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Dexamethasone impairs muscle energetics, studied by ^{31}P NMR, in rats.

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Abstract

Aims/hypothesis. Glucocorticoid treatments are associated with increased whole-body oxygen consumption. We hypothesised that an impairment of muscle energy metabolism can participate in this increased energy expenditure.

Methods. To investigate this possibility, we have studied muscle energetics of dexamethasone-treated rats ($1.5 \text{ mg}\cdot\text{kg}^{-1}\cdot\text{d}^{-1}$ for 6 days), *in vivo* by ^{31}P NMR spectroscopy. Results were compared with control and paired-fed (PF) rats before and after overnight fasting.

Results. Dexamethasone treatment resulted in decreased phosphocreatine (PCr) concentration and PCr:ATP ratio, increased ADP concentration and higher PCr to γ -ATP flux but no change in β -ATP to β -ADP flux in gastrocnemius muscle. Neither 4 days of food restriction (PF rats) nor 24-hours fasting affected high-energy phosphate metabolism. In dexamethasone-treated rat, there was an increase in plasma insulin and non-esterified fatty acid concentration.

Conclusion/interpretation. We conclude that dexamethasone treatment altered resting *in vivo* skeletal muscle energy metabolism, by decreasing oxidative phosphorylation, producing ATP at the expense of PCr.

Keywords: ATP, food restriction, glucocorticoid, oxidative phosphorylation, phosphocreatine

Abbreviations: β -OHB, β -hydroxybutyrate; CON, control; DEX, dexamethasone; GCTC, glucocorticoid; NEFA, non-esterified fatty acid; NMR, nuclear magnetic resonance; PF, paired-fed; PCr, phosphocreatine

Type 2 diabetes mellitus is a chronic metabolic disorder characterised by insulin resistance, hyperglycaemia, hyperinsulinaemia and elevated non-esterified fatty acids (NEFA). Energy metabolism is altered in Type 2 diabetes mellitus. In the diabetic heart, glucose and lactate oxidation are decreased while fatty acid oxidation is increased, raising the oxygen requirement per ATP molecule produced [1]. ³¹P nuclear magnetic resonance spectroscopy (NMR) studies performed in Type 2 diabetic patients, whose cardiac morphology, mass and function appeared to be normal, showed significantly lower phosphocreatine (PCr):ATP ratios (as compared to normal subjects). The cardiac PCr:ATP ratio negatively correlated with fasting plasma NEFA concentrations [2]. Furthermore, patients with Type 2 diabetes mellitus have limited exercise tolerance which is associated with significantly faster muscle PCr loss and pH decrease during exercise, while PCr recovery after exercise is slower [2].

Insulin stimulates oxidative phosphorylation capacity [3] while insulin resistance appears to inhibit respiration and decrease the efficiency of ATP production. Indeed, non-alcoholic steatohepatitis elicits mitochondrial dysfunction leading to a reduction of 30–60 % in the activities of the 5 respiratory chain complexes in the liver. Activities of these complexes thereby negatively correlate with HOMA, an indicator of insulin-sensitivity [4]. Furthermore, Petersen et al [5] have shown an increased fat accumulation in the muscle and liver of healthy elderly subjects which is associated with a 40 % reduction in mitochondrial oxidative and phosphorylative activity. These authors suggest that an age-associated decline in mitochondrial function contributes to insulin resistance in the elderly [5].

Increased glucocorticoid (GCTC) plasma concentration induces an insulin resistant state, which affects energy metabolism. GCTC-induced insulin resistance results in decreased insulin-stimulated glucose uptake in muscle [6], and increased plasma concentrations of NEFA, insulin and leptin [7, 8], with troglitazone antagonising this effect [7]. At the whole body level, increased GCTC concentrations are known to increase oxygen consumption [9,

10, 11], and decrease food intake [12, 13] resulting in a more negative energy balance than in pair-fed animals. We have shown that there is a decreased efficiency of oxidative phosphorylation in the liver mitochondria of dexamethasone treated rats [13]. We failed to show similar abnormalities *in vitro* within skeletal muscle mitochondria. GCTC secretion is increased and plays a pivotal role in metabolic adaptation to stress [14, 15]. ³¹P NMR studies performed in septic rats (which display corticosterone concentrations 2-fold greater than controls) show that PCr breakdown rates and Na-K ATPase activities were higher than those of control animals. These changes were antagonised by mifepristone, a GCTC receptor antagonist [15].

Therefore, in all these insulin resistant states, there are arguments to suggest that muscle energy metabolism is impaired. We hypothesise that in dexamethasone (DEX)-treated rats, *in vivo* skeletal muscle energy metabolism is directed toward the production of ATP from PCr because of a relative failure of mitochondrial oxidative phosphorylation. Muscle energetics of DEX-treated rats were therefore studied using ³¹P NMR both under fasting and fed conditions.

Materials and Methods

Animals. The present investigation was performed in accordance with the French guiding principles in the care and use of animals. Eighty male Sprague-Dawley rats (10 weeks old; 300-350g) were supplied by IFFA CREDO (L'Arbresle, France). After arrival in our animal facilities, animals were provided with water *ad libitum* and a standard diet (U.A.R A04, IFFA CREDO, L'Arbresle, France) consisting (by weight) of 16% protein, 3% fat, 60% carbohydrate and 21% water, fiber, vitamins and minerals. The metabolisable energy content was 2.9 kcal/g. They were kept in a controlled environment (constant temperature 22°C, and a 12/12 h light-dark cycle). They were acclimatised in individual cages for 8 days. Rats were divided into 3 groups of 6 as follows: dexamethasone (DEX)-treated rats received a daily intraperitoneal injection of $1.5 \text{ mg}\cdot\text{kg}^{-1}\cdot\text{d}^{-1}$ of dexamethasone for 6 days. Given the fact that dexamethasone treatment induces anorexia, pair-fed (PF) rats were used to differentiate between the effect of anorexia and the effect of dexamethasone itself on the parameters tested. PF rats received the same quantity of food as was consumed by DEX-treated rats the previous day, and were injected daily with an isovolumic solution of 0.9% NaCl. Rats from the control group (CON) were healthy, received no treatment, and were fed *ad libitum*. Experiments were conducted over a 6-day period. The dose and duration of the dexamethasone treatment was chosen with reference to the literature from what is known to induce a reproducible maximum hypercatabolic state [16]. NMR measurements were performed for the first time on the 5th day, when animals were fed. On the 6th day, NMR measurements were conducted among the same animals although that they had been fasted for 24 hours.

Furthermore, dexamethasone being a highly reproducible model of hypermetabolic stress [12, 13, 17], a similar experiment was conducted in a parallel group of rats to test plasma

substrate and hormone concentrations. In a third experiment, rats were anaesthetised with isoflurane, the skin over the leg was dissected away and the gastrocnemius was rapidly freeze-clamped, removed, frozen in liquid nitrogen and subsequently stored at $-80\text{ }^{\circ}\text{C}$ until analysis of PCr and creatine concentration.

In vivo ^{31}P NMR spectroscopy. On the day of the NMR experiment, rats were anaesthetised with 1.8 ml/h isoflurane throughout the duration of the measurement. The rat was wrapped in a blanket to preserve core body temperature. The ^{31}P NMR experiments were performed at 81.07 MHz using the Bruker Biospec (Karlsruhe, Germany) 4.7 T system (horizontal/40-cm-diameter bore magnet). The $^1\text{H}/^{31}\text{P}$ Bruker surface coil (50 mm) was positioned over the rat's hind limb. Shimming was performed by optimizing the proton signal from water. Usually, a half-height line width of the water signal of 70 Hz was achieved. Spectra were acquired with a hard pulse of 55 μs length to perform 90° at the coil center. Each spectrum represented an average of 128 scans with a recycle time of 25 s, accumulated over a total period of 60 min. A sweep width of 5000 Hz and 16 k data points were used.

Free induction decay was multiplied by 20 Hz line broadening constant before Fourier transformation.

Magnetisation transfer. In the saturation transfer experiment, a gaussian selective pulse was applied for 6 s on the signal. In the spectrum without γ -ATP saturation, the same pulse was applied symmetrically to γ -ATP/ β -ADP at high-field side of β -ATP.

A saturation transfer experiment has been fully developed previously [18, 19]. In our experiment, the rate constants k_a and k_b between PCr and γ -ATP and between β -ATP and β -ADP respectively, were determined as to: $k_i = (1/T_1)((M_0-M)/M)$

where T_1 is intrinsic longitudinal relaxation of PCr or β -ATP, M_0 is the signal intensity of PCr or β -ATP without saturation and M is the signal intensity of PCr or β -ATP with γ -ATP/ β -ADP saturation.

The flux from PCr to ATP was calculated as $k_a \times [\text{PCr}]$ while that from β -ATP to β -ADP was $k_b \times [\beta\text{-ATP}]$.

Ex vivo ^1H NMR spectroscopy. Muscle energetics calculated from ^{31}P NMR spectroscopy parameters rely on hypotheses, one of which is that total creatine (PCr + creatine) content in muscle is strictly proportional to the NMR signal, regardless the circumstances. To make sure that total creatine content was constant in DEX-treated, PF and CON rats, we have measured this content in perchloric acid extracts with ^1H NMR spectroscopy, which is the reference technique [20, 21].

Frozen muscle was pulverized using a mortar and pestle precooled in liquid nitrogen and homogenized and deproteinized in ice-cold 1 mol/l perchloric acid (1:5). The supernatant was neutralized with 2 mol/l potassium bicarbonate and then lyophilized. ^1H spectra from perchloric acid extracts prepared immediately after excision were obtained from 128 accumulations with trimethylsilylpropionic $-2,2,3,3\text{d}_4$ -acid as reference (2 mmol/l). A 90° flip angle; 10 s repetition time, 2000 Hz sweep width/16K and 0.3 Hz exponential broadening were used.

This shows that total creatine did not differ in the three groups: DEX-treated rats (44.7 ± 5 mmol/g), PF rats (49.2 ± 7 mmol/g) and CON rats (44.8 ± 2 mmol/g); $p = \text{NS}$.

Calculations. Intracellular pH was determined from the chemical shift of Pi (δ) according to the following formula [19, 22]:

$$\text{pH} = 6.73 + \log (\delta - 3.37) / (5.66 - \delta)$$

NMR spectra were fitted by using Peakfit® software to determine the area of peaks of Pi, PCr and ATP.

Ratios of PCr:ATP and Pi:PCr were calculated from the respective areas.

Assuming that the total level of phosphorylated metabolites detected by ^{31}P NMR is visible [23] and remains constant as a function of diet and the PCr level is 35.8 mmol/l at rest for the fed control rats [24], we can calculate the dependence of the concentration for each metabolite versus diet.

The values of ATP and Pi used for the final analysis were calculated from the ratios of PCr:ATP and Pi:PCr. Thus $\text{ATP} = \text{PCr}:\text{ATP} / [\text{PCr}]$, and $\text{Pi} = \text{Pi}:\text{PCr} \times [\text{PCr}]$.

ADP level was calculated from the creatine kinase reaction, according to Lawson and Veech [22], taking into account intracellular pH changes and assuming a free Mg^{2+} concentration of 0.86 mmol/l. The equilibrium constants of the creatine kinase reaction (K_{eq}) were calculated from the intracellular pH:

$$\log K_{\text{eq}} = 8.31 - (0.87 \times \text{pH})$$

ADP was then calculated using the formula:

$$\text{ADP (mmol/l)} = [\text{ATP}] \times [\text{creatine}] / [\text{PCr}] \times K_{\text{eq}}$$

pH and K_{eq} calculations are sensitive to Mg^{2+} concentration. Free Mg^{2+} concentration affects the distance between the alpha and the beta ATP peaks. In the present experiment the chemical shift separation between the alpha and the beta ATP peaks did not change ruling out the possibility of changes in Mg^{2+} concentration (data not shown).

The free-energy change of ATP hydrolysis can be calculated as follows:

$$\Delta G_{\text{ATP}} (\text{kJ / mol}) = \Delta G^{\circ} + 2.58 \ln [\text{ADP}][\text{Pi}] / [\text{ATP}]$$

Plasma concentrations. Non-esterified fatty acids plasma concentrations were determined using a COBAS analyser (Roche Diagnostics, Grenoble, France) by using commercially available kits from Boeringher (Grenoble, France).

Plasma levels of β -hydroxybutyrate (β -OHB) were determined by standard colorimetric and enzymatic methods adapted for the COBAS analyzer (Roche Diagnostics).

Plasma insulin concentrations were determined using a commercially available enzyme immunoassay (Biotrak (EIA) System, Amersham Biosciences, Uppsala, Sweden). Plasma leptin levels were determined by radioimmunoassay (Linco Research, St. Charles, MO, USA).

Statistical analysis. Results were expressed as mean \pm SD. Repeated measurements were compared by ANOVA with the Bonferroni/Dunn test as a post-hoc test. For the other parameters measured, means were compared by ANOVA and using the Fisher post-hoc test. A P value < 0.05 was considered significant.

Results

Dexamethasone induced a significant reduction in food intake from day 2 (Fig. 1). Animals in the 3 groups did not differ with respect to body weight at the beginning of the experimental procedure (325 ± 29 vs. 334 ± 13 vs. 322 ± 12 g in DEX-treated, PF and CON rats). Body weight decreased in DEX-treated rats from day 1 and PF animals from day 2 (Fig. 2). This decrease was significantly ($P < 0.0001$) greater in DEX-treated rats than in PF animals, corresponding to 18% (DEX-treated) and 4% (PF) of initial body mass on the 5th day of treatment. At the same time, CON rats increased their body mass by 8% (Fig. 2). The 24-hour fast, between days 5 and 6, decreased body weight by 6% ($P < 0.0001$) in the 3 rat groups and there was no interaction with treatment (Fig. 2).

Phosphoryl compounds.

(i) fed rats.

PCr concentration was significantly decreased (-9%; $P < 0.0001$) by dexamethasone in comparison to CON and PF rats, while ADP (+57%; $P < 0.0001$) and ATP (+13%; $P < 0.0005$) concentrations were increased (Table 1). PCr:ATP ratio and free energy change of ATP hydrolysis (ΔG_{ATP}) was lower in DEX-treated rats than in CON and PF rats. There was no difference in pH, Pi concentration or Pi:PCr ratio between the 3 rat groups (Table 1).

(ii) fasted rats.

PCr was significantly lower in DEX-treated rats than in CON (-10%; $P < 0.0001$) and PF rats (-6%; $P < 0.005$). PCr was also lower in PF rats than in CON rats (-5%; $P < 0.005$). ADP was higher in DEX-treated rats than in CON (+55%; $P < 0.0001$) and PF rats (+27%;

P<0.001). ADP was also higher in PF rats than in CON rats (+22%; P<0.05). Pi concentration was significantly increased by dexamethasone (Table 1). ATP content was higher in PF and DEX-treated rats than in CON rats (+8%; P<0.05 and +6%; P<0.05). ΔG_{ATP} was lower in DEX-treated rats than in PF and CON rats. PCr:ATP ratio was lower in DEX-treated and PF rats than in CON rats (Table 1). Pi:PCr ratio was significantly increased by dexamethasone (Table 1). There was no difference in pH across the 3 rats groups (Table 1).

(iii) the effect of a 24-hour fast (animals were fasted for the final 24 hours of the experiments allowing the paired comparison of muscle energetics between these two states).

Twenty-four hours fasting decreased ATP content in DEX-treated rats (-7%; P<0.05) and increased it in PF rats (+5%; P<0.05). Fasting significantly increased Pi concentration and Pi:PCr ratio in DEX-treated rats (Table 1). ΔG_{ATP} was decreased by the 24-hour fast in the single DEX-treated rat group (Table 1). In PF rats, fasting significantly decreased PCr:ATP ratio and increased (P<0.05) free ADP concentration by 16% (Table 1).

Magnetisation transfer.

(i) fed rats.

In DEX-treated rats, the pseudo-first order rate constant of γ -ATP synthesis from PCr (k_a) and the PCr to γ -ATP flux were significantly higher than in CON and PF rats (Table 2). The pseudo-first order rate constant of β -ADP synthesis from β -ATP (k_b) and the β -ATP to β -ADP flux were not affected by dexamethasone (Table 2).

(ii) fasted rats.

k_a and the PCr to γ -ATP flux were significantly higher in DEX-treated rats than in CON and PF rats (Table 2). Regardless of the rat group, k_b and the β -ATP to β -ADP flux did not differ (Table 2).

(iii) the effects of a 24-hour fast.

Fasting did not affect all parameters tested (Table 2).

Hormones and metabolites.

(i) fed rats.

NEFA (+115%) and insulin (+204%) concentrations were significantly increased by dexamethasone in comparison to CON and PF rats, while there was no difference in concentrations between CON and PF rats (Table 3). Leptin was higher in DEX-treated rats than in CON (+46%; $P < 0.05$) and PF rats (+590%; $P < 0.0005$). Leptin concentration was decreased by 80% by food restriction. Whatever the rat group, β -OHB did not differ (Table 3).

(ii) fasted rats.

NEFA (+92%) insulin (+186%) and leptin (+830%) concentrations were significantly increased by dexamethasone in comparison to CON and PF rats, while there was no difference in these concentrations between CON and PF rats (Table 3). β -OHB was lower in DEX-treated rats than in CON (-57%; $P < 0.01$) and PF rats (-67%; $P < 0.0005$) (Table 3).

(iii) the effect of a 24-hour fast.

Fasting increased NEFA concentration in all rat groups (Table 3). Whatever the rat group, β -OHB was significantly increased by the 24-hour fast. Leptin levels were significantly

decreased (-75%) in the only CON rat group (Table 3). There was no effect of fasting on insulin concentration (Table 3).

Discussion

The present study shows that dexamethasone-induced insulin resistance (as shown by increased insulin and NEFA concentrations), affects muscle energetics at rest. Dexamethasone induced a decreased PCr concentration and PCr:ATP ratio, increased ADP concentration and led to higher PCr to γ -ATP flux but no change in β -ATP to β -ADP flux in gastrocnemius muscle. In contrast, neither 4 days of food restriction (PF rats) nor 24-hours fasting affected high-energy phosphate metabolism. This suggests that insulin resistance affects muscle energetics by impairing *in vivo* oxidative phosphorylation, and partially redirecting ATP synthesis from PCr flux.

^{31}P NMR calculation of muscle energetics rely in a constant total creatine (PCr + creatine) content in the three groups. ^1H NMR of perchloric acid extracts shows that this is the case. Furthermore it had been shown that total creatine is proportional to creatine content in other circumstances [21, 25]. Therefore, the conclusion drawn with ^{31}P NMR on muscle energetics (PCr, PCr:ATP ratio and PCr to γ -ATP flux) appear to be valid.

The finding that PCr content was decreased in DEX-treated rats is of particular interest. In fasted DEX-treated rats, the resulting decrease in PCr:ATP ratio was mainly due to a decrease in PCr levels, as ATP levels remained unchanged. On the contrary, during the fed state, ATP concentration was increased, thereby further decreasing the ratio. Such a decrease in PCr and an increase in ADP levels have also been noted with fatigue [26]. However, there are major differences between those and the present results. In fatigue, there is a significant fall in pH and a rise in P_i , which was not the case here, not surprisingly as the present animals were studied at rest. The decrease in PCr and increase in ADP could also be due to a change in fibre type i.e. a decreased proportion of type II fibres in comparison to an increased proportion of type I fibres. NMR studies in rat skeletal muscle have shown that type II fibres display higher ATP and PCr concentrations and

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lower Pi and ADP concentrations when compared with type I fibres [27]. In general, animal studies show that long-term treatment (2-10 weeks) with glucocorticoids results in skeletal muscle atrophy, with a greater decline in type II fibre than in type I fibre area, appearing in the last 7 weeks of treatment [28, 29, 30, 31, 32]. Therefore, it is unlikely that 5 days of dexamethasone would be sufficient to decrease type II fibre proportion in comparison to type I fibre. Equally, in accordance with previous *in vitro* and *in vivo* results [27, 33, 34], the spectra of the gastrocnemius in all rat groups in our study showed high PCr:Pi ratios, a characteristic feature of type II fibres. Furthermore, since acute-treatment with dexamethasone (present study) was not associated with a change in Pi or a decrease in ATP, an alteration in fibre type, if any, cannot entirely explain the observed change in PCr and free ADP levels. These results suggest therefore that either ATP was maintained at the expense of PCr or total creatine (PCr + Cr) content was decreased in muscle [35]. A lower PCr:ATP ratio, has already been found during malnutrition [35, 36, 37, 38]. In contrast, in calorie restricted rats (20% intake of control rats for 7 days), where PCr:ATP was decreased, there was a rise in free ADP concentration in comparison with control rats. It has been argued [35] that in 2-day food deprived rats, the lower PCr:ATP ratio is related to a decrease in total creatine content of skeletal muscle, while in hypocalorically fed rats, ATP levels are maintained at the expense of PCr. Here, we did not find a decrease in total creatine. Taken together, these results suggest that dexamethasone treatment was accompanied by a decrease in energy stores in order to maintain ATP levels of resting muscle. The fact that PCr to γ -ATP flux was significantly higher in DEX-treated rats reinforces such a notion.

A decrease in PCr can be the result of either an increased ATP turnover (which was not found here) or a decrease in oxidative ATP production. However, we cannot totally rule out a decrease in glycolytic ATP production since the rate of glycolysis has been found to

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be either unchanged or decreased in the muscle of rats treated with dexamethasone [39, 40]. However, increased ADP concentration is known to stimulate glycolysis. Finally, as ADP is a powerful regulator of respiration [41], we would therefore expect that higher free ADP would result in increased oxidative phosphorylation, hence sufficient ATP synthesis. As this is not the case here, it suggests that oxidative phosphorylation is impaired. In support of this, a decreased ADP/O ratio has been reported, suggesting an impairment of oxidative phosphorylation, in isolated skeletal muscle mitochondria treated *in vitro* by GCTC [42].

GCTC induces an insulin resistant state resulting in decreased insulin-stimulated glucose uptake in muscle [6], and increased plasma NEFA, insulin and leptin concentrations [7]. This is consistent with the significant increase in plasma NEFA, insulin and leptin levels in the DEX-treated rats in the present studies. Interestingly, dexamethasone-induced insulin resistance was found to perturb the equilibrium between the use of fat and glucose as energetic fuels, resulting in increased NEFA oxidation in muscle [43]. Therefore, the higher proportion of FADH₂ reducing equivalents arising from fatty acid oxidation than from glucose metabolism results in a shift in electron supply toward a two coupling site system instead of a three coupling site system. This in turn would lead to a decrease in intrinsic respiratory coupling, and a decrease oxidative phosphorylation yield. Collectively, these results suggest that ATP levels were maintained at the expense of lower PCr levels, and that ATP production from oxidative, and possibly non-oxidative, metabolism in gastrocnemius muscle of DEX-treated rats is impaired.

In the present study, *in vivo* muscle energetics are not affected by food restriction (55% for 4 days or fasting for 24 hours), as there was no difference between PF and CON rats or fasted and fed CON rats, regardless of the parameter tested. This result is in agreement with the effect of long-term caloric restriction (30% for 10 months) on high-energy

phosphate metabolism in rat skeletal muscle [44]. However, our results are in contrast to the effect of short-term caloric restriction [35]. Indeed, those authors observed that PCr:ATP ratio was lower in restricted rats (80% for 7 days) and fasted rats (48 hours) than in control rats [35]. However, the food restriction protocol used in that study [35] resulted in a 20% weight loss in restricted rats and 10% in 48-hour fasted rats. In the present study, the mean body weight loss was 4% in food-restricted rats and 6% in 24-hour fasted rats. Collectively, these results suggest that while food restriction results in a decrease in energy expenditure at the whole-body level [45], this adaptation is not always accompanied by a modification of resting energy state (PCr:ATP ratio) in skeletal muscle. Indeed, it appears that the PCr:ATP ratio is decreased when the food restriction-related weight loss is relatively high i.e. more than 9%. This notion is further reinforced by the fact that while we found that a 24-hour fast (which resulted in a 6% relative mass loss) did not alter PCr:ATP ratio, the combination of 4 days of food restriction and 24-hour fast (that resulted in a 11% loss of initial weight, PF rats in the fasted state) decreased the PCr:ATP ratio ($P < 0.05$ Anova interaction between treatment and fasting). Therefore, the decrease in bioenergetic reserves in resting skeletal muscle reported elsewhere [35] could be the consequence of the intensity of weight loss rather than that of the food restriction itself. Interestingly, DEX-treated rats (present study) lost 18% of their initial weight. However, in DEX-treated rats ATP concentration was increased, unlike that reported for food restricted rats [35]. Moreover, in our study, where the fasted DEX-treated rats had a significantly greater weight loss than the DEX-treated rats in the fed state (24 in comparison to 18%), energy status was no more affected (NS Anova interaction between treatment and fasting). This result therefore clearly indicates that the decrease in PCr:ATP ratio was mainly due to dexamethasone treatment per se. This agrees with a previous study [46] where it was found

that muscle energy status was more severely affected by triamcinolone treatment than by food restriction.

In conclusion, insulin resistance induced by dexamethasone treatment altered resting *in vivo* muscle energetics, by decreasing oxidative phosphorylation, orientating ATP synthesis from PCr.

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Table 2.

Magnetisation transfer measurements of γ -ATP synthesis from PCr (k_a) and β -ADP synthesis from β -ATP (k_b) in skeletal muscle of dexamethasone (DEX)-treated, pair-fed (PF) and control (CON) rats in fed and fasted states.

	DEX-treated		PF		CON	
	Fed	Fasted	Fed	Fasted	Fed	Fasted
Rate constant k_a (s^{-1})	0.57 ± 0.03^a	0.61 ± 0.13^a	0.42 ± 0.04	0.40 ± 0.07	0.39 ± 0.04	0.38 ± 0.04
PCr to γ -ATP flux (mmol/l. s^{-1})	18.7 ± 1.1^a	19.8 ± 4.3^a	14.8 ± 1.8	13.9 ± 2.4	14.1 ± 1.4	13.7 ± 1.2
Rate constant k_b (s^{-1})	0.79 ± 0.18	0.63 ± 0.22	0.77 ± 0.26	0.82 ± 0.02	0.87 ± 0.2	0.67 ± 0.16
β -ATP to β -ADP flux (mmol/l. s^{-1})	7.6 ± 1.7	5.8 ± 3.1	6.6 ± 2.1	7.6 ± 0.7	7.6 ± 1.7	5.7 ± 1.4

Values are mean \pm SD; n = 6; ^a P < 0.005 compared with PF and CON rats of the identical nutritional status.

Table 1.

Phosphorylated compounds in skeletal muscle of dexamethasone (DEX)-treated, pair-fed (PF) and control (CON) rats in fed and fasted states.

	DEX-treated			PF		CON	
	Fed	Fasted		Fed	Fasted	Fed	Fasted
pH	7.15 ± 0.05	7.15 ± 0.02		7.15 ± 0.04	7.15 ± 0.04	7.15 ± 0.03	7.16 ± 0.02
PCr content (mmol/l)	32.7 ± 1.1 ^a	32.4 ± 0.8 ^a		35.5 ± 0.9	34.3 ± 0.5 ^b	35.8 ± 0.7	36.0 ± 0.9
ATP content	9.8 ± 0.4 ^a	9.0 ± 0.6 ^{b c}		8.7 ± 0.3	9.2 ± 0.6 ^{b c}	8.7 ± 0.3	8.5 ± 0.4
ADP (mmol/l)	0.029 ± 0.003 ^a	0.028 ± 0.004 ^a		0.019 ± 0.003	0.022 ± 0.003 ^{b c}	0.018 ± 0.003	0.018 ± 0.002
Pi (mmol/l)	3.6 ± 0.8	5.9 ± 1.5 ^{a c}		3.7 ± 0.3	3.4 ± 0.4	3.3 ± 0.6	3.9 ± 0.7
ΔGATP (kJ/mol)	-44.08 ± 0.64 ^a	-42.65 ± 0.7 ^{a c}		-44.80 ± 0.37	-44.71 ± 0.54	-45.22 ± 0.61	-44.67 ± 0.58

Values are mean ± SD; n = 6; ^a P < 0.05 compared with PF and CON rats of the identical nutritional status; ^b P < 0.05 compared with CON rats of the identical nutritional status; ^c P < 0.05 Anova interaction between treatment and fasting.

Table 3.

Plasma hormone and metabolite concentration of dexamethasone (DEX)-treated, pair-fed (PF) and control (CON) rats in fed and fasted states.

	DEX-treated		PF		CON	
	Fed	Fasted	Fed	Fasted	Fed	Fasted
Non-esterified fatty acids (μM)	885 \pm 199 ^a	1213 \pm 196 ^{a c}	485 \pm 146	615 \pm 46	348 \pm 80	646 \pm 73 ^c
β -hydroxybutyrate (μM)	146 \pm 42	250 \pm 66 ^{a c}	126 \pm 74	759 \pm 263 ^c	116 \pm 53	578 \pm 158 ^c
Insulin (nM)	6.1 \pm 3.5 ^a	5.9 \pm 3.9 ^a	1.9 \pm 0.5	2.2 \pm 1.1	2.2 \pm 1.6	1.9 \pm 1.0
Leptin (ng/ml)	5.3 \pm 1.3 ^a	6.7 \pm 3.6 ^a	0.8 \pm 0.6 ^b	0.5 \pm 0.1	3.6 \pm 1.2	0.9 \pm 0.5 ^c

Values are mean \pm SD; n = 6; ^a P < 0.05 compared with PF and CON rats of the identical nutritional status; ^b P < 0.05 compared with CON rats of the identical nutritional status. ^c P < 0.05 Anova interaction between treatment and fasting.

Fig. 1. Daily food intake over the first 5 days of treatment (mean \pm SD; n = 6; † P < 0.01 vs. control). Δ , control; \blacklozenge , dexamethasone-treated rats

Fig. 2. Body weight changes over the 6 treatment days (mean \pm SD; n = 6; † P < 0.0001 vs. control rats; ‡ P < 0.0001 vs. pair-fed rats). ■, pair-fed; Δ , control; \blacklozenge , dexamethasone-treated rats.

fig. 1

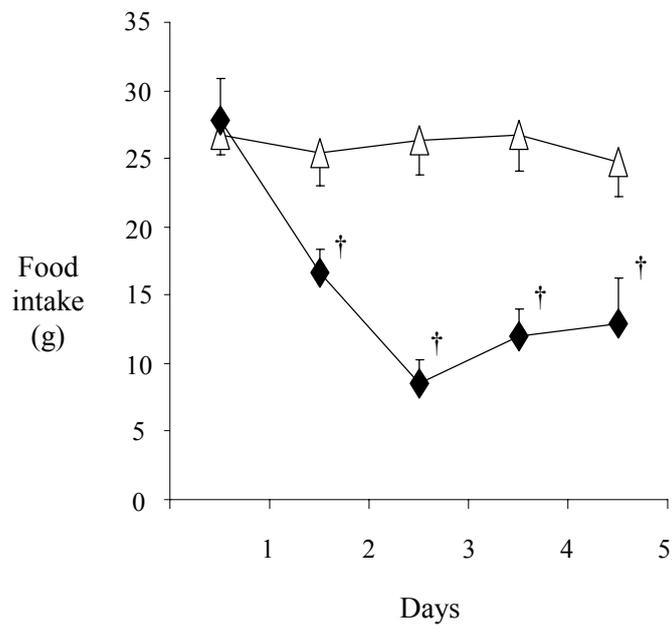


fig. 2

