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Substrate availability and transcriptional regulation of metabolic genes in human skeletal muscle during recovery from exercise☆

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Abstract

In skeletal muscle of humans, transcription of several metabolic genes is transiently induced during recovery from exercise when no food is consumed. To determine the potential influence of substrate availability on the transcriptional regulation of metabolic genes during recovery from exercise, 9 male subjects (aged 22-27) completed 75 minutes of cycling exercise at 75% $\dot{V}O_2$max on 2 occasions, consuming either a high-carbohydrate (HC) or low-carbohydrate (LC) diet during the subsequent 24 hours of recovery. Nuclei were isolated and tissue frozen from vastus lateralis muscle biopsies obtained before exercise and 2, 5, 8, and 24 hours after exercise. Muscle glycogen was restored to near resting levels within 5 hours in the HC trial, but remained depressed through 24 hours in the LC trial. During the 2- to 8-hour recovery period, leg glucose uptake was 5- to 15-fold higher with HC ingestion, whereas arterial plasma free fatty acid levels were ~3- to 7-fold higher with LC ingestion. Exercise increased ($P < .05$) transcription and/or mRNA content of the pyruvate dehydrogenase kinase 4, uncoupling protein 3, lipoprotein lipase, carnitine palmitoyltransferase I, hexokinase II, peroxisome proliferator activated receptor γ coactivator-1α, and peroxisome proliferator activated receptor α. Providing HC during recovery reversed the activation of pyruvate dehydrogenase kinase 4, uncoupling protein 3, lipoprotein lipase, and carnitine palmitoyltransferase I within 5 to 8 hours after exercise, whereas providing LC during recovery elicited a sustained/enhanced increase in activation of these genes through 8 to 24 hours of recovery. These findings provide evidence that factors associated with substrate availability and/or cellular metabolic recovery (eg, muscle glycogen restoration) influence the transcriptional regulation of metabolic genes in skeletal muscle of humans during recovery from exercise.

1. Introduction

Exercise activates transcription and increases the mRNA content of several metabolic genes in human skeletal muscle [1-4]. An interesting feature of this response is that both transcription and mRNA content may remain elevated or continue to increase during the initial hours of recovery, depending in part on the intensity and duration of the exercise bout [5], but then return to near baseline levels within 24 hours after exercise. The magnitude and timing of the response also varies among genes [2,3,5], reflecting differences in the regulatory sensitivity of each gene to exercise. Although not as well studied, exercise-induced increases in mRNA are generally followed by acute increases in the corresponding protein [1,6-9]. Taken together, these findings suggest that the recovery period after exercise represents the time frame during which the molecular responses to endurance exercise training occur in skeletal muscle [10].

The transient nature of the molecular response during recovery from exercise is similar to the timing of a number

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of other metabolic adjustments in skeletal muscle. These include an elevation in resting oxygen consumption [11], an initial enhanced glucose uptake independent of insulin [12,13], a prolonged and marked increase in the sensitivity and responsiveness of glucose transport to insulin [12-14], and an increase in glycogen synthase activity [15,16]. The initial elevation in postexercise glucose uptake and glycogen synthase activation, both in the absence and presence of insulin, is inversely related to muscle glycogen content [17,18]. Whereas the noninsulin-dependent phase of glycogen resynthesis reverses within the first several hours after exercise [12,19], the enhanced sensitivity of muscle to insulin persists until muscle glycogen stores are replenished [19-21]. These findings suggest that muscle glycogen content may play a significant role in regulating the activity of several intracellular signaling pathways [14,17,22,23]. Muscle glycogen content also appears to influence the regulation of gene transcription, as we have previously found in humans that lowering muscle glycogen content before exercise enhances the exercise-induced transcriptional activation of exercise-responsive genes [24,25]. Alternatively, other factors associated with dietary manipulation, including substrate availability and/or insulin/counterregulatory hormone levels, may contribute to the regulation of metabolic genes in skeletal muscle.

In the present study, we sought to further examine the potential association between metabolic state and the regulation of metabolic gene expression in skeletal muscle by investigating the effect of dietary intake during recovery from exercise. Specifically, we tested in humans the hypothesis that limiting metabolic recovery by restricting carbohydrate intake during the initial 24-hour period after exercise enhances and/or prolongs the activation of exercise-responsive metabolic genes in skeletal muscle as compared with when a high-carbohydrate (HC) diet is ingested. The regulation of gene expression was assessed at the level of both transcription (direct index of gene activation) and mRNA concentration. Several genes previously shown to be acutely activated in skeletal muscle by exercise and/or other metabolic challenges were selected for transcription/mRNA analysis. Particular attention was given to the pyruvate dehydrogenase kinase (PDK4) gene, the product of which has been suggested to play an important role in minimizing the oxidation of glucose in skeletal muscle under conditions in which glucose is needed for muscle glycogen resynthesis [26]. Other exercise-responsive genes examined included uncoupling protein 3 (UCP3), 3 glucose metabolism genes (GLUT4, hexokinase II [HKII], glycogen synthase [GS]), 3 lipid metabolism genes (lipoprotein lipase [LPL], carnitine palmitoyltransferase I [CPT I], fatty acid translocase [CD36]), and 3 transcriptional regulatory factors (peroxisome proliferator activated receptor gamma coactivator 1α [PGC-1α], peroxisome proliferator activated receptor α [PPARα], and forkhead homolog in rhabdomyosarcoma [FOXO1]).

2. Materials and methods

2.1. Subjects

Nine healthy male subjects (age, 22-33 years; height, 178 ± 2 cm; weight, 73 ± 2 kg; $\dot{V}O_2$max, 4.2 ± 0.2 l/min [mean ± SE]) participated in the present study. The subjects were all physically active but had not participated in any regular physical training program during the 6 months before the study. The subjects were given both written and verbal information about the experimental protocol and procedures involved and informed about any discomfort that might be associated with the experiment before they gave their written consent. The study was performed according to the Declaration of Helsinki and approved by the Copenhagen and Frederiksberg Ethics Committee (Denmark) and the Human Investigations Committee, Yale University.

2.2. Experimental design

The study was conducted initially with 6 subjects with 3 subjects added during a second phase of experiments. Both phases of the study were conducted at the Copenhagen Muscle Research Center, Copenhagen, Denmark. All subjects completed 2 trials (separated by ~3 weeks), each consisting of 75 minutes of bicycling exercise at 75% $\dot{V}O_2$max followed by 24 hours of recovery during which either a low-carbohydrate (LC) (LC trial) or HC (HC trial) diet was consumed. To simulate a typical endurance training program, the subjects completed a daily exercise session (bicycling, 75% $\dot{V}O_2$max for 75 minutes) for the 4 days preceding each trial, the fifth day representing the experimental day. The subjects consumed the same food during the 4-day period before each trial and were provided with standard HC meals on the evening before each experimental day.

On the day of the experiment, the subjects arrived to the laboratory overnight fasted, and a resting muscle biopsy was obtained from the middle portion of the vastus lateralis muscle of one leg using the percutaneous needle biopsy technique with suction [27]. Immediately after the exercise bout (75 minutes, 75% $\dot{V}O_2$max), catheters were inserted under the inguinal ligament in one femoral artery and one femoral vein allowing simultaneous blood sampling over one leg. Blood samples were taken 40 minutes after the end of exercise (premeal) and every hour until 8 hours of recovery. Subjects remained supine during the 8-hour recovery period. Additional muscle biopsies were obtained at 2, 5, 8, and 24 hours of recovery. Biopsies were alternately obtained from each leg at sites at least 2 cm from any previous site to avoid the potential mitigating effects of local inflammatory cytokines. To minimize the number of biopsies, no muscle sample was obtained immediately after exercise (0 hour). Femoral arterial blood flow was measured before exercise and every hour throughout the first 8 hours of recovery by the ultrasound Doppler technique [28]. Immediately after the initial blood sample (40 minutes after the end of exercise) and