Exercise-induced pyruvate dehydrogenase activation is not affected by 7 days of bed rest

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J Appl Physiol 111: 751–757, 2011. First published June 16, 2011; doi:10.1152/japplphysiol.00063.2011.—To test the hypothesis that physical inactivity impairs the exercise-induced modulation of pyruvate dehydrogenase (PDH), six healthy normally physically active male subjects completed 7 days of bed rest. Before and immediately after the bed rest, the subjects completed an oral glucose tolerance test (OGTT) and a one-legged knee extensor exercise bout [45 min at 60% maximal load (Wmax)] with muscle biopsies obtained from vastus lateralis before, immediately after exercise, and at 3 h of recovery. Blood samples were taken from the femoral vein and artery before and after 40 min of exercise. Glucose intake elicited a larger (P ≤ 0.05) insulin response after bed rest than before, indicating glucose intolerance. There were no differences in lactate release/uptake across the exercising muscle before and after bed rest. PDH-E1α protein content did not change in response to bed rest or in response to the exercise intervention. Exercise increased (P ≤ 0.05) the activity of PDH in the active form (PDHa) and induced (P ≤ 0.05) dephosphorylation of PDH-E1α on Ser203, Ser205 and Ser206, with no difference before and after bed rest. In conclusion, although 7 days of bed rest induced whole body glucose intolerance, exercise-induced PDH regulation in skeletal muscle was not changed. This suggests that exercise-induced PDH regulation in skeletal muscle is maintained in glucose-intolerant (e.g., insulin resistant) individuals.

It is well established that skeletal muscle is a highly plastic tissue capable of adapting quickly to both use and disuse (3). Such skeletal muscle adaptations affect muscle substrate choice and utilization (31) and can have a major impact on whole body metabolism and hence the health of the individual (4).

Insulin-mediated glucose uptake by resting skeletal muscle is influenced by both increases and reductions in physical activity (20, 28, 30). Physical activity level also influences substrate utilization during exercise with increased fat oxidation after a period of exercise training (14, 39). Such changes in metabolism render the muscle more metabolically efficient and consequently enhance muscle endurance (14). But the impact of reductions in normal physical activity level on skeletal muscle substrate utilization during exercise has been less investigated, although this is the major problem in a Western lifestyle. It is possible that a reduction in physical activity level induces an opposite change in skeletal muscle substrate utilization to that observed with training. However, as physical inactivity has been shown to induce insulin resistance and hence reduce glucose uptake at a given insulin concentration at rest (27), it is also possible that carbohydrate use during exercise is compromised when the physical activity level is reduced. This remains to be clarified.

The mechanism underlying modifications in substrate utilization during exercise following a change in physical activity level is unresolved. This may involve adjustments in the expression/activity of membrane transporters and metabolic enzymes (9, 11), as well as capillarization (13), but changes in the acute regulation of substrate choice in skeletal muscle during exercise may indeed also contribute to the observed changes in substrate oxidation. An important regulation of carbohydrate metabolism in skeletal muscle during exercise is GLUT4 translocation to the plasma membrane enhancing the capacity for glucose uptake as well as intracellular removal of glucose by hexokinase II. However, when examining the choice of substrate, the regulation of the PDH complex is of special interest, as it is responsible for catalyzing the decarboxylation of pyruvate to acetyl-CoA, and represents the only entry of carbohydrate-derived substrate into the mitochondria for oxidation, and thus determines which metabolic pathway the carbohydrate undergoes (10). Therefore, regulation of the PDH complex is believed to be important for the mitochondrial choice of substrate both at rest and during exercise. The PDH component of the complex catalyzes the decarboxylation of pyruvate to form acetyl-CoA, and the activity of PDH determines the overall activity of the complex.

PDH activity is regulated by a phosphorylation/dephosphorylation cycle catalyzed by PDH kinases (PKD) (of which there exist 4 isoforms: PKD1, PKD2, PKD3, and PKD4) and PDH
phosphatases (PDP) (of which there exist 2 isoforms: PDP1 and PDP2) (23). Especially PDK has proven to be highly regulated at the mRNA level in skeletal muscle in recovery from exercise (33, 35), and acute regulation of PDK4 expression has been suggested to play a role in restoring muscle glycogen stores after exercise (34). The activity of PDH in the active form (PDHa) increases in human skeletal muscle during exercise as first demonstrated by Ward et al. (45) and an associated dephosphorylation of PDH-E1α has been shown more recently (16, 17, 32). The exercise-induced PDHa activation increases with increasing power output (12), and the upregulation of PDHa activity in the initial part of an exercise bout occurs concomitant with increased carbohydrate use (17, 47). A reduction in PDHa activity toward the resting level is observed in the later part of both prolonged low-intensity exercise (29, 32, 46) and high-intensity exercise (16), potentially reflecting reduced carbohydrate oxidation. In accordance with a potential role of PDH in the observed changes in fat utilization in skeletal muscle with training (14), the exercise-induced increase in PDHa activity has been shown to be lower after a period of endurance exercise training than before, when exercising at the same absolute intensity (21). Such a reduced PDHa activity during exercise may indicate that less carbohydrate is oxidized in the trained muscle during exercise at a given absolute intensity, although a study examining dog muscle reported that PDHa activity does not necessarily reflect the level of carbohydrate oxidation during steady-state contractions (42). The impact of physical inactivity on exercise-induced PDH regulation is however unknown. The response may be opposite of the training effect (21), but as insulin resistance has been shown to be associated with a reduced insulin-stimulated increase in PDH activity in skeletal muscle (26) and physical inactivity to induce insulin resistance (20, 28), exercise-induced PDH regulation may also be impaired.

Therefore the aim of the present study was to investigate the impact of physical inactivity on exercise-induced PDH regulation. Recreationally physically active male subjects underwent 7 days of bed rest. An acute exercise trial with muscle biopsies obtained before and after exercise and with leg arteriovenous (a-v) differences was performed before and after the bed rest period.

MATERIALS AND METHODS

Subjects. Six healthy normally physically active male subjects with an average age, weight, height, body mass index, and maximal oxygen consumption of 28.7 ± 5.3 yr, 82.2 ± 12.3 kg, 183.1 ± 7.6 cm, 24.4 ± 2.2 m², and 4.1 ± 1.0 l/min (means ± SD), respectively, completed 7 days of bed rest. 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-A-2008–024).

Bed rest. Seven days of bed rest was used as a physical inactivity intervention. The subjects were placed in hospital beds with manual head and leg elevation adjustments. During the 7-day bed rest period, the subjects were allowed to sit up for 5 h/day, and they were transported to the restroom, the TV lounge, and outside in a wheelchair. During the bed rest period, subjects were served regular healthy food (10–20% energy from protein, 50–60% energy from carbohydrate, 25–35% energy from fat) ad libitum, from the kitchen at Rigshospitalet, Denmark.

Performance tests. Six to ten days before the onset of bed rest and at the end of the bed rest period, whole body maximal oxygen uptake (VO₂max) was determined by an incremental bicycle test, and leg muscle endurance was determined by a one-legged knee extensor exercise test starting with 15 min at 75% of maximal load (Wmax; see below) (before bed rest level) followed by 90% Wmax (before bed rest level) until exhaustion; thus the same absolute intensity was used before and after bed rest. The leg used in the endurance test was the opposite of the leg used in the experimental protocol.

Body composition. Six to ten days before and immediately after the bed rest period, the subjects body composition was measured using a dual-energy X-ray absorptiometry (DEXA) scanner (Lunar Prodigy Advance, GE Healthcare, Madison, WI).

Oral glucose tolerance test and HOMA-IR. An oral glucose tolerance test (OGTT) was performed between 6 and 10 days before the onset of bed rest and 6 days into the bed rest. After an overnight fast each subject consumed 1 g of glucose/kg body mass, with each gram of glucose dissolved in 6.67 ml water. Blood was sampled 30, 60, and 120 min after glucose intake, and the samples were subsequently analyzed for plasma insulin and glucose (Department of Clinical Biochemistry, Rigshospitalet, Denmark). Homeostatic model assessment of insulin resistance (HOMA-IR) was calculated as HOMA-IR = [glucose (mmol/l) - insulin (mU/l)]/22.5.

Pretesting. Before the first exercise trial each of the six subjects performed a one-legged knee extensor exercise performance test to determine the workload to be used during the experiments. The workload was gradually increased every 2 min and the highest load, which could be sustained for 2 min, was set as the maximal load (Wmax) (35). In the exercise trial performed before and after the bed rest, the subjects exercised at 60% of Wmax, pre-bed rest level.

Experimental protocol. After consuming a standardized dinner the evening before and breakfast on the experimental day with energy content adjusted for body weight (30 kJ/kg), the subject reported to the laboratory between 0700 and 0900. The food consumed was identical before and after bed rest. Under local anesthesia (2% lidocaine) a catheter was placed in one femoral vein and artery, with the Seldinger technique. Using a modified ergometer bicycle, a one-leg knee extensor exercise bout (45 min at 60% Wmax) was performed. Muscle biopsies were obtained from vastus lateralis before (Pre) and immediately after exercise (Post) and 3 h into recovery (3 h Rec) (Fig. 1), using the percutaneous needle biopsy technique (2) with suction. All muscle biopsies were taken through separate incisions, and quickly frozen in liquid nitrogen (<15 s) and stored at −80°C until analyzed. Blood samples were taken from the femoral vein and artery simultaneously at 40 min of exercise to determine a-v differences over the vastus lateralis. Respiratory exchange ratio (RER) was measured before exercise and at 40 min of exercise using COSMED Quark b2.

This protocol was completed between 6 and 10 days before and immediately after the 7 days of bed rest. Three of the subjects worked with their dominant leg and three with their nondominant leg.

Blood analyses. Plasma insulin was measured with an insulin enzyme-linked immunosorbent assay (ELISA) kit (DAKO, Glostrup). Plasma lactate concentration was measured immediately upon sampling using a blood analyzer (ABL 725, Radiometer, Denmark). Glucose concentrations were measured using an automatic spectrophotometer (Cobas FARA 2, Roche Diagnostic, Switzerland). Plasma palmitate concentrations were determined by gas chromatography (43).

Muscle glycogen. Muscle glycogen content was determined on freeze-dried muscle as glycetyl units after acid hydrolysis (24) using an automatic spectrophotometer.

RNA isolation and reverse transcription. RNA isolation was performed on muscle tissue with the guanidinium thiocyanate-phenol-chloroform method (6) with modifications (35). Reverse transcrip-
tion was performed using the Superscript II RNase H− system (Invitrogen) as previously described (35) and was diluted in nuclease-free H2O.

**PCR.** Real-time PCR was performed with an ABI 7900 sequence-detection system (Applied Biosystems, Foster City, CA). Target gene mRNA content was for each sample normalized to single-stranded cDNA determined by OliGreen reagent (Molecular Probes, Leiden, The Netherlands) as previously described (25). The mRNA expression of PDP1, PDP2, PDK1, PDK2, PDK3, and PDK4 was measured, using self-designed primers and probes as previously reported (32).

**Muscle lyase.** A freeze-dried portion of the vastus lateralis muscle biopsy was 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 Na3VO4, 3 mM benzamidine, pH 7.5) for ~2 min using a tissue lyser with 30 oscillations/s (TissueLyser II; QIAGEN, Germany). Homogenates were rotated end over end for 1 h at 4°C. Lysates were generated by centrifugation (16,000 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 precursor PDH-E1α protein has a 29-amino acid-long transit peptide, and when these amino acids are included in the sequence, the previously reported phosphorylation sites (18, 19) are found at Ser232 (site 1), Ser293 (site 2), Ser295, and Ser300 are thought to be the dominant in skeletal muscle (17, 19, 32). In addition to these three sites, a new phosphorylation site has recently been identified at PDH Ser295 (15). PDH-E1α and PDK4 protein expression, phosphorylation of PDH-E1α Ser293, Ser295, and Ser300 were determined by SDS-PAGE and Western blotting as previously described (15, 32).

**PDHa activity.** The activity of PDHa was determined as previously described (5, 7, 37) with modifications (32). The PDHa activity was adjusted to total creatine in each muscle sample (41).

**Calculations and statistics.** The phosphorylation of PDH-E1α Ser293, Ser295, and Ser300 is expressed relative to the PDH-E1α content. The release/uptake of lactate and glucose by vastus lateralis was determined by the difference in the arterial and venous blood concentration and the blood flow. The blood flow was at rest determined by ultrasound Doppler (38) and calculated during exercise by estimating the oxygen consumption from the work performed (1) and using the Fick principle. Due to low number of samples, combined with inconsistent values, we have chosen not to present the respiratory quotient (RQ) results. Values presented are means ± SE. Two-way

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**Table 1. Anthropometric and performance data before and after the bed rest**

<table>
<thead>
<tr>
<th></th>
<th>Before Bed Rest</th>
<th>After Bed Rest</th>
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<tbody>
<tr>
<td>Body mass, kg</td>
<td>81.6 ± 4.9</td>
<td>81.4 ± 5.0</td>
</tr>
<tr>
<td>Leg adipose tissue, g</td>
<td>5,538 ± 529</td>
<td>5,679 ± 582</td>
</tr>
<tr>
<td>Total adipose tissue, g</td>
<td>9,407 ± 1,729</td>
<td>9,351 ± 1,790</td>
</tr>
<tr>
<td>Leg muscle mass, g</td>
<td>21,693 ± 1,891</td>
<td>21,020 ± 1,711*</td>
</tr>
<tr>
<td>Total muscle mass, g</td>
<td>61,230 ± 4,583</td>
<td>60,970 ± 4,336</td>
</tr>
<tr>
<td>VO2max, l/min</td>
<td>4.11 ± 0.42</td>
<td>3.87 ± 0.36*</td>
</tr>
<tr>
<td>Endurance test, min to exhaustion, min</td>
<td>28.2 ± 7.5</td>
<td>14.6 ± 6.0</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>0.94 ± 0.22</td>
<td>1.75 ± 0.28(*)</td>
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</table>

Values are means ± SE. VO2max, maximal oxygen consumption. HOMA-IR, homeostatic model assessment of insulin resistance. *Significantly different from before bed rest, P < 0.05; (*) indicates a tendency, 0.05 < P < 0.10.
ANOVA was applied to evaluate the effect of bed rest and exercise. The Student-Newman-Keuls post hoc test was used to locate differences. Differences were considered significant at $P \leq 0.05$, and a tendency is reported when $0.05 < P \leq 0.1$. Statistical calculations were performed using SigmaStat version 3.11.

RESULTS

**Body composition.** Total body weight and total fat and muscle mass did not change in response to 7 days of bed rest, but leg muscle mass was on average reduced ($P \leq 0.05$) by 650 g by bed rest (Table 1).

**Performance.** $\dot{V}O_2\text{max}$ was lower ($P \leq 0.05$) after bed rest than before (Table 1), and time to exhaustion decreased (non-significantly) in response to bed rest (Table 1). There were no differences in RER during exercise before and after bed rest (data not shown).

OGTT and HOMA-IR. The area under the curve (AUC) was calculated for the plasma glucose and insulin in response to the OGTT, before and after bed rest. The AUC for the glucose response was unchanged, while insulin AUC was greater after bed rest than before ($P \leq 0.05$) (Fig. 2). HOMA-IR tended to increase ($0.05 < P \leq 0.10$) in response to the bed rest (Table 1).

**Blood parameters.** Exercise induced a release of lactate from the leg both before ($P \leq 0.05$) and after bed rest ($0.05 < P \leq 0.10$) with no significant differences before and after bed rest (Fig. 3). The glucose uptake over the leg increased ($P \leq 0.05$) in response to exercise both before and after bed rest (Fig. 3), but with lower ($P \leq 0.05$) glucose uptake during exercise after bed rest than before (Fig. 3). Plasma palmitate concentration tended to be lower ($0.05 < P \leq 0.10$) after bed rest than before (Fig. 3).

**Muscle glycogen.** The muscle glycogen level was higher ($P \leq 0.05$) before and tended to be higher ($0.05 < P \leq 0.10$) after bed rest, but muscle glycogen breakdown ($P \leq 0.05$) during exercise was similar before (139 mmol/kg dry wt) and after bed rest (145 mmol/kg dry wt) (Fig. 4).

Fig. 4. Muscle glycogen level before (Pre) and immediately after 45 min of one-legged knee extensor exercise (Post) performed before (black bars) and after (open bars) 7 days of bed rest. Values are means ± SE. *Significantly different from Pre, $P \leq 0.05$; †significantly different from before bed rest at same time point, $P \leq 0.05$. Parenthesis marks a tendency, $0.05 < P \leq 0.10$.

Table 2. **PDK and PDP mRNA**

<table>
<thead>
<tr>
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<th>Before Bed Rest</th>
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<tr>
<td></td>
<td>Pre 3 h Rec</td>
<td>Pre 3 h Rec</td>
</tr>
<tr>
<td>PDP1</td>
<td>1 ± 0.44</td>
<td>0.86 ± 0.46</td>
</tr>
<tr>
<td>PDP2</td>
<td>1 ± 0.29</td>
<td>1.10 ± 0.34</td>
</tr>
<tr>
<td>PDK1</td>
<td>1 ± 0.52</td>
<td>2.17 ± 1.02</td>
</tr>
<tr>
<td>PDK2</td>
<td>1 ± 0.23</td>
<td>1.11 ± 0.23</td>
</tr>
<tr>
<td>PDK3</td>
<td>1 ± 0.31</td>
<td>2.42 ± 0.83</td>
</tr>
<tr>
<td>PDK4</td>
<td>1 ± 0.54</td>
<td>0.56 ± 0.33</td>
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</table>

Values are means ± SE. mRNA content of the PDP and PDK isoforms in vastus lateralis before (Pre) and at 3 h of recovery (3 h Rec) from 45 min of one-legged knee extensor exercise performed before and after bed rest. mRNA content is normalized to the content of single-stranded DNA. (*)Tends to be significantly different from Pre, $0.05 < P \leq 0.10$. 
Ser295 phosphorylation in human skeletal muscle.
and after bed rest. This is the first time to demonstrate the

Fig. 6. Phosphorylation of PDH-E1 and Ser300 (Fig. 6) with no difference in the response before and phosphorylation (Ser588, Thr642, and Ser751) were unaffected by exercise and bed rest (data not shown).

PDK and PDP mRNA expression. To investigate if bed rest influenced exercise-induced regulation of PDK1–4 and PDP1–2 mRNA, a biopsy was also sampled 3 h after exercise. There were no significant effects on these mRNAs in response to the acute exercise bout in any of the investigated genes either before or after the bed rest period, except that PDK4 mRNA tended to increase (0.05 < P ≤0.10) ~12 fold at 3 h of recovery relative to Pre, both before and after bed rest (Table 2). In addition, bed rest did not significantly affect the PDK 1–4 and PDP1–2 mRNA level at Pre or at 3 h of recovery.

PDHa activity and PDH-E1α phosphorylation. Exercise increased (P ≤ 0.05) the PDHa activity (Fig. 5) and induced (P ≤ 0.05) dephosphorylation of PDH-E1α on Ser293, Ser295, and Ser300 (Fig. 6) with no difference in the response before and after bed rest. This is the first time to demonstrate the Ser295 phosphorylation in human skeletal muscle.

DISCUSSION

The main findings of the present study are that although 7 days of bed rest induced whole body glucose intolerance and reduced glucose uptake by the exercising muscle, exercise-induced PDH regulation in skeletal muscle was unchanged, suggesting that the capacity for glucose oxidation during exercise was not impaired.

The present finding that 7 days of bed rest tended to increase the HOMA-IR index and increased the insulin response to a given oral glucose challenge reflects the need for an increased insulin secretion to maintain a given removal of glucose from the blood due to decreased insulin sensitivity. This is in line with previous studies showing both impaired glucose tolerance and decreased insulin sensitivity in skeletal muscle in response to bed rest and reduced physical activity (20, 27, 28, 30). However, the present study is the first to demonstrate that glucose uptake during exercise is also reduced by 7 days of bed rest. Because glucose uptake during exercise was reduced with ~30% and leg muscle mass only by 3% after bed rest, the lower glucose uptake cannot be completely explained by less muscle mass. This may indicate that exercise-induced GLUT4 translocation was affected by bed rest, or that the glucose gradient across the membrane was decreased by bed rest. To further examine this unexpected result, AS160 phosphorylation was determined on three different sites. But the observation that exercise-induced AS160 phosphorylation was unaffected by bed rest does not support that impaired GLUT 4 translocation was the mechanism behind the reduced glucose uptake after bed rest. However, the finding that 7 days of bed rest tended to reduce the HK-II protein content in vastus lateralis (unpublished data) suggests that a reduced removal of intracellular glucose and hence potentially a reduced transmembrane glucose gradient could be a likely explanation. Although no significant changes were observed in lactate release it should be noted that the absolute reduction after bed rest is substantial (Fig. 3), with an average 427 μmol/min lower lactate release after bed rest, and glucose uptake 273 μmol/min lower after bed rest. Notably, the energetic value of these differences is very close to equalizing one another, suggesting that the smaller amount of glucose taken up is matched by a smaller lactate release. Carbohydrate oxidation of blood-derived glucose therefore appears to have been the same before and after bed rest and because glycogen use was similar before

Fig. 5. The activity of PDH in the active form (PDHa) before (Pre) and immediately after (Post) 45 min of one-legged knee extensor exercise performed before (black bars) and after (open bars) 7 days of bed rest. Values are means ± SE. *Significantly different from Pre, P ≤ 0.05.

Fig. 6. Phosphorylation of PDH-E1α Ser293, Ser295, and Ser300, relative to the total PDH-E1α, before (Pre) and immediately after (Post) 45 min of one-legged knee extensor exercise performed before (black bars) and after (open bars) 7 days of bed rest. Representative blot from one subject is presented above. Values are means ± SE. *Significantly different from Pre, P ≤ 0.05.

AS160 protein and phosphorylation. AS160 protein content and phosphorylation (Ser88, Thr42, and Ser751) were unaffected by exercise and bed rest (data not shown).

PDHa activity and PDH-E1α phosphorylation. Exercise increased (P ≤ 0.05) the PDHa activity (Fig. 5) and induced (P ≤ 0.05) dephosphorylation of PDH-E1α on Ser293, Ser295, and Ser300 (Fig. 6) with no difference in the response before and after bed rest. This is the first time to demonstrate the Ser295 phosphorylation in human skeletal muscle.
and after bed rest, no changes in pyruvate delivery to the mitochondria or in carbohydrate oxidation are expected. The observed similar RER values during exercise before and after bed rest further support this notion. Finally, the observed elevated resting muscle glycogen concentration after bed rest might be due to the lack of physical activity.

The observed exercise-induced increase in PDHa activity and reduction in PDH-E1α phosphorylation in skeletal muscle before bed rest is in accordance with previous studies (16, 17, 32). However, the finding that the exercise-induced PDH regulation was unaffected by 7 days of bed rest demonstrates that skeletal muscle maintains the ability to regulate PDH in response to exercise after 1 wk of physical inactivity. Thus, despite reduced resting (based on the OGTT results) and exercise-induced glucose uptake, skeletal muscle still has the ability to elicit acute changes in PDH regulation with exercise, which may reflect that although physically inactive individuals become glucose intolerant they will still possess the capability to increase the PDH activity when exercising, and would therefore be expected to be able to increase carbohydrate oxidation as glucose-tolerant subjects are.

Based on previous reports that exercise at a given absolute intensity increased PDH activity less after a period of endurance exercise training (12, 21), and that physical inactivity reduces exercise performance (40), it was expected that the exercise bout performed after the 7 days of bed rest would elicit a higher PDH activity and a more marked PDH-E1α dephosphorylation than before bed rest. The similar exercise-induced changes observed before and after bed rest may indicate that bed rest did not attenuate the performance capacity of the muscle as expected, thus resulting in similar relative exercise intensity before and after bed rest. However, the observation that time to exhaustion during one-legged knee extensor exercise was reduced (nonsignificantly) after bed rest relative to before does not support this possibility and rather indicates that the exercise-induced increase in PDH activity was smaller than would be expected based on the relative exercise intensity. Hence it cannot be ruled out that a bed rest-induced impairment of the exercise-induced PDH activation is masked by a larger response of PDH to exercise at a higher relative exercise intensity after bed rest. We have previously observed that reduced muscle glycogen levels were associated with lower exercise-induced PDH activity in human skeletal muscle (17) indicating that the elevated glycogen levels in the present study may have caused the PDH activity to increase. One suggested mechanism behind glycogen-dependent regulation of PDH involves glycogen regulatory enzymes, such as protein phosphatase 1 (PP1), which have been suggested to be bound to the glycogen scaffold, but released when the glycogen content decreases (33). In addition, elevated plasma FFA concentrations have been shown to be associated with smaller exercise-induced PDH activation in human skeletal muscle (17), suggesting that the lower plasma palmitate after bed rest than before in the present study may have enhanced the PDH activity. A likely mechanism behind elevated plasma FFA resulting in lower PDH activity is an increased NADH:NAD+ ratio in the mitochondria due to β-oxidation, leading to PDK4 upregulation and eventually downregulation of PDHα (10). Thus, despite a possible combined effect of elevated muscle glycogen and reduced palmitate concentrations to increase PDHα activity, exercise-induced PDHα activity remained similar before and after bed rest. An alternative possibility is that PDHα activity is only dependent on glycogen content below a certain threshold, where changes in pyruvate concentra-

tion and the ADP/ATP ratio likely are present. Of note, glucose uptake, lactate release, and muscle glycogen utilization taken together indicate that pyruvate delivery during exercise was the same before and after bed rest and it is possible that this is the determining factor for the similar PDH regulation before and after bed rest, although there are controversies regarding the role of pyruvate in the regulation of PDHα activity (8).

The total amount of PDH-E1α did not change in response to bed rest, which was expected, because an earlier study with 5 wk of immobilization of the arm did not affect the total amount of PDH-E1α in triceps (44). Of note is that 7–8 wk of training has been shown to increase the content of PDH-E1α (22, 31), suggesting that reductions and increases in physical activity level do not induce opposite changes in PDH-E1α protein content. The PDK and PDP mRNA data with exercise-induced increases in PDK4 mRNA are overall as expected based on previous findings (36). In addition the observation that the bed rest intervention had no effect on the PDK and PDP mRNA expression is in accordance with the present findings, that exercise-induced PDH regulation was unaffected by bed rest.

In conclusion, 7 days of bed rest induced whole body glucose intolerance, and reduced exercise-induced glucose uptake, but exercise-induced PDH activation in skeletal muscle was still present. This suggests that while resting and exercise-mediated glucose uptake is impaired, exercise-induced glucose oxidation is preserved after short-term physical inactivity, underlining the beneficial effect of physical activity for glucose-intolerant individuals.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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