Effects of glucocorticoid excess on the sensitivity of glucose transport and metabolism to insulin in rat skeletal muscle

George DIMITRIADIS[§], Brendan LEIGHTON^{*}, Mark PARRY-BILLINGS^{*}, Shlomo SASSON[†], Martin YOUNG^{*}, Ulrike KRAUSE[‡], Samantha BEVAN^{*}, Terrence PIVA^{*}, Gerhard WEGENER[‡] and Eric A. NEWSHOLME^{*}

^{*}Department of Biochemistry, University of Oxford, South Parks Road, Oxford OX1 3QU, U.K., [†]Department of Pharmacology, Hebrew University School of Medicine, P.O. Box 12272, Jerusalem 91120, Israel, and [‡]Institut für Zoologie, Johannes Gutenberg-Universität, D-55099 Mainz, Germany

This study examines the mechanisms of glucocorticoid-induced insulin resistance in rat soleus muscle. Glucocorticoid excess was induced by administration of dexamethasone to rats for 5 days. Dexamethasone decreased the sensitivity of 3-*O*-methylglucose transport, 2-deoxyglucose phosphorylation, glycogen synthesis and glucose oxidation to insulin. The total content of GLUT4 glucose transporters was not decreased by dexamethasone; however, the increase in these transporters in the plasma membrane in response to insulin (100 m-units/litre) was lessened. In contrast, the sensitivity of lactate formation to insulin was normal. The content of 2-deoxyglucose in the dexamethasone-treated muscle was decreased at 100 m-units/litre insulin, while the contents of glucose 6-phosphate and fructose 2,6-bisphosphate were normal at all concentrations of insulin studied. The maximal activity of hexokinase in the soleus muscle was not

affected by dexamethasone; however, inhibition of this enzyme by glucose 6-phosphate was decreased. These results suggest the following. (1) Glucocorticoid excess causes insulin resistance in skeletal muscle by directly inhibiting the translocation of the GLUT4 glucose transporters to the plasma membrane in response to insulin; since the activity of hexokinase is not affected, the changes in the sensitivity of glucose phosphorylation to insulin seen under these conditions are secondary to those in glucose transport. (2) The sensitivity of glycogen synthesis and glucose oxidation to insulin is decreased, but that of glycolysis is not affected: a redistribution of glucose away from the pathway of glycogen synthesis and glucose oxidation could maintain a normal rate of lactate formation although the rate of glucose transport is decreased.

INTRODUCTION

Insulin resistance is a well-known effect of glucocorticoid excess [1–4]; however, the mechanisms are still unclear. Studies using blood cells, fibroblasts and adipocytes have shown that glucocorticoids decrease the insulin-stimulated rate of glucose transport and the binding of insulin to its receptors [5–10]. Although muscle is quantitatively most important for glucose disposal in response to insulin [11], there are very few reports on the effects of glucocorticoids on this tissue. In rats, administration of cortisol did not affect the number or affinity of insulin receptors in skeletal muscle but reduced the phosphorylation of receptor tyrosine kinase by insulin, suggesting post-receptor effects [12]. In man, cortisol excess decreased insulin-mediated whole-body glucose uptake under euglycaemic conditions [1,2], which reflects glucose uptake primarily into skeletal muscle [11]. The decrease in the sensitivity of glucose uptake to insulin could be caused by a direct effect of glucocorticoids on the glucose transporters, although this possibility has never been studied. Another possibility is that glucocorticoids may inhibit the rate of glucose phosphorylation, leading to an indirect decrease in the rate of insulin-mediated glucose transport. Indeed experiments in rats, using the isolated diaphragm or heart, have suggested that corticosteroids do not affect the transport of metabolizable or non-metabolizable sugars [13–15]. Recent studies in rats showed that glucocorticoid excess did not decrease the content of GLUT-4 transporters in skeletal muscle [16,17], suggesting that the inhibition of glucose transport caused by glucocorticoids may be indirect.

The present study was therefore undertaken to examine the effects of glucocorticoid excess on the sensitivity of transport, phosphorylation and metabolism of glucose to insulin in skeletal

muscle. These experiments were done in the soleus muscle isolated from rats treated with dexamethasone. In order to separate any possible effects of dexamethasone on glucose phosphorylation from those on glucose transport, two glucose analogues were used: 3-*O*-methylglucose, which is transported but not phosphorylated, and 2-deoxyglucose which is transported and phosphorylated but not further metabolized.

MATERIAL AND METHODS

Animals and treatments

Male Wistar rats of 160–180 g were used (Olac, Bicester, U.K.). Rats were injected with dexamethasone (0.5 mg/day per rat; intraperitoneal injection) for 5 days; the rats were fasted for 12 h prior to each experiment and were killed by cervical dislocation. Aprotinin, antipain, BSA, dGlc, glycine, PMSF and streptavidin–agarose beads were obtained from Sigma Chemicals (St. Louis, MO, U.S.A.), NHS-LC biotin [sulphosuccinimidyl 6-(biotin-amido)hexanoate] from Pierce (Rockford, IL, U.S.A.), leupeptin and α -macroglobulin from Boehringer Mannheim (Germany) and Triton X-100 from BDH Chemicals (Poole, Dorset, U.K.). All other chemicals and radiolabelled substances were obtained from sources given previously [18,19].

Muscle incubations

Soleus muscle strips were prepared as previously described and tied to stainless-steel clips to maintain their resting tension [18]. The isolated muscles were transferred into silicone-treated 25 ml Erlenmeyer flasks with 3.5 ml of Krebs–Ringer bicarbonate

Abbreviations used: NHS-LC biotin, sulphosuccinimidyl 6-(biotin-amido)hexanoate; dGlc, 2-deoxy-D-glucose.

[§] To whom correspondence should be addressed. Present address: Athens University, 103-105 Aegeou Pelagous Str., 15342-Agia Paraskevi, Greece.

buffer containing 1 mM of pyruvate, succinate and L-glutamate and 5.5 mM D-glucose. Defatted BSA was added to a final concentration of 1.5 % (w/v) and the pH was adjusted to 7.3. The medium was gassed with O₂/CO₂ (19:1) during preparation and during the incubation, which was carried out at 37 °C. After 30 min, the muscles were transferred into other flasks containing 3.5 ml of identical Krebs–Ringer bicarbonate buffer, except that pyruvate, succinate and L-glutamate were omitted but insulin was added (1–1000 m-units/litre). After 60 min incubation, the muscles were removed, blotted and freeze-clamped in liquid N₂; the frozen muscles were processed for determination of metabolites.

Glycolysis, glycogen synthesis and glucose oxidation

The rate of glycolysis was estimated from that of lactate formation. The concentration of lactate in the incubation medium was assayed enzymically [20]. For experiments in which the rate of glycogen synthesis was measured, [U-¹⁴C]glucose was added to the incubation medium at a final specific radioactivity of 0.4 µCi/ml. Glycogen was precipitated with ethanol [19,21]. For measurements of glucose oxidation, at the end of the incubation and after the muscles were removed, the flasks were rapidly resealed and the incubation was acidified with perchloric acid (final concentration 4 %, w/v); flasks were left on ice for 60 min. The ¹⁴CO₂ was trapped in pieces of filter-paper moistened with 200 µl of 2-phenethylamine/methanol (1:1, v/v) [22].

Rates of glucose transport, glucose phosphorylation and flux of glucose to hexose monophosphate

The rate of glucose transport was measured with 3-O-methyl [³H]glucose. Muscles were incubated in Krebs–Ringer bicarbonate buffer identical with that described above except that D-glucose was omitted. After 30 min preincubation, the muscles were further incubated for 10 min in buffer containing 3-O-methylglucose (5.5 mM), 3-O-methyl [³H]glucose and [U-¹⁴C]sucrose (final specific radioactivities were 2 µCi/ml and 0.1 µCi/ml respectively), 1 mM of pyruvate, succinate and L-glutamate, and insulin. After incubation, muscles were removed, washed briefly in ice-cold Krebs–Ringer bicarbonate buffer, blotted dry and freeze-clamped in liquid N₂. The frozen muscles were digested in 0.5 ml of 1 M KOH at 70 °C for 20–30 min. The muscle digest was transferred into scintillation vials and the tubes were rinsed once with 0.5 ml of distilled water. Scintillation fluid was added to the vials, the mixture was acidified with glacial acetic acid and radioactivity was measured [23].

The rate of glucose phosphorylation was measured by following the accumulation of 2-deoxy [³H]glucose 6-phosphate in muscles. In addition, the intracellular level of non-phosphorylated 2-deoxy [³H]glucose was measured. Trace amounts of 2-deoxy [³H]glucose and [U-¹⁴C]sucrose were added to the incubation medium (see above) which did not contain D-glucose but did contain 5.5 mM 2-deoxyglucose and 1 mM pyruvate, succinate and L-glutamate; the final specific radioactivities were 0.5 µCi/ml and 0.1 µCi/ml for 2-deoxy [³H]glucose and [U-¹⁴C]sucrose respectively. Muscles were incubated for 60 min as described above. The frozen muscles were powdered in liquid N₂, homogenized in 6 % (w/v) perchloric acid and centrifuged to remove precipitated protein. The supernatant was neutralized with 2 M KOH/0.5 M triethanolamine and placed on Dowex-2 (formate form) ion-exchange columns. The columns were washed, first with 20 ml of distilled water containing 5 mM D-glucose, to elute [¹⁴C]sucrose and 2-deoxy [³H]glucose,

and then with 10 ml of 0.3 M ammonium formate/1 M formic acid to elute 2-deoxy [³H]glucose 6-phosphate [23–25].

The net uptake of 2-deoxy [³H]glucose and 3-O-methyl [³H]glucose into the muscles was calculated after correcting for the ³H radioactivity trapped in the extracellular space; this was measured by the [¹⁴C]sucrose radioactivity. The rate of 3-O-methylglucose transport in the soleus muscle is linear for at least 20 min in the presence of 10000 m-units/litre insulin [26].

The flux from glucose to hexose monophosphate (representing the sum of glucosyl units converted into glycogen and metabolized through the glycolytic pathway) was calculated as previously described [24]. [¹⁴C]Lactate was separated from other labelled compounds in the incubation medium using ion-exchange chromatography [25]. Incubation medium was placed on Dowex-2 (formate form) ion-exchange columns, which were washed with 20 ml of distilled water containing 5 mM D-glucose to elute [¹⁴C]glucose, and then with 10 ml of 0.5 M formic acid to elute [¹⁴C]lactate.

Activity of hexokinase

For these measurements muscles were incubated as described above, in the absence of insulin [27]. After 30 min incubation, muscle strips were removed from clips and homogenized for 10–15 s in ice-cold buffer [250 mM sucrose/5 mM Hepes, pH 7.4/5 mM MgCl₂/1 mM dithiothreitol/5 % dextran 70] using a Polytron homogenizer at half-maximal speed. As hexokinase activity is regulated by glucose 6-phosphate, two separate measurements were made in each muscle homogenate: total hexokinase activity, i.e. the activity measured in the absence of glucose 6-phosphate (glucose 6-phosphate dehydrogenase was added in the assay medium), and fractional hexokinase activity, i.e. the activity measured in the presence of accumulating concentrations of glucose 6-phosphate (glucose 6-phosphate dehydrogenase was not added). To measure fractional hexokinase activity the assay medium contained 75 mM triethanolamine, pH 7.4, 7.5 mM MgCl₂, 1.5 mM KCl, 2 mM dithiothreitol, 0.8 mM EDTA, 2.5 mM ATP, 1 mM glucose ([U-¹⁴C]glucose, 0.2 µCi/ml), 10 mM phosphocreatine and 7 units/ml creatine kinase (final concentrations). For measurements of total hexokinase activity the buffer was identical, with the addition of 3 mM NADP⁺ and 3 units/ml glucose 6-phosphate dehydrogenase (final concentrations). The reaction was initiated with the addition of 1 vol. of homogenate to 1 vol. of assay buffer. After 3 min, 2 vol. of 90 % (v/v) ethanol were added to stop the reaction. An aliquot of the final sample was pipetted on to DE81 ion-exchange discs (Whatman, Maidstone, Kent, U.K.). The discs were washed with an excess of water, and the amount of phosphorylated product was quantified using a Beckman liquid-scintillation counter. One unit of activity is defined as the amount that can convert 1 µmol of glucose/min at 30 °C.

Muscle contents of 2-deoxyglucose, glucose 6-phosphate and fructose 2,6-bisphosphate

Glucose 6-phosphate [28] was measured after extraction of the muscles with 6 % perchloric acid. Fructose 2,6-bisphosphate was measured as previously described [29,30].

Measurements of GLUT4 glucose transporters

On the morning of the experiment, rats were anaesthetized with ketamine hydrochloride (150 mg/kg body weight; intraperitoneal injection) and the soleus muscles were removed. These were either homogenized and used for Western-blot analysis to

estimate GLUT4 abundance (total homogenates), or divided and incubated as described above: one half was incubated in the absence and the other in the presence (100 m-units/l) of insulin; after incubation, muscles were processed for the biotinylation procedure and Western blots. The biotinylation procedure measures the cell-surface content of protein, in this case GLUT4 glucose transporters; the method was adapted from methods described previously [31,32].

Preparation of muscle homogenates and surface biotinylation procedure

Muscles were washed in BSA-free PBS (in mmol/litre: 137 NaCl, 2.7 KCl, 9.6 Na₂HPO₄, 1.5 KH₂PO₄, 0.5 MgCl₂), pH 7.4, blotted and incubated in 1 ml of 0.5 mg/ml NHS-LC biotin in PBS for 30 min at 4 °C. The reaction was stopped by rinsing the muscles three times with 15 mmol/litre glycine in ice-cold PBS. The muscles were then washed once in PBS, blotted and frozen in liquid N₂. The frozen muscles were pulverized and homogenized in solubilization buffer (150 mmol/litre NaCl/50 mmol/litre Hepes, pH 7.4/1% Triton X-100, containing 2 mmol/l PMSF, 2 µg/ml aprotinin, 0.1 mmol/litre antipain, 0.2 mmol/litre leupeptin and 0.5 unit/ml α-macroglobulin. The homogenate was solubilized for 30 min on ice with frequent shaking. The supernatant was separated by a 1 min centrifugation in an Eppendorf centrifuge and mixed with 50 µl of streptavidin-agarose beads (1 mg of streptavidin/1 ml of gel in solubilization buffer containing 0.1 mmol/litre PMSF). The suspension was gently mixed for 30 min at 4 °C and the beads were sedimented by centrifugation. The bead pellet was washed three times with 150 mmol/litre NaCl/10 mmol/litre Tris, pH 7.0, containing 2 mmol/l PMSF and 0.2 mmol/l leupeptin. The final pellet was resuspended in 120 µl of Laemmli buffer (1.2-fold concentrated, without Bromophenol Blue) and incubated for 30 min at 65 °C. The supernatant was separated from the beads by centrifugation, collected, and kept at -70 °C until used.

Western blot analysis

Protein content in samples was determined by the Bio-Rad DC protein assay (Bio-Rad Laboratories, Hercules, CA, U.S.A.) using BSA as standard. Proteins were diluted in Laemmli buffer, separated by SDS/PAGE (10%), transferred electrophoretically to nitrocellulose membranes and analysed by Western blotting, as previously described [33], with specific rabbit antiserum against synthetic rat GLUT4 C-terminus at a dilution of 1:500. Immuno-reactive species were detected by enhanced chemiluminescence (ECL; Amersham). The densities of the Western-blot bands were measured by computer-assisted scanning analysis of the gels and are expressed in arbitrary units.

Results are presented as mean ± S.E.M. Statistical analysis was done with a two-tailed non-paired Student's *t*-test.

RESULTS AND DISCUSSION

Glucose transport and phosphorylation

Treatment with dexamethasone decreased the body weight of the rats (148 ± 4 g versus 170 ± 8 g in controls).

Dexamethasone decreased the rates of 3-*O*-methylglucose transport in the isolated soleus muscle at physiological, but not at maximal, concentrations of insulin (Figure 1). 3-*O*-Methylglucose is a glucose analogue which is transported like glucose but is not further metabolized, and therefore provides a direct estimate of the rate of glucose transport [34]. These results suggest that dexamethasone decreases the sensitivity of muscle

Figure 1 Rates of 3-*O*-methylglucose transport (top) and 2-deoxyglucose phosphorylation (bottom) at various concentrations of insulin in soleus muscles isolated from control (○) and dexamethasone-treated rats (●)

Values are presented as means ± S.E.M. of 4–6 separate incubations. Statistically significant differences are indicated by **P* < 0.01 and ***P* < 0.001.

glucose transport to insulin through an effect on the glucose transporters. These findings support previous studies in man showing that glucocorticoids decrease the sensitivity of whole-body glucose uptake to insulin [1,2].

In skeletal muscle, insulin increases the rate of glucose utilization, mainly by stimulating the translocation of the GLUT4 glucose transporters from an intracellular pool to the plasma membrane [35,36]. Therefore, the effect of dexamethasone on insulin-mediated glucose transport may be via a decrease in the net translocation of the GLUT4 transporters. This is also suggested by two recent studies which show that the total content of GLUT4 in skeletal muscle, following glucocorticoid excess, is unaltered [16,17]. On the Western blot, the GLUT4 protein was detected as a doublet of approx. 54 and 44 kDa, which represents two glycosylation states of the transporter; neither dexamethasone nor insulin changed the ratio of these two species in the muscle cell surface (Figure 2). In agreement with other reports [16,17], dexamethasone did not decrease the abundance of GLUT4 transporters in the soleus muscles (Figure 2, bottom): 1089 ± 30 versus 1226 ± 39 for the 54 kDa and 413 ± 59 versus 461 ± 30 for the 44 kDa in control and dexamethasone-treated rats respectively (mean ± S.E.M. in arbitrary units; *n* = 4).

Using the biotinylation procedure [31–33] we measured the increase in density of the GLUT4 transporter proteins in the muscle cell surface in response to insulin (100 m-units/litre). Treatment of rats with dexamethasone reduced insulin-stimulated increases in GLUT4 in the soleus by about 60% (results are presented as fold increases in density; mean ± S.E.M.,

Figure 2 Effect of dexamethasone (DEX) on translocation of GLUT4 to the plasma membrane in response to insulin (top and middle panels), and on the total number of GLUT4 transporters (bottom panel) in rat soleus muscle ($n = 4$)

Western blot analysis of the muscle homogenates was done either before (total homogenate) or after incubation of the soleus muscles with insulin (100 m-units/l) and surface biotinylation (Control and Dex) (see the Materials and methods section). C, controls; D, dexamethasone; — or + denotes the absence or presence of insulin. Upper bands correspond to the 54 kDa and lower bands to the 44 kDa GLUT4.

$n = 4$): 1.68 ± 0.04 and 1.77 ± 0.06 in control and 1.07 ± 0.06 and 1.08 ± 0.10 in dexamethasone-treated rats for the 54 kDa and 44 kDa bands respectively (differences between control and dexamethasone were significant, $P < 0.01$). These results suggest that the effect of dexamethasone on insulin-mediated glucose transport is via a decrease in the translocation of the GLUT4 glucose transporters from cytosol to the plasma membrane.

Previous studies have suggested that glucocorticoids may decrease the sensitivity of glucose uptake to insulin, not by effects on glucose transporters but by inhibiting the rate of glucose phosphorylation [13–15]. This could be achieved by inhibition of hexokinase activity, either directly or by inhibiting 6-phosphofructokinase: the latter would lead to an increase in the level of glucose 6-phosphate, which is a potent inhibitor of hexokinase. Decreased hexokinase activity would lead to an increased level of intracellular glucose, which would increase the rate of glucose efflux and, hence, cause a decrease in the net rate of glucose transport. Indeed, treatment of rats with cortisol decreased the rate of phosphorylation of glucose and fructose 6-phosphate in the diaphragm or heart, suggesting that hexokinase and 6-phosphofructokinase were inhibited [13,15]; and, in the rat diaphragm, the rate of transport of non-metabolizable sugars was not decreased, suggesting that glucose transport was not affected by corticosteroids [13]. In the present study, dexamethasone decreased the insulin sensitivity of 2-deoxyglucose phosphorylation and of the flux of glucose to hexose monophosphate in the soleus muscle (Figures 1 and 3). These changes are similar to those observed in the rate of 3-*O*-methylglucose transport, but the contents of 2-deoxyglucose and glucose 6-phosphate in these muscles (and presumably that of fructose 6-phosphate with which glucose 6-phosphate is in equilibrium) were not increased (Table 1): this should be the case if hexokinase was inhibited either directly or as a consequence of a reduced 6-phosphofructokinase activity by dexamethasone treatment. However, it should be pointed out that this reasoning may be valid only if the entry of glucose was similar under both conditions; since it was decreased after dexamethasone, this suggestion was further tested by measurement of the activity or

Figure 3 Top left: flux of glucose to hexose monophosphate (4–5), and rates of glycogen synthesis (4–7) (top right), [^{14}C]lactate formation (4–7) (bottom left) and glucose oxidation (4–7) (bottom right) at various concentrations of insulin in soleus muscles isolated from control (○) and dexamethasone-treated rats (●)

Values are presented as means $\pm$ S.E.M. Number of separate incubations is given in parenthesis. Statistically significant differences are indicated by * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$.

regulatory properties of hexokinase in the soleus muscle (Table 2). The total hexokinase activity was the same in muscles from control and dexamethasone-treated rats, indicating that the expression of hexokinase in the soleus muscle was not affected by dexamethasone. However, the fraction of hexokinase that could be inhibited by glucose 6-phosphate was significantly lower in the dexamethasone-treated rats than in controls. Less inhibition of hexokinase by glucose 6-phosphate may help to increase the flux through glucose phosphorylation under these conditions. Hence, in contrast with previous suggestions [13–15], the changes seen in the rate of glucose phosphorylation by dexamethasone in skeletal muscle may be secondary to those observed in glucose transport.

Glycolysis, glucose oxidation and glycogen synthesis

After dexamethasone treatment, the rates of lactate formation were normal at all concentrations of insulin (Figure 4); however, since the rates of glucose transport and phosphorylation were decreased, it would be expected that the flux through glycolysis should also be decreased. This apparent paradox can be explained by the decrease in the rates of glycogen synthesis caused by dexamethasone treatment (Figure 3) [17,37,38], so that there is a shift of glucose metabolism towards glycolysis. The finding that the content of glucose 6-phosphate was normal at all concen-

Table 1 Contents of 2-deoxyglucose, glucose 6-phosphate and fructose 2,6-bisphosphate in soleus muscles isolated from rats treated with dexamethasone at various concentrations of insulin

Values are means $\pm$ S.E.M. of 4–9 separate experiments. Statistically significant differences from control muscles are indicated by * $P < 0.001$.

Insulin (m-units/litre)	2-Deoxyglucose ($\mu\text{mol/g}$)		Glucose 6-phosphate ($\mu\text{mol/g}$)		Fructose 2,6-bisphosphate (nmol/g of muscle)	
	Control	Treated	Control	Treated	Control	Treated
1	0.219 $\pm$ 0.05	0.289 $\pm$ 0.09	—	—	—	—
10	0.331 $\pm$ 0.05	0.219 $\pm$ 0.03	0.186 $\pm$ 0.03	0.135 $\pm$ 0.03	0.528 $\pm$ 0.07	0.549 $\pm$ 0.05
100	0.546 $\pm$ 0.04	0.327 $\pm$ 0.04*	0.204 $\pm$ 0.01	0.168 $\pm$ 0.02	0.715 $\pm$ 0.05	0.591 $\pm$ 0.06
1000	0.623 $\pm$ 0.06	0.781 $\pm$ 0.08	0.300 $\pm$ 0.02	0.319 $\pm$ 0.02	0.825 $\pm$ 0.07	0.867 $\pm$ 0.09

Table 2 Activity of hexokinase in homogenates of soleus muscle isolated from control and dexamethasone-treated rats

The activity of the enzyme was measured in the presence (total or maximal activity) or in the absence of glucose 6-phosphate dehydrogenase (fractional activity; see the Materials and methods section). Values are means $\pm$ S.E.M. of four separate experiments. Statistically significant differences from control muscles are indicated by * $P < 0.05$.

	Activity of hexokinase ($\mu\text{mol/min per g}$)	
	Total	Fractional
Control	1.53 $\pm$ 0.13	0.55 $\pm$ 0.04
Dexamethasone	1.43 $\pm$ 0.07	0.83 $\pm$ 0.10*

Figure 4 Rates of lactate formation at various concentrations of insulin in soleus muscles isolated from control (○) and dexamethasone-treated rats (●)

Values are presented as means $\pm$ S.E.M. of 4–7 separate incubations.

trations of insulin (Table 1) is also consistent with this suggestion. This is also supported by the measurements of [^{14}C]lactate formation (Figure 3). The rate of conversion of [^{14}C]glucose into [^{14}C]lactate in resting muscle is considered to be regulated by the activity of the glucose transport process. Therefore, since the rate of glucose transport was decreased, the rate of [^{14}C]lactate formation should also be decreased: this rate was normal, suggesting that, following transport and phosphorylation, glucose was metabolized almost entirely via glycolysis to maintain a

normal rate of lactate formation, despite the decrease in the rate of glucose transport. In addition, the content of fructose 2,6-bisphosphate, which is a potent activator of 6-phosphofructokinase from muscle [30,39], and may be part of the mechanism by which insulin increases glycolytic flux [23], was not changed by dexamethasone treatment (Table 1). The effect of dexamethasone to decrease the insulin sensitivity of glucose transport, while maintaining a 'normal' rate of glycolysis, means that the apparent sensitivity of glycolysis to insulin in the muscle was actually increased.

Glucocorticoid excess increases the rate of proteolysis [40]. Administration of dexamethasone to rats for 5 days increased muscle concentration of alanine [41]: it is possible that some of these carbons may feed back through pyruvate and contribute to lactate formation. However, by comparison with lactate formation from glucose, this contribution may be trivial, since the rates of [^{14}C]lactate formation in the soleus muscle were not affected by dexamethasone treatment.

Treatment with dexamethasone decreased the sensitivity of glucose oxidation to insulin in the soleus muscle (Figure 3). In rats, administration of cortisol increased plasma levels of pyruvate and impaired pyruvate tolerance, suggesting a decrease in the rate of pyruvate oxidation in the tissues [15]. In man, administration of dexamethasone decreased whole-body insulin-stimulated glucose oxidation (measured with glucose–insulin clamps and indirect calorimetry); this decrease was considered secondary to the increase in plasma levels of non-esterified fatty acids [42]. Previous studies have shown that glucocorticoids increase the rate of lipolysis, suggesting that insulin resistance in muscle may be caused by the increased oxidation of non-esterified fatty acids [17,43]. Although, *in vivo*, such a possibility is likely to play a role, the present results suggest that this may not be the main mechanism by which glucocorticoids induce insulin resistance. Since our experiments were done using an isolated muscle, the effects are presumably independent of any increase in plasma non-esterified fatty acids. Moreover, non-esterified fatty acids would be expected to inhibit glycolysis and glucose oxidation at all concentrations of insulin [44].

Lactate is an important substrate for gluconeogenesis, and skeletal muscle is an important source of this substrate [45]. The present results suggest that preservation of the rate of lactate formation in muscle helps to ensure that this tissue produces lactate at a normal rate, despite a decreased rate of glucose transport. This may be part of the mechanism by which glucocorticoids regulate the rate of gluconeogenesis from lactate [46–48].

In summary, dexamethasone causes insulin resistance in muscle by inhibiting glucose transport. In addition, dexamethasone

decreases the sensitivity of glycogen synthesis and glucose oxidation to insulin, but does not affect glycolysis: a redistribution of glucose residues away from glycogen synthesis and glucose oxidation maintains a normal rate of lactate formation by muscle, although the rate of glucose transport is decreased.

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REFERENCES

- Rizza, R., Mandarino, L. and Gerich, J. (1982) *J. Clin. Endocrinol. Metab.* **54**, 131–138
- Nosadini, R., Del Prato, S. and Tiengo, A. (1983) *J. Clin. Endocrinol. Metab.* **57**, 529–536
- Baron, A., Wallace, P. and Brechtel, G. (1987) *Diabetes* **36**, 1230–1237
- Bowes, S., Benn, J., Scobie, I., Umpleby, A., Lowry, C. and Sonksen, P. (1991) *Clin. Endocrinol.* **34**, 311–316
- Olefsky, J. (1975) *J. Clin. Invest.* **56**, 1499–1508
- Grunfeld, C., Baird, K., Van Obberghen, E. and Kahn, R. (1981) *Endocrinology* **109**, 1723–1730
- Malchoff, D., Maloff, B., Livingston, J. and Lockwood, D. (1982) *Endocrinology* **110**, 2081–2087
- Horner, H., Munck, A. and Lienhard, G. (1987) *J. Biol. Chem.* **262**, 17696–17702
- Carter-Su, C. and Okamoto, S. (1987) *Am. J. Physiol.* **252**, E441–E453
- Tanaka, H., Akama, H., Ichikawa, Y., Homma, M. and Makino, I. (1992) *Acta Endocrinol.* **126**, 29–36
- Baron, A., Brechtel, G., Wallace, P. and Edelman, S. (1988) *Am. J. Physiol.* **255**, E769–E774
- Block, N. and Buse, M. (1989) *Am. J. Physiol.* **256**, E39–E48
- Kipnis, D. (1959) *Ann. N. Y. Acad. Sci.* **82**, 354–365
- Morgan, H., Regen, D., Henderson, M., Sawyer, T. and Park, C. (1961) *J. Biol. Chem.* **236**, 2162–2168
- Randle, P. and Morgan, H. (1962) *Vitam. and Horm.* **20**, 199–249
- Haber, R. and Weinstein, S. (1992) *Diabetes* **41**, 728–735
- Guillaume-Gentil, C., Assimacopoulos-Jeannet, F. and Jeanrenaud, B. (1993) *Diabetologia* **36**, 899–906
- Espinal, J., Dohm, L. and Newsholme, E. (1983) *Biochem. J.* **212**, 453–458
- Dimitriadis, G., Parry-Billings, M., Dunger, D., Bevan, S., Colquhoun, A., Taylor, A., Calder, P., Krause, U., Wegener, G. and Newsholme, E. (1992) *J. Endocrinol.* **133**, 37–43
- Engel, P. and Jones, J. (1978) *Anal. Biochem.* **88**, 475–484
- Guendet, G., Loten, E., Jeanrenaud, B. and Renold, A. (1976) *J. Clin. Invest.* **58**, 1078–1088
- Leighton, B., Budohoski, L., Lozeman, F., Challiss, J. and Newsholme, E. (1985) *Biochem. J.* **227**, 337–340
- Dimitriadis, G., Parry-Billings, M., Bevan, S., Dunger, D., Piva, T., Krause, U., Wegener, G. and Newsholme, E. (1992) *Biochem. J.* **285**, 269–274
- Challiss, R., Arch, J. and Newsholme, E. (1984) *Biochem. J.* **221**, 153–161
- Hammerstedt, R. (1980) *Anal. Biochem.* **109**, 443–448
- Dimitriadis, G., Parry-Billings, M., Leighton, B., Piva, T., Dunger, D., Calder, P., Bond, J. and Newsholme, E. (1994) *Eur. J. Clin. Invest.* **24**, 161–165
- Sanderson, A. (1996) Ph.D. Thesis, University of Oxford
- Michal, G. (1984) in *Methods of Enzymatic Analysis* (Bergmeyer, U., ed.), pp. 191–198, Verlag Chemie, Weinheim
- Van Schaftigen, E. (1984) in *Methods of Enzymatic Analysis* (Bergmeyer, U., ed.), pp. 335–341, Verlag Chemie, Weinheim
- Wegener, G., Krause, U. and Thuy, M. (1990) *FEBS Lett.* **267**, 257–260
- Shetty, M., Loeb, J., Vikstrom, K. and Beigi, I. (1993) *J. Biol. Chem.* **268**, 17225–17232
- Levy-Toledano, R., Caro, R., Hindman, L. and Taylor, S. (1993) *Endocrinology* **133**, 1803–1808
- Wertheimer, E., Sasson, S., Cerasi, E. and Ben-Neriah, Y. (1991) *Proc. Natl. Acad. Sci. U.S.A.* **88**, 2525–2529
- Narahara, H. and Ozand, P. (1963) *J. Biol. Chem.* **238**, 40–49
- Klip, A. and Paquet, M. (1990) *Diabetes Care* **13**, 228–243
- Gould, G. and Holman, D. (1993) *Biochem. J.* **295**, 329–341
- Li, C., Kalman, C. and Evans, H. (1949) *Arch. Biochem.* **23**, 512–514
- Leighton, B., Lozeman, F., Challiss, R. and Newsholme, E. (1987) *Biochem. J.* **246**, 551–554
- Krause, U. and Wegener, G. (1996) *Am. J. Physiol.* **270**, R821–R829
- Simmons, P., Miles, J., Gerich, J. and Haymond, M. (1984) *J. Clin. Invest.* **73**, 412–420
- Parry-Billings, M., Leighton, B., Dimitriadis, G., Bond, J. and Newsholme, E. (1990) *Biochem. Pharmacol.* **40**, 1145–1148
- Tappy, L., Randin, D., Vollenweider, P., Vollenweider, L., Paquout, N., Scherrer, U., Schneiter, P., Nicod, P. and Jequier, E. (1994) *J. Clin. Endocrinol. Metab.* **79**, 1063–1069
- De Feo, P., Perriello, G., Torlone, E., Ventura, M., Fanelli, F., Santeusano, F., Brunetti, P. and Gerich, J. (1989) *Am. J. Physiol.* **257**, E35–E42
- Newsholme, E. and Leech, A. (1983) *Biochemistry for the Medical Sciences*, J. Wiley, Chichester, New York, Brisbane, Toronto
- Consoli, A., Nurjhan, N., Gerich, J. and Mandarino, L. (1992) *Metabolism* **41**, 176–179
- Exton, J. (1972) *Metabolism* **21**, 945–990
- Chan, T. (1984) *J. Biol. Chem.* **259**, 7426–7432
- Sistare, F. and Haynes, R. (1985) *J. Biol. Chem.* **260**, 12754–12760