lateral gastrocnemius muscle before, immediately after, and 1, 2, and 7 days after a race.

METHODS

Subjects. Seven healthy well-trained male runners participating in the Copenhagen Marathon 1996 (May 19, 1996), aged 28–37 yr, in good health, and especially with no 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 72.8 kg (range 60.0-79.5 kg) and 182.6 cm (range 175.5–186.3 cm), respectively, and the average maximal O₂ consumption determined on a treadmill 2 wk before the race was 58.5 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, 9 min (range 2 h, 39 min-3 h, 38 min). The study was approved by the Copenhagen Ethics Committee and conforms with the code of Ethics of the World Medical Association (Declaration of Helsinki). Subjects were covered by state medical insurance and, in addition, by the same insurance covering hospitalized patients in case of complications.

Diet. Two days before the marathon, subjects commenced a standard weight-maintenance diet, containing at least 7 g carbohydrate·kg body weight, and kept a constant activity level, in which slow walking and bicycling were allowed, but the subjects abstained from any other forms of exercise. The carbohydrate-rich diet was consumed daily for 7 days after the race, and the subjects were instructed to avoid alcohol, tobacco, and drugs during this period. 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.

Biopsies. Muscle biopsies were obtained from the lateral gastrocnemius under local anesthesia (Xylocaine: 20 mg/ml, Astra, Sweden), and the specimens were immediately frozen in liquid nitrogen and kept at -80°C. Muscle and blood samples were taken at least 3 h after subjects consumed an individual light breakfast similar to the meal on the marathon day. The Copenhagen Marathon is primarily a level running course, and the gastrocnemius was chosen rather than the quadriceps muscle because glycogen depletion after level endurance running is more pronounced in the former than the latter (11). To avoid the taking of muscle biopsies just before the marathon, before-race muscle samples were taken 9, 10, or 11 days earlier, preceded by a similar routine as that followed during the 2 days before the race. The postrace biopsy was obtained within 15 min after subjects crossed the finish line. Samples were obtained in a random manner from the nondominant and dominant leg, from the ipsilateral muscle before and on days 1 and 2 and from the contralateral muscle immediately after 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 (13). On days 3–5 after the race the subjects were encouraged to run 5–10 km slowly, whereas on the days before muscle and blood samples were taken (days 1 and 6) the runners kept the constant low activity level, abstaining from exercise.

Analytic procedures. 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. Glycogen was measured by a hexokinase method after acid hydrolysis (21). Glycogen synthase activity was measured with a modification of the filter-paper method of Thomas et al. (28). Assay conditions were 37°C, uridine diphosphate glucose at 1.5 mM (saturating), and glucose 6-phosphate (G-6-P) at 0.17 and 8.0 mM, the latter concentration saturating. Maximal activity was measured at saturating (8 mM) G-6-P concentration. The percentage of fractional velocity was calculated as activity at the submaximal G-6-P concentration (0.17 mM) in percent maximal activity. The GLUT-4 protein content in skeletal muscle was quantified in duplicate by Western blot by using a mouse monoclonal primary antibody directed against the COOH-terminal amino acids of GLUT-4 and a horseradish peroxidase-labeled goat secondary anti-mouse antibody as described previously (5).

Statistics. To compare mean values in muscle, a one-way analysis of variance for repeated measures was used. Student's paired t-test with Bonferroni correction was used as a post hoc test. Because the CK data were not distributed normally, a nonparametric test was used (Wilcoxon) for these data. The level of significance was set at P < 0.05 during all tests. The figure displays values as means ± SE (n = 5–7 observations).

RESULTS

The plasma CK concentration peaked 1 day after the race at 2,360 U/l (range 595–5,150 U/l) and remained elevated at 1,193 U/l (range 306–2,086 U/l) on day 2, whereas no difference was found between the before-race value at 225 U/l (range 91–458 U/l) and the level on day 7 [241 U/l (range 125–453 U/l)].

The muscle glycogen concentration before the race was 758 ± 53 mmol/kg dry weight, and it was reduced to 148 ± 39 mmol/kg dry weight immediately afterward. The concentration remained lower than the before-race value at 514 ± 35 and 531 ± 58 mmol/kg dry weight on days 1 and 2, respectively, whereas on day 7 the concentration of glycogen had returned to the before-race level (734 ± 33 mmol/kg dry wt) (Fig. 1).

Total GLUT-4 protein content was unaltered over the experimental period. The content was 89 ± 4, 89 ± 4, 89 ± 5, 88 ± 6, and 91 ± 5% of a rat heart standard before, after, and on days 1, 2, and 7 after the race, respectively (Fig. 1).

The maximal activity of glycogen synthase was unchanged over the experimental period at 20.1 ± 0.5, 18.3 ± 3.3, 22.2 ± 1.9, 20.4 ± 1.7, and 24.4 ± 3.4 nmol·min⁻¹·mg⁻¹ before, after, and on days 1, 2, and 7 after the race, respectively. The fractional velocity of glycogen synthase had increased dramatically from 24.3 ± 6.4 to 80.3 ± 1.8% immediately after the race, and it remained elevated at 52.1 ± 5.8% on day 1 after the race. The values on days 2 and 7 after the race at 29.6 ± 10.1 and 27.1 ± 6.2%, respectively, were not different from the before-race value.

DISCUSSION

The principle findings in this study are that the GLUT-4 content is unaltered in the lateral gastrocnemius after a competitive marathon and that the slow resynthesis of muscle glycogen after the race apparently involves factors other than changes in the total content of GLUT-4.

A competitive marathon is one of the most strenuous physical events in sports and is followed by morphological signs of muscle damage (19, 30), elevated muscle protein levels in blood (1), insulin resistance (29), and delayed glycogen resynthesis (27). The mechanism(s) behind the impaired postmarathon glycogen resynthesis remains obscure, but changes in the activities of glycogen-synthesizing enzymes cannot explain the phenomenon (27). Unaccustomed eccentric exercise (involving forced lengthening of active muscle) is another type of muscle activity that induces muscle damage and postexercise changes very similar to what are found after a marathon (4, 12, 14, 16, 20, 24, 31). Furthermore, studies have revealed that the content of the predominant glucose transporter in skeletal muscle fibers (GLUT-4) is decreased 1–2 days after unaccustomed eccentric exercise, and this decrease has been suggested to play a role in the delayed glycogen resynthesis (5, 6). By analogy, a decrease in the total content of muscle GLUT-4 protein after a marathon could be involved in the delayed postrace glycogen resynthesis. A recent study (29) detected no changes in protein or mRNA content of GLUT-4 in the vastus lateralis 16 h after a competitive marathon, but changes in GLUT-4 protein content might be delayed as seen after eccentric contractions (5, 6), and glycogen depletion during primarily level endurance running like the Copenhagen Marathon is more pronounced in the gastrocnemius than the quadriceps muscle (11). The latter finding suggests that the gastrocnemius muscle may be more

fig. 1. Muscle glycogen and muscle glucose transporter (GLUT-4) content after a marathon. Values are means ± SE; n = 5–7 observations. Glycogen concentrations (○) and GLUT-4 contents (●) were determined before and immediately after race and 1, 2, and 7 days (D) later. Before-race (B) muscle biopsy was obtained at least 9 days before race, preceded by 2 days without exercise and by ingestion of a carbohydrate-rich diet. Postrace (P) muscle biopsy was obtained within 15 min after subjects crossed finish line. GLUT-4 data are expressed as content per microgram protein relative to a rat heart standard (2 µg protein) run on same gel. *Significantly different from before-race value, P < 0.05.
involved than the thigh muscles during prolonged running on a relatively flat course (11).

It is well described that muscle glycogen restores within 2 days after glycogen-depleting concentric exercise of shorter duration when a large amount of carbohydrates (~8 g·kg⁻¹·24 h⁻¹) is consumed (8, 26), whereas this process is delayed after muscle-damaging exercise (5, 6, 9, 12, 14, 25, 27, 31). In accordance with an earlier study (27), the muscle glycogen concentration in the present study remained 30% lower than before-race values 1 and 2 days after the marathon despite a carbohydrate-rich diet (containing at least 7 g carbohydrate·kg body mass⁻¹·day⁻¹), and no net glycogen synthesis was observed from day 1 to day 2. The present study also reveals that the total GLUT-4 content in the lateral gastrocnemius is unchanged after a marathon. Taken together, these findings indicate that the delayed glycogen resynthesis pattern after the marathon must involve factors other than a decrease in the muscle glycogen content. It should be borne in mind, however, that the absence of changes in the total GLUT-4 content does not exclude that the translocation of GLUT-4 from the intracellular depot to the sarclemma was impaired in the recovery period after the marathon. Sherman et al. (27) found no changes in the activities of glycogen-synthesizing enzymes (hexokinase, glycogen synthase) from before-race values in the period after a marathon that could account for the delayed glycogen resynthesis, and the present unchanged maximal activity of glycogen synthase and increased fractional velocity of glycogen synthase immediately after and on day 1 after the race are in accordance with these findings. Our laboratory has previously found (4) that muscle lactate release is increased 2 days after a bout of unaccustomed eccentric exercise. Increased lactate release may reflect increased muscle glycolysis, possibly because of activation of glycogen phosphorylase caused by increased cytosolic Ca²⁺ concentration in the damaged muscle (7). By analogy, such a mechanism might also be operative after the marathon. However, despite many similar features in the period after a bout of unaccustomed eccentric exercise and a marathon, differences exist, and there might also be different mechanisms behind the sustained decreased muscle glycogen concentrations after the two muscle contraction patterns.

Two different mechanisms have been offered to explain the initiation of muscle damage after exercise (3, 15). One is based on a disturbance in metabolic function as the primary event, and the other addresses mechanical disruption of the cell as the initiator of muscle damage (15). Thus it has been hypothesized that long-term concentric exercise (the calf muscles performing mixed concentric/eccentric contractions during a marathon) induces injury mainly because of the metabolic stress imposed on the muscle, whereas high-force eccentric exercise initially may cause a disruption of fibers because of physical stress to the muscle. The present study included well-trained male runners accustomed to distance running (all ran at least 70 km a week close to the race), whereas our study of eccentric exercise investigated the effect of unaccustomed eccentric contractions in untrained male subjects (5). The frequent endurance running by the marathon runners probably induced a protective adaptation, diminishing the magnitude of muscular trauma during the race. In contrast, muscle damage can be obtained after eccentric exercise of much shorter duration, provided the subjects are unaccustomed to eccentric exercise, because at least some of the damaging effects are reduced or abolished by repeated bouts (18, 22). Another observation that might reflect the fundamentally different contraction pattern and the way muscle damage is initiated during the two contraction types is the shape of the plasma CK curve. It is well described that the plasma concentration of CK increases after different types of muscle-damaging exercise (10, 17, 23). In the present study the peak occurred 1 day after the race, which is in accordance with what has been described previously as occurring after a competitive race (2), whereas the peak after unaccustomed eccentric exercise seems more delayed (5).

We conclude that the total gastrocnemius GLUT-4 protein content is unaltered after a competitive marathon in well-trained runners and that the slow recovery of muscle glycogen stores after the race involves mechanisms other than changes in the total content of this protein in the muscles.

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