Regulation of PDH in human arm and leg muscles at rest and during intense exercise

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Arm and leg skeletal muscles in humans differ in their metabolic response to exercise. Examples are a larger glucose extraction in arm than in leg muscles and a markedly higher net lactate release at similar relative exercise intensities (1). Moreover, the fatty acid uptake as well as the contribution of lipids for the energy yield are less in the arms (27). Also, the action of insulin appears to be most pronounced in the arm muscles with a larger insulin-stimulated glucose uptake and a further lowering of the free fatty acid (FFA) uptake (19, 27). These differences in metabolism comparing arm and leg muscles can hardly be explained by fiber type, blood flow, or oxygen delivery alone, as these variables are quite similar in the muscles of the upper and lower limbs (1). The interest in understanding especially the difference in lactate release is emphasized by lactate being a central player in both cellular and whole body metabolism (12). Training status could play a role, but even in the most well-trained endurance athletes using their arms just as much as their legs, a high dependency of carbohydrates and high lactate production are still observed in the arm muscles (8). This indicates that the difference in metabolic response to exercise between arm and leg muscles could be related to the metabolic profile of the involved muscles, regulation of the glycolytic flux, and/or entry of carbohydrate-derived fuel into the mitochondria.

Muscle type-related distributions of metabolic enzymes have been demonstrated in human muscles, with higher phosphofructokinase (PFK) activity in the arm muscle triceps than in the leg muscles soleus and vastus lateralis (25) and higher citrate synthase (CS) and 3-hydroxyacyl-CoA dehydrogenase (HAD) activity in soleus and vastus lateralis than in triceps (11, 25). Oxidative muscles are typically characterized by a high proportion of myosin heavy chain (MHC) type I fibers and glycolytic muscles by a high proportion of MHC type II fibers. However, the different adaptability of metabolic enzymes and MHC isoforms (13) indicates that a given MHC muscle fiber type may have a wide range of metabolic capacity (21, 25), and that metabolically related parameters may not necessarily show a tight relationship with MHC composition. The activities of the metabolic enzymes mentioned above are all sensitive to endurance exercise training, with upregulation of CS (7) and HAD (28) activity and downregulation of PFK activity (28), underlining the major impact of physical activity on skeletal muscle metabolic profile (11, 23, 24, 28, 29). It may be that the differences in substrate use between arm and leg muscles solely can be explained by the lower respiratory capacity of arm than leg muscles. This could be the case, even in highly trained cross-country skiers, for whom very intense use of the arm muscles still renders the oxidative enzyme capacity of the arm muscles lower than that of the leg muscles (8).

The pyruvate dehydrogenase complex (PDC) regulates the entry of carbohydrate-derived fuel into the mitochondria for oxidation by catalyzing the decarboxylation of pyruvate to acetyl-CoA. Lactate production will be expected to be related to the capacity and ability of the PDC to convert pyruvate to acetyl-CoA. PDC is composed of three catalytic proteins (E1, E2, and E3), a structural protein (E3BP), and two regulatory proteins, pyruvate dehydrogenase kinase (PDK) and pyruvate dehydrogenase kinase; pyruvate dehydrogenase activity; pyruvate dehydrogenase phosphorylation; muscle type
dehydrogenase phosphatase (PDP). The E1 subunit, pyruvate dehydrogenase (PDH), is responsible for catalyzing the decarboxylation of pyruvate. Therefore, regulation of this PDH component of the enzyme complex could be important for the mitochondrial choice of substrate at rest and during exercise.

Thus, to provide information on mechanisms underlying differences in carbohydrate metabolism in human muscles, the aim of the present study was to test the hypothesis that differences in exercise-induced activation of PDH in part can explain the dissimilarity in carbohydrate dependency between arm and leg skeletal muscles during exercise. This is examined by investigating PDH regulation in human arm and leg muscles characterized by different metabolic and MHC profiles.

**MATERIALS AND METHODS**

**Subjects.** Eight healthy male subjects with normal physical activity level, an average age of 26 yr (range: 23–30 yr), weight 85 kg (range: 71–96 kg), and height 185 cm (range: 179–192 cm) participated in the study. The maximum power (wattmax) was 121 ± 5 and 303 ± 12 W (average ± SE) for arm and leg, respectively.

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.

**Experimental protocol.** Approximately 1 wk before the first trial, the subjects performed preexperimental tests to determine wattmax for arm and leg exercise separately. The wattmax was determined for arm cycling and leg cycling separately using incremental tests where the resistance was increased (arm, 12 W; leg, 30 W) every second minute until exhaustion. Wattmax was estimated from the time to exhaustion and workload. Each subject completed three experimental days, one day with leg cycling using an electronic ergometer (Monark 839E) and two days with arm cycling using an arm crank ergometer (Monark 891E), which was adjusted so that the shoulder of the subject leveled the crank. The day before each experimental trial, the subjects refrained from exercise. On the experimental day, the subjects arrived at the laboratory 2.5 h after consuming a standardized breakfast [77 percent energy (E%) carbohydrate; 11 E% protein; 12 E% fat], regulated for bodyweight and activity level (34). Muscle biopsies were obtained from either deltoid or triceps on the arm exercise days and from vastus lateralis on the day of leg cycling. Three incisions for muscle biopsies were made under local anesthesia (lidocaine and epinephrine). After a resting muscle biopsy was obtained using the percutaneous needle biopsy technique (3) with suction, a blood lactate sample was taken using finger prick and analysis of mixed blood lactate with Lactate Pro LT-1710 (Arkray). Thereafter, the subjects performed 30 min of exercise. The aim of the protocol was to elicit considerable glycogen use, and the exercise protocol therefore aimed at reaching exhaustion after 30 min of exercise. Pilot tests showed that an intensity of 80% wattmax for arm and 70% wattmax for leg exercise was a suitable starting intensity. The exercise intensity was adjusted if needed to ensure exhaustion after 30 min of exercise, based on reports from the subject on exertion level. Whereas the two arm exercise trials increased the heart rate to 155 ± 6 and 156 ± 4 beats/min at the end of exercise, the heart rate reached 174 ± 3 beats/min at 30 min of exercise in the leg cycling trial. Additional blood lactate samples and biopsies were taken after 10 and 30 min of exercise. The biopsies were taken from separate incisions and were rapidly frozen in liquid nitrogen and stored at −80°C until analysis.

**Muscle fiber types.** Fiber analyses were carried out with ATPase histochemistry, as previously described (4, 5). Tema (Scanbeam, Hadsund, Denmark) was used as the image program. Because of a lack of tissue, only samples from four to five subjects were analyzed.

**Muscle glycogen, lactate, and enzyme activity.** 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 using an automatic spectrophotometer as previously described (16).

Muscle lactate concentrations of freeze-dried samples were determined fluorometrically (16).

The activity of CS, HAD, lactate dehydrogenase (LDH), and PFK was analyzed spectrophotometrically as previously described (11).

**Muscle lysate.** Muscle pieces were homogenized in an ice-cold buffer (10% glycerol, 20 mM Na-pyrophosphate, 150 mM NaCl, 50 mM HEPES, 1% NP-40, 20 mM β-glycerophosphate, 10 mM NaF, 1 mM EDTA, 1 mM EGTA, 2 mM PMSF, 10 µg/ml aprotinin, 10 µg/ml leupeptin, 2 mM NαVO₃, 3 mM benzamidine, pH 7.5) for 20 s using a homogenizer (PT 3100, Kinematica). Homogenates were rotated end over end for 1 h at 4°C. Lysates were generated by centrifugation (17,500 g) for 20 min at 4°C. Protein content in lysates was measured by the bicinchoninic acid method (Pierce Chemical).

**SDS-PAGE and Western blotting.** The protein expression and phosphorylation of sites 1 and 2 were measured in muscle lysate by SDS-PAGE (Tris–HCl 10% gel, Bio-Rad) and Western blotting using polyvinylidene difluoride (PVDF) membrane and semi-dry transfer. After the transfer, the PVDF membrane was blocked for 1 h at room temperature [TBS with Tween (TBST) + 2% skim milk], followed by incubation with primary antibody overnight at 4°C (TBST + 2% skim milk).

The following day, the membrane was incubated with horseradish peroxidase-conjugated secondary antibody (Dako) for 1 h at room temperature (TBST + 2% skim milk). Immunoblot Western (Millipore) was used as a detection system. Bands were visualized using an Eastman Kodak Image Station 2000MM. Bands were quantified using Kodak Molecular Imaging Software v.4.0.3, and protein content was expressed in units relative to control samples loaded on each gel.

**Protein levels of the PDH-E1α subunit and phosphorylation of sites 1 (Ser⁷⁰⁹) and site 2 (Ser⁷⁰⁹) of PDH-E1α were determined using antibodies generated in sheep as previously described (22).**

**Activity of the active form of PDH.** The activity of the active form of PDH (PDH₄) was determined as previously described (9, 10, 26) after homogenizing ~10 mg of muscle tissue for 50 s in the modified glycogen synthase kinase-3 buffer given above, using a glass homogenizer (Kontes), and quickly (10–15 s) freezing the samples in liquid nitrogen.

It is normal procedure that PDH₄ activity is adjusted to total creatine. However, because of previous findings in rodents that glycolytic type II-rich muscles have a higher content of creatine than oxidative type I muscles (14), the PDH₄ activity was in the present study normalized to total PDH-E1α protein content as measured by Western blotting.

**Statistics.** Values presented are means ± SE. Two-way ANOVA for repeated measures was applied to evaluate the effect of muscle type and time. One-way ANOVA for repeated measures was used to test for differences in fiber type distribution and enzyme activity between muscle types. The Student-Newman-Keuls post hoc test was used to locate differences. Differences were considered significant at P ≤ 0.05, and a tendency is reported when 0.05 ≤ P ≤ 0.1. Statistical calculations were performed using SigmaStat v.2.03.

**RESULTS**

**Muscle fiber composition.** The percent occurrence of type I fibers was similar in vastus lateralis (52%) and deltoid (54%), whereas triceps (32%) contained less type I fibers than vastus and deltoid (P ≤ 0.05; Table 1).

**Enzyme activity.** The activity of CS was ~50% higher in vastus lateralis (31.3 mmol·min⁻¹·kg dry wt⁻¹) than in both triceps (18.8 mmol·min⁻¹·kg dry wt⁻¹) and deltoid (17.1
mmol·min⁻¹·kg dry wt⁻¹) (P ≤ 0.05) (Table 1). The HAD activity was ∼20% higher in deltoid (29.9 mmol·min⁻¹·kg dry wt⁻¹) than in triceps (25.8 mmol·min⁻¹·kg dry wt⁻¹) (P ≤ 0.05) and ∼50 and ∼30% higher in vastus lateralis (39.5 mmol·min⁻¹·kg dry wt⁻¹) than in triceps and deltoid, respectively (P ≤ 0.05) (Table 1). There were no differences in total LDH (752–1,023 mmol·min⁻¹·kg dry wt⁻¹) or PFK (370–411 mmol·min⁻¹·kg dry wt⁻¹) activity among the three muscles (Table 1).

**Muscle glycogen.** The glycogen concentration at rest was lower in deltoid (366 mmol/kg dry wt) than in triceps (457 mmol/kg dry wt) and vastus lateralis (454 mmol/kg dry wt) (Table 2). Net glycogen utilization during exercise was similar in vastus (205 mmol/kg dry wt) and triceps (269 mmol/kg dry wt) but lower (P ≤ 0.05) in deltoid (90 mmol/kg dry wt). The average net glycogen utilization rate from onset of exercise to 10 min of exercise was 11.4 and 8.4 mmol·kg⁻¹·min⁻¹ in triceps and vastus lateralis, respectively, and for the last 20 min of exercise was 7.2 mmol·kg⁻¹·min⁻¹ in both muscles.

On the basis of these findings, it is concluded that both triceps and vastus lateralis were intensely activated during the full 30 min of exercise, and a direct comparison on PDH regulation during this exercise protocol is only reasonable for these two muscles, assuming that net muscle glycogen use is a reflection of muscle recruitment. Therefore, the exercise data below are only statistically analyzed for vastus lateralis and triceps.

**Muscle lactate.** There was at rest no difference in the lactate concentration in triceps (18.5 mmol/kg dry wt), vastus (12.6 mmol/kg dry wt), and deltoid (17.3 mmol/kg dry wt) (Table 2). Exercise increased muscle lactate concentration after both 10 (∼315% increase) and 30 min (∼380% increase) of exercise compared with rest for triceps and vastus (P ≤ 0.05) (Table 2). The level of muscle lactate was higher in triceps than in vastus lateralis after both 10 and 30 min of exercise (P ≤ 0.05) (Table 2).

**PDH-E1α protein and phosphorylation state.** PDH-E1α protein content was higher in vastus lateralis than in triceps and deltoid (P ≤ 0.05) (Table 1). Phosphorylation of the PDH-E1α subunit at sites 1 and 2 was higher in vastus lateralis than in triceps and deltoid at rest (P ≤ 0.05) (Table 1). Considering the relative phosphorylation (normalized to PDH-E1α content) of sites 1 and 2, there was no difference between vastus lateralis and deltoid, but a more marked phosphorylation of the two sites was still evident in vastus relative to triceps (P ≤ 0.05) (Table 1).

Exercise induced a pronounced dephosphorylation of sites 1 and 2 in both triceps and vastus lateralis (P ≤ 0.05). After 10 and 30 min of exercise, phosphorylation of site 1 was 21 and 14% of the resting level in triceps and 29 and 24% in vastus lateralis, respectively (Fig. 1C). A more pronounced phosphorylation of the PDH-E1α subunit on site 1 was present in vastus lateralis compared with triceps during exercise (P ≤ 0.05) (Fig. 1B).

Relative to the level at rest, the phosphorylation level of site 2 was reduced (P ≤ 0.05) in triceps to 6 and 4% and in vastus lateralis to 19 and 16% after 10 and 30 min of exercise,
respectively (Fig. 1D). No difference was apparent between the muscles in site 2 phosphorylation during exercise (Fig. 1D). When converting the absolute phosphorylation to the relative phosphorylation, no difference was evident between triceps and vastus lateralis during exercise.

PDH$_E$ activity. There was no difference in the PDH$_E$ activity among the three muscles at rest (0.82–1.0 mmol·min$^{-1}$·kg wet wt$^{-1}$) (Table 1). In triceps, an $\approx$160% increase in PDH$_E$ activity was evident after 10 min of exercise compared with the activity at rest (Fig. 1A) ($P \leq 0.05$), with no significant difference between rest and 30 min of exercise (Fig. 1A). In vastus lateralis, the PDH$_E$ activity increased $\approx$246 and $\approx$170% after 10 and 30 min of exercise, respectively, relative to rest (Fig. 1A) ($P \leq 0.05$).

After 10 min of exercise, the absolute PDH$_E$ activity was $\approx$45% higher in vastus lateralis than in triceps ($P \leq 0.05$) (Fig. 1A), but after 30 min of exercise, there was no longer a difference between the two muscles (Fig. 1A). Normalizing the PDH$_E$ activity to PDH-E1$\alpha$ protein content resulted, however, in similar PDH$_E$ activity in vastus lateralis and triceps.

The relationship between phosphorylation on sites 1 and 2 and the PDH$_E$ activity shows that a high PDH$_E$ activity is associated with an extremely low phosphorylation on both site 1 and site 2 (Fig. 2, A and B). Within the lower level of PDH$_E$ activity, there was, however, a large range in phosphorylation and, therefore, not the same strong relation between activity and phosphorylation. Analyzing the data for phosphorylation and activity using a monoexponential model gives, for triceps, $r^2 = 0.23$ and $r^2 = 0.34$ for sites 1 and 2, respectively. For vastus, $r^2 = 0.54$ and $r^2 = 0.67$ for sites 1 and 2, respectively.

**DISCUSSION**

The main findings of the present study are that the PDH content follows the metabolic profile of the muscle rather than the MHC fiber type distribution, and that a smaller exercise-induced increase in PDH$_E$ activity in triceps than in vastus lateralis can be explained by a lower content of PDH in triceps rather than differences in activation of each PDH molecule. In addition, the lower PDH$_E$ activity in triceps...
the PDH-E1α protein content was the same in triceps and vastus lateralis at each of the three time points indicates that each PDH molecule is activated to the same degree in triceps and vastus lateralis, meaning that the higher absolute PDH$_a$ activity and likely flux through PDH in vastus lateralis was due to greater PDH capacity in vastus lateralis than in triceps rather than differences at the molecule level. Thus it is suggested that, because of a lower capacity of PDH, the flux through the PDH complex is lower in triceps, resulting in the conversion of pyruvate to lactate. However, it may be noted that triceps showed a more marked dephosphorylation of site 1 than vastus lateralis during exercise, indicating that there may indeed be some differences in regulation of the phosphorylation state of PDH in triceps and vastus lateralis.

The present finding of more pronounced phosphorylation of sites 1 and 2 in vastus lateralis than in triceps at rest, as an absolute value as well as when normalized to PDH protein content, would be expected to cause a lower PDH$_a$ activity in vastus lateralis than in triceps. The fact that no differences in the PDH$_a$ activity were observed among the three muscles at rest indicates that the PDH$_a$ activity is not regulated only by these two phosphorylation sites, which is supported by previous findings (22) where intralipid infusion resulted in an increased phosphorylation of PDH without an effect on the PDH$_a$ activity. Because regulation of PDH$_a$ activity at site 3 usually is considered negligible in human skeletal muscle (20), these findings may indicate that other covalent regulations of PDH exist. While a discrepancy seems to exist between PDH$_a$ activity and the degree of phosphorylation at rest, a stronger correlation exists at higher PDH$_a$ activities, but still the decline in PDH$_a$ activity from 10 to 30 min of exercise was not associated with a stronger phosphorylation of sites 1 and 2. Thus the covalent regulation of PDH$_a$ activity in skeletal muscle during exercise also seems to be more complex and, perhaps, is not only explained by phosphorylation of sites 1 and 2.

The exercise-induced activation of PDH$_a$ activity corresponds well with previous human studies on vastus lateralis (18, 22, 26, 31, 32). In these studies, the intensity has in general been moderate [45–55% maximal oxygen consumption (VO$_2$ max) or wattmax], and only one study examined PDH$_a$ activity during intense exercise (6), where subjects performed graded 10-min sessions of exercise at 60 and 90% VO$_2$ max. Previous studies (32, 33) have reported a PDH$_a$ activity of $\approx$2.8 mmol·min$^{-1}$·kg wet wt$^{-1}$ after 10 min of exercise at 55% VO$_2$ max, corresponding to a 3-fold increase compared with rest, and an $\approx$3.5-fold increase in PDH$_a$ activity was found after 5 min of exercise at 55% VO$_2$ max (30). This is rather similar to the level obtained in the present study, where the PDH$_a$ activity in vastus lateralis was $\approx$3.1 mmol·min$^{-1}$·kg wet wt$^{-1}$ after 1 min of exercise at 70–80% W$_{max}$, corresponding to an $\approx$3.5-fold increase relative to rest. Thus the PDH$_a$ activity and the relative increase in PDH$_a$ activity do not seem to be higher in the present study with more intense exercise than in protocols with low and intermediate exercise intensities. This observation can probably be explained by an upper limit of total PDH activity, which has been reported to be $\approx$3.6 mmol·min$^{-1}$·kg wet wt$^{-1}$ (17). This number is, however, sensitive to aerobic exercise training, as 8 wk of aerobic exercise was found to increase total PDH activity from $\approx$3.8 to $\approx$4.9 mmol·min$^{-1}$·kg wet wt$^{-1}$ (15). It may therefore be...
spected that the normally physically active subjects in the present study were close to the maximal PDH activity after 10 min of exercise. Furthermore, no study has reported PDH activity reaching total PDH activity during exercise. Studies with low-intensity exercise suggest that a high PDH activity found after 10 min of exercise (32) is sustained during the initial 2 h of exercise, followed by a decline toward resting level as exercise duration proceeds beyond 2 h (18, 22). In the present study with high-intensity exercise, the PDH activity was highest after 10 min of exercise, and for triceps, the PDH activity returned to resting level after 30 min of exercise. Thus it seems that the exercise-induced upregulation of PDH activity is rather independent of exercise intensity, while the intensity is important for the timing of the downregulation of PDH activity in both types of muscles investigated. The reduction in PDH activity may be related to decreasing glycogen levels in the exercising muscle, and, as high-intensity exercise consumes a great amount of muscle glycogen, this may contribute to the early downregulation of PDH activity observed in both vastus lateralis and triceps at 30 min of exercise in the present study.

As explained, the deltoid muscle was not included in the comparison of exercise-induced PDH regulation between muscles because of less marked net glycogen usage in deltoid and thus, most likely, less recruitment of deltoid. Interestingly, however, the smaller net glycogen utilization in deltoid was associated with less marked changes in PDH activity and PDH-E1α phosphorylation in response to arm cycling, indicating that local factors rather than systemic factors are critical in regulating PDH during this type of exercise. Whether the level of muscle glycogen itself could be a determining factor is presently not known.

In conclusion, the present findings show that, in humans, PDH protein content follows the metabolic profile of the muscle rather than MHC fiber type distribution. While PDH protein content and exercise-induced PDH activation are higher in vastus lateralis than in triceps, the PDH molecule seems to be regulated similarly in the two muscles. This suggests that a lower PDH capacity in triceps, rather than less PDH activation of the PDH molecule, is likely a contributing factor to differential carbohydrate metabolism in triceps vs. vastus lateralis.

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