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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

after 2, 5, and 8 hours of recovery, meals, either low in carbohydrate (LC trial) or high in carbohydrate (HC trial), were consumed. The meals were pairwise isocaloric and designed to provide a minimum of carbohydrate ~0.5 g kg^{-1} body weight per 8 hours (LC trial) or adequate carbohydrate ~5 g kg^{-1} body weight per 8 hours (HC trial). After the 8 hours of recovery, subjects were prescribed an evening meal and an evening snack that had a similar macronutrient composition as the meals consumed during the 8 hours in the laboratory. The meals in the LC trial averaged a macronutrient composition (of total energy consumed) of 7% carbohydrate, 71% fat, and 22% protein across the day. The HC meals in the HC trial averaged a macronutrient composition of 80% carbohydrate, 8% fat, and 12% protein across the day.

2.3. Blood variables

Venous plasma concentrations of insulin were measured using a radioimmunoassay kit (Insulin RIA 100, Pharmacia, Sweden). Arterial and venous plasma glucose and arterial plasma free fatty acid (FFA) concentrations were measured using an automatic spectrophotometer (Cobas FARA 2, Roche Diagnostic, Switzerland), as previously described [29]. Glucose uptake was calculated using Fick's principle, and total glucose uptake was determined for specific time intervals during recovery (40 minutes to 2 hours, 2 to 5 hours, and 5 to 8 hours) as the area under the uptake vs time curves. Venous plasma FFA and FFA uptake across the leg proved unreliable, likely because of contribution of FFA to the venous sampling site from nonskeletal muscle tissues (skin and subcutaneous adipose tissue) as previously described [30].

2.4. Transcription, mRNA, and glycogen analysis

To determine the molecular responses to the exercise and diet regime at the level of both the gene (transcription) and mRNA (reflecting the balance between synthesis and degradation), both nuclei and total RNA were isolated from each muscle biopsy. Muscle samples were immediately placed on an ice-cold aluminum block, cleaned of connective tissue and blood, weighed, and separated for isolation of nuclei (~110-140 mg) or frozen in liquid N_2 for subsequent isolation of total RNA (15-25 mg) and analysis of glycogen content (5-10 mg) as previously described [3]. Relative transcriptional activity of selected genes was determined by a reverse transcriptase polymerase chain reaction–based nuclear run-on technique as previously described in detail [3,31]. Reverse transcription of both nascent RNA from the nuclear run-on reactions and of total RNA (from whole muscle) was performed using the Superscript II RNase H^- system (Invitrogen, Carlsbad, Calif) as previously described [3,31]. Quantification of nuclear run-on and mRNA cDNAs of target genes was performed either by conventional PCR (initial 6 subjects) as previously described [3] or by real-time PCR using a fluorogenic 5′ nuclease assay with TaqMan probes (ABI PRISM 7700 Sequence Detection System, Applied Biosystems, Foster City, Calif) (remaining 3 subjects) as previously described [2]. For conventional PCR analysis, prior testing was used to establish optimal annealing temperature, $MgCl_2$ concentration, and cycle number for each gene to ensure that analysis was performed in the linear range of PCR amplification. Polymerase chain reaction products were separated by gel (2.5% agarose) electrophoresis, stained with ethidium bromide, and visualized by ultraviolet exposure using a CCD integrating camera (Gel Do, Bio-Rad, Hercules, Calif) and quantified under nonsaturating conditions using an analysis software (Molecular Analyst, Bio-Rad). For quantification by real-time PCR, a standard curve was constructed for each target gene by performing real-time PCR on serial dilutions of a representative sample (same sample dilutions used to establish standard curves for all target genes) and plotting the critical threshold cycle number (C_t value) vs log dilution. The average C_t value of unknown samples was converted to relative expression data using the appropriate standard curve. For both conventional and real-time PCR analysis, all samples for each subject were analyzed simultaneously to establish expression data relative to preexercise control. The sequences used for amplifying the various target transcripts/mRNAs have been previously reported [2,32] with the exception of PDK1, PDK2, PDP1 (pyruvate dehydrogenase phosphatase 1), CD36 (fatty acid translocase), and FOXO1, which are provided in Table 1. Transcriptional activity and mRNA content for each sample were normalized to β-actin transcription and mRNA content, respectively. Total RNA/mg muscle weight and β-actin mRNA/μg RNA (2 μg RNA in each reverse transcription reaction) were unaffected by either the exercise or diet regime.

2.5. Statistics

The statistical analyses on transcriptional and mRNA data were conducted on logarithmic transformed ratios (target/endogenous control). Two-way analysis of variance

Table 1
New primer and probe sequences used for real-time PCR

Gene	Forward primer	Reverse primer	Probe
PDK1	TTCTACATGAGTCGCATTTCAATTAGA	TGTTTTCGATGAGATGGACTTCCT	TTGCCTTTTCCACCAAACAATAAAGAGTGCTGA
PDK2	ATCGCACCCTGAGCCAGTT	ATCGCCGTAGGTGTCCTTGTAC	CGCCCTGGTCACCATCCGGAA
PDP1	TGTCTAATGACCACAATGCTCAAA	CGACACTCTTGGCCTCACTCT	TGGATGTTCCAATTTCAGCCGTTCTAGTTCT
CD36	AGTCACTGCGACATGATTAATGGT	CTGCAATACCTGGCTTTTCTCA	ACAGATGCAGCCTCATTTCCACCTTTTG
FOXO1	ACCGAACAGGATGATCTTGGA	TTGCTTATCTCAGACAGACTGGGTAA	CCATCTGCCGCAAAGATGGCCTCTA

Table 2
Glucose uptake (μmol/min), arterial plasma FFA concentration (μmol/L), and venous insulin concentration (pmol/L)

	Time after exercise								
	40 min	1 h	2 h	3 h	4 h	5 h	6 h	7 h	8 h
Insulin									
HC trial	20 ± 4	137±83[a]	233 ± 47[a]	223 ± 66[a]	123 ± 23	77 ± 13	162 ± 27[a]	139 ± 50	48 ± 13
LC trial	23 ± 4	54 ± 17[b]	53 ± 16[b]	52 ± 13[b]	49 ± 14[b]	45 ± 13	72 ± 18	67 ± 17	67 ± 22
Arterial FFA									
HC trial	1089 ± 68	874 ± 68[a]	151 ± 19[a]	126 ± 18[a]	136 ± 21[a]	191 ± 37[a]	132 ± 17[a]	122 ± 11[a]	144 ± 9[a]
LC trial	1293 ± 101[b]	909 ± 76[a]	513 ± 75[a,b]	534 ± 62[a,b]	774 ± 107[a,b]	759 ± 42[a,b]	743 ± 35[a,b]	824 ± 62[a,b]	813 ± 55[a,b]
Glucose uptake									
HC trial	0.25 ± 0.03	0.44 ± 0.15	1.36 ± 0.10[a]	1.83 ± 0.21[a]	1.34 ± 0.12[a]	0.87 ± 0.20	1.32 ± 0.16[a]	1.50 ± 0.08[a]	0.69 ± 0.13
LC trial	0.30 ± 0.11	0.31 ± 0.08	0.27 ± 0.11[b]	0.15 ± 0.03[b]	0.10 ± 0.03[b]	0.12 ± 0.08[a,b]	0.15 ± 0.07[b]	0.07 ± 0.04[a,b]	0.05 ± 0.02[a]

Blood samples were obtained from an arterial and venous femoral catheter 40 minutes, 1, 2, 3, 4, 5, 6, 7, and 8 hours after the end of 75-minute bicycling at 75% $\dot{V}O_2$max. An HC (HC trial) or an LC (LC trial) diet was provided immediately after the 40-minute, 2-, and 5-hour samples. Values are mean ± SE.
[a] Significantly different from 40 minutes in the same trial.
[b] Significantly different from the HC trial at that time point.

for repeated measures was applied to evaluate the effect of time and diet on transcription, mRNA content, and blood parameters using Student-Newman-Keuls post hoc test to locate differences. A 1-way analysis of variance was also applied to evaluate the effect of recovery time separately for each trial. Differences were considered significant at $P < .05$. For presentation in figures, samples are expressed relative to the corresponding trial control sample (Pre), which is set to 1. The values presented are means ± SE.

3. Results

3.1. Blood variables

Providing carbohydrate-rich meals (HC trial) during recovery from exercise resulted in significantly ($P < .05$) higher plasma glucose and insulin concentrations as compared with when LC meals (LC trial) were provided, particularly in the first hour after each meal (Table 2). In the HC trial, glucose uptake across the leg increased ($P < .05$) from 2 to 7 hours of recovery relative to the initial postexercise blood sample (ie, 40 minutes after exercise), whereas glucose uptake decreased ($P < .05$) from 5 to 8 hours of recovery in the LC trial. Glucose uptake was markedly higher ($P < .05$) in HC than in LC during recovery with total glucose uptake being 65 ± 12 mmol (HC) and 18 ± 7 mmol (LC) from 40 minutes to 2 hours, 218 ± 27 mmol (HC) and 25 ± 5 mmol (LC) from 2 to 5 hours, and 167 ± 19 mmol (HC) and 10 ± 5 mmol (LC) from 5 to 8 hours of recovery. As expected, arterial plasma FFA concentrations were highest in the initial postexercise blood sample (due to lipolysis during exercise) and then decreased dramatically in both trials during recovery from exercise. However, arterial FFA levels were markedly higher (2.4- to 5.8-fold; $P < .05$) in the LC trial than in the HC trial from 2 through 8 hours of recovery.

3.2. Muscle glycogen

Two hours after exercise (first biopsy time point during exercise recovery) (Table 3), muscle glycogen concentration was similar in the 2 trials, averaging 222 mmol kg^{-1} dry weight (dw) (HC) and 204 mmol kg^{-1} dw (LC) below ($P < .05$) preexercise levels. In the HC trial, muscle glycogen concentration returned to near resting levels within 5 hours of recovery as glycogen increased by 103 mmol kg^{-1} dw from 2 to 5 hours, whereas no muscle glycogen resynthesis occurred for the entire 24-hour recovery period in the LC trial. As a result, muscle glycogen concentration in the LC trial was 37% lower ($P = .057$) after 8 hours and 40% lower ($P < .05$) after 24 hours of recovery than in the HC trial.

3.3. Transcription and mRNA

In both the HC and LC trial, PDK4 transcription and mRNA content were significantly ($P < .05$) elevated (5- to 15-fold) 2 hours after exercise (Fig. 1). The response pattern, however, was different (significant interaction) between the 2 trials during the ensuing 22 hours of recovery. In the HC trial, PDK4 transcription and mRNA content quickly reversed, returning to near resting levels within 5 hours after exercise and remaining at this level for the rest of the 24-hour recovery period. In the LC trial, PDK4 transcription and mRNA content followed a biphasic pattern, also returning to near resting levels within 5 hours after exercise, but then undergoing a second rise reaching ~6- to 8-fold at 8 hours of recovery and remaining elevated through 24 hours of recovery (~4- to 6-fold; transcription,

Table 3
Muscle glycogen concentration (mmol kg dw^{-1})

	Pre	Time after exercise			
		2 h	5 h	8 h	24 h
HC trial	560 ± 29	338 ± 24[a]	441 ± 42	531 ± 62	576 ± 52
LC trial	552 ± 44	348 ± 44[a]	320 ± 43[a]	336 ± 27[a]	348 ± 23[a,b]

Muscle glycogen concentration before and after 75 minutes of cycling at 75% $\dot{V}O_2$max with either HC (HC trial) or LC (LC trial) meals provided at 40 minutes, 2, 5, and 8 hours of recovery as well as dinner and evening snack. Muscle biopsies were obtained before exercise (Pre) and at 2, 5, 8, and 24 hours of recovery. Values are mean ± SE.
[a] Significantly different from Pre in the same trial ($P < .05$).
[b] Significantly different from HC trial ($P < .05$).

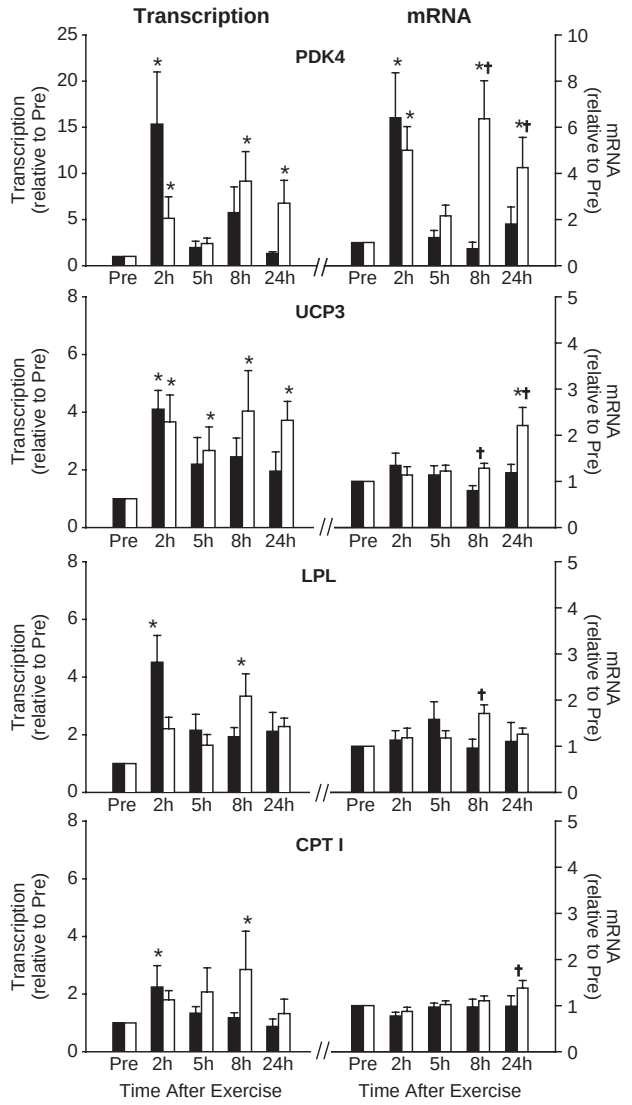

Fig. 1. Effect of HC (solid bars) vs LC intake (open bars) on transcriptional activity of select metabolic genes during recovery from exercise. Transcription (left portion) and mRNA content for the PDK4, UCP3, LPL, and CPT I genes were determined from muscle biopsies obtained from the vastus lateralis muscle before (Pre) exercise (75 minutes of bicycling at 75% $\dot{V}O_2$max) and at the indicated time points during recovery. High-carbohydrate (HC trial) and low carbohydrate (LC trial) meals were provided immediately after the 40-minute, 2-hour, and 5-hour samples. Data for each gene were normalized to β-actin and expressed as the mean ± SE relative to Pre (set to 1.0). *Significantly ($P < .05$) different from Pre value in the same trial. †Significantly ($P < .05$) different from the HC trial at that time point.

$P < .05$ for time; mRNA, $P < .05$ for both time and trial) (Fig. 1). PDK1 and PDK2 mRNA did not change in response to exercise in either trial (data not shown). Interestingly, PDP1 mRNA decreased ($P = .002$ for time) by ~30% to 70% in response to exercise in both trials, remaining depressed through the 24 hours of recovery in both trials (data not shown).

Similar to PDK4, UCP3 transcription was increased ($P < .05$) by ~3.5 to 4.0-fold at 2 hours of recovery in both trials. During the remainder of the 24-hour recovery period, UCP3 transcription was consistently elevated ($P < .05$ for time) over preexercise levels in the LC trial but returned to near resting levels in the HC trial. Lipoprotein lipase and CPT I transcription also increased ($P < .05$ for time) during recovery from exercise (Fig. 1), peaking in the HC trial at 2 hours of recovery (4.5- and 2.2-fold, respectively) and in the LC trial at 8 hours of recovery (3.3 and 2.9-fold, respectively). For UCP3 and the 3 lipid metabolism genes LPL, CPT I, and CD36, the LC trial also resulted in higher ($P < .05$ for time and/or trial) mRNA levels late in recovery (8 and/or 24 hours of recovery), ranging from 1.4- to 2.7-fold, than the HC trial (Figs. 1 and 2). Interestingly, the diet consumed during recovery did not affect the glucose metabolism gene HKII, as HKII mRNA increased ($P < .05$) by 3.8-fold in the HC trial and by 2.5-fold in the LC trial with no significant difference between trials (Fig. 2). GLUT4 and GS mRNA did not change during recovery from exercise in either trial (Fig. 2).

The effect of exercise and diet during recovery on the mRNA expression of 3 transcriptional regulatory factors was also investigated. Exercise induced an increase ($P < .05$) in PGC-1α mRNA at 5 hours after exercise in both trials (>5-fold, $P < .05$ for time), a response that was maintained through at least 8 hours of recovery in the LC trial but reversed in the HC trial (4.8-fold vs 1.6-fold at 8 hours, respectively) (Fig. 3). FOXO1 mRNA increased during recovery from exercise only in the LC trial ($P < .05$ for time), increasing 2.3-fold by 8 hours and remaining elevated (1.9-fold) through 24 hours. PPARα mRNA increased ($P < .05$ for time) by >2.5-fold at 8 hours,

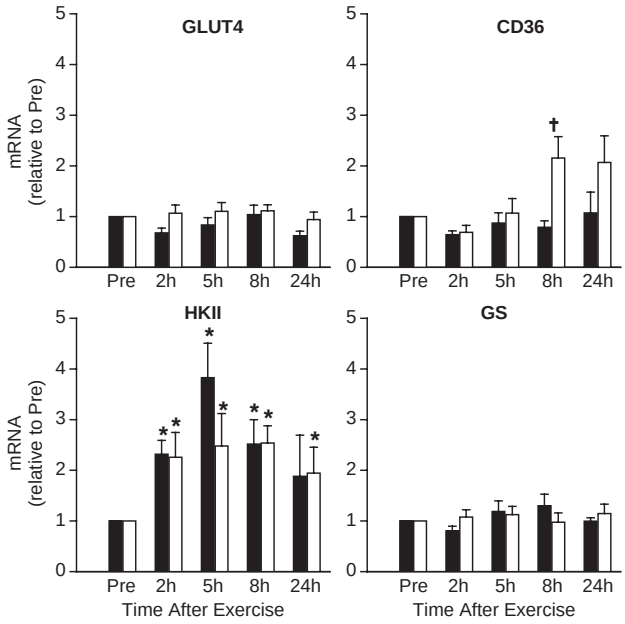

Fig. 2. Effect of HC (solid bars) vs LC intake (open bars) during recovery from exercise on the mRNA content of GLUT4, CD36, HKII, and GS from experiments as described in Fig. 1. *Significantly ($P < .05$) different from Pre value in the same trial. †Significantly ($P < .05$) different from the HC trial at that time point.

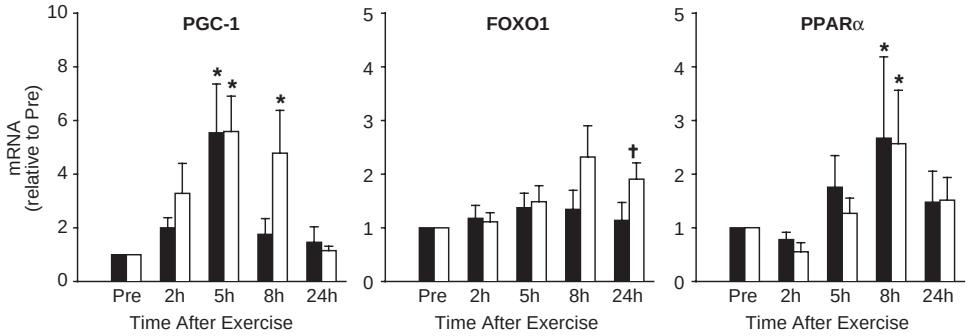

Fig. 3. Effect of HC (solid bars) vs LC intake (open bars) during recovery from exercise on the mRNA content of PGC-1α, FOXO1, and PPARα from experiments as described in Fig. 1. *Significantly ($P < .05$) different from Pre value in the same trial. †Significantly ($P < .05$) different from the HC trial at that time point.

returning to near resting levels 24 hours after exercise, irrespective of the diet consumed during recovery.

4. Discussion

The major finding of the present study is that the type of diet consumed during recovery from exercise influences the postexercise regulation of metabolic gene expression in skeletal muscle. Specifically, LC feeding during recovery from exercise elicits a prolonged and/or secondary activation of select exercise-responsive metabolic genes (eg, PDK4, UCP3, LPL, CPT I, CD36, and FOXO1), whereas HC feeding elicits a reversal of the exercise-induced activation of these genes. The difference in response between the 2 trials was particularly evident at the mRNA level, possibly reflecting combined regulation of both transcription and mRNA stability. As expected, restricting carbohydrate intake during exercise recovery prevented the resynthesis of muscle glycogen, raising the possibility that replenishment of muscle glycogen reserves may be linked to the regulation of metabolic gene expression during recovery from exercise. Alternatively, signaling mechanisms affected by differences in circulating substrate availability (ie, glucose and/or FFA, insulin, counterregulatory hormones) may be primary factors contributing to the transcriptional regulation of metabolic genes during recovery from exercise.

In humans, exercise activates transcription and increases the mRNA expression of several metabolic genes in skeletal muscle, a response that persists or even increases through the initial hours of recovery [1-3,25,33]. In each of these previous studies, food was not provided to the subjects after exercise. The present study was undertaken to determine whether the pattern and/or duration of the exercise-induced activation of metabolic genes during recovery from exercise is sensitive to differences in dietary carbohydrate intake. During recovery from exercise, skeletal muscle is characterized by a dramatic increase in insulin action and glycogen synthase activity, both of which promote the rapid resynthesis of muscle glycogen [12,13,15,16]. Reversal of this exercise-induced altered metabolic state in muscle generally occurs within several hours after exercise, coincident with the replenishment of muscle glycogen content [15]. Conversely, when muscle glycogen resynthesis is limited by restricting carbohydrate intake, muscle insulin sensitivity and glycogen synthase activity remain elevated for as long as 48 hours after exercise [13,20,21,34]. Several studies have provided evidence that muscle glycogen content directly affects the sensitivity of signaling pathways mediating control over both glucose transport and glycogen resynthesis [16,17]. Moreover, we have previously found that lowering muscle glycogen content before exercise accelerates and/or enhances the transcriptional activation of select genes in response to exercise. [24,25]. In the present study, the exercise-induced increase in transcription and mRNA for a number of genes remained elevated or continued to increase when muscle glycogen resynthesis was limited by LC intake. However, when muscle glycogen resynthesis was accelerated and glycogen content restored by HC intake, the exercise-induced activation of metabolic genes reversed and returned to near preexercise levels. These findings are therefore consistent with the idea that the activation and subsequent deactivation of metabolic genes in response to exercise may be regulated by signaling mechanisms sensitive to muscle glycogen content.

An alternative possibility is that the effects of the 2 different diets on gene regulation during recovery from exercise may have been related to differences in plasma FFA levels between the 2 trials. Plasma FFA levels were significantly higher during exercise recovery in the LC trial, raising the possibility that FFA-mediated signaling events, possibly via members of the lipid-activated PPAR family of transcription factors, may have contributed to the elevated transcription/mRNA content of lipid-related genes. The apparent biphasic induction pattern of PDK4 mRNA in the LC trial, together with the significantly higher levels of UCP3, LPL, and CD36 mRNA late in the LC trial (ie, 8 and/or 24 hours), all of which are known to be PPAR responsive target genes, lends credence to this possibility. Moreover, other experimental conditions that evoke increases in plasma FFA levels (fasting, high fat feeding, lipid infusion) have been found to increase the expression of most of these genes in skeletal muscle [32,35-37]. However, both the HC

and LC trials elicited a similar increase in PPARα mRNA somewhat late in recovery (8 hours), suggesting that an increase in PPARα expression was not required for the selective activation of UCP3, LPL, or CD36 in the LC trial. The extent to which existing levels of PPARα protein, other isoforms of PPAR, and/or other transcription factors participate in the regulation of metabolic genes during recovery from exercise will require further investigation. However, in this regard, forced expression of the PPAR δ-isoform in skeletal muscle has recently been shown in transgenic mice to induce a near doubling in muscle mitochondrial content and treadmill running capacity [38], lending support to the notion that PPAR transcription factors may contribute to the regulation of exercise-responsive metabolic genes.

Consistent with 2 previous studies in humans [2,33], recovery from exercise was associated with a marked increase in PGC-1α mRNA (Fig. 3). There is now considerable evidence suggesting that this nuclear coactivator, interacting with specific transcription factors, plays a role in coordinating the transcriptional activation of exercise-responsive genes. Interestingly, regulation of PGC-1α in response to exercise may be sensitive to the metabolic state of muscle, as evidenced by the prolonged elevation in PGC-1α mRNA in the LC trial of the present study and under conditions in which blood flow is restricted to the working muscle [33].

The rapid reversal in transcription and mRNA content of exercise-responsive genes that occurred during recovery from exercise when a HC diet was provided may also be related to insulin-mediated regulation. Plasma insulin levels were generally 2- to 4-fold higher when carbohydrate meals were provided during recovery from exercise. In liver, insulin signaling opposes the catabolic states induced by exercise, fasting, and diabetes, exerting a dominant suppressive effect over the transcriptional regulation of several gluconeogenic genes [39]. Similar regulation appears to be present in skeletal muscle as insulin has recently been shown to reverse the fasting-induced elevation in PDK4 mRNA and protein in muscle of rats [40].

The manner in which the various genes were regulated in the present study may provide some insight as to the function of each gene product. PDK4 catalyzes the phosphorylation and inactivation of PDH, thereby inhibiting the oxidation of glucose-derived fuel. The striking and persistent induction of PDK4 in the LC trial of the present study is in line with the idea that PDK4 induction may represent an important mechanism for inhibiting the terminal oxidation of glycolytic products in skeletal muscle when whole-body carbohydrate availability is low. Interestingly, exercise also caused a marked reduction in PDP1 mRNA, providing evidence that the phosphorylation state/activity of PDH in skeletal muscle is determined, at least in part, via coordinated regulation of PDK4 and PDP1 expression. Low carbohydrate availability during recovery from exercise also resulted in increased activation of the LPL, CD36, and CPT I genes, all 3 of which encode for proteins involved in the uptake and transport of fatty acids to the mitochondria. The increased expression of these genes therefore seems to be part of a mechanism leading to an enhanced capacity for fatty acid uptake and oxidation during conditions in which carbohydrate availability is limited. Indeed, triglyceride clearance after a high fat meal has been found to be significantly improved when exercise is performed ~12 to 24 hours before the meal [41,42]. Finally, HKII mRNA increased by ~3- to 4-fold irrespective of the diet consumed during recovery, suggesting that increasing the capacity to phosphorylate glucose after exercise also serves to facilitate the resynthesis of muscle glycogen independent of carbohydrate availability. Other genes, including both GLUT4 and GS, appear not to be induced to any significant extent under these conditions.

In conclusion, substrate availability during recovery from exercise influenced the expression pattern of several metabolic genes in human skeletal muscle. Restricting carbohydrate intake during the 24-hour period after exercise enhanced and/or prolonged the transcriptional activation/mRNA increase of the PDK4 and UCP3 genes as well as several genes involved in lipid metabolism. In contrast, ingestion of HC meals during recovery from exercise restricted and/or reversed the exercise-induced activation of these same metabolic genes. These data add to the findings from an earlier study [25] in which it was found that limiting substrate availability before exercise (ie, lowering muscle glycogen content) also enhanced the transcriptional activation/mRNA content during and/or after exercise of these same metabolic genes. Collectively, these studies provide evidence that the regulation of metabolic gene expression in response to exercise is particularly sensitive to substrate availability and, thus, provides the basis for more mechanistic studies to examine the role of muscle glycogen content, plasma FFA levels, and/or circulating insulin/counterregulatory hormones on the regulation of metabolic gene expression in skeletal muscle during and after exercise.

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