

Low Muscle Glycogen and Elevated Plasma Free Fatty Acid Modify but Do Not Prevent Exercise-Induced PDH Activation in Human Skeletal Muscle

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OBJECTIVE—To test the hypothesis that free fatty acid (FFA) and muscle glycogen modify exercise-induced regulation of PDH (pyruvate dehydrogenase) in human skeletal muscle through regulation of PDK4 expression.

RESEARCH DESIGN AND METHODS—On two occasions, healthy male subjects lowered (by exercise) muscle glycogen in one leg (LOW) relative to the contra-lateral leg (CON) the day before the experimental day. On the experimental days, plasma FFA was ensured normal or remained elevated by consuming breakfast rich (low FFA) or poor (high FFA) in carbohydrate, 2 h before performing 20 min of two-legged knee extensor exercise. Vastus lateralis biopsies were obtained before and after exercise.

RESULTS—PDK4 protein content was ~2.2- and ~1.5-fold higher in LOW than CON leg in high FFA and low FFA, respectively, and the PDK4 protein content in the CON leg was approximately twofold higher in high FFA than in low FFA. In all conditions, exercise increased PDHa (PDH in the active form) activity, resulting in similar levels in LOW leg in both trials and CON leg in high FFA, but higher level in CON leg in low FFA. PDHa activity was closely associated with the PDH-E1 α phosphorylation level.

CONCLUSIONS—Muscle glycogen and plasma FFA attenuate exercise-induced PDH regulation in human skeletal muscle in a nonadditive manner. This might be through regulation of PDK4 expression. The activation of PDH by exercise independent of changes in muscle glycogen or plasma FFA suggests that exercise overrides FFA-mediated inhibition of PDH (i.e., carbohydrate oxidation), and this may thus be one mechanism behind the health-promoting effects of exercise. *Diabetes* 59:26–32, 2010

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Insulin resistance has been suggested to be associated with dysregulation of the pyruvate dehydrogenase complex in skeletal muscle, but the underlying mechanism remains unclear (1–3). However, it has been suggested that elevated plasma free fatty acid (FFA) concentrations is the initial triggering event leading to downregulation of PDH (pyruvate dehydrogenase) activity and thus potentially contributing to insulin resistance (1).

The pyruvate dehydrogenase complex occupies a central role in carbohydrate metabolism, catalyzing the first irreversible step in mitochondrial glucose metabolism, and hence determines the fate of carbohydrates in skeletal muscle metabolism (4). Regulation of PDH activity in human skeletal muscle is believed mainly to be mediated through changes in the phosphorylation state of site one (Ser²⁹³) and two (Ser³⁰⁰) on the PDH-E1 α subunit, where dephosphorylation activates (5). The known regulatory kinases and phosphatases include four isoforms of PDH kinase (PDK1–4) and two PDH phosphatases (PDP1–2) (6–8). Of these, PDK2, PDK4, and PDP1 are thought to be the important isoforms in skeletal muscle (4,8,9).

Skeletal muscle PDH activity is affected by fasting, high-fat diet, and exercise (4,10–13). Factors responsible for this regulation have been suggested to include changes in plasma FFA concentration (1,12,14), muscle glycogen content (15–17), plasma insulin levels (14,18–20), and intracellular Ca²⁺ concentration (21,22). Thus, regulation of PDH seems to be under both local and systemic control (17). Insulin activates PDH at least in part through downregulation of PDK4 expression as shown at the protein level in rat skeletal muscle (14). Also based on findings in rat skeletal muscle, plasma FFA is also believed to reduce PDH activity through a peroxisome proliferator-activated receptor (PPAR)- α -mediated upregulation of PDK4 protein (14). Similarly, manipulation of the muscle glycogen content in humans has indicated that lowering of muscle glycogen upregulates PDK4 at the transcriptional and mRNA level (15,16). As previously suggested, such a glycogen-dependent regulation of gene expression may take place through glycogen regulatory enzymes such as protein phosphatase 1 (PP1) and glycogen synthase kinase 3 (GSK3), which are bound to the glycogen scaffold, but released when the glycogen content decreases (15). However, in these studies, plasma FFA and muscle glycogen were manipulated simultaneously, making it impossible to discriminate between the role of muscle glycogen and FFA.

Therefore, the aim of the present study was to test the

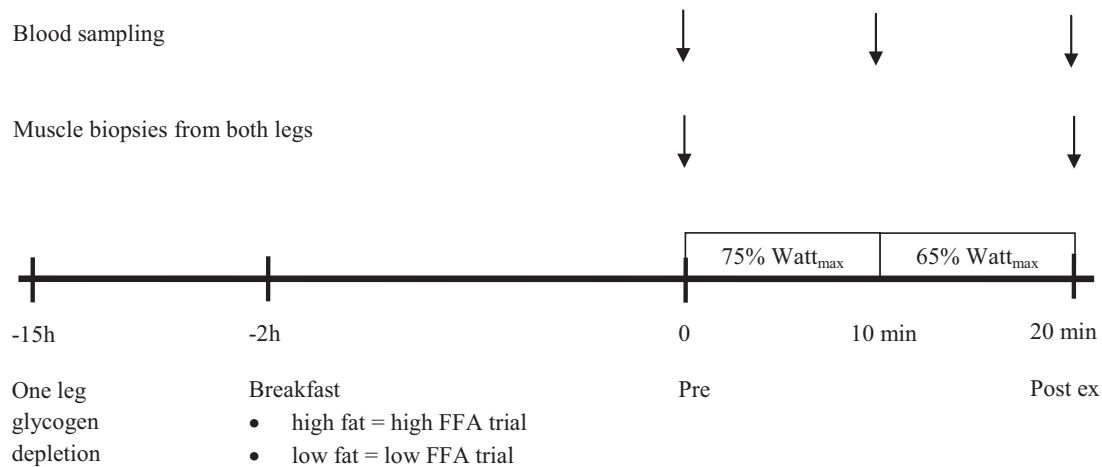


FIG. 1. A schematic overview of the experimental setup. Each subject completed the experiment on two separate days, with the only difference being the breakfast consumed. Therefore, each subject completed both the high FFA and low FFA trial. Exercise (ex) was performed as two-legged knee extensor exercise.

hypothesis that both low muscle glycogen and elevated FFA modify exercise-induced PDH regulation in human skeletal muscle independent of each other, potentially through regulation of PDK4 expression.

RESEARCH DESIGN AND METHODS

Eight healthy normally physical active male subjects with an average age of 26.5 years (range 22–31), weight 80.6 kg (60.4–99.8), and stature of 184.6 cm (175–193) participated in the study. The average peak oxygen uptake of the subjects was $51.8 \text{ ml O}_2 \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$ (47.9–55).

The subjects were given both written and oral information about the experimental protocol and procedures and were 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 was approved by the Copenhagen and Frederiksberg Ethics Committee, Denmark (H-C-2007-0085).

Experimental protocol. Approximately 2 weeks before the first trial, peak oxygen uptake and $Watt_{max}$ of the subjects were determined by an incremental bicycle test. Furthermore, $Watt_{max}$ during two-legged knee extensor exercise was determined by an incremental test, with a starting resistance of 48–60 W and increasing the load by 12 W every 2 min. The maximal resistance that could be sustained for 2 min was set as $Watt_{max}$.

Each subject completed two experimental trials, which consisted of identical exercise protocols, but differed in the dietary protocol.

The subjects were instructed to eat food rich in carbohydrates 5 days before a glycogen depletion protocol. Before reporting at the laboratory on the day of glycogen depletion, the subjects consumed a prepackaged standardized meal regulated to body weight and activity level (23), with 77% energy (%E) carbohydrate, 10%E protein, and 13%E fat.

The day before each experimental trial, the subjects arrived at the laboratory between 4:00 and 6:00 P.M. To reduce muscle glycogen in one leg, the subjects performed a one-legged cycling exercise protocol, consisting of 20 min continuous cycling (10 min 65% $Watt_{max}$ and 10 min 55% $Watt_{max}$) followed by intermittent one-legged cycling as previously described (15). The depletion leg was randomly selected. To lower glycogen stores in the liver, and thus to minimize glycogen resynthesis, the subjects furthermore performed 30 min of arm cycling. After the glycogen depletion, they were given a dinner low in carbohydrates (1%E carbohydrate, 26%E protein, and 73%E fat) to prevent muscle glycogen resynthesis.

On the experimental day (Fig. 1), the subjects arrived at the laboratory in the morning 2 h after intake of a prepackaged breakfast either high in fat (high FFA) (3%E carbohydrate, 18%E protein, and 79%E fat) or high in carbohydrate (low FFA) (74%E carbohydrate, 12%E protein, and 14%E fat). This breakfast was the only difference between the trials and aimed at obtaining similar insulin levels, whereas FFA levels were different in the two trials.

The two trials were separated by at least 10 days and were performed in random order. A venous catheter was inserted in either v. cephalica or v. mediana cubiti, and a resting blood sample was taken. Furthermore, two incisions were made in the middle part of vastus lateralis of each leg under local anesthesia (lidocaine), and a resting biopsy was obtained from the glycogen depleted leg (LOW) and the nonexercised leg (CON) using the

percutaneous needle biopsy technique (24), with suction. Thereafter, the subjects performed a two-legged knee extensor exercise bout at 75% $Watt_{max}$ for 10 min followed by 10 min at 65% $Watt_{max}$. Immediately at the end of the 20-min exercise period, a muscle biopsy was obtained simultaneously from each leg through the prior made new incisions. Additional blood samples were taken after 10 and 20 min of exercise. The work that each leg performed was evaluated using strain gauge. The LOW and CON leg did an equal amount of work in both trials.

Blood parameters. Plasma FFA was measured with a Wako FA kit (Wako Chemical, Neuss, Germany) and an automatic spectrophotometer (Cobas FARA 2; Roche Diagnostic, Basel, Switzerland). Plasma insulin was measured with an insulin enzyme-linked immunosorbent assay (ELISA) kit (DakoCytomation, Glostrup, Denmark).

Muscle glycogen. Muscle specimens were freeze-dried and dissected free of blood, fat, and connective tissue under the microscope, and muscle glycogen content was determined as glycosyl units after acid hydrolysis (25) using an automatic spectrophotometer (Cobas FARA 2, Roche Diagnostic, Switzerland).

Muscle lysate. Muscles pieces were homogenized in an ice-cold buffer (10% glycerol, 20 mmol/l Na-pyrophosphate, 150 mmol/l NaCl, 50 mmol/l HEPES, 1% NP-40, 20 mmol/l β -glycerophosphate, 10 mmol/l NaF, 1 mmol/l EDTA, 1 mmol/l EGTA, 2 mmol/l phenylmethylsulfonyl fluoride, 10 $\mu\text{g/ml}$ aprotinin, 10 $\mu\text{g/ml}$ leupeptin, 2 mmol/l Na_3VO_4 , 3 mmol/l benzamide, pH 7.5) for 20 s using a polytron (PT 1200; Kinematica AG, Switzerland). Homogenates were rotated end over end for 1 h at 4°C. Lysates were generated by centrifugation (17,500g) for 20 min at 4°C. Protein content in lysates was measured by the bicinchoninic acid method (Pierce, Rockford, IL).

SDS-PAGE and Western blotting. PDH-E1 α and PDK4 protein expression and phosphorylation of PDH-E1 α site 1 and 2 were measured in muscle samples by SDS-PAGE (Tris-HCl 10% gel, Bio-Rad, Denmark) and Western blotting using PVDF membrane and semi-dry transfer. After the transfer, the PVDF membrane was blocked overnight at 4°C (Tris-buffered saline with Tween [TBST] + 2% skim milk). The following day, the membrane was incubated with primary antibody (in TBST + 2% skim milk) for 2 h at room temperature and thereafter washed in TBST and incubated with horseradish peroxidase-conjugated secondary antibody (Dako, Denmark) for 1 h at room temperature (TBST + 2% skim milk). Immobilon Western (Millipore, Billerica, MA) was used as a detection system. Bands were visualized using an Eastman Kodak Image Station 2000MM. Bands were quantified using Kodak Molecular Imaging Software version 4.0.3, and protein content was expressed in units relative to control samples loaded on each gel.

Protein levels of the PDH-E1 α subunit and phosphorylation of site 1 and 2 of PDH-E1 α were determined using antibodies generated in sheep as previously described (12) and PDK4 protein by in-house-made antibodies generated in rabbit (26).

PDHa activity. The activity of PDHa (PDH in the active form) was determined as previously described (27–29) after homogenizing ~ 10 mg muscle tissue for 50 s in a glass homogenizer (Kontes) and quickly (10–15 s) freezing the samples in liquid nitrogen. The PDHa activity was adjusted to total creatine in each muscle sample.

Statistical analysis. Values presented are means \pm SE. Two-way ANOVA for repeated measures was applied to evaluate the effect of exercise and trial (low

TABLE 1

FFA and insulin before exercise, 10 min into exercise, and immediately after 20 min of two-legged knee extensor exercise

	High FFA	Low FFA
Plasma FFA ($\mu\text{mol/l}$)		
Pre-exercise	714 \pm 142*	193 \pm 30
10 min exercise	497 \pm 61*†	188 \pm 24
Post-exercise	564 \pm 70*	223 \pm 29
Plasma insulin (pmol/l)		
Pre-exercise	24 \pm 3	39 \pm 9
10 min exercise	13 \pm 1	19 \pm 3
Post-exercise	35 \pm 11	25 \pm 5

Data are means \pm SE. The experimental protocol was performed on two occasions with either fat-rich (high FFA) or carbohydrate-rich (low FFA) breakfast 2 h prior to the first blood samples. *Significant difference between the trials, $P \leq 0.05$. †Significantly different from pre-exercise, $P \leq 0.05$.

FFA vs. high FFA) as well as the effect of exercise and leg (CON versus LOW). The Student-Newman-Keuls post hoc test was used to locate differences. Differences were considered significant at $P \leq 0.05$. Statistical calculations were performed using SigmaStat Version 2.03.

RESULTS

Plasma FFA and insulin. The plasma FFA concentration was in the high FFA trial \sim 3.7-fold higher ($P \leq 0.05$) at rest and \sim 2.5-fold higher ($P \leq 0.05$) during exercise than in the low FFA trial. In the high FFA trial, the plasma FFA concentration was at 10 min of exercise reduced ($P \leq 0.05$) relative to pre-exercise. There was no difference in plasma insulin levels between the trials or over time (Table 1).

Muscle glycogen. Within each trial, muscle glycogen concentration was in the LOW leg \sim 46 and \sim 37% of the level in the CON leg before and after exercise, respectively ($P \leq 0.05$). Exercise lowered ($P \leq 0.05$) muscle glycogen in both legs in both trials (Fig. 2).

Muscle lactate. The muscle lactate concentration was similar in CON and LOW leg before exercise in both trials (Table 2). Muscle lactate concentration was 2.5-fold higher ($P \leq 0.05$) after exercise than before exercise in the low FFA trial. Muscle lactate concentration was after exercise in low FFA trial 2.4-fold higher ($P \leq 0.05$) in CON leg than in LOW leg. In addition the muscle lactate concentration after exercise in CON leg was 1.1 fold higher ($P \leq 0.05$) in high FFA trial than low FFA trial.

Muscle glucose-6-phosphate. The muscle glucose-6-phosphate concentration was similar in LOW and CON leg before exercise in both trials. No changes were observed over time in muscle glucose-6-phosphate concentration in the LOW leg in either trial, whereas the glucose-6-phosphate concentration in the CON leg was increased ($P \leq 0.05$) \sim 2.5-fold after exercise compared with before exercise in both trials. The concentration of muscle glucose-6-phosphate was \sim 2.6-fold higher ($P \leq 0.05$) in the CON leg than LOW leg after exercise in the low FFA trial (Table 2).

Muscle glucose. The muscle glucose concentration was similar in the LOW and CON leg before exercise in both trials. No changes were observed over time in muscle glucose concentration in the LOW leg in either trial, whereas an approximately threefold increase ($P \leq 0.05$) was observed after exercise in the CON leg relative to before exercise in both trials. The muscle glucose concentration was \sim 2.6-fold higher ($P \leq 0.05$) in the CON leg than in the LOW leg in both trials (Table 2).

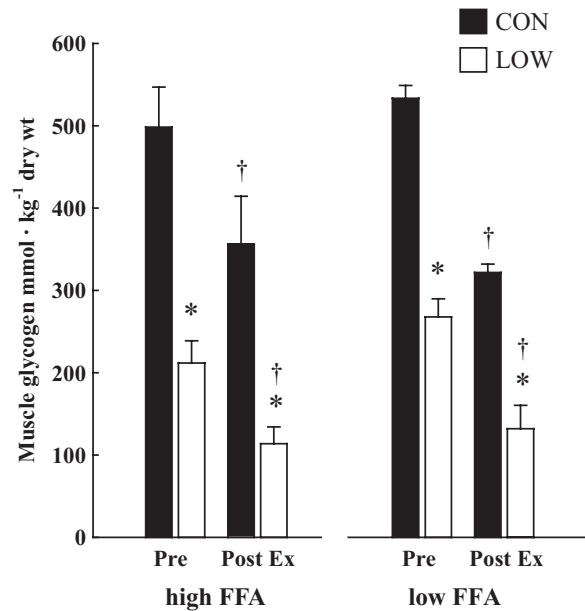


FIG. 2. Content of muscle glycogen in vastus lateralis before and after 20 min of two-legged knee extensor exercise (Ex). At the initiation of this exercise, muscle glycogen was reduced in one leg (LOW leg) (\square) by one-legged exercise the day before (14 h prior) and high-fat diet overnight, whereas the other leg (CON leg) (\blacksquare) had normal glycogen levels. The experimental protocol was performed on two occasions with either fat-rich (high FFA) or carbohydrate-rich (low FFA) breakfast 2 h before the first biopsies. Values are means \pm SE. *Significantly different from CON at given time point and within given trial, $P \leq 0.05$. †Significantly different from Pre within given leg and trial.

PDHa activity. Before exercise, PDHa activity was similar in the two legs in both trials. Exercise increased ($P \leq 0.05$) the PDHa activity in both trials and legs. In the high FFA trial, the increase in PDHa activity with exercise was similar in the CON leg (3.8-fold) and LOW leg (3.7-fold; Fig. 4A). In the low FFA trial, exercise increased ($P \leq 0.05$) PDHa activity 5.7-fold in the CON leg and 5.1-fold in the LOW leg, resulting in higher ($P \leq 0.05$) PDHa activity level in the CON than LOW leg after exercise.

TABLE 2

Muscle lactate, glucose-6-phosphate, and muscle glucose concentrations (in mmol/kg dry wt) in vastus lateralis muscle before and immediately after 20 min of two-legged knee extensor exercise

	High FFA		Low FFA	
	CON	LOW	CON	LOW
Muscle lactate				
Pre-exercise	15 \pm 2	13 \pm 2	16 \pm 2	13 \pm 2
Post-exercise	35 \pm 10‡	24 \pm 5	40 \pm 10†	17 \pm 2*
Glucose-6-phosphate				
Pre-exercise	1.0 \pm 0.2	0.8 \pm 0.2	0.8 \pm 0.1	0.7 \pm 0.2
Post-exercise	2.3 \pm 0.2†	1.1 \pm 0.2	2.3 \pm 0.5†	0.9 \pm 0.2*
Muscle glucose				
Pre-exercise	1.8 \pm 0.3	1.8 \pm 0.3	1.7 \pm 0.3	1.8 \pm 0.3
Post-exercise	5.3 \pm 1.2†	2.1 \pm 0.3*	5.1 \pm 1.0†	1.9 \pm 0.4*

Data are means \pm SE. The LOW leg had reduced muscle glycogen due to one-legged exercise the day before (14 h prior) and high-fat diet overnight, whereas the prior nonexercised CON leg had normal glycogen levels. The experimental protocol was performed on two occasions with either fat-rich (high FFA) or carbohydrate-rich (low FFA) breakfast 2 h before the first biopsies. *Significant difference between CON and LOW, $P \leq 0.05$. †Significantly different from pre-exercise, $P \leq 0.05$. ‡Significant difference between trials, $P \leq 0.05$.

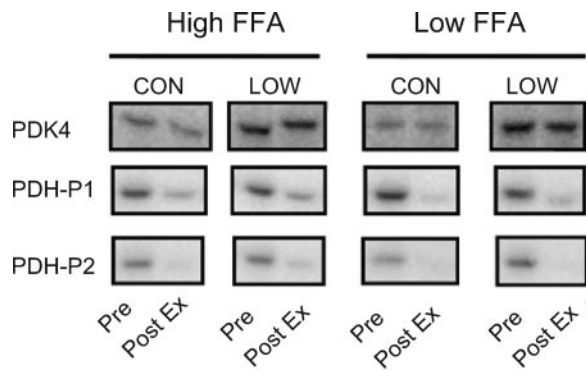


FIG. 3. Representative Western blots for PDK4 protein and for the phosphorylation of PDH-P1 and PDH-P2 shown for the samples of one subject. Ex, exercise.

Furthermore, the PDHa activity in the CON leg after exercise was 1.3-fold higher ($P \leq 0.05$) in the low FFA trial than in the high FFA trial.

PDK4 protein. The PDK4 protein content was higher ($P \leq 0.05$) in the LOW leg than in the CON leg before and after exercise in both trials (~ 1.5 -fold higher [$P \leq 0.05$] level in the high FFA trial and ~ 2.2 -fold higher [$P \leq 0.05$] level in the low FFA trial). PDK4 protein content in the CON leg was 19% lower ($P \leq 0.05$) after exercise than before in the high FFA trial, and PDK4 protein content in the LOW leg was 21% lower ($P \leq 0.05$) after exercise than before in the low FFA trial (Fig. 3 and Fig. 4B). Before exercise, PDK4 protein content in the CON leg was approximately twofold higher ($P \leq 0.05$) in the high FFA trial than in the low FFA trial.

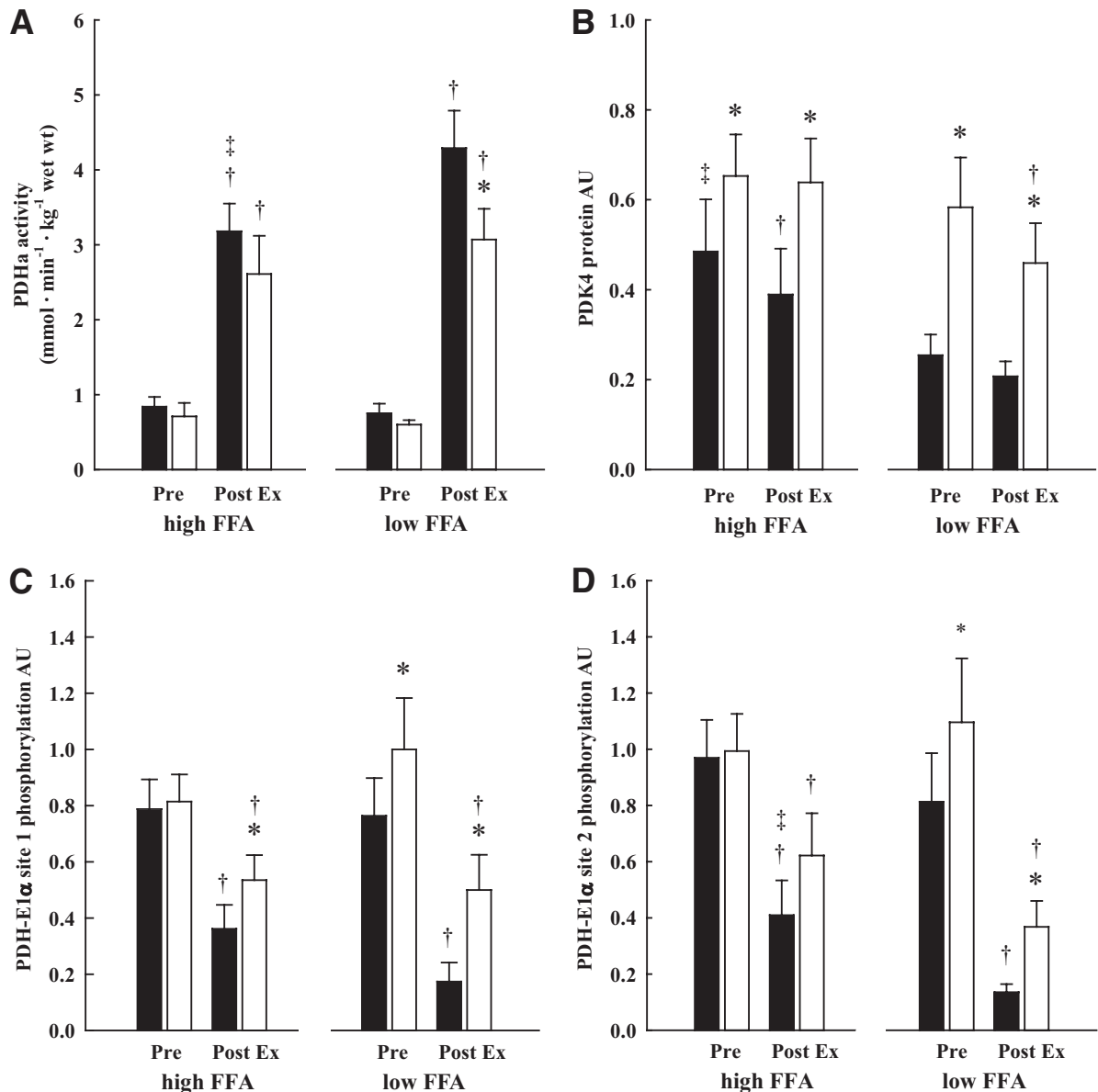


FIG. 4. **A:** Activity of PDH in the active form (PDHa activity). **B:** PDK4 protein expression. **C:** PDH-E1 α site 1 phosphorylation. **D:** PDH-E1 α site 2 phosphorylation in both vastus lateralis muscles before and immediately after 20 min of two-legged knee extensor exercise. At the initiation of this exercise, muscle glycogen was reduced in one leg (LOW leg) (□) by one-legged exercise the day before (14 h prior) and high-fat diet overnight, whereas prior non-exercised leg (CON leg) (■) had normal glycogen levels. The experimental protocol was performed on two occasions with either fat-rich (high FFA) or carbohydrate-rich (low FFA) breakfast 2 h before the first biopsies. Values are means \pm SE. *Significantly different from CON at given time point and within given trial, $P \leq 0.05$. †Significantly different from Pre within given leg and trial, $P \leq 0.05$. ‡Significantly different from low FFA trial, $P \leq 0.05$.

PDH-E1 α protein and phosphorylation. The PDH-E1 α protein content was the same in the two legs throughout each protocol. The phosphorylation data are presented as relative phosphorylation (normalized to the PDH-E1 α content).

Exercise induced ($P \leq 0.05$) a dephosphorylation of PDH-P1 (to 20–45% of prelevel) and PDH-P2 (34–65% of prelevel) in both trials and legs (Fig. 3 and Fig. 4C and D).

In the low FFA trial, phosphorylation on PDH-P1 and PDH-P2 was greater ($P \leq 0.05$) in the LOW leg than in the CON leg at all time points, with ~ 1.4 -fold before exercise and ~ 2.8 -fold after exercise, respectively.

No trial effect was apparent for PDH-P1 phosphorylation in the CON leg. After exercise, phosphorylation on PDH-P2 was in the CON leg ~ 2.9 -fold higher ($P \leq 0.05$) in the high FFA trial than in the low FFA trial.

DISCUSSION

The main findings of the present study are that exercise increases PDHa activity in human skeletal muscle despite enhanced plasma FFA levels, but both reduced muscle glycogen concentration and elevated plasma FFA levels are associated with reduced exercise-induced PDH activation. In addition, the results support that the observed relationship between these metabolic parameters and regulation of PDH may be mediated through effects on PDK4 expression before exercise.

The present finding that exercise increased the PDHa activity at least threefold independent of differences in muscle glycogen concentration, and despite enhanced plasma FFA levels, demonstrates that mechanisms other than muscle glycogen and plasma FFA dominate exercise-induced PDH regulation in human skeletal muscle. Increases in mitochondrial calcium levels are likely important, since mitochondrial calcium concentration increases during exercise (4,21) and calcium has been shown to activate PDP1 leading to dephosphorylation and activation of PDH (30). The impact of exercise on PDH regulation in skeletal muscle unrelated to the metabolic status of the cell and body may reflect that exercise can overcome potential inhibition of carbohydrate utilization present in resting skeletal muscle when circulating FFA levels are increased (2). Thus, although there was no effect of elevated FFA on PDHa activity at rest in the present study, maybe due to the overnight high-fat diet in both trials, elevated FFA levels have been shown to induce insulin resistance (31,32), and individuals with enhanced circulating FFA levels like type 2 diabetic subjects may experience FFA-mediated insulin resistance at rest (33), potentially in part because of FFA-induced downregulation of PDH (1). Therefore, the observed upregulation of PDHa activity by exercise despite elevated plasma FFA in the present study in accordance with previous findings (26,34,35) supports that a beneficial effect of physical activity may include that FFAs do not prevent exercise-induced PDH activation.

However, the observation that the highest exercise-induced PDHa activity and largest PDH dephosphorylation were present when the muscle glycogen level was only moderately reduced and when FFA remained close to baseline levels (193 $\mu\text{mol/l}$) indicates that metabolic factors do adjust the exercise-induced activation of PDH. In addition, the smaller increase in PDHa activity and the smaller decline in PDH dephosphorylation in response to exercise when muscle glycogen was reduced or plasma

FFA concentration was elevated initially suggest that each of these factors may modify exercise-induced PDH regulation. Such an effect of FFA is in accordance with previous studies, indicating that FFA downregulates PDHa activity or increases PDH phosphorylation in rat (1) and human (12) skeletal muscle at rest. A relation between muscle glycogen levels and PDHa activity and PDH phosphorylation is in line with our previous finding that further lowering of muscle glycogen during high-intensity exercise was associated with reduced PDHa activity (17). Of notice is also that the same degree of repression of PDHa activity and PDH dephosphorylation changes was evident when muscle glycogen was lowered to 268 mmol/kg dry wt or plasma FFA was elevated to 714 $\mu\text{mol/l}$, and when both these changes were present simultaneously, no further effect was found. Thus, the impact on PDH regulation associated with low muscle glycogen and elevated plasma FFA was not additive, which may suggest that a similar underlying mechanism could be involved in exerting this effect on PDHa activity.

The possibility that changes in PDK4 protein expression may have mediated the observed association between muscle glycogen and PDH regulation as well as between plasma FFA and PDH regulation is supported by the observation that the highest PDH activation and largest dephosphorylation occurred in the leg and trial with lowest PDK4 protein expression. The downregulation of PDK4 protein content when plasma FFA was reduced by a carbohydrate-rich breakfast in the present study supports previous studies showing that an elevated FFA level is associated with increased PDK4 expression (1,36). Although plasma insulin was at basal level and similar in the two trials when the pre-exercise biopsy was obtained, a transient increase in plasma insulin in response to the meal has without doubt occurred and cannot be excluded to have had a contributing influence on the observed PDK4 expression, as previous studies have demonstrated that insulin can regulate PDK4 expression (3,14). However, PDK4 mRNA downregulation in human skeletal muscle is typically not observed until after 3 h of insulin infusion (unpublished data, from our laboratory), and although posttranscriptional regulation cannot be ruled out, these findings do suggest that insulin changes elicited by the meal unlikely have been important in the quick reduction in PDK4 protein content observed in the present study.

The current finding that PDK4 protein content was higher in the muscle with low glycogen than the control muscle both before and after exercise and in both trials indicates that reduced muscle glycogen levels could be an initiating signal to increase PDK4 expression. These findings are in accordance with previous human studies showing a similar association between lowered muscle glycogen and regulation of PDK4 mRNA expression, but changes in muscle glycogen was in these previous studies accompanied by changes in plasma insulin and/or plasma FFA (15,16). In the present study, however, the carbohydrate-rich breakfast meal in one trial ensured lowered plasma FFA levels and normalized plasma insulin, leaving only muscle glycogen different. Of notice is that the low muscle glycogen leg had exercised intensively ~ 14 h before, and thus it cannot be ruled out that other exercise-associated signals have initiated the induction of PDK4 expression in this muscle. In addition, we have recently shown that PDK4 protein content is increased 6 h after a prolonged exercise session, and although this change was associated with reduced muscle glycogen, a decline in

muscle glycogen was not required to obtain an increase in PDK4 protein expression after prolonged exercise in that study (26). On the other hand, lowering of plasma FFA by the carbohydrate-rich breakfast in the present study was associated with a 50% reduction in PDK4 protein content in the control leg but not in the low glycogen leg, supporting that reduced muscle glycogen may indeed have induced PDK4 protein expression. Thus, taken together, the association between changes in muscle glycogen and PDK4 protein as well as between plasma FFA levels and PDK4 protein content supports that PDK4 expression may be regulated by each of these metabolic parameters. Moreover, the association between PDK4 protein content and both PDH phosphorylation and PDHa activity also supports that such PDK4 expression changes may have a functional role in regulating substrate utilization in human skeletal muscle to match availability.

The lowering of PDK4 protein content just 2 h after the carbohydrate-rich meal, and the reduction in response to 20 min of exercise, clearly shows that regulation of PDK4 protein content is fast. To our knowledge, no previous studies have reported such quick regulation of PDK4 protein content in skeletal muscle, although changes have been reported in PDK4 protein after 48 h of fasting in rats (14) and 1 day of high-fat diet in humans (37), and we recently have shown an upregulation of PDK4 protein 6 h after a single exercise session (26).

Glycolytic flux has been suggested to be one factor regulating PDH and hence the flux through the pyruvate dehydrogenase complex (29,35,38). Based on measurements of muscle glucose and muscle glucose-6-phosphate concentrations, the current findings may suggest that glycolytic flux did not entirely determine the PDH activity during exercise. Thus, the clear differences in exercise-induced muscle glucose-6-phosphate and muscle glucose responses in the normal and the low muscle glycogen muscles in the high FFA trial were not associated with differences in PDH regulation, and despite similar muscle glucose-6-phosphate and muscle glucose levels in the normal glycogen leg in the two trials, PDHa activity was higher in the low FFA trial than in the high FFA trial. Such interpretation is supported by the observation that lower glycogen utilization in the low glycogen muscle than in the normal glycogen muscle occurred without influence on PDH regulation in the high FFA trial. But at the same time, the present data are also consistent with the previous indications (29,36,37) that PDH activation plays a role in determining the balance between glycolytic/glycogenolytic flux and oxidation. Hence, accumulation of glycolytic intermediates may depend on how well glycolytic flux and PDH activity match, and the lack of accumulation of intermediates in the low leg in both trials may reflect a balanced glycolytic flux and PDH activity, whereas glycolytic flux may have exceeded PDH activity in the CON leg, leading to accumulation of glucose-6-phosphate.

In conclusion, muscle glycogen and plasma FFAs modify exercise-induced PDH regulation in human skeletal muscle in a nonadditive manner, which might be through glycogen and FFA-mediated regulation of PDK4 expression. However, of notice is that marked exercise-induced activation of PDH was still present when plasma FFA was elevated, which suggests that beneficial effects of physical activity include that exercise overrules FFA-mediated inhibition of carbohydrate oxidation.

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No other potential conflicts of interest relevant to this article were reported.

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