Effects of Exercise Training on Regulation of Skeletal Muscle Glucose Metabolism in Elderly Men

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Abstract

Background. The aim was to investigate the molecular mechanisms behind exercise training-induced improvements in glucose regulation in aged subjects.

Methods. Twelve elderly male subjects completed 8 weeks of exercise training. Before and after the training period, the subjects completed an oral glucose tolerance test (OGTT) and a muscle biopsy was obtained from the vastus lateralis before and 45 minutes into the OGTT. Blood samples were collected before and up to 120 minutes after glucose intake.

Results. Exercise training increased Hexokinase II, GLUT4, Akt2, glycogen synthase (GS), pyruvate dehydrogenase (PDH)-E1α, PDK2 protein, and glycogen content in skeletal muscle. Furthermore, in response to glucose, GS activity was increased and the dephosphorylation of GS site 2 + 2a and 3a was enhanced after the training intervention. The glucose-mediated insulin stimulation of TBC1D4 Thr642 phosphorylation was increased after exercise training. In the trained state, the PDHa activity was reduced following glucose intake and without changes in phosphorylation level of PDH-E1α.

Conclusions. The present results suggest that exercise training improves glucose regulation in elderly subjects by enhancing the capacity and acute regulation of glucose uptake and by enhancing intracellular glucose removal to glycogen synthesis rather than glucose oxidation.

Key Words: Physical activity—Aging—Pyruvate dehydrogenase—Glycogen synthase—Insulin signaling.

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Aging is associated with impaired glucose metabolism as demonstrated both by reduced glucose tolerance during an oral glucose tolerance test (OGTT; (1)) and reduced glucose disposal during a hyperinsulinemic euglycemic clamp (2,3). Such changes may predispose to diseases like type 2 diabetes (4) and prevention of age-related dysfunctional glucose metabolism through physical activity is therefore important. Regular physical activity has been shown to increase glucose tolerance (1,5) and insulin sensitivity in elderly subjects (3) to a similar extent as in young subjects (6,7). Aging and exercise training associated changes in glucose tolerance and insulin sensitivity involve several tissues, but changes in skeletal muscle metabolism have a particularly large impact on whole body metabolism (8).

Insulin-stimulated glucose uptake in skeletal muscle involves glucose transporter (GLUT)4 translocation to the plasma membrane...
regulated through phosphorylation and activation of Akt and con-
comitant phosphorylation and inactivation of Akt substrate of 160 kD (AS160/TBC1D4) (9). Glucose 6 phosphate (G-6-P) will in a
resting muscle cell either be incorporated into glycogen or be ox-
dized within the mitochondria. The rate limiting enzyme in glycogen
formation is glycogen synthase (GS), while the pyruvate dehydroge-
nase (PDH) complex converts pyruvate to acetyl CoA in an irrevers-
ible step, which represents the only entry of carbohydrate-derived
substrates into the mitochondria for oxidation (10). GS is stimu-
lated allosterically by G-6-P (11) and inactivated by phosphoryla-
tion (12,13). Insulin is known to increase the activity of GS through
insulin-stimulated activation of Akt and concomitant phosphoryla-
tion and inactivation of GS kinase (12,13). Activity of PDH in the active
form (PDHa) is primarily determined by the phosphorylation level
of the PDH-E1α subunit regulated by PDH kinases (PDK), which
phosphorylate and inactivate PDH, and PDH phosphatases (PDP),
which dephosphorylate and activate PDH (14,15). PDHa activity
has previously been shown to increase in human skeletal muscle in
response to oral glucose intake (16), and to either increase (17) or
remain unchanged (18) during a hyperinsulinemic euglycemic clamp
in humans. In accordance with an insulin-mediated regulation of
PDHa activity, insulin has been reported to increase PDP activity
(19) and to reduce PDK4 protein in rat skeletal muscle (20).

Previous studies have observed either reduced or unchanged
GLUT4 protein content in human skeletal muscle with increasing
age (21,22). Moreover, the recent finding that aging was associ-
ated with impaired insulin-induced TBC1D4 phosphorylation in
skeletal muscle (2) suggests that reduced insulin-mediated GLUT4
translocation may contribute to insulin resistance in aged subjects.
Furthermore, skeletal muscle HKII activity has been shown to be
lower in elderly than in young subjects (23) indicating that a reduced
ability to maintain the transmembrane glucose gradient also may
affect skeletal muscle glucose uptake in aged subjects. Knowledge
about potential age-related effects on GS and PDH regulation in skel-
etal muscle is scarce. However, total GS activity has been reported
to be reduced with age (24). Moreover, insulin-stimulated GS activa-
tion has been demonstrated to be impaired in Type 2 diabetes (T2D)
patients (22,25). Similarly, an insulin-induced increase in PDHa
activity has been reported to be abolished in T2D patients (17).
In line with this observation, T2D has been shown to be associated
with impaired insulin-mediated down-regulation of PDK4 in skeletal
muscle suggesting that PDK4-induced inhibition of PDH and hence
inhibition of glucose oxidation may contribute to insulin resistance
(26). Together, this indicates that both oxidative and nonoxidative
glucose removal are reduced in skeletal muscle of T2D patients. This
may suggest that GS and PDH dysregulation also contributes to age-
associated glucose intolerance and insulin resistance.

Exercise training has previously been shown to increase skeletal
muscle protein content of GLUT4, HKII, Akt, TBC1D4, GS, PDK2,
and PDH-E1α in young subjects (7,22,27) and similarly for GLUT4
and Akt2, but not TBC1D4 in elderly subjects (2,28). Furthermore,
exercise training has been demonstrated to enhance insulin-mediated
TBC1D4 and GS regulation in skeletal muscle of young subjects as
well as to reverse the age-associated impairment of insulin-stimu-
lated TBC1D4 phosphorylation in skeletal muscle (2,22).

Therefore, the purpose of the present study was to test the
hypotheses that exercise training-induced improvements in whole
body glucose metabolism in elderly men are associated with
increased content of key factors involved in GS and PDH regulation
in skeletal muscle, and enhanced acute regulation of GS and PDH in
skeletal muscle upon glucose intake.

Methods
Cardiovascular and skeletal muscle analyses from the current exper-
iment have previously been published (29,30).

Subjects
Twelve physically inactive but healthy male subjects, 60–72 years of age
with an average body mass index of 26.0 ± 0.5 kg/m² participated in the
study. Initially 13 subjects were included as previously published (29,30),
but one of the subjects did not complete the current protocol described
below. All subjects were nonsmokers and underwent a medical examina-
tion. None had been diagnosed with cardiovascular disease, hyperten-
sion, renal dysfunction, insulin resistance or type 2 diabetes, and all subjects
had normal ECG. One subject was diagnosed with hypercholesterolemia
regulated by his own physician (medication was maintained during the
experimental period). The other participants had normal cholesterol lev-
els. Subject characteristics have previously been published (29).

Exercise Training
The subjects exercise trained for 8 weeks, 4 days a week. The training
was a combination of supervised high intensity cycling exercise (spin-
ing) on cycle ergometers performed two times per week, strength and
mobility training (crossfit) once per week and a 5 km walk once a week
as previously described (29). The intensity of the exercise was controlled
by TEAM2 WearLink+ heart rate monitors (Polar, Kempele, Finland).

Experimental Setup
Before and after the intervention period, the subjects were challenged
with an OGTT (1 g/kg body mass). A muscle biopsy was obtained from
vastus lateralis before and 45 minutes after glucose intake using the
Bergström needle biopsy method with suction. Blood samples were
obtained before and up to 2 hours after glucose intake (see supplemen-
tary material for details). After the training period, the OGTT was per-
formed 48 hours after the last exercise bout.

Plasma and Muscle Analyses
See Supplementary material.

Statistics
Values are presented as mean ± SE. A student paired t-test was used
to test the effect of exercise training on fasting plasma insulin and
glucose as well as skeletal muscle protein content. Two-way ANOVA
with repeated measures was used to test the effect of exercise training
and glucose intake on plasma glucose, insulin and c-peptide as well
as skeletal muscle protein and protein phosphorylation levels. When
a main effect was observed, a Student–Newman–Keuls post hoc test
was used to locate differences between groups. The data set was log
transformed if the results did not pass the equal variance test. The data
were considered significant at p < .05 and a trend is reported when
.05 ≤ p < .1. Statistical calculations were performed in SigmaPlot 11.0.

Results
Performance
Exercise training increased (p < .05) VO2max and muscle endurance as
previously published (29,30).

Blood analyses
Fasting glucose and insulin
Fasting plasma insulin and plasma glucose concentrations were not
different before and after the 8 weeks of exercise training as in part
previously reported (29).
OGTT
Plasma glucose
The plasma glucose response during the OGTT was similar in the untrained and trained state increasing ($p < .05$) in both conditions to approximately 8 mM 30 minutes after glucose intake and returning to the basal level (~3 mM) at 120 minutes after intake. There was no difference in the plasma glucose concentration between the untrained and the trained state (Figure 1A).

Plasma insulin
The plasma insulin concentration increased ($p < .05$) ~10-fold 60 minutes after the glucose intake and remained elevated ($p < .05$) 120 minutes after intake relative to before both in the untrained and trained state. Moreover, the plasma insulin concentration was at 15 minutes after glucose intake higher ($p < .05$) and at 90 and 120 minutes lower ($p < .05$) in the trained than in the untrained state (Figure 1B). The insulin area under the curve (AUC) was ~15% lower ($p < .05$) after exercise training than before exercise training. While there was no difference in the HOMA-IR index before and after exercise training, the Matsuda index was higher ($p < .05$) after exercise training (12.3 ± 3.6) than before (8.0 ± 2.3).

Plasma C-peptide
The plasma C-peptide concentration increased ($p < .05$) similarly in the untrained and trained state to ~5-fold at 60 minutes after glucose intake relative to before and remained in both conditions elevated ($p < .05$) at 120 minutes relative to before intake. Moreover, as for the plasma insulin concentration, the C-peptide concentration was at 90 and 120 minutes after glucose intake lower ($p < .05$) in the trained than in the untrained state (Figure 1C). The C-peptide AUC was ~15% lower ($p < .05$) after exercise training than before.

Muscle Analyses
Muscle glycogen
The muscle glycogen concentration increased ($p < .05$) from 496 ± 29 mmol/kg dw before to 686 ± 43 mmol/kg dw after exercise training.

Protein content
Skeletal muscle protein content of GLUT4, Akt2, GS, PDK2, and PDH-E1α (all $p < .05$) and HKII ($0.5 < p < 1$) increased 1.2- to 1.8-fold with exercise training. There was no difference in Akt1, TBC1D4, GSK-3β, or PDK4 protein content before and after exercise training (Table 1).

Intracellular Signaling
Akt phosphorylation
Forty-five minutes after glucose intake, the absolute Akt Thr308 phosphorylation increased ($p < .05$) 2.8- and 3.7-fold relative to before glucose intake in the untrained and trained skeletal muscle, respectively, with no difference between training status (Table 2). The absolute phosphorylation on Akt Ser473 increased ($p < .05$) 2.1- and 3-fold in response to glucose intake in the untrained and trained muscle.

Table 1. Skeletal Muscle Protein Content of Hexokinase (HK)II, Glucose Transporter (GLUT)4, Protein Kinase B (Akt)1, Akt2, Akt Substrate of 160 kD (AS160/TBC1D4), Glycogen Synthase, Pyruvate Dehydrogenase (PDH)-E1α, Glycogen Synthase Kinase (GSK)-3β in Untrained (UT) and Exercise-Trained (T) Aged Male Subjects. Values are given in arbitrary units (AU).

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<tr>
<td>HK (AU)</td>
<td>1 ± 0.2</td>
<td>1.5 ± 0.2‡</td>
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<tr>
<td>GLUT4 (AU)</td>
<td>1 ± 0.1</td>
<td>1.2 ± 0.1†</td>
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<td>Akt1 (AU)</td>
<td>1 ± 0.1</td>
<td>1.1 ± 0.1</td>
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<tr>
<td>Akt2 (AU)</td>
<td>1 ± 0.1</td>
<td>1.3 ± 0.1†</td>
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<tr>
<td>TBC1D4 (AU)</td>
<td>1 ± 0.1</td>
<td>0.9 ± 0.1</td>
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<tr>
<td>GS (AU)</td>
<td>1 ± 0.1</td>
<td>1.1 ± 0.1†</td>
</tr>
<tr>
<td>PDH-E1α (AU)</td>
<td>1 ± 0.1</td>
<td>1.2 ± 0.1†</td>
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<tr>
<td>GSK-3β (AU)</td>
<td>1 ± 0.1</td>
<td>1.0 ± 0.1</td>
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Values are presented as mean ± SE.
‡Significantly different from UT, $p < .05$.
†Tends to be significantly different from UT, $0.05 < p < .1$. 
Table 2. Skeletal Muscle Akt<sup>Thr<sub>183,283</sub></sup>, Akt<sup>Ser<sub>473</sub></sup>, Akt Substrate of 160 kD (AS160/TBC1D4)<sup>Thr<sub>842</sub></sup>, Glycogen Synthase Kinase (GSK)-3β<sup>Ser<sub>9</sub></sup>, Glycogen Synthase (GS) site 2 + 2a, GS Site 3a Phosphorylation (p) and GS Activity (I-form) Before (Pre) and 45 Minutes into an Oral Glucose Tolerance Test (Post) in Untrained (Untrained) and Exercise-Trained (Trained) Aged Male Subjects. Values are given in arbitrary units (AU)

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<tr>
<td>p-Akt&lt;sup&gt;Thr&lt;sub&gt;183,283&lt;/sub&gt;&lt;/sup&gt; (AU)</td>
<td>0.6 ± 0.1 1.2 ± 0.2&lt;sup&gt;*&lt;/sup&gt;</td>
<td>0.4 ± 0.1 1.5 ± 0.2&lt;sup&gt;*&lt;/sup&gt;</td>
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<td>p-Akt&lt;sup&gt;Ser&lt;sub&gt;473&lt;/sub&gt;&lt;/sup&gt; (AU)</td>
<td>0.6 ± 0.1 1.2 ± 0.1†</td>
<td>0.7 ± 0.1 2.0 ± 0.3&lt;sup&gt;‡&lt;/sup&gt;</td>
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<tr>
<td>p-TBC1D4&lt;sup&gt;Thr&lt;sub&gt;842&lt;/sub&gt;&lt;/sup&gt; (AU)</td>
<td>0.5 ± 0.1 0.9 ± 0.1&lt;sup&gt;‡&lt;/sup&gt;</td>
<td>0.5 ± 0.1 1.1 ± 0.1&lt;sup&gt;‡&lt;/sup&gt;</td>
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<tr>
<td>p-GSK&lt;sup&gt;β&lt;/sup&gt;-&lt;sup&gt;Ser&lt;sub&gt;21&lt;/sub&gt;&lt;/sup&gt; (AU)</td>
<td>0.9 ± 0.1 1.2 ± 0.1&lt;sup&gt;‡&lt;/sup&gt;</td>
<td>0.8 ± 0.1 1.3 ± 0.2†</td>
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<td>p-GS site 2 + 2a (AU)</td>
<td>0.8 ± 0.1 1.1 ± 0.1</td>
<td>1.5 ± 0.2&lt;sup&gt;‡&lt;/sup&gt; 1.2 ± 0.2&lt;sup&gt;‡&lt;/sup&gt;</td>
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<tr>
<td>p-GS site 3a (AU)</td>
<td>0.7 ± 0.1 0.5 ± 0.1&lt;sup&gt;‡&lt;/sup&gt;</td>
<td>1.2 ± 0.1&lt;sup&gt;‡&lt;/sup&gt; 0.7 ± 0.1&lt;sup&gt;‡&lt;/sup&gt;</td>
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<tr>
<td>GS activity (I-form)</td>
<td>21.5 ± 1.3 22.2 ± 1.3</td>
<td>18.2 ± 1.0 24.0 ± 1.7&lt;sup&gt;‡&lt;/sup&gt;</td>
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All values are presented as mean ± SE.
<sup>*</sup>Significantly different from Pre within given group, p < .05.
<sup>†</sup>Significantly different from UT, p < .05.
<sup>‡</sup>Tends to be significantly different from UT, .05 < p < .1.

state, respectively, reaching a 2-fold higher (p < .05) level in the trained than untrained state (Table 2).

TBC1D4 phosphorylation
The absolute TBC1D4 Thr<sup>842</sup> phosphorylation in skeletal muscle increased (p < .05) 1.8-fold in the untrained state and 2.5-fold in the trained state in response to glucose intake reaching a 1.2-fold higher (p < .05) level in the trained than the untrained state (Table 2).

GSK3β phosphorylation
Absolute GSK3β Ser<sup>9</sup> phosphorylation in skeletal muscle increased (p < .05) similarly 1.3- to 1.4-fold 45 minutes after glucose intake relative to before both before and after the exercise training period with no difference between the two conditions (Table 2).

GS Regulation
GS site 2 + 2a
Glucose intake had no effect on the absolute GS site 2 + 2a phosphorylation in skeletal muscle before exercise training, but after exercise training the absolute GS site 2 + 2a phosphorylation decreased (p < .05) 20% in response to glucose intake. Furthermore, the absolute GS site 2 + 2a phosphorylation was in the trained state 1.4-fold higher (p < .05) in the basal state and tended to be 1.2-fold higher (.05 < p < .1) 45 minutes after glucose intake than in the untrained state (Table 2).

GS site 3a
The absolute GS site 3a phosphorylation in skeletal muscle tended to decrease (.05 < p < .1) 25% in response to glucose intake before exercise training and decreased (p < .05) 40% with glucose intake after exercise training. The absolute GS site 3a phosphorylation was 1.3- to 1.7-fold higher (p < .05) in the trained state than in the untrained state (Table 2).

GS activity
The GS activity (I-form) in skeletal muscle did not change with glucose intake before exercise training, but increased (p < .05) 1.3-fold 45 minutes after glucose intake in the exercise-trained state. There was no difference in GS activity between the untrained and trained state (Table 2).

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<td>Pre Post</td>
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<tr>
<td>PDH Ser&lt;sup&gt;Ser&lt;sub&gt;21&lt;/sub&gt;&lt;/sup&gt; (AU)</td>
<td>0.9 ± 0.1 0.9 ± 0.1</td>
<td>1.2 ± 0.1&lt;sup&gt;‡&lt;/sup&gt; 1.2 ± 0.1&lt;sup&gt;‡&lt;/sup&gt;</td>
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<tr>
<td>PDH Ser&lt;sup&gt;Ser&lt;sub&gt;9&lt;/sub&gt;&lt;/sup&gt; (AU)</td>
<td>1.1 ± 0.2 1.0 ± 0.2</td>
<td>1.6 ± 0.2&lt;sup&gt;†&lt;/sup&gt; 1.4 ± 0.2&lt;sup&gt;†&lt;/sup&gt;</td>
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<td>PDHa activity/total Cr (mmol·min&lt;sup&gt;-1&lt;/sup&gt;·kg&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>0.9 ± 0.1 1.0 ± 0.2</td>
<td>0.9 ± 0.2 0.7 ± 0.1&lt;sup&gt;‡&lt;/sup&gt;</td>
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<tr>
<td>PDK2 (AU)</td>
<td>0.6 ± 0.01 0.6 ± 0.01</td>
<td>0.9 ± 0.01&lt;sup&gt;†&lt;/sup&gt; 0.9 ± 0.01&lt;sup&gt;†&lt;/sup&gt;</td>
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<td>PDK4 (AU)</td>
<td>0.8 ± 0.1 0.7 ± 0.1&lt;sup&gt;‡&lt;/sup&gt;</td>
<td>0.8 ± 0.1 0.7 ± 0.1&lt;sup&gt;‡&lt;/sup&gt;</td>
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All values are presented as mean ± SE.
<sup>§</sup>Significantly different from Pre within given group, p < .05.
<sup>†</sup>Significantly different from UT at given time point, p < .05.
<sup>‡</sup>Tends to be significantly different from UT at given time point, .05 ≤ p < .1.
<sup>†</sup>Tendency for an overall effect of glucose intake, .05 ≤ p < .1.

PDH Regulation
PDH phosphorylation
There was no effect of glucose intake on the absolute PDH site 1 and site 2 phosphorylation in skeletal muscle. Exercise training increased (p < .05) the absolute phosphorylation level of both sites with 1.3- to 1.8-fold, but this effect was removed by normalization to PDH-E1α protein, except for site 2 phosphorylation in the basal state (Table 3; Supplementary Figure 3A).

PDHa activity
There was no effect of glucose intake on skeletal muscle PDHa activity before exercise training, but glucose intake decreased (p < .05) the PDHa activity 30% after the exercise training period (Table 3).

PDK4 Protein
PDK4 protein content in skeletal muscle overall (untrained and trained state together) tended to be lower (.05 ≤ p < .1) after glucose intake than before (Table 3).

Discussion
The main findings of the present study are that exercise training-improved whole body glucose regulation in elderly healthy subjects was associated with increased skeletal muscle HKII, GLUT4, Akt2, PDK2, GS, and PDH-E1α protein content. Moreover, exercise training resulted in an enhanced response of TBC1D4 and GS and a reduced PDHa activity in skeletal muscle after glucose intake relative to before training.

The present observation that the plasma glucose response to an oral glucose intake was similar before and after the exercise training period shows that glucose tolerance was unaffected by the exercise training intervention. However, the lower insulin and C-peptide plasma concentrations as well as the lower AUC for insulin and C-peptide during the OGTT after training than before training indicate that exercise training improved the insulin sensitivity in the present study. This is further supported by the higher Matsuda index after exercise training than before in the current study. Such an exercise training effect is in line with the observations in previous
studies using hyperinsulinemic euglycemic clamp in elderly subjects demonstrating enhanced whole body insulin action (1,2) as well as improved insulin sensitivity in skeletal muscle after exercise training (2,6).

The finding in the current study that glucose intake induced a more marked absolute TBC1D4 phosphorylation after exercise training than before is in accordance with a recent study demonstrating that insulin-stimulated TBC1D4 phosphorylation during a hyperinsulinemic euglycemic clamp was higher after a training period than before in aged subjects (2). This indicates that exercise training enhances skeletal muscle GLUT4 translocation upon glucose intake in elderly subjects as previously reported in young subjects (2). Although the effect of exercise training on TBC1D4 phosphorylation normalized to TBC1D4 protein did not reach statistical significance in the present study, the lack of change in TBC1D4 protein content with exercise training both in the present and the previous study (2,22) suggests that the observed training effect on TBC1D4 phosphorylation upon glucose intake is due to acute regulation rather than a change in TBC1D4 protein content. Although Akt Thr173 phosphorylation after glucose intake was similar in the untrained and trained state, the present observation that the level of Akt Ser473 phosphorylation after glucose intake was higher in the trained muscle than the untrained is in line with the higher TBC1D4 phosphorylation after the training period. This indicates that Akt mediated the exercise training effect on TBC1D4 phosphorylation in the present study, which is different from a previous study (2) reporting that insulin-stimulated Akt Ser473 phosphorylation could not explain the observed exercise training-induced changes in TBC1D4 phosphorylation. This difference between the two studies may be related to the use of hyperinsulinemic euglycemic clamp (2) and an oral glucose intake. In addition, the observed higher plasma insulin concentration in the trained state than in the untrained 15 minutes after glucose intake may have contributed to the enhanced Akt Ser473 phosphorylation response in the trained state in the present study. The observed increase in GLUT4 and HKII protein content with exercise training in the current study is in line with previous studies showing that exercise training increases skeletal muscle GLUT4 protein content in aged subjects (1,28). Together these observations suggest that increased capacity for glucose transport and phosphorylation as well as acute regulation of glucose uptake in skeletal muscle will contribute to exercise training-induced improved regulation of skeletal muscle glucose uptake in elderly subjects.

The observation that glucose intake did not affect GS activity in the elderly subjects in the untrained state is in contrast with previous observations in young subjects (16) suggesting an age-associated impairment in GS regulation (24). However, the finding that glucose intake induced an increase in GS activity in the elderly subjects after the exercise training period in the present study may indicate that exercise training restored the ability of the aged muscle to activate GS in response to glucose intake. This is in accordance with a previous study reporting improved nonoxidative glucose metabolism in elderly subjects after a training period (32).

The observation that glucose intake in the untrained state did not change GS site 2 + 2a phosphorylation is in contrast to the previously observed GS site 2 + 2 dephosphorylation in skeletal muscle of middle aged (25) and young subjects (24,25,33), but is in line with the unchanged GS activity in the present study. Furthermore, the observed dephosphorylation of GS site 2 + 2a in response to glucose intake after the exercise training period is in accordance with the observed increase in GS activity upon glucose intake in the exercise trained state. The present observation that glucose uptake-stimulated phosphorylation of GSK-3β was similar before and after the exercise training period suggests that a difference in GSK-3 is not a likely explanation for the changes in GS regulation with exercise training. However, previous studies have indicated that GS site 2 + 2a hyperphosphorylation may contribute to impairment of insulin-induced GS activation in T2D patients (25) and in young subjects after 1 week of bed rest (33). This suggests that the exercise training-induced change in glucose intake-stimulated site 2 + 2a phosphorylation may explain the associated changes in GS activation in the present study. The increased muscle glycogen concentration with exercise training is a possible reason for the elevated level of GS 2 + 2a phosphorylation after exercise training, because GS site 2 + 2a is regulated by the glycogen level (34). But the lack of change in muscle glycogen after glucose intake does not support that muscle glycogen influenced the acute changes in GS site 2 + 2a phosphorylation in the present study.

The finding that PDHa activity was unaffected by the glucose intake in the untrained state is not in accordance with the previously reported increase in PDHa activity in young subjects during an OGTT (16). It is possible that the elderly subjects in the present study had an impaired PDH regulation in response to glucose intake in the untrained state, but this needs to be further investigated. The finding, that glucose intake even reduced skeletal muscle PDHa activity in the trained state, was unexpected and has not previously been reported. However, it may suggest that downregulation of PDHa activity combined with increased GS activity after glucose intake in the trained state serves to reduce carbohydrate oxidation ensuring that carbohydrate enters glycogenesis rather than oxidation.

The observation that the exercise training-induced elevation in basal PDH-E1α phosphorylation was associated with a similar increase in PDH-E1α protein content indicates that the same fraction of PDH-E1α molecules was phosphorylated in the trained and the untrained state. Furthermore, the concomitant exercise training-induced increase in PDK2 protein suggests that the enhanced PDH-E1α phosphorylation was executed by PDK2. In addition, the observed overall tendency for reduction in PDK4 protein in response to glucose intake may be expected to influence the phosphorylation status of PDH-E1α and thus affect PDHa activity. However the lack of change in PDH-E1α phosphorylation with glucose intake shows that the regulation of PDHa activity in the trained state was independent of the phosphorylation status of site 1 and 2 on PDH-E1α. As PDH-E1α has been shown to be phosphorylated on at least two further sites (35) as well as being regulated by acetylation (35), additional post-translational modifications may explain the observed regulation of PDHa activity in the trained state.

While a hyperinsulinemic euglycemic clamp is seen as the golden standard in investigating insulin-stimulated glucose regulation and can provide highly important information, it is also an un-physiological situation. The OGTT was therefore used in the current study to reflect glucose metabolism during a physiological setting with an endogenous insulin response to a standard glucose load. Furthermore, the present findings that exercise training induced adaptations in several metabolic parameters underline that aged skeletal muscle can adapt to exercise training. However, it should be mentioned that the subjects were healthy elderly men and whether similar effects will be present in healthy elderly women or T2D patients remain to be determined.

In conclusion, the present findings suggest that exercise training-improved glucose regulation in elderly subjects is associated with enhanced potential for GLUT4 translocation, glucose phosphorylation and intracellular glucose removal to glycogen synthesis rather than oxidation.
Supplementary Material
Supplementary material can be found at: http://biomedgerontology.oxfordjournals.org/

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Conflict of Interest
None declared.

References
