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Mitochondrial energy metabolism in an experimental dexamethasone malnutrition model.

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Short title: Mitochondrial energetics in DEX-treated rats.

Keywords: glucocorticoid, mitochondrion, oxidative phosphorylation, and respiratory chain complexes.

Abstract

This investigation was undertaken to evaluate whether mitochondrial energy metabolism is altered in a malnutrition model associated with dexamethasone treatment (1.5mg/kg/day for 5 days). Gastrocnemius and liver mitochondria were isolated from dexamethasone (DEX)-treated, pair-fed (PF) and control (CON) rats. Body weight was significantly more reduced in DEX-treated group (-16%) than in PF group (-9%). Dexamethasone increased the liver mass (+59% vs. PF and +23% vs. CON) and decreased gastrocnemius mass. Moreover, in DEX-treated rats, liver mitochondria exhibited an increased rate of non-phosphorylative oxygen consumption with all substrates (approximately +42%). There was no difference in enzymatic complex activities in liver mitochondria between rat groups. Collectively, these results suggest an increased proton leak and/or redox slipping in liver mitochondria of DEX-treated rats. In addition, dexamethasone decreased the thermodynamic coupling and efficiency of oxidative phosphorylation. We therefore suggest that this increase in the proton leak and/or of redox slip in liver is responsible for the decrease in the thermodynamic efficiency of energy conversion. In contrast, none of the determined parameters of energy metabolism were altered by dexamethasone in gastrocnemius mitochondria. Therefore, it appears that dexamethasone specifically affects mitochondrial energy metabolism in liver.

Introduction

Malnutrition is highly prevalent among patients, especially elderly people. It is responsible for an increased mortality, significant hospital expenditure, reduced muscle and immune function, and decreased quality of life (Wallace *et al.* 1995; Tucker & Miguel, 1996; Chima *et al.* 1997; Lesourd & Mazzari, 1997; Landi *et al.* 2000). Malnutrition results from a negative energy balance, a situation where energy intake fails to meet energy requirements. Although anorexia and a reduced energy intake are always associated with malnutrition, in some clinical circumstances, an increased resting oxygen consumption rate (referred to as an increased energy requirements) can be shown (Nguyen *et al.* 1999). On the contrary, most human and non-human studies show that energy restriction decreases energy expenditure (Ramsey *et al.* 2000). Therefore it appears that in these clinical states, adaptive mechanisms which lead to a reduction of energy requirements, fail to operate. However, the biochemical nature of this negative energy balance phenomenon is poorly understood at the present time. A wasting of energy may be a possible explanation. In other words, oxidative phosphorylation may be less efficient.

High-dose glucocorticoid treatment affects body weight and body composition (Kochakian & Robertson, 1951; Hausberger & Hausberger, 1958). It has also been found to induce a hypercatabolic state which leads to a reduced muscle mass (Marone *et al.* 1994; Minet-Quinard *et al.* 1999), suppressed protein synthesis, a transient increase in protein degradation and a negative nitrogen balance (Bowes *et al.* 1996; Max *et al.* 1988; Odedra *et al.* 1983). Moreover, it is a model of hypercortisolism, which occurs during metabolic stress in humans and which is associated with an increased energy expenditure (Woodward & Emery, 1989; Brillon *et al.* 1995; Tataranni *et al.* 1996). On the other hand, acute high-dose treatment (<1 week) of rats with dexamethasone decreases food intake (Kaur *et al.* 1989; Minet-Quinard *et al.* 1999). Therefore, a negative energy balance ensues, both because of an increased energy expenditure and a decreased energy intake. The mechanisms leading to this increased energy expenditure are not fully understood. There are arguments to suggest that mitochondrial energy production could be affected. However, the effects of glucocorticoids on cellular energy metabolism depend on the tissues being investigated and on

28 the duration of treatment. In the liver for example, short-term administration (<24 hours) of
29 dexamethasone appears to increase oxidative phosphorylation while having no effect on non-
30 phosphorylative respiration (Wakat & Haynes, 1977; Allan *et al.* 1983). On the contrary, longer
31 administration (<1 week) of glucocorticoids, results in decreased liver oxidative phosphorylation
32 and ATP synthesis when fuelled through complex I, however remaining unchanged when fuelled
33 through complex II or IV (Kerppola, 1960; Kimura & Rasmussen, 1977; Jani *et al.* 1991). In
34 isolated skeletal muscle mitochondria, studies show either no change, a decrease or an increase in
35 oxidative capacity (Cytochrome c oxidase activity) or in oxidative phosphorylation (Vignos &
36 Greene, 1973; Koski *et al.* 1974; Capaccio *et al.* 1985; Marone *et al.* 1994; Weber *et al.*, 2002).

37 Since liver and muscle contribute approximately 50% of body oxygen consumption (Rolfe
38 & Brown, 1997), most of it being coupled with ATP synthesis, we undertook this study to
39 investigate the effects of 5 days of high dose (1.5mg/kg/day) dexamethasone on energy metabolism
40 particularly in muscle and liver mitochondria. The comparison with caloric restriction helps in the
41 understanding of the increased energy expenditure observed at the whole body level.

42

Materials and methods

Animals

The present investigation was performed in accordance with the French guiding principles in the care and use of animals. Thirty-two male Sprague-Dawley rats, born and bred in our animal facility, were housed in individual cages at 9 weeks of age (300-350g). Animals were provided with water *ad libitum* and a standard diet (U.A.R A04) consisting (% weight) of 16% protein, 3% fat, 60% carbohydrate and 21% water, fibre, vitamins and minerals. The metabolizable energy content was 12 kJ/g. Rats were divided into 4 groups of 8 as follows: dexamethasone(DEX)-treated rats received a daily intraperitoneal injection of 1.5 mg/kg of dexamethasone for 5 days. Due to the fact that dexamethasone treatment induces anorexia, pair-fed (PF) rats were used to discriminate between the effect of anorexia and the effect of dexamethasone itself on the parameters measured. PF rats were pair-fed with DEX-treated animals (rats received the same food quantity 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*. Animals of the control injected group (CI) were fed *ad libitum* and were injected with an isovolumic solution of 0.9% NaCl. This group was used to study the effects of NaCl injection. As results were similar between the two control groups, the CI group was omitted in the presentation of data. Experiments were conducted over a 5-day period. The dose and duration of the dexamethasone treatment was chosen with reference to the literature and is known to induce a reproducible maximum hypercatabolic state (Minet-Quinard *et al.* 2000). On the 4th day, following an overnight fast, the animals were killed by decapitation. Gastrocnemius, liver and interscapular brown adipose tissue were removed rapidly and weighed. Some tissue samples were immediately used for respiratory measurements and the remainder were frozen in liquid nitrogen and stored at -80°C in order to measure enzyme activity levels. Gastrocnemius muscle was chosen because this mixed-fibers tissue is representative of muscle fiber types contained in the hindlimb of the Sprague-Dawley rat (Armstrong & Phelps, 1984).

Mitochondrial enzyme activities

69
70 Frozen liver and gastrocnemius (10-30 mg) were thawed and homogenized with a Potter-Elvehjem
71 homogeniser (7 strokes) in an isolation medium consisting of 220 mM mannitol, 75 mM sucrose, 10
72 mM Tris and 1 mM EGTA, pH 7.2. Each homogenate was centrifuged at 600 g for 10 min and the
73 resulting supernatants were filtered through cheesecloth. All procedures were performed at 4°C.
74 The activity of citrate synthase, succinate dehydrogenase and complexes I, III and IV was measured
75 spectrophotometrically at 37°C in the supernatant fraction via an adaptation of that as described by
76 Malgat *et al.* (1999), and in agreement with the Mitochondrial Diseases Group of the Association
77 Française de Myopathie (AFM). Protein concentration was determined using the Bicinchoninic acid
78 Assay kit (Interchim, Montluçon, France) with bovine serum albumin (BSA) used as a control.

79 The activity of citrate synthase (CS) was measured in a reaction medium consisting of 100
80 mM Tris/HCl, 40 µg/ml 5,5'-dithio-bis(2-nitrobenzoic acid), 1 mM oxaloacetate, 0.3 mM acetyl
81 CoA and 4 % of Triton X 100, pH 8.1. After 3 min of incubation, the reaction was initiated by
82 adding the homogenate (20 to 50 µg proteins) and the change in optical density at 412 nm was
83 recorded for 3 min.

84 The activity of succinate dehydrogenase was measured by following the reduction of 2,6-
85 dichlorophenolindophenol (DCPIP), in the presence of phenazine methosulfate (PMS) at 600 nm.
86 Homogenate (20 to 50 µg proteins) was preincubated in a buffer containing 50 mM KH₂PO₄, 16
87 mM succinate, 1.5 mM KCN, 100 µM PMS, pH 7.5 for 5 min. The reaction began with the addition
88 of 103 µM DCPIP and the optical density was recorded for 3 min.

89 The activity of complex I was determined by monitoring the oxidation of NADH at 340 nm.
90 Homogenate (40 to 100 µg proteins) was preincubated for 3 min in 820 µl of distilled water.
91 Following this, 10 mM KH₂PO₄, 2 mM KCN, 5 µg/ml antimycin A, 100 µM decylubiquinone, 1.3
92 mg/ml BSA, 5 mM MgCl₂, pH 7.5 were added. The reaction was initiated by the addition of 200
93 µM NADH and the change in the optical density was analysed for 3 min. The NADH
94 decylubiquinone reductase activity was also measured in the presence of 12.7 µM rotenone. The

95 specific activity of complex I represented the difference between NADH oxidation activity, both
96 with and without the rotenone.

97 The activity of complex III was determined by monitoring the reduction of cytochrome c at
98 550 nm. Homogenate (20 to 50 μg proteins) was incubated for 30 sec in a reaction medium
99 consisting of 35 mM KH_2PO_4 , 5 mM MgCl_2 , 2.5 mg/ml BSA, 1.8 mM KCN, 125 μM oxidized
100 cytochrome c, 12.5 μM rotenone and 62.5 mM EDTA, pH 7.5. The reaction was initiated by adding
101 80 μM decylubiquinol and the optical density was measured for 3 min. The nonenzymatic reduction
102 of cytochrome c was measured under identical conditions after the addition of 10 $\mu\text{g}/\text{ml}$ antimycin
103 A. The specific activity of complex III was calculated by subtracting the activity of the
104 nonenzymatic reaction from that of the total activity of complex III.

105 The activity of complex IV was measured by monitoring the oxidation of reduced
106 cytochrome c at 550 nm. A 50 μM solution of reduced cytochrome c (92 to 97% reduced using
107 dithionite) in 10 mM KH_2PO_4 , pH 7.0 was preincubated for 5 min. The reaction was initiated by
108 adding the homogenate (20 to 50 μg proteins) and the change in optical density was measured for
109 1.5 min.

110

111 *Mitochondrial isolations*

112 Gastrocnemius muscle and liver were removed, weighed and immediately placed in an ice-cold
113 isolation medium consisting of 250 mM sucrose, 1 mM EGTA and 10 mM Tris/HCl, pH 7.4.
114 Muscle mitochondria were isolated from gastrocnemius via an adaptation of the differential
115 centrifugation procedure, as used previously by Roussel *et al.* (2000). All steps were performed at
116 4°C.

117 Muscles (2-2.5 g) were cut with scissors, minced using a Polytron (4 to 5 sec.) in an isolation
118 medium (20 ml/g tissue) then homogenized with a Potter-Elvehjem homogeniser (7 strokes). The
119 homogenate was centrifuged at 600 g for 10 min. The resulting supernatant was filtered through
120 cheesecloth and then centrifuged at 10 000 g for 10 min. The pellet was resuspended in the isolation
121 medium and then centrifuged at 10 000 g for 10 min. Finally, the pellet was resuspended in a

122 minimal volume of respiratory medium consisting of 120 mM KCl, 5 mM KH₂PO₄, 3 mM
123 HEPES, 1 mM EGTA, 2 mM MgCl₂ and 0.3 % (w/v) BSA, pH 7.4. Liver mitochondria were
124 isolated by using a modification of the differential centrifugation procedure used previously by
125 Krahenbuhl *et al.* (1994). The liver (6-7 g) was cut with scissors and homogenized using a Potter-
126 Elvehjem homogeniser (7 strokes) in the isolation medium (8 ml/g tissue). The homogenate was
127 centrifuged at 600 g for 10 min. The resulting supernatant was filtered through cheesecloth, then
128 centrifuged at 7 000 g for 10 min. The pellet was resuspended in the isolation medium (10 ml/g
129 tissue) and then centrifuged at 3 500 g for 10 min. The resulting pellet was resuspended in a minute
130 volume of respiratory medium. The lower g force of the last centrifugation step (3500 g) gives a
131 more homogeneous mitochondrial preparation and is known to minimize the cytoplasmic
132 contamination and mitochondrial damage done to the pellet (< 10% in the present study) in addition
133 to maximizing the respiratory parameters (Goglia *et al.* 1988; Lanni *et al.* 1996).

134

135

Mitochondrial respiration

136 Oxygen was measured using a Clark oxygen electrode (oxygraph Hansatech), in a 2 ml glass cell,
137 via continuous stirring at a constant temperature of 30°C. Mitochondria (0.4-0.6 mg protein/ml)
138 were incubated in the respiratory reaction medium as described above and saturated with room air.
139 Substrate concentrations from liver measurements were 5 mM glutamate, 5 mM succinate and 5
140 mM ascorbate + 0.5 mM *N,N,N',N'*-tetramethyl-*p*-phenylenediamine (TMPD). With regards to
141 gastrocnemius measurements, 5 mM pyruvate + 5 mM malate, 5 mM succinate and 2 mM ascorbate
142 + 0.5 mM TMPD were used. Inhibitor concentrations included 5 µM rotenone (to inhibit complex I
143 of the respiratory chain), and 3 mM myxothiazole (to inhibit complex III). The active state of
144 respiration (state 3) was initiated by the addition of ADP (150 µM to the liver mitochondria or 200
145 µM to the gastrocnemius mitochondria). The basal non-phosphorylating respiration rate (state 4)
146 was obtained by the addition of 3 µg/ml of oligomycin. RCR was the ratio of oxygen consumed
147 after the addition of ADP to that consumed in the presence of oligomycin. The uncoupled state of
148 respiration was initiated by the addition of 2 µM of FCCP (carbonyl cyanide *p*-

149 trifluoromethoxyphenylhydrazine). The respiratory parameters measured in isolated mitochondria
 150 were normalized in relation to the specific activity of citrate synthase.

151

152 *Calculation of thermodynamic coupling and efficiency of oxidative phosphorylation*

153 Parameters were calculated using the methodology of Cairns *et al* (1998). The thermodynamic
 154 coupling of the energy conversion is designated by the dimensionless parameter q , known as the
 155 degree of coupling of oxidative phosphorylation.

156
$$q = (1 - (J_{sh}/J_{unc}))^{1/2}$$

157 J_{sh} is the net oxygen consumption in state 4-oligomycin respiration, in the presence of oligomycin
 158 that inhibits ATP synthase. J_{unc} is the uncoupled rate of oxygen uptake induced by the addition of
 159 FCCP, which dissipates the transmembrane proton gradient, and as a result ATP production
 160 becomes nil.

161 Kedem and Caplan (1965) have defined the efficiency of the energy conversion for oxidative
 162 phosphorylation (η). Between state 4-oligomycin and state 3-uncoupled respiration, which represent
 163 two steady states, an optimal thermodynamic efficiency of the energy conversion (η_{opt}) can be
 164 discerned for any value of q (Stucki, 1980).

165
$$\eta_{opt} = \tan^2 (\alpha/2), \text{ where } \alpha = \arcsin q$$

166 Oxidative phosphorylation should operate at a steady state for optimal efficiency for any given
 167 degree of coupling. In addition, q can represent several well-defined values depending on the
 168 energetic needs of the cell (Stucki, 1980). This theory is based on the thermodynamic trade-off of
 169 reducing efficiency to produce the maximum phosphate potential or increasing the efficiency to
 170 economize phosphate potential. Stucki (1980) has defined some physiological meanings for the
 171 degrees of mitochondrial oxidative coupling. The specific thermodynamic degrees of coupling
 172 correspond to the following set points with a unique maximal value of q : q_p^{ec} (0.972) which is the
 173 economic net output power (phosphate potential) at optimal efficiency, q_f^{ec} (0.953) being the
 174 economic net output flow (ATP), q_p (0.910) as the maximal net output power and q_f (0.786) as the
 175 maximal net output flow at optimal efficiency.

176 In comparison to conventional measurements (RCR, ATP/O), non-equilibrium thermodynamics
177 analysis provides a quantitative description and a better estimation of stoichiometry and the
178 efficiency of energy conversion.

179

180 *Statistical analysis*

181 Results were expressed as mean \pm standard deviation (SD). Means were compared by ANOVA
182 using a Fisher Post-hoc test. A P value of < 0.05 was considered significant in all cases. All
183 analyses were performed using StatView version 5.0 (SAS Institute, Cary, NC, USA).

184

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. Body weight decreased in DEX-treated rats from day 1, and PF animals from day 2 (Fig. 2). This decrease was significantly greater in DEX-treated rats than in PF animals, corresponding to 16% (DEX-treated) and 9% (PF) of initial body mass on the 4th day of treatment. At the same time, CON rats increased their body mass by 3.2% (Fig. 2).

The overnight fasting decreased body weight in the 3 rats groups (Table 1). Liver weight was increased in DEX-treated animals by 23% vs. CON ($P < 0.01$) and by 59% vs. PF ($P < 0.01$) (Table 1). Conversely food restriction significantly decreased liver mass by 20% (PF rats vs. CON rats). This difference in liver mass was not related to a change in hydration, as the dexamethasone-treatment or the food restriction did not affect relative water contents (62.0 ± 3.0 vs. 66.0 ± 8.4 vs. 62.1 ± 4.3 % per liver in DEX-treated, PF and CON rats). In DEX-treated rats, gastrocnemius mass was significantly decreased in comparison with PF rats (-19%) and CON animals (-19%) (Table 1). There was a significant increase in interscapular BAT mass in the DEX-treated group (+117% compared to PF and 90% compared to CON) (Table 1).

In the liver, complex I activity was significantly higher in the DEX-treated group than in the PF group (+120%), but it was not different in comparison to the CON group (Table 2). Dexamethasone treatment significantly decreased the specific activity of complex IV (-28% compared to PF rats), although it was not different when compared to controls. There were no significant difference in the specific activities of citrate synthase, succinate dehydrogenase and complex III between the DEX-treated and other groups (Table 2).

In gastrocnemius, none of the enzymatic activities were significantly affected by glucocorticoid treatment (Table 3).

In the isolated liver mitochondria, the specific citrate synthase activity was significantly lower in the DEX-treated group than in the CON group (-28%) and PF group (-20%) (data not shown). Basal non-phosphorylative respiration (state 4-oligomycin respiration) rates, normalized by

211 the specific citrate synthase activity, are shown in Figure 3. With succinate and TMPD/ascorbate
212 used as substrates, the state 4-oligomycin respiration was significantly increased (+46%) in isolated
213 liver mitochondria from DEX-treated rats compared to other groups. In the liver glutamate-respiring
214 mitochondria, dexamethasone treatment significantly increased (+33%) the state 4-oligomycin
215 oxygen consumption in comparison to PF rats, but not when compared to CON rats.

216 Table 4 shows that the respiratory parameters in the liver, expressed per mg of mitochondria
217 protein, were similar, across groups regardless of the respiratory substrate.

218 In gastrocnemius none of the respiratory parameters were affected by dexamethasone
219 treatment whatever the substrate used (Table 5). When gastrocnemius respiratory parameters were
220 normalized by the CS activity, no difference was observed between groups (data not shown).

221 In liver succinate-respiring mitochondria, q was significantly decreased by dexamethasone
222 treatment. With glutamate, q was marginally ($P=0.12$) lower in DEX-treated and PF than in CON
223 rats (Table 6). Similar results were obtained for the determined thermodynamic optimal efficiency
224 of oxidative phosphorylation (η_{opt}).

225 In gastrocnemius, q and η_{opt} were similar in the 3 rats groups (Table 7).

226

Discussion

The present study reports that the induction of a catabolic state, by dexamethasone, results in an increased liver mass and increased non-phosphorylative oxygen consumption in liver mitochondria. In addition, we found a decreased thermodynamic coupling and efficiency of the oxidative phosphorylation in the complex I and II respiratory pathways in the liver mitochondria of DEX-treated rats. In contrast, dexamethasone induced gastrocnemius mass atrophy without affecting mitochondrial energy metabolism.

While the citrate-synthase activity was not modified in the 600 g homogenate it was decreased in isolated mitochondria of liver in DEX-treated rats. Moreover, we found a lower percentage of intact mitochondria in isolated liver mitochondria of DEX-treated rats (91% compared to 96% and 94% in CON and PF groups). Therefore, for the measurement of parameters in isolated mitochondria preparations, we used the specific citrate synthase activity as a mitochondrial marker enzyme. These parameters/CS ratios may reflect a change originating from the mitochondria themselves, rather than from the homogeneization or measurement procedures.

Of particular interest is the finding that dexamethasone treatment significantly increased liver non-phosphorylative oxygen consumption using succinate (+46%) and ascorbate as substrates (+46%; versus CON and PF). With regard to glutamate, state 4-oligomycin respiration was not different compared to control rats, but it was higher (+33%) in DEX-treated relative to PF animals. Therefore, it appears that there is a specific catabolic state-related increase (+42%) in the non-phosphorylative oxygen consumption of liver mitochondria. Indeed, state 4-oligomycin respiration was increased in DEX-treated rats compared to CON rats, except in complex I (effect of food restriction and/or hypercatabolism), and consistent increases were found when DEX-treated animals were compared to PF animals (effect of hypercatabolism). On the other hand, food restriction appears to reduce state 4-oligomycin oxygen consumption (via complex I) in PF compared to CON rats. Such a change in these non-phosphorylative conditions could be due to modifications in respiratory chain activity, inner membrane conductance (proton leak) or the intrinsic coupling of the respiratory chain ($H^+/2e^-$). The present acute treatment using dexamethasone did not modify

253 respiratory chain complex activities in the liver. As a result, this strongly suggests that basal
254 proton conductance and/or the efficiency at the level of the respiratory chain did change in the liver
255 mitochondria. In the latter case, we can speculate an effect located on the cytochrome oxidase as
256 state 4-oligomycin respiration by using ascorbate/TMPD as substrate was increased. Moreover, it
257 has been demonstrated that cytochrome oxidase $H^+/2e^-$ stoichiometry is variable and represents a
258 possible location for intrinsic uncoupling at the level of the respiratory chain (Capitanio *et al.* 1991;
259 Papa *et al.* 1991; Piquet *et al.* 2000). However, further experiments are necessary to confirm these
260 possibilities. Whatever the mechanism involved, this represents a substantial increase in state 4
261 energy wastage. Moreover, it is interesting to note that proton leak and redox slipping may affect
262 the oxidative phosphorylation yield. Our results agree with data obtained from the long-term
263 administration (3-7 days) of high-dose glucocorticoids, which are reported to decrease liver
264 oxidative phosphorylation (state 3 respiration) via complex I respiratory substrates while remaining
265 unchanged when fuelled through complex II or IV (Kerppola, 1960; Kimura & Rasmussen, 1977;
266 Jani *et al.* 1991). Our findings that state 4 respiration is increased are therefore complementary to
267 those studies since it has never been studied in DEX-treated rats, although inconsistencies were
268 shown in corticosterone-treated rats (Jani *et al.* 1991).

269 Mitochondria can vary the efficiency of oxidative phosphorylation in order to respond to one
270 of four physiological missions: 1) maximizing ATP production with a corresponding value q_f for
271 the thermodynamic degree of coupling, 2) maximizing the cellular phosphate potential (q_p), 3)
272 minimizing the cost of production (q^{ec}_f) and 4) a combination of all three (q^{ec}_p) (Stucki JW, 1980).
273 In the control animals used in our study, the experimentally derived q value for the complex I and II
274 pathways were between that of q_p and q^{ec}_f . Therefore, the liver mitochondria of the control animals
275 adapted their function for the economic production of ATP in addition to maintaining the phosphate
276 potential. Dexamethasone decreases the degree of thermodynamic coupling of oxidative
277 phosphorylation in both complex I and II respiratory pathways. Their values were between q_f and q_p
278 and nearly that of q_p . In relation to this result, the thermodynamic optimal efficiency of energy
279 conversion (η_{opt}) was also decreased by dexamethasone. These results therefore suggest that in

280 DEX-treated rats, liver mitochondria adapt their function for maximum ATP production and also
281 to maintain cellular phosphate potential at the expense of the energy conversion efficiency. Similar
282 adaptive reductions in the efficiency of oxidative phosphorylation has already been noted by
283 Nogueira *et al* (2001) who showed that cellular respiratory rate increases in liver mitochondria of
284 either hyperthyroid rats or animals exposed to a polyunsaturated fatty acid deficient diet.

285 It is well known that liver mass is decreased during energy restriction, which in turn could
286 partly explain why energy expenditure falls in response to a reduced food intake (Ramsey *et al.*
287 2000). In the present study, liver weight was decreased by food restriction (PF versus control rats)
288 and higher (23 to 59%) in DEX-treated rats compared with other rat groups. Such an increase in
289 liver mass could result from an increased glycogen content (Weber & Kletzien, 1982; Michaels &
290 Cardell, 1997; Bollen *et al.* 1998) although we did not find any difference in hydration.
291 Alternatively, hepatic lipid content is increased in DEX-treated rats, but is insufficient to fully
292 explain the increased liver mass (Kaur N *et al.* 1989; Palacios *et al.* 1995; Franco-Colin *et al.* 2000).
293 Finally, dexamethasone treatment increase liver protein synthesis and therefore metabolic tissue
294 (Odedra *et al.* 1983; Savary *et al.* 2001). This suggests that both maintenance and growth costs may
295 be increased.

296 Our study demonstrated that a dexamethasone-induced hypercatabolic state results in
297 skeletal muscle atrophy without any change in mitochondrial energy metabolism. Indeed, none of
298 the respiratory complex activities, oxygen consumption rates or the thermodynamic degree of
299 coupling of oxidative phosphorylation, were altered in the mitochondria of DEX-treated rats. This is
300 in agreement with previously reported effects of glucocorticoid treatment (Vignos & Greene, 1973;
301 Capaccio *et al.* 1985; Marone *et al.*, 1994). These results suggest that the mitochondrial metabolism
302 of skeletal muscle produces enough ATP to fulfil either the cellular energy requirement and/or the
303 energy-dependent pathways induced by glucocorticoids, such as the energy-ubiquin-dependent
304 proteolytic pathway (Tiao G *et al.* 1996; Mitch *et al.* 1999), and the energy-dependent glutamine
305 synthase activity pathway (Max SR *et al.*, 1988; Minet-Quinard *et al.* 1999, 2000).

306 The main thermogenic tissue in rats is brown adipose tissue, the weight of which is largely
307 increased (+100%, present study) by glucocorticoid injection. Previous studies have clearly
308 demonstrated that such an increase in the BAT mass was due to increased lipid storage rather than
309 an increased thermogenic capacity of this tissue (Mazzuccheli *et al.* 1960; Strack *et al.* 1995).
310 Furthermore, glucocorticoids are known to reduce the activity or the gene expression of UCP1
311 (Tokuyama & Himms-Hagen, 1989; Moriscot *et al.* 1993; Strack *et al.* 1995). Moreover, in our
312 study we found no effect of dexamethasone on mitochondrial oxidative capacity (cytochrome c
313 oxidase activity; data not shown). Therefore, it is unlikely that interscapular BAT increases energy
314 expenditure in DEX-treated rats.

315 A reduction in body size (a 16% weight loss in the present study) generally results in
316 lowering of energy expenditure per whole rat, because of the reduced maintenance requirement
317 (Ramsey *et al.* 2000). In the present study, the dexamethasone-related decrease in the body weight
318 was greater than that observed in the PF rats, highlighting an increased whole body energy
319 expenditure. This is paradoxical in view of the reducing effect of food restriction on energy
320 expenditure (Ramsey *et al.* 2000). Furthermore, in our study, dexamethasone treatment increased
321 the liver-to-body weight ratio while the relative skeletal muscle mass remained unchanged. Since
322 liver and muscle are the main contributors to standard metabolic rate in rats (Rolfe & Brown, 1997),
323 it is likely that the liver would effectively contribute to the increased energy expenditure despite the
324 body weight loss (Woodward & Emery, 1989). Indeed, if we assume that liver contributes 20% to
325 the metabolic rate of a rat (Rolfe & Brown, 1997), then the 60-80% gain in the liver-to-body weight
326 ratio reported herein, could increase total energy expenditure by 12-16%. Glucocorticoids
327 administered in humans and rats results in an increased energy expenditure by 10-20% (Woodward
328 & Emery, 1989; Brillon *et al.* 1995; Tataranni *et al.* 1996). Obviously, we can not rule out the
329 influence of other biochemical mechanisms or possible determinants of energy balance which have
330 accounted also for the weight loss.

331 In conclusion, 5 days of high-dose dexamethasone treatment induced a significant increase
332 in liver mass, an increase in liver mitochondrial non-phosphorylative oxygen consumption rate from

333 all substrates used, and a decrease in the thermodynamic coupling of oxidative phosphorylation in
334 liver respiratory pathways. We suggest therefore that dexamethasone increases proton leak and/or
335 redox slipping in liver mitochondria, which in turn is probably responsible for the decrease in the
336 thermodynamic efficiency of energy conversion. Thus, rats would adapt their mitochondrial energy
337 functions to a dexamethasone-induced hypermetabolic state by maximizing ATP production in
338 addition to maintaining their cellular energy state, regardless of the cost. This treatment has no
339 effect on energy metabolism in the muscle. Together with a decreased food intake, the increase in
340 energy expenditure induced by high dose of dexamethasone results in a negative energy balance and
341 thus weight loss.

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Textes footnotes

BAT, brown adipose tissue; CS, citrate synthase; DCPIP, 2,3-dichlorophenolindophenol; FCCP, carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone; PMS, phenazine methosulfate; RCR, respiratory control ratio; TCA, tricarboxylic acid; TMPD, *N,N,N',N'*-tetramethyl-*p*-phenylenediamine; TNB, thio-bis(2-nitrobenzoic acid).

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Figure 1. Daily food intake during the last 4 days of treatment, for control (CON) (▲) and dexamethasone (DEX)-treated (◆) rats. For details of procedure see Materials and methods section. Values expressed as the mean for eight rats per group (standard deviation denoted by vertical bars). Significant differences indicated: † P < 0.01 vs. CON.

Figure 2. Body weight changes during the first 4 days of treatment for pair-fed (PF) (■), control (CON) (▲) and dexamethasone (DEX)-treated (◆) rats. For details of procedure see Materials and methods section. Values expressed as the mean for eight rats per group (standard deviation denoted by vertical bars). Significant differences indicated: † P < 0.01 vs. CON; ** P < 0.05 vs. PF.

Figure 3. State 4-oligomycin respiration normalized by the specific citrate synthase activity in liver isolated mitochondria from pair-fed (PF), control (CON) and dexamethasone (DEX)-treated rats. For details of procedure see Materials and methods section. Values expressed as the mean for eight rats per group (standard deviation denoted by vertical bars). Significant differences indicated: ‡ P < 0.05 vs. CON; ** P < 0.05 vs. PF.

Table 1. Body weight and organ mass: liver, gastrocnemius muscle and interscapular brown adipose tissue in pair-fed (PF), control (CON) and dexamethasone (DEX)-treated rats.
(Values expressed as mean \pm standard deviation for eight rats per group)

	DEX-treated	PF	CON	ANOVA
Initial body weight, g	310 \pm 28	329 \pm 38	329 \pm 32	NS
Sacrifice body weight, g	242 \pm 19 †*	285 \pm 37	315 \pm 33	P = 0.0006
Liver, g	13.38 \pm 2.30 †*	8.58 \pm 1.12 †	10.84 \pm 1.47	P < 0.0001
g/100g body weight	5.49 \pm 0.66 †*	3.04 \pm 0.28	3.44 \pm 0.24	P < 0.0001
Gastrocnemius, g	3.27 \pm 0.21 †*	4.04 \pm 0.50	4.25 \pm 0.27	P < 0.0001
g/100g body weight	1.36 \pm 0.07	1.41 \pm 0.06	1.36 \pm 0.09	NS
Brown adipose tissue, g	0.76 \pm 0.20 †*	0.35 \pm 0.10	0.40 \pm 0.14	P < 0.0001
g/100g body weight	0.31 \pm 0.08 †*	0.12 \pm 0.04	0.12 \pm 0.04	P < 0.0001

Significant differences indicated: * P < 0.01 vs. PF, † P < 0.01 vs. CON; NS: not significant.

For details of procedure see Materials and methods.

Table 2. Mitochondrial enzyme activities in liver 600 g homogenate from pair-fed (PF), control (CON) and dexamethasone (DEX)-treated rats.

(Values expressed as mean \pm standard deviation for eight rats per group)

Enzymatic activity (nmol/min per mg of protein)	DEX-treated	PF	CON	ANOVA
Citrate Synthase	151 \pm 27	158 \pm 23	152 \pm 26	NS
Succinate Dehydrogenase	101 \pm 26	107 \pm 30	109 \pm 26	NS
NADH-ubiquinone reductase (Complex I)	44 \pm 11 *	20 \pm 8 †	44 \pm 20	P < 0.01
Ubiquinol-cytochrome c reductase (Complex III)	88 \pm 57	86 \pm 52	101 \pm 81	NS
Cytochrome c oxidase (Complex IV)	91 \pm 31 **	126 \pm 29	100 \pm 21	P < 0.05

Significant differences indicated: * P < 0.01 vs. PF, † P < 0.01 vs. CON; ** P < 0.05 vs. PF; NS: not significant.

For details of procedures see materials and methods section.

Table 3. Mitochondrial enzyme activities in gastrocnemius muscle 600 g homogenate from pair-fed (PF), control (CON) and dexamethasone (DEX)-treated rats.

(Mean values with their standard deviation for eight rats per group)

Enzymatic activity (nmol/min per mg of protein)	DEX-treated	PF	CON	ANOVA
Citrate Synthase	602 ± 239	653 ± 314	538 ± 183	NS
Succinate Dehydrogenase	130 ± 65	147 ± 63	125 ± 49	NS
NADH-ubiquinone reductase (Complex I)	111 ± 57	99 ± 76	85 ± 32	NS
Ubiquinol-cytochrome c reductase (Complex III)	382 ± 190	363 ± 208	409 ± 170	NS
Cytochrome c oxidase (Complex IV)	166 ± 66	213 ± 112	194 ± 84	NS

NS: not significant.

For details of procedures see materials and methods section.

Table 4. Respiratory parameters of liver isolated mitochondria from pair-fed (PF), control (CON) and dexamethasone (DEX)-treated rats.

(Values expressed as mean \pm standard deviation for eight rats per group)

Respiratory substrates	Parameters	DEX-treated	PF	CON	ANOVA
Glutamate	State 3	35 \pm 16	26 \pm 13	43 \pm 19	NS
	State 4	5 \pm 1	5 \pm 1	6 \pm 1	NS
	RCR	7 \pm 3	5 \pm 3	7 \pm 3	NS
	Uncoupled state	29 \pm 17	24 \pm 13	46 \pm 24	NS
Succinate	State 3	76 \pm 29	79 \pm 28	98 \pm 16	NS
	State 4	18 \pm 5	14 \pm 3	18 \pm 2	NS
	RCR	4.2 \pm 1.4	5.6 \pm 1.4	5.4 \pm 0.8	NS
	Uncoupled state	93 \pm 26	103 \pm 31	121 \pm 21	NS
TMPD/ascorbate	State 3	131 \pm 34	122 \pm 13	147 \pm 27	NS
	State 4	98 \pm 26	81 \pm 16	96 \pm 16	NS
	RCR	1.3 \pm 0.1	1.5 \pm 0.2	1.5 \pm 0.1	NS
	Uncoupled state	167 \pm 39	163 \pm 30	190 \pm 41	NS

NS: not significant.

State 3, state 4 and uncoupled state respiration expressed as nanomoles of oxygen/min per mg mitochondrial protein. For details of procedures see materials and methods section.

Table 5. Respiratory parameters of gastrocnemius isolated mitochondria from pair-fed (PF), control (CON) and dexamethasone (DEX)-treated rats.

(Values expressed as mean \pm standard deviation for eight rats per group)

Respiratory substrates	Parameters	DEX-treated	PF	CON	ANOVA
Pyruvate + malate	State 3	70 \pm 39	105 \pm 48	85 \pm 30	NS
	State 4	8 \pm 4	9 \pm 2	7 \pm 2	NS
	RCR	9 \pm	11.7 \pm	12.8 \pm	NS
	Uncoupled state	123 \pm 74	174 \pm 37	133 \pm 41	NS
Succinate	State 3	88 \pm 29	118 \pm 26	120 \pm 44	NS
	State 4	24 \pm 13	35 \pm 21	27 \pm 16	NS
	RCR	3.7 \pm	3.4 \pm	4.4 \pm	NS
	Uncoupled state	123 \pm 46	156 \pm 53	128 \pm 59	NS
TMPD/ascorbate	State 3	260 \pm 117	289 \pm 75	231 \pm 61	NS
	State 4	172 \pm 84	186 \pm 58	156 \pm 39	NS
	RCR	1.5 \pm 0.2	1.5 \pm 0.2	1.5 \pm 0.3	NS
	Uncoupled state	385 \pm 128	354 \pm 108	403 \pm 121	NS

NS: not significant.

State 3, state 4 and uncoupled state respiration expressed as nanomoles of oxygen/min per mg mitochondrial protein. For details of procedures see materials and methods section.

Table 6. Thermodynamic degree of coupling (q) and optimal efficiency (η_{opt}) of the oxidative phosphorylation in liver isolated mitochondria from pair-fed (PF), control (CON) and dexamethasone (DEX)-treated rats.

(Values expressed as mean \pm standard deviation for eight rats per group)

Respiratory substrates	Parameters	DEX-treated	PF	CON	ANOVA
Glutamate	q	0.903 ± 0.044	0.896 ± 0.040	0.942 ± 0.013	NS
	η_{opt}	0.413 ± 0.092	0.396 ± 0.080	0.500 ± 0.039	NS
Succinate	q	$0.896 \pm 0.019 \ddagger^{**}$	0.923 ± 0.022	0.922 ± 0.011	$P < 0.05$
	η_{opt}	$0.387 \pm 0.038 \ddagger^{**}$	0.451 ± 0.052	0.439 ± 0.028	$P < 0.05$
Ascorbate/TMPD	q	$0.656 \pm 0.028 \dagger^*$	0.708 ± 0.024	0.706 ± 0.028	$P < 0.005$
	η_{opt}	$0.140 \pm 0.016 \dagger^*$	0.173 ± 0.016	0.173 ± 0.018	$P < 0.005$

Significant differences indicated: ** $P < 0.05$ vs. PF; \ddagger $P < 0.05$ vs. CON; * $P < 0.01$ vs. PF; \dagger $P < 0.01$ vs. CON. NS: not significant.

For details of procedures see materials and methods section.

Table 7. Thermodynamic degree of coupling (q) and optimal efficiency (η_{opt}) of the oxidative phosphorylation in gastrocnemius isolated mitochondria from pair-fed (PF), control (CON) and dexamethasone (DEX)-treated rats.

(Values expressed as mean \pm standard deviation for eight rats per group)

Respiratory substrates	Parameters	DEX-treated	PF	CON	ANOVA
Pyruvate + malate	q	0.966 ± 0.011	0.972 ± 0.016	0.972 ± 0.011	NS
	η_{opt}	0.593 ± 0.059	0.635 ± 0.089	0.628 ± 0.059	NS
Succinate	q	0.889 ± 0.039	0.880 ± 0.052	0.887 ± 0.036	NS
	η_{opt}	0.400 ± 0.076	0.374 ± 0.105	0.377 ± 0.064	NS
Ascorbate/TMPD	q	0.749 ± 0.073	0.713 ± 0.025	0.774 ± 0.050	NS
	η_{opt}	0.214 ± 0.083	0.176 ± 0.017	0.229 ± 0.051	NS

NS: not significant.

For details of procedures see materials and methods section.





