Contraction activates glucose uptake and glycogen synthase normally in muscles from dexamethasone-treated rats

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Ruzzin, Jérôme, and Jørgen Jensen. Contraction activates glucose uptake and glycogen synthase normally in muscles from dexamethasone-treated rats. Am J Physiol Endocrinol Metab 289: E241–E250, 2005. First published March 1, 2005; doi:10.1152/ajpendo.00587.2004.—Glucocorticoids cause insulin resistance in skeletal muscle. The aims of the present study were to investigate the effects of contraction on glucose uptake, insulin signaling, and regulation of glycogen synthesis in skeletal muscles from rats treated with the glucocorticoid analog dexamethasone (1 mg·kg−1·day−1 ip for 12 days). Insulin resistance in dexamethasone-treated rats was confirmed by reduced insulin-stimulated glucose uptake (~35%), glycogen synthesis (~70%), glycogen synthase activation (~80%), and PKB Ser473 phosphorylation (~40%). Chronic dexamethasone treatment did not impair glucose uptake during contraction in soleus or epitrochlearis muscles. In epitrochlearis (but not in soleus), the presence of insulin during contraction enhanced glucose uptake to similar levels in control and dexamethasone-treated rats. Contraction also increased glycogen synthase fractional activity and dephosphorylated glycogen synthase at Ser645, Ser649, Ser653, and Ser657 normally in muscles from dexamethasone-treated rats. After contraction, insulin-stimulated glycogen synthesis was completely restored in epitrochlearis and improved in soleus from dexamethasone-treated rats. Contraction did not increase insulin-stimulated PKB Ser473 or glycogen synthase kinase-3 (GSK-3) phosphorylation. Instead, contraction increased GSK-3β Ser2 phosphorylation in epitrochlearis (but not in soleus) in muscles from control and dexamethasone-treated rats. In conclusion, contraction stimulates glucose uptake normally in dexamethasone-induced insulin resistant muscles. After contraction, insulin’s ability to stimulate glycogen synthesis was completely restored in epitrochlearis and improved in soleus from dexamethasone-treated rats.

insulin signaling; glycogen synthase kinase-3; protein kinase B; glucocorticoids; AMP-activated protein kinase

GLUCOCORTICOIDS INDUCE INSULIN RESISTANCE as observed in patients with Cushing’s syndrome and during glucocorticoid treatment (38, 39). It is also assumed that glucocorticoids mediate insulin resistance induced by mental stress (42) and high-fat diet (27). Recently, glucocorticoids have been attracted new interest, as 11β-hydroxysteroid dehydrogenase, which converts inactive cortisone to the biological active cortisol, seems to play a physiological role for development of obesity and insulin resistance (49). Furthermore, muscle cells with high expression of glucocorticoid receptor have reduced insulin sensitivity (54). Skeletal muscles dispose of the major part of glucose during insulin stimulation (9), and glucocorticoids impair metabolic regulation, at least in part, by reducing insulin-stimulated glucose uptake in skeletal muscles (11, 52).

Contraction, like insulin, stimulates glucose uptake by translocation of GLUT4 from intracellular vesicles to the cell membrane (31). The two stimuli, however, stimulate glucose uptake via different mechanisms. Whereas insulin-stimulated glucose uptake requires activation of phosphatidylinositol 3-kinase (PI 3-kinase), contraction-stimulated glucose uptake occurs independently of PI 3-kinase (31, 53). The fact that contraction stimulates glucose uptake by an insulin-independent mechanism may also explain why some studies have reported that contraction normally stimulates glucose uptake in insulin-resistant skeletal muscles from obese Zucker rats (5, 14). However, reduced contraction-stimulated glucose uptake has also been reported in obese Zucker rats (12, 19) and when insulin resistance is induced by a diet rich in fat or sucrose (17, 18, 25, 41).

For physiological regulation of blood glucose concentration, excess glucose has to be taken up by skeletal muscles and incorporated into glycogen (47). Exercise stimulates glycogen breakdown and increases glucose uptake and glycogen synthase fractional activity for several hours (16, 22). Part of the glycogen is replenished even in the absence of insulin. However, probably the most important effect of exercise for type 2 diabetes patients is the improvement of insulin’s ability to stimulate glucose uptake and activate glycogen synthase for many hours after exercise. Regulation of glycogen synthase is complex and involves the phosphorylation of at least nine serine residues by different kinases (7). Particularly important seem to be the sites 3a, 3b, 3c, and 4 (Ser645, Ser649, Ser653, and Ser657 in human glycogen synthase), which are phosphorylated by GSK-3. In muscles from dexamethasone-treated rats, insulin is unable to activate glycogen synthase (20). Curiously, although a hallmark of type 2 diabetes is impaired glycogen synthase activation (47), few studies have focused on glycogen synthase activation and dephosphorylation in insulin-resistant muscles after exercise. In contrast to the lack of knowledge about metabolic regulation by contraction in muscles exposed to glucocorticoids, the effects of glucocorticoids on insulin signaling are well documented. Dexamethasone treatment does not impair insulin-stimulated tyrosine phosphorylation of the insulin receptor, but activation of PI 3-kinase is severely impaired (43).

A number of studies have reported that muscle contraction and insulin have an additive effect on muscle glucose uptake (3, 16, 53). Interestingly, the combination of contraction and insulin has even been reported to stimulate glucose uptake to the same extent in insulin-resistant muscles from obese Zucker...
rats (14) and in muscles from high-fat diet-fed rats (18). However, not all studies find that insulin-stimulated glucose uptake after contraction is increased to the same level in muscles from obese and lean Zucker rats, although insulin and contraction stimulate glucose uptake additively (12, 19). In humans, both epidemiological and intervention studies also support the idea that exercise improves insulin sensitivity (46), and the mechanisms for improved metabolic regulation after exercise has attracted large attention. Whether contraction is able to improve insulin action and insulin signaling in dexamethasone-treated rats remains to be elucidated.

Exercise is a cornerstone for treatment of insulin resistance. Knowledge about the effects of contraction on metabolic regulation in muscles made insulin resistant by glucocorticoid is lacking. Therefore, the aims of the present study were to investigate in muscles from dexamethasone-treated rats 1) whether contraction normally stimulates glucose uptake, 2) whether contraction dephosphorylates and activates glycogen synthase, 3) whether muscle contraction enhances insulin action and insulin signaling, and 4) whether insulin’s ability to stimulate glycogen synthesis is improved after contraction.

MATERIALS AND METHODS

Materials. Dexamethasone, fraction V bovine serum albumin (BSA), HEPES, and pyruvate were obtained from Sigma Chemical (St. Louis, MO). Insulin (Actrapid) was from Novo Nordisk (Bagvaerd, Denmark). d-Glucose was obtained from Merck (Darmstadt, Germany). Amyloglucosidase was from Boehringer Mannheim (Indianapolis, IN). 2-Deoxy-d-[3H]glucose (25.5 Ci/mmol), [U-14C]glucose (298 Ci/mmol), and [1-14C]mannitol (51.5 Ci/mmol) were purchased from DuPont-New England (Boston, MA). Anti-phospho-NSK-3α/β (Ser21/Ser9), anti-phospho-PKB (Ser473), and anti-phospho-5’-AMP-activated protein kinase-α (AMPKα)/1/2 (Thr172) were from Cell Signaling (Beverly, MA). Anti-phospho-glycogen synthase (Ser465, Ser469, Ser463, Ser672) was from Oncogene (San Diego, CA). Anti-rabbit horseradish peroxidase (HRP)-linked secondary antibody was from New England Biolabs (Beverly, MA). DC protein assay kit was from Bio-Rad Laboratories (Hercules, CA).

Animals. Male Wistar rats weighing ~250 g were obtained from B & K Universal (Bk1:West, Nittedal, Norway). The rats were housed individually and fed chow and water ad libitum. The animal room was maintained at 21°C with a 12:12-h light-dark cycle.

After arrival, animals were acclimatized for 1 wk and weight matched. Rats were assigned to receive a daily (10 AM) intraperitoneal injection of dexamethasone (1 mg/kg) dissolved in 0.9% NaCl or saline for 12 days. This concentration is known to cause insulin-resistant skeletal muscles (43). All procedures were approved and performed in accordance with the laws and regulations controlling experiments on live animals in Norway and the European Convention for the Protection of Vertebrate Animals used in Experimental and Other Scientific Purposes.

Muscle preparation. Rats were given 5 g of chow at 4 PM the day preceding the experiment. Between 10 AM and 12 noon, rats were anesthetized by an intraperitoneal injection of ~0.5 ml of pentobarbital sodium (50 mg/ml). Intact epichonchleins and soleus muscle strips were dissected out and mounted at their approximate resting lengths in normal and contraction apparatuses. Muscles were preincubated for ~30 min in 3.5 ml of Krebs-Henseleit buffer containing 5.5 mM glucose, 2 mM pyruvate, 5 mM HEPES, and 0.1% bovine serum albumin, pH 7.4. Contracted muscles were stimulated electrically with impulse trains of 200 ms (100 Hz; square wave pulses of 0.2 ms duration and 10 V amplitude) delivered every 2 s for 30 min, as described previously (3). Incubation buffers were continuously gassed with 95% O2-5% CO2 and maintained at 30°C in a water bath. Immediately after incubation, muscles were removed from the apparatuses, blotted on filter paper, frozen in liquid nitrogen, and stored at −70°C until analysis.

Glucose uptake. After preincubation, glucose uptake was measured in resting muscles or during contraction for 30 min in Krebs-Henseleit buffer containing 0.25 μCi/ml 2-deoxy-d-[3H]glucose and 0.1 μCi/ml [1-14C]mannitol with or without 60 nmol/l insulin. Muscles were freeze-dried, weighed, and dissolved in 600 μl of 1 M KOH for 20 min at 70°C. Then, 400 μl of dissolved muscles were added to 3 ml of scintillation solution (Hionic Fluor; Packard Bioscience, Groningen, The Netherlands) mixed, and counted for radioactivity (TRICARB 1900 TR, Packard).

Glycogen synthesis. Glycogen synthesis was measured after 30 min of contraction or rest. For measurement of glycogen synthesis, skeletal muscles were incubated for 60 min in Krebs-Henseleit buffer containing 0.2 μCi/ml d-[1-14C]glucose with or without 60 nmol/l insulin, as described previously (15). Freeze-dried muscles were weighed and dissolved in 600 μl of 1 M KOH at 70°C for 20 min. To 500 μl of KOH digest, 100 μl of saturated Na2SO4 and 100 μl of 12 mM G-6-P were added and mixed. Cold ethanol (~70°C, 1.5 ml) was added and the glycogen precipitated overnight at −20°C. After centrifugation (3,000 g for 20 min at 4°C) the supernatant was discarded and the precipitate dissolved in 500 μl of distilled water for 20 min at 70°C, and the glycogen was reprecipitated with cold ethanol (1 ml) for 60 min at ~20°C. The new precipitates were dissolved in 300 μl of distilled water, and 250 μl of the solution were added to 3 ml of scintillation solution (Hionic Fluor) and counted for radioactivity (TRICARB 1900 TR).

Glycogen synthase activity. Glycogen synthase activity was measured in muscles incubated with and without insulin. Glycogen synthase activity was analyzed as described before (15). Glycogen synthase activity was measured with 0.17 mM glucose 6-phosphate (G-6-P) and with 12 mM G-6-P (total activity). Glycogen synthase fractional activity (%) was calculated as the activity of the enzyme with 0.17 mM G-6-P divided by the total activity and multiplied by 100.

Glycogen concentration. For analysis of skeletal muscle glycogen content, 100 μl of the KOH digest were neutralized with 25 μl of 7 M acetic acid, and 500 μl of 0.3 M acetate buffer (pH 4.8) containing 0.2 U/ml amylglucosidase were added. The glycogen was hydrolyzed at 37°C for 3 h, and the glucose formed was determined according to Lowry and Passonneau (30).

Western blot. Skeletal muscles were weighed and homogenized (1 mg wet wt: 25 μl) in ice-cold buffer containing 50 mM HEPES, 150 mM NaCl, 10 mM Na2PO4, 30 mM NaF, 1 mM Na2VO4, 10 mM EDTA, 2.5 mM benzamidine, 0.5 μg/10 mg muscle of protease inhibitor cocktail (Sigma P-8430), and 1% Triton X-100 (41). Homogenates were rotated for 1 h at 4°C and centrifuged (11,500 g at 4°C for 10 min), as described before (53). Protein concentration in supernatants was determined by DC protein assay (Bio-Rad Laboratories) according to the instruction manual. Supernatants were diluted to a protein concentration of 3 μg/μl aliquoted, and stored at −70°C.

For electrophoresis, aliquots were prepared with Laemmli buffer (28), heated at 95°C for 5 min to completely dissociate proteins, and centrifuged at 22,000 g for 15 s. Muscle proteins were separated by electrophoresis in 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Transfer of proteins from the gel onto the polyvinylidene difluoride (PVDF) membrane was performed for 1 h at 200 V in aMini-trans-Blot cell with Bio-Ice cooling unit (Bio-Rad laboratories). The transfer buffer contained 25 mM Tris, 192 mM glycine, and 10% methanol. Membranes were washed (3 × 10 min) in PBS-T (80 mM Na2HPO4, 20 mM NaH2PO4, 100 mM NaCl, 0.1% Tween 20). To avoid nonspecific binding of antibody to the PVDF, membranes were blocked in PBS-T containing 5% nonfat milk for 2 h at room temperature. After blocking, membranes were washed in PBS-T (2 × 30 s), and incubated overnight at 4°C with the primary antibody. After a wash in PBS-T (6 × 10 min), membranes were incubated with the appropriate secondary antibody conjugated to HRP for 1 h at room
temperature and washed again (6 × 10 min). Antibody binding was detected by enhanced chemiluminescence (Amersham Pharmacia Biotech, Buckinghamshire, UK), as described by the manufacturer. Blots were scanned (Scan Jet Ilcx scanner, Hewlett-Packard) and signals quantified by densitometry with Scion Image software (Scion, Frederick, MD).

Statistics. Data are presented as means ± SE. One-way analysis of variance (ANOVA) was performed to determine the differences between experimental groups. When ANOVA revealed significant differences, further analysis was performed using least significant difference post hoc tests. P values <0.05 were considered as significant.

RESULTS

Glucose uptake during muscle contraction. Insulin-stimulated glucose uptake was reduced by ~38% in soleus and by ~32% in epitrochlearis muscles from dexamethasone-treated rats compared with control rats (Fig. 1). On the other hand, glucose uptake during contraction was similar in muscles from control and dexamethasone-treated rats (Fig. 1). In epitrochlearis, the combination of contraction and insulin stimulated glucose uptake additively. Interestingly, when insulin was present during contraction, a similar rate of glucose uptake was achieved in epitrochlearis from control and dexamethasone-treated rats (Fig. 1 B). In soleus muscles, the combination of contraction and insulin did not have an additive effect on glucose uptake (Fig. 1 A).

Muscle glycogen concentration. Glycogen content was slightly higher in muscles from dexamethasone-treated rats than in the corresponding muscles from control rats (Fig. 2). Contraction decreased the glycogen content to similar level in soleus from control and dexamethasone-treated rats (Fig. 2A). In epitrochlearis muscles from control and dexamethasone-treated rats, contraction decreased basal glycogen by ~110 mmol/kg dry wt (Fig. 2B). Insulin did not prevent contraction-stimulated glycogen breakdown in any muscle from control and dexamethasone-treated rats.

PKB phosphorylation. PKB Ser473 phosphorylation was barely detectable in the absence of insulin in muscles from...
control and dexamethasone-treated rats (Fig. 3, A and B). Dexamethasone treatment decreased insulin-stimulated PKB phosphorylation in soleus (Fig. 3C) and epitrochlearis muscles (Fig. 3D). Contraction did not increase PKB Ser\textsuperscript{473} phosphorylation in soleus (Fig. 3A) or in epitrochlearis muscles (Fig. 3B) from control or dexamethasone-treated rats. In soleus muscles, contraction did not influence insulin-stimulated PKB phosphorylation (Fig. 3C). Despite the improvement of insulin action observed in epitrochlearis muscles from dexamethasone-treated rats, contraction did not increase insulin-stimulated PKB phosphorylation (Fig. 3D). In fact, contraction slightly reduced insulin-stimulated PKB phosphorylation in epitrochlearis muscles from control rats (Fig. 3D).

**GSK-3α phosphorylation and GSK-3β phosphorylation.** In the absence of insulin, GSK-3β Ser\textsuperscript{373} phosphorylation was similar in muscles from control and dexamethasone-treated rats (Fig. 4A and B). Insulin-stimulated GSK-3β phosphorylation was slightly reduced in soleus (Fig. 4C) but not in epitrochlearis muscles (Fig. 4D) from dexamethasone-treated rats. Similar results were found for GSK-3α Ser\textsuperscript{21} phosphorylation (Table 1). In soleus, contraction did not increase the phosphorylation of GSK-3β (Fig. 4A) or GSK-3α (Table 1) in muscles from control or dexamethasone-treated rats. In epitrochlearis muscles, contraction increased phosphorylation of GSK-3β (Fig. 4B) and GSK-3α (Table 1). In both soleus and epitrochlearis muscles, contraction decreased insulin-stimulated phosphorylation of GSK-3β (Fig. 4C and D) and GSK-3α (Table 1) in muscles from control rats as well as in muscles from dexamethasone-treated rats.

**Glycogen synthesis after contraction.** In soleus muscles, basal glycogen synthesis was similar in control and dexamethasone-treated rats (Fig. 5A). Dexamethasone treatment reduced insulin-stimulated glycogen synthesis in soleus (Fig. 5A) and epitrochlearis muscles (Fig. 5C). After contraction, glycogen synthesis was increased to similar levels in soleus muscles from control and dexamethasone-treated rats in the absence of insulin (Fig. 5A). In soleus muscles from control rats, the combination of contraction and insulin did not increase glycogen synthesis above insulin alone (Fig. 5A). In soleus muscles

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**Fig. 3.** PKB Ser\textsuperscript{473} phosphorylation in rested or contracted muscles from control and dexamethasone-treated rats incubated with or without insulin. Skeletal muscles from control (open bars) and dexamethasone-treated rats (filled bars) were incubated for 30 min in the absence or presence of 60 nmol/l insulin when contracted or rested. Muscles from control rats incubated with 60 nmol/l insulin and without contraction were used as 100%, and all values were calculated as percentage of this. Blots show phosphorylation of PKB (pPKB) in control (C) and dexamethasone-treated rats (D). A: effects of dexamethasone treatment on PKB phosphorylation in rested or contracted soleus; n = 6–7. B: effects of dexamethasone treatment on PKB phosphorylation in rested or contracted epitrochlearis; n = 4–7. C: effects of dexamethasone treatment on insulin-stimulated PKB phosphorylation in rested or contracted soleus; n = 6–7. D: effects of dexamethasone treatment on insulin-stimulated PKB phosphorylation in rested or contracted epitrochlearis; n = 6–8. Values are means ± SE. \(a\) P < 0.0001 vs. other groups; \(b\) P < 0.04 vs. muscle from control rats incubated similarly; \(c\) P < 0.05 vs. rested muscle incubated with insulin from rats exposed to similar treatment.

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**Fig. 4.** GSK-3β phosphorylation in rested or contracted muscles from control and dexamethasone-treated rats incubated with or without insulin. Skeletal muscles from control (open bars) and dexamethasone-treated rats (filled bars) were incubated for 30 min in the absence or presence of 60 nmol/l insulin when contracted or rested. Muscles from control rats incubated with 60 nmol/l insulin and without contraction were used as 100%, and all values were calculated as percentage of this. Blots show phosphorylation of GSK-3β (pGSK-3β) in control (C) and dexamethasone-treated rats (D). A: effects of dexamethasone treatment on GSK-3β phosphorylation in rested or contracted soleus; n = 6–7. B: effects of dexamethasone treatment on GSK-3β phosphorylation in rested or contracted epitrochlearis; n = 4–7. C: effects of dexamethasone treatment on insulin-stimulated GSK-3β phosphorylation in rested or contracted soleus; n = 6–7. D: effects of dexamethasone treatment on insulin-stimulated GSK-3β phosphorylation in rested or contracted epitrochlearis; n = 6–8. Values are means ± SE. \(a\) P < 0.0001 vs. other groups; \(b\) P < 0.04 vs. muscle from control rats incubated similarly; \(c\) P < 0.05 vs. rested muscle incubated with insulin from rats exposed to similar treatment.
from dexamethasone-treated rats, on the other hand, contraction and insulin had an additive effect on glycogen synthesis. However, the rate of glycogen synthesis in the presence of insulin after contraction remained slightly lower in dexamethasone-treated rats than in control rats (Fig. 5A). When insulin was present after contraction in epitrochlearis, the rate of glycogen synthesis was similar in muscles from control and dexamethasone-treated rats (Fig. 5C).

**Glycogen synthase fractional activity.** Basal glycogen synthase fractional activity was similar in soleus muscles from control and dexamethasone-treated rats (Fig. 5B). In soleus muscles, the combination of contraction and insulin enhanced glycogen synthase fractional activity above that observed by contraction or insulin alone (Fig. 5B). However, glycogen synthase fractional activity remained lower in soleus from dexamethasone-treated rats than in control rats when contraction and insulin were combined (Fig. 5B). In epitrochlearis muscles, combined contraction and insulin increased glycogen synthase fractional activity to similar levels in muscles from control and dexamethasone-treated rats (Fig. 5D).

**Glycogen synthase phosphorylation.** In the absence of insulin, glycogen synthase Ser645, Ser649, Ser653, and Ser657 phosphorylation was similar in soleus muscles from control and dexamethasone-treated rats (Fig. 6A) and in epitrochlearis muscles (Fig. 6B). Contraction decreased glycogen synthase Ser645, Ser649, Ser653, and Ser657 phosphorylation in soleus (Fig. 6A) to a similar low level in dexamethasone-treated rats (Fig. 6B). In soleus muscles, the combination of contraction and insulin enhanced glycogen synthase fractional activity above that observed by contraction or insulin alone (Fig. 5B). However, glycogen synthase fractional activity remained lower in soleus from dexamethasone-treated rats than in control rats when contraction and insulin were combined (Fig. 5B). In epitrochlearis muscles, combined contraction and insulin increased glycogen synthase fractional activity to similar levels in muscles from control and dexamethasone-treated rats (Fig. 5D).

**Fig. 4. Glycogen synthase kinase-3β Ser9 phosphorylation (pGSK-3β) in rested or contracted muscles from control and dexamethasone-treated rats incubated with or without insulin.** Skeletal muscles from control (open bars) and dexamethasone-treated rats (filled bars) were incubated for 30 min in the absence or presence of 60 nmol/l insulin when contracted or rested. Muscles from control rats incubated with 60 nmol/l insulin and without contraction were used as 100%, and all values were calculated as percentage of this. Blots show phosphorylation of GSK-3β in control (C) and dexamethasone-treated rats (D). A: effects of dexamethasone treatment on GSK-3β phosphorylation in rested or contracted soleus; n = 6–7. B: effects of dexamethasone treatment on GSK-3β phosphorylation in rested or contracted epitrochlearis; n = 4–9. C: effects of dexamethasone treatment on insulin-stimulated GSK-3β phosphorylation in rested or contracted soleus; n = 6–7. D: effects of dexamethasone treatment on insulin-stimulated GSK-3β phosphorylation in rested or contracted epitrochlearis; n = 6–8. Values are means ± SE. aP < 0.015 vs. other groups; bP < 0.006 vs. muscle from control rats incubated similarly; cP < 0.05 vs. rested muscle incubated with insulin from rats exposed to similar treatment; dP < 0.0001 vs. contracted muscle incubated without insulin from rats exposed to similar treatment.
muscles from control and dexamethasone-treated rats. In epitrochlearis muscles, contraction also decreased glycogen synthase phosphorylation to a low level in muscles from control and dexamethasone-treated rats (Fig. 6B). In soleus and epitrochlearis muscles, the combination of contraction and insulin did not decrease glycogen synthase phosphorylation more than contraction alone (Fig. 6, C and D).

AMPKα phosphorylation. AMPKα phosphorylation was not detectable in resting muscles from control and dexamethasone-treated rats (Fig. 7). Contraction increased AMPKα phosphorylation to a similar level in soleus muscles from control and dexamethasone-treated rats (100.0 ± 25.7 vs. 102.0 ± 13.0%, respectively, n = 6; Fig. 7A). Surprisingly, AMPKα Thr172 phosphorylation was lower in epitrochlearis muscles from dexamethasone-treated rats than from control rats (100.0 ± 18.0 vs. 42.6 ± 10.8%, P < 0.002, n = 7; Fig. 7B).

**DISCUSSION**

This is the first demonstration that glucose uptake is stimulated normally during contraction in insulin-resistant muscles from dexamethasone-treated rats. Following contraction, furthermore, glycogen synthase fractional activity was increased and glycogen synthase Ser645, Ser649, Ser653, and Ser657 phosphorylation decreased to a similar extent in muscles from control and dexamethasone-treated rats. Contraction and insulin together had an additive effect on glucose uptake in epitrochlearis and increased glucose uptake to similar levels in muscles from control and dexamethasone-treated rats. Despite the fact that glucose uptake and glycogen synthesis were normalized in epitrochlearis muscles from dexamethasone-treated rats when contraction and insulin were combined, insulin-stimulated phosphorylation of PKB was still reduced. In soleus from dexamethasone-treated rats, insulin-stimulated glycogen synthesis and glycogen synthase activation were increased after contraction, whereas contraction did not improve insulin-stimulated glucose uptake.

Interestingly, glucose uptake during contraction reached the same level in both the slow-twitch soleus muscles and the

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**Table 1. Effects of contraction and insulin on GSK-3α phosphorylation in skeletal muscles**

<table>
<thead>
<tr>
<th>GSK-3α Ser21 Phosphorylation</th>
<th>Soleus</th>
<th>Epitrochlearis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Dexamethasone</td>
<td>Control</td>
</tr>
<tr>
<td>Basal</td>
<td>&lt;5*(4)</td>
<td>&lt;5*(4)</td>
</tr>
<tr>
<td>Insulin</td>
<td>100.0±4.2 (6)</td>
<td>77.4±4.3† (6)</td>
</tr>
<tr>
<td>Contraction</td>
<td>&lt;5*(4)</td>
<td>&lt;5*(4)</td>
</tr>
<tr>
<td>Con+Ins</td>
<td>81.0±7.8*(6)</td>
<td>56.2±5.0*† (6)</td>
</tr>
</tbody>
</table>

Values are means ± SE; nos. of muscles in each group are given in parentheses. Muscles from control rats incubated with insulin were set to 100% and all other values were calculated as % of this. GSK-3α, glycogen synthase kinase-3α; Con+Ins, insulin present during contraction. Muscles were incubated as described in Fig. 4. *P < 0.05 vs. insulin from rats exposed to similar treatment. †P < 0.03 vs. muscle from control rats incubated similarly. ‡P < 0.006 vs. basal from rats exposed to similar treatment.

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fast-twitch epitrochlearis muscles from dexamethasone-treated rats as in the respective muscles from control rats. Insulin-stimulated glucose uptake, on the other hand, was reduced as expected (11, 20, 52). Some studies have reported that contraction normally stimulates glucose uptake in muscles from obese Zucker rats (5, 14), whereas others have reported slightly reduced contraction-stimulated glucose uptake in muscles from obese Zucker rats (12, 19). Contraction-stimulated glucose uptake is also reduced when insulin resistance is induced by a high-fat diet (17, 18, 41) or a high-sucrose diet (25). Contraction, in contrast to insulin, stimulates glucose uptake via PI 3-kinase-independent mechanisms (31, 53). Even so, contraction-stimulated glucose uptake is impaired in some models of insulin resistance, but the reason for these differences in contraction-stimulated glucose uptake is not obvious. Glucocorticoids regulate expression of a number of genes, and our data show that the signaling pathway for contraction-stimulated glucose uptake remains intact during dexamethasone treatment.

The above-mentioned conclusion may at first glance seem to contradict studies in rats fed a high-fat diet where contraction-stimulated glucose uptake was impaired (17, 18, 41), and insulin resistance could be prevented by blockade of glucocorticoid receptors (27). However, it has never been shown that contraction-stimulated glucose uptake remains normal when glucocorticoids are blocked during fat feeding. Therefore, it is possible that fat feeding impairs insulin- and contraction-stimulated glucose uptake via different mechanisms. Furthermore, it cannot be excluded that in vivo blockade of glucocorticoid receptors also influences metabolic regulation in skeletal muscles indirectly, which makes comparisons between the two situations complex.

To further investigate the effects of contraction, AMPKα Thr172 phosphorylation was investigated. In soleus, contraction increased AMPKα phosphorylation to a similar extent in control and dexamethasone-treated rats, whereas AMPKα phosphorylation was lower in epitrochlearis muscles from dexamethasone-treated rats. This reduced AMPKα phosphorylation in epitrochlearis from dexamethasone-treated rats was unexpected. However, glycogen content was slightly higher in these muscles, and elevated glycogen content has been reported to reduce AMPK activation (10). Furthermore, the fact that...
AMPKα phosphorylation was reduced in epitrochlearis from dexamethasone-treated rats, despite normal glucose uptake, is not a contradiction, because contraction normally stimulates glucose uptake in muscles lacking AMPKα (24, 34). On the other hand, hypoxia and 5-aminoimidazole-4-carboxamide-1-β-d-ribofuranoside do not stimulate glucose uptake in muscles lacking AMPKα activity (24, 34). The fact that contraction is able to stimulate glucose uptake via other mechanisms than hypoxia may also explain why hypoxia-induced glucose uptake is reduced in dexamethasone-treated rats (52) whereas we find normal glucose uptake during contraction.

After dexamethasone treatment, insulin was unable to activate glycogen synthase, and this supports previous studies (20, 29). Importantly, muscle contraction caused normal activation of glycogen synthase in muscles from dexamethasone-treated rats. Contraction is a potent activator of glycogen breakdown, and glycogen content strongly influences glycogen synthase fractional activity (8, 36). Contraction decreased the glycogen content in soleus from control and dexamethasone-treated rats to similar levels and caused a substantial decrease in epitrochlearis from both groups, and we believe that the reduced glycogen content is important for the activation of glycogen synthase after contraction. Contraction reduced glycogen synthase phosphorylation at Ser645, Ser649, Ser653, and Ser657, which are target sites for GSK-3, to a similar extent in muscles from control and dexamethasone-treated rats. Dephosphorylation of these serines, corresponding to sites 3a, 3b, 3c, and 4, are critical for the activation of glycogen synthase (40), and their normal dephosphorylation supports the normal activation of glycogen synthase in muscles from dexamethasone-treated rats.

Contraction increased GSK-3 phosphorylation in epitrochlearis. Contraction could therefore, theoretically activate glycogen synthase by decreasing GSK-3 activity. However, several points argue against this idea. First, insulin caused normal phosphorylation of GSK-3 in epitrochlearis from dexamethasone-treated rats without triggering glycogen synthase dephosphorylation and activation. Second, glycogen synthase is dephosphorylated and activated in control, even though GSK-3 is not phosphorylated. Glycogen synthase is dephosphorylated by protein phosphatase-1 (PP-1) (40), and contraction does not activate glycogen synthase in mice lacking the RGL regulatory subunit of PP-1 (1). We therefore suggest that activation of PP-1 causes dephosphorylation of glycogen synthase and that contraction activates PP-1 normally in dexamethasone-treated rats.

The combination of muscle contraction and insulin had an additive effect on glucose uptake in epitrochlearis from control rats, which agrees with previous studies (3, 35, 53). Interestingly, when insulin was present during contraction, glucose uptake reached similar levels in epitrochlearis muscles from dexamethasone-treated rats and normal rats. This normalization of insulin action has previously been reported in obese Zucker rats and high-fat-fed rats (14, 18). In soleus from control rats, contraction and insulin did not have an additive effect on glucose uptake. Although contraction stimulates glucose uptake normally in soleus muscles from dexamethasone-treated rats, the combination of contraction and insulin did not increase glucose uptake above that of insulin alone in muscles from dexamethasone-treated rats. Training has consistently been shown to improve insulin sensitivity in fast-twitch muscles from obese Zucker rats (13, 21, 48). Increased insulin action after physical training is less evident in soleus muscles, and studies have reported increased (44, 48) or unchanged (13, 21) insulin-stimulated glucose uptake in soleus from obese Zucker rats after training. The intensity of contraction determines glucose uptake (2, 31), and we cannot exclude the possibility that more intense electrical stimulations would have restored insulin-stimulated glucose uptake in soleus from dexamethasone-treated rats.

A new important finding of the current study is that contraction completely reversed insulin’s ability to stimulate glycogen synthesis and glycogen synthase activation in epitrochlearis muscles from dexamethasone-treated rats. Contraction also improved insulin-stimulated glycogen synthesis and glycogen synthase activation in soleus from dexamethasone-treated rats but not quite to the same level as in control rats. Exercise is a powerful tool to prevent insulin resistance, and the search for the mechanisms behind exercise-mediated improvement of insulin action is consequently important. Recently, it has been hypothesized that activation of glucocorticoid receptors plays a physiological role in development of insulin resistance (54).

The fact that contraction normally stimulates glucose uptake and improves insulin action after dexamethasone treatment, as is the case in insulin-resistant humans, supports this hypothesis.

Dexamethasone treatment decreased insulin-stimulated PKB phosphorylation in skeletal muscles, which agrees with our previous observation (Ruzzin J, Wagman AS, and Jensen J, unpublished observations). PKB is important for insulin-stimulated glucose uptake (23, 50, 51), and a number of studies have shown reduced PKB phosphorylation in insulin-resistant muscles (26, 37). We hypothesized that contraction might restore insulin-stimulated PKB phosphorylation in muscles from dexamethasone-treated rats; however, muscle contraction did not enhance insulin-stimulated PKB phosphorylation in skeletal muscles from dexamethasone-treated rats. Our results agree with previous data showing that exercise training increases insulin-stimulated glucose uptake in obese Zucker rats without increasing PKB phosphorylation (6, 44).

PKB was not phosphorylated by contraction in muscles from control or dexamethasone-treated rats, which supports the idea that contraction stimulates glucose uptake independently of PKB (4, 32). Contraction on the other hand, increased GSK-3α Ser21 and GSK-3β Ser32 phosphorylation in the fast-twitch epitrochlearis muscle but not in the slow-twitch soleus. It was
initially reported that contraction deactivates GSK-3 without increasing GSK-3 phosphorylation (33). However, a more recent study has shown that contraction increases GSK-3α and GSK-3β phosphorylation in the fast-twitch extensor digitorum longus muscle (45). The present study is the first to compare the effects of contraction on GSK-3 phosphorylation in the fast-twitch epitrochlearis and the slow-twitch soleus muscles, and our results show that contraction-stimulated GSK-3α and GSK-3β phosphorylation is muscle fiber type dependent.

Phosphorylation of GSK-3β was not improved when contraction and insulin were combined. Indeed, insulin-stimulated GSK-3α and GSK-3β phosphorylation was slightly reduced by muscle contraction in both control and dexamethasone-treated rats. The fact that contraction decreased insulin-stimulated GSK-3 phosphorylation in skeletal muscles from both normal and insulin-resistant muscles may seem paradoxical. However, in humans, acute exercise increases insulin-stimulated glucose uptake without elevated GSK-3α phosphorylation (55). The present data do not explain how contraction restores activation of insulin-stimulated glucose uptake. However, contraction restores insulin’s ability to activate glycogen synthase after exercise without increasing insulin-stimulated PKB or GSK-3β phosphorylation.

In conclusion, dexamethasone treatment did not impair glucose uptake during contraction. After contraction, insulin-stimulated glycogen synthesis and glycogen synthase activation were improved in muscles from dexamethasone-treated rats. Muscle contraction improved insulin action in muscles from dexamethasone-treated rats without enhancing insulin-stimulated phosphorylation of PKB and GSK-3β. Furthermore, the fact that contraction normally stimulates glucose uptake and improves insulin action in muscles made insulin resistant by dexamethasone suggests exercise as a potential treatment for glucocorticoid-induced insulin resistance.

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