EFFECTS OF CORTICOSTERONE ON MUSCLE MITOCHONDRIA
IDENTIFYING DIFFERENT SENSITIVITY TO GLUCOCORTICOIDS IN LEWIS
AND FISCHER RATS

RUNNING HEAD: MITOCHONDRIAL SENSITIVITY TO CORTICOSTERONE

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Key words: skeletal muscle, cytochrome-c-oxidase, tissue sensitivity to glucocorticoids, Lewis and Fischer rats
Abstract

Previous studies in rat have demonstrated decreased number of mitochondria and uncoupling of oxidative phosphorylation after administration of glucocorticoids but at supraphysiological doses and using synthetic glucocorticoids. To analyze the relationships between corticosterone levels - the natural glucocorticoid in rat - and muscle mitochondrial metabolism, Lewis and Fischer 344 rats were bilaterally adrenalectomized and implanted with different corticosterone pellets (0, 12, 50, 100 and 200mg corticosterone).

Rats bearing a corticosterone pellet delivering corticosterone at concentrations in the range of chronic stress-induced levels presented a lower amount of functional muscle mitochondria with a decrease in cytochrome c oxidase and citrate synthase activities and a depletion of mitochondrial DNA. Moreover, a strain difference in tissue sensitivity to corticosterone was depicted both in end-organ sensitive to glucocorticoids (body weight, thymus and adrenals weights) and in muscle mitochondrial metabolism (Lewis > Fischer). Interestingly, this strain difference was also observed in the absence of corticosterone with a deleterious effect on muscle mitochondrial metabolism in Fischer rats whereas no effects were observed in Lewis rats. We therefore postulate that corticosterone is necessary to muscle mitochondrial metabolism exerting its effects in Fischer rats with an inverted U curve, whereby too little (only Fischer) or too much (Fischer and Lewis) corticosterone is deleterious to muscle mitochondrial metabolism. In conclusion, we propose a general model of co-ordinate regulation of mitochondrial energetic metabolism by glucocorticoids.

Key words: skeletal muscle, cytochrome-c-oxidase, tissue sensitivity to glucocorticoids, Lewis and Fischer rats
Introduction

Glucocorticoids (GC), hormones released by the adrenal cortex, have numerous homeostatic and stress-response functions. A delicate balance exists between the protective effects of adrenal steroids secreted in response to stressful experiences, and the negative consequences that GC may have for many processes (20). For example, in skeletal muscles, GC insufficiency leads to debilitating fatigue, myalgia and general muscle weakness (27), whereas excess GC causes muscular atrophy and a decreased rate of muscle protein synthesis (2). Among several mechanisms leading to muscle wasting in GC-induced myopathy, defects in mitochondrial function appear to be involved (21, 22, 35). With the administration of supraphysiological doses of GC in rat, electron microscopic and biochemical studies have demonstrated enlarged mitochondrial volume, decreased number of mitochondria, decreased substrate utilization, and uncoupling of oxidative phosphorylation (21). Studies of the effects of GC on mitochondrial oxidative phosphorylation (ATP production) have been relatively numerous, consisting of reports describing the in vitro direct effects of GC on mitochondrial energy metabolism (22, 33). However, high concentrations of synthetic GC (dexamethasone, triamcinolone, methylprednisolone, etc.) have been used in these studies (21, 22, 35). To determine the effects of endogenous increases in plasma corticosterone, the natural GC in rat, on muscle mitochondrial metabolism, in a previous study we have submitted rats to eight weeks of daily stress (novel environment or forced exercise), and we have demonstrated that plasma corticosterone concentrations were negatively correlated with mitochondrial metabolism (9). Although such correlations do not necessarily indicate a direct effect of the change in corticosterone concentration on mitochondrial function, this does suggest that plasma corticosterone and functional muscle mitochondrial amount are related. The present experiment was designed to demonstrate that corticosterone at physiological concentrations (from 25 to 210 ng/ml) is involved in the regulation of muscle mitochondrial metabolism in
the plantaris muscle. Rats were adrenalectomized and implanted with subcutaneous pellets with various amounts of corticosterone to maintain constant corticosterone concentrations (24). Moreover, since in the previous study we reported a strain effect in the response of muscle mitochondrial amount to variations in plasma corticosterone levels, Lewis and Fischer strains were selected because their differ considerably in the response of their hypothalamo-pituitary-adrenal (HPA) axis to acute or chronic stress. Fischer rats are more reactive than standard rat lines to a variety of stressful stimuli while the Lewis HPA axis is hyporesponsive to a wide range of acute or chronic stress compared to Sprague-Dawley rats (7). The Fischer and Lewis strains could be thought of as representing two ends of a spectrum of HPA axis responsiveness to stress. For these reasons, we were interested in determining if similar plasma corticosterone concentrations in Lewis and Fischer strains could be differentially translated at the level of muscle mitochondrial metabolism. Two other markers were used in support of the different sensitivity to corticosterone of Lewis and Fischer strains of rat: body weight changes and thymus weight. Indeed, thymus is a corticosteroid target tissue and thymus weight is sensitive to integrated corticosterone secretion in previous days (1), and a good index of corticosterone levels (3).

We confirm our previous results (9) demonstrating a dose effect of corticosterone from low levels to those reached after stressful stimuli on functional muscle mitochondrial amount and a strain effect on tissue sensitivity to glucocorticoids.

Materials and methods

Animals

Experiments were conducted on 37 male Lewis and 36 male Fischer rats (IFFA CREDO, Les Oncins, France). The animals were four-week old upon arrival at our laboratory, and were housed one per cage in an animal quarter at constant temperature (23-25°C) with a
12h/12h light-dark cycle (lights on at 07.00 h). Food and water were provided *ad libitum*. All rats were allowed to adapt to the animal room for two weeks before the beginning of the experiments. This study was conducted in conformity with the French recommendations on animal experimentation.

*Experimental design*

Lewis and Fischer rats were randomly assigned to experimental groups and either bilaterally adrenalectomized (ADX) or sham adrenalectomized under intraperitoneal pentobarbital anaesthesia (day 0 : D0). In ADX rats, subcutaneous pellets of 100 mg of wax or a fused mixture of corticosterone (12 mg, 50 mg or 100 mg) and cholesterol were inserted at the time of surgery ~ 2 cm rostral to the skin incision in the nape of the neck. Pellets were made using an established technique (1). Briefly, corticosterone (Sigma-Aldrich, St Quentin Fallavier, France) was liquefied by heating and pipetted into a mold designed specifically for manufacturing the pellets. Therefore, in order to obtain a final weight of 100mg, the 12, 50 and 100 mg corticosterone pellets consisted of 12, 50 and 100 mg corticosterone fused with 88, 50 and 0 mg cholesterol (Sigma-Aldrich, St Quentin Fallavier, France), respectively. Consequently, there was in each strain six experimental groups: sham-operated (wax pellet), ADX with wax pellet or 12 mg, 50 mg, 100 mg, 2x100 mg corticosterone pellets, named respectively, ADX + 0 mg, ADX + 12 mg, ADX + 50 mg, ADX + 100 mg, ADX + 200 mg corticosterone (n=6 per experimental group). After surgery all rats were provided with both water and 0.9% saline solution to drink.

Body weight and food intake were measured daily in all rats (initial weight: Fischer: 111 ± 10 g ; Lewis: 142 ± 16 g). Twelve days after adrenal surgery and pellet insertion (D12), the animals were sacrificed by decapitation in an adjacent room (09.00-12.00) within 30 seconds of removal from their home cage. Immediately after decapitation, trunk blood (4 ml)
was collected in 10 ml polyethylene tubes containing 0.1 ml of 10% EDTA, and stored in ice until centrifugation. Plasma samples were stored at –80 °C until analysis. Thymus was collected and weighed. The superficial portion of left and right plantaris muscles was removed and muscle samples were immediately frozen at –80 °C. The superficial portion of the plantaris, a fast-twitch muscle, was chosen because this region contains predominantly type II fibers (fast-twitch fibers), whereas the region close to the bone (deep region) contains a mixture of fiber types (10). Because fast-twitch muscles are highly susceptible to the atrophying actions of GC whereas little or no effects are observed in slow-twitch muscles such as the soleus which are more resistant to GC treatment (5), we hypothesized that the effects of corticosterone on mitochondrial metabolism would be more marked in fast-twitch fibers.

**Plasma corticosterone**

Plasma corticosterone was measured after alcohol extraction by a competitive protein binding assay (4) using rhesus monkey serum as the source of transcortine, [3H]corticosterone as the tracer, and dextran-coated charcoal to absorb the unbound fraction (sensitivity 4 ng/ml, specificity >95%, interassay coefficient of variation 8.0%). Samples were analyzed in duplicate.

**Mitochondrial enzymatic activities**

*Analysis of mitochondrial metabolism:* To understand the variations of mitochondrial activity and amount of functional mitochondria, we monitored two specific activities: i) cytochrome c oxidase (COX) activity which represents one of the activities of oxidative phosphorylation and can be used to evaluate the mitochondrial ability to produce ATP; ii) succinate dehydrogenase (SDH) activity which is specific for mitochondrial metabolism.
(Krebs cycle) but independent of oxidative phosphorylation. We could therefore use these activities to evaluate the amount of functional mitochondria in cells (16, 17). The normalization of COX activity by SDH allowed us to distinguish two types of adaptation mechanisms: (i) a variation in the amount of functional mitochondria (in this case, COX/SDH ratio remains constant); (ii) a direct effect on the COX kinetic parameters (modification of the COX/SDH ratio whereas SDH remains constant).

Tissue preparation: Left muscles were cut in thin pieces and homogenized at 4°C in 10 volumes of a buffer (225 mM mannitol, 75 mM sucrose, 10 mM Tris-HCl, 0.1 mM EDTA, pH 7.2). Homogenates were then centrifuged for 20 min at 2500 g (4 °C). Supernatants were frozen at – 80 °C. The enzymatic activities of cytochrome c oxidase and succinate dehydrogenase were measured on the supernatant by observing the transfer of electrons from a natural or artificial substrate to a natural or artificial respiratory chain acceptor (16, 17). The rate of appearance of the reduced acceptor was measured spectrophotometrically (Kontron 922). All the enzymatic activities were measured at 37 °C in a final volume of 1 ml and expressed in nmoles of product formed per min and per mg of protein. Protein concentrations were determined spectrophotometrically by the procedure of Lowry (18).

Cytochrome c oxidase (COX) activity (complex IV): complex IV activity was determined by monitoring the cytochrome c oxidation, measured spectrophotometrically by the decrease of absorbency at 550 nm, for 1 minute (38). The kinetics of cytochrome c oxidation was linear for 1 minute. Activity was calculated using ε (cytochrome c)=18500 M⁻¹ cm⁻¹. Cytochrome c from horse heart (Sigma C-7752) at 1% (0.8 mM) was diluted 1/20 in phosphate buffer (KH₂PO₄, 25 mM, pH = 7.4). One ml of this solution was reduced by a light excess of sodium dithionite and 1 ml was completely oxidized by an excess of potassium ferricyanide to serve as a reference. The value of the OD ratio (reduced cytochrome c) / (oxidized cytochrome c) at 550 nm was around 0.7. Then, small quantities of the completely
reduced solutions were added to the 8 ml of the initial 50 µM oxidized solution until 80-90% reduction was reached (checked spectrophotometrically). This ensured that in the solution there was no excess dithionite that could interfere with the reaction itself.

**Succinate dehydrogenase activity** (SDH) was measured by the following method: in phosphate buffer (KH₂PO₄, 60 mM, pH = 7.4) with succinate (100 mM) as the substrate, and PMS (Phenazine methosulfate, Sigma P-9625)(130 mM) and DCIP (2,6-dichloroindophenol, Sigma D-1878)(7.2 mM) as electron acceptors, enzymatic activity was determined by monitoring the absorbency increase at 600 nm for 2 minutes at 37°C, which corresponds to the DCIP reduction. For the calculation of the activity, ε(DCIP) = 21000 M⁻¹cm⁻¹.

**Southern blot analysis**

Total genomic DNA was isolated from homogenates of plantaris muscle according to standard procedures. Approximately 5 µg of DNA was digested with XhoI, a restriction enzyme that linearizes the covalently closed circular mitochondrial DNA molecule by cutting at a unique site. Samples were resolved on 0.7% agarose gels by electrophoresis, transferred to nylon membranes and hybridized simultaneously with ³²P-labeled probes for mitochondrial DNA (mtDNA) and the nuclear 18S rRNA gene to correct for uneven loading of the samples (25). Signals were quantified with a PhosphorImager™ using ImageQuant™ software (Molecular Dynamics, Inc.). To correct for quantitative variations among the samples, mtDNA signals were normalized relative to nuclear DNA signals. The results of mtDNA were expressed in means ± SEM or in percentage of ADX + corticosterone rats compared to sham-operated rats.
Statistics

The final number of rats analyzed was 67 instead of 73 rats, due to the exclusion of six rats: 2 Lewis and 4 Fischer (see Table 1) (2 rats died after ADX, loss of its 100mg pellet in one rat and bad corticosterone diffusion from three 200mg pellets).

Data are presented as means ± SEM. Body weight and thymus weight were analyzed by means of a two-way ANOVA with strain (two levels) and experimental group (six levels) as between-subject factors. For the comparisons of plasma corticosterone values and muscle mitochondrial metabolism between experimental groups, sham-operated rats were not used for calculation because the plasma corticosterone levels measured in these rats do not correspond to their 24-h mean levels but instead only to their nadir (morning) levels. Therefore, for the above mentioned parameters, between-subject factors included five levels (experimental groups). When significant, analysis of variance was followed by post-hoc individual comparisons (Newman-Keuls test). Relationships between corticosterone levels and enzymatic activity or thymus weight were examined by computing logarithmic non linear regression and the Pearson product-moment correlation coefficient (r), respectively. Statistical significance was accepted at p<0.05.

Results

Effectiveness of corticosterone pellets

Plasma corticosterone. Plasma corticosterone levels in samples collected from the six experimental groups of Lewis and Fischer rats at 1500 h by decapitation on D12 are shown in table 1. In ADX rats, ANOVA revealed an experimental group effect (F4,45=146.19, p<10^-6) and a strain by experimental group effect (F4,45=4.04, p<0.005). Plasma corticosterone levels were significantly higher in Fischer ADX + 12 mg and ADX + 100mg subgroups compared to their Lewis counterparts (p=0.03 and 0.04, respectively), whereas ADX + 200 mg yielded to
lower plasma corticosterone levels in Fischer than in Lewis rats (p=0.001). Moreover, in both
strains, serum corticosterone concentrations were roughly proportional to pellet composition
for 50, 100 and 200 mg corticosterone (present study, 1, 3, 24). However the serum level
produced by the 12 mg corticosterone pellet was only 50% lower than that produced by a 50
mg corticosterone pellet. This may be due to differences in diffusion and/or peripheral
metabolism of corticosterone between the 12 mg and 50 mg pellets. Such differences have
also been reported by Akana et al. (1). As expected, after implantation of 12 mg
corticosterone pellet, serum corticosterone levels are within the range found in sham-operated
rats on the morning in both Lewis and Fischer rats (11, 30) whereas 50 mg corticosterone
pellet leads to corticosterone levels similar to those reached during the first hours of light off
in Lewis rats (62.3 vs 80 ng/ml, Lewis ADX + 50 mg corticosterone vs [11], respectively), but
not in Fischer rats (72.9 vs 130 ng/ml, Fischer ADX + 50 mg corticosterone vs [30]).
Consequently, in Lewis rats, taking into account the entire 24h-period, plasma corticosterone
levels obtained after implantation of the 12 mg pellets are lower than the mean 24 h range of
corticosterone found in sham-operated rats, as they are equivalent to the nadir (light on) levels
of corticosterone of rats with normal circadian variation of plasma corticosterone (sham-
operated rats). On the other hand, the 50 mg pellets produced plasma corticosterone levels
that were higher than the 24-h mean corticosterone levels produced during the normal
circadian rhythm as equivalent to the peak (dark) levels of corticosterone found in sham-
operated rats. This is also true for Fischer rats, albeit less marked as concerns the 50 mg
corticosterone pellet.

Body weight variation. The time course of body weight variation is shown in figure 1A for
sham-operated and ADX rats implanted with different corticosterone pellets. Before surgery
(D-1 and D0) both strains displayed the same body weight gain independently of the
experimental group. After surgery, ANOVA revealed a day effect (day effect: $F_{15,825}=827.29$;
p<0.0001), a strain effect (F<sub>1,55</sub>=17.58; p<0.0001), an experimental group effect (F<sub>5,55</sub>=21.59; p<0.0001) and a day x strain x group interaction (F<sub>75,825</sub>=5.86; p<0.0001). In Lewis rats, from day 3 to the end of the experiment, body weight increase in sham-operated rats was significantly higher than in all the other groups (p<0.01). Moreover, ADX + 0 mg, ADX + 100 mg and ADX + 200 mg corticosterone groups showed significantly less body weight increase than their counterparts with 12 mg and 50 mg corticosterone pellets since day 2 (p<0.01). In Fischer rats, no significant inter-group difference could be observed up to the fourth post-surgical day. Compared to their sham-operated counterparts, ADX + 100 mg and ADX + 200 mg corticosterone groups showed less body weight increase since day 4 and day 6, respectively (p<0.05). Finally, ADX + 50 mg corticosterone group displayed less body weight gain than their sham-operated counterparts only in day 11 and day 12.

To determine the effects of variations of corticosterone concentration on body weight gain and taking into account the differences obtained in plasma corticosterone levels between strains within the same corticosterone pellet, body weight variations were also calculated comparatively with the group with no corticosterone (ADX + 0 mg) and represented with reference to the plasma corticosterone levels actually reached (Figure 1B). For plasma corticosterone values over 100 ng/ml, weight loss induced by increased plasma corticosterone levels was lower in Fischer rats compared to their Lewis counterparts.

**Thymus weight.** Strain (F<sub>1,55</sub>=16.19, p<0.01) and experimental group (F<sub>5,55</sub>=90.06, p<0.01) had significant effects on relative thymus weight (figure 2A). No inter-strain thymus weight difference was observed in sham-operated rats, but *post-hoc* comparisons revealed, comparatively to sham-operated rats, a significant increase in thymus weight of ADX + 0 mg and ADX + 12 mg corticosterone groups in Lewis rats only (p<0.01). In both strains, 50 mg, 100 mg and 200 mg corticosterone pellets insertion resulted in a significant decrease in
thymus weight. Moreover, a significant negative correlation was measured between plasma corticosterone and thymus weight in Lewis rats ($r=-0.41; p<0.01$) and in Fischer rats ($r=-0.61; p<0.01$).

When the variation of thymus weight was calculated with regard to the thymus weight obtained in condition with no corticosterone (ADX + 0 mg), thymolysis induced by increased plasma corticosterone levels was lower in Fischer rats compared to their Lewis counterparts (figure 2B).

It is clear from the data presented in figures 1 and 2 that in the Lewis strain, 50 mg, 100 mg and 200 mg corticosterone pellets insertion resulted in significant decreases in body weight gain and in thymus weight, suggesting that the 50 mg, 100 mg and 200 mg pellets provide larger than physiological amounts of corticosterone. This is also true for Fischer rats, albeit less marked as concerns the 50 mg pellet. Finally, as previously reported (1), the absence of corticosterone replacement (ADX + 0 mg corticosterone) or insufficient corticosterone replacement (ADX + 12 mg corticosterone) resulted in a decreased rate of body weight gain and a marked increase in thymus weight in Lewis only.

**Muscle mitochondrial metabolism**

COX activity. As table 1 shows, ANOVA revealed a strain effect ($F_{1,45}=29.61, p<10^{-5}$) with higher COX activities in Lewis rats than in Fischer rats and a group effect ($F_{4,45}=4.94, p<0.005$) on COX activity.

SDH activity. A strain effect ($F_{1,45}=42.09, p<10^{-6}$) with higher SDH activities in Lewis rats than in Fischer rats, a group effect ($F_{4,45}=9.58, p<10^{-4}$) and a tendency to a strain x group interaction ($F_{4,45}=2.29, p=0.07$) were noted on SDH activity.

COX/SDH activity. A group effect ($F_{4,45}=3.83, p<0.01$) was noted on COX/SDH activity. Two hundred mg corticosterone replacement induced a significant decrease in COX/SDH ratio of ADX rats ($p<0.05$).
**Relationships between plasma corticosterone and muscle mitochondrial metabolism**

When the Fischer rats are considered in terms of individuals instead of different experimental groups, plasma corticosterone is correlated with COX activity (p=0.002) (figure 3A) and SDH activity (p=0.0008) (figure 4A). In Lewis rats, no significant correlation was found between plasma corticosterone and COX activity (figure 3B) or SDH activity (figure 4B). By contrast, when only supraphysiological values of corticosterone are taken into account, plasma corticosterone was negatively correlated with COX activity (r=-0.44, p<0.001) and SDH activity (r=-0.54, p<0.001) in Lewis rats (pooled data from ADX + 50 mg, 100 mg and 200 mg corticosterone groups). In Fischer rats, above 100 ng/ml of plasma corticosterone concentrations (ADX + 100 mg and ADX + 200 mg corticosterone groups) a strong negative correlation was also evidenced (COX: r=-0.91, p=0.0005 ; SDH: r=-0.85, p=0.003).

In the absence or in low plasma corticosterone levels (ADX + 0 and +12 mg), COX and SDH activities were decreased in Fischer (Table 1 and Figures 3A and 4A) but not in Lewis rats (Table 1 and Figures 3B and 4B).

Lastly, no correlation was found between COX/SDH ratio and plasma corticosterone in both strains (Fischer: r=-0.40, p=0.07; Lewis: r=0.022, p=0.92). These results suggest that in Fischer and in Lewis rats, plasma corticosterone at physiological ranges (upon low to high concentrations) are correlated to significant changes in the amount of functional mitochondria in plantaris muscle.

**mtDNA analysis.**

Since the biochemical data showed a partial deficiency of enzyme complexes containing subunits encoded by mtDNA, we examined mtDNA for rearrangements and determined mtDNA levels by Southern blot hybridization only for subgroups with high
corticosterone plasma concentrations (ADX + 50 mg, + 100 mg and + 200 mg) (figure 5). The rat muscle contained mtDNA of the same size as sham-operated but the mtDNA signal relative to the nuclear DNA signal was, in the case of Lewis ADX + 50 mg, + 100 mg and + 200 mg corticosterone groups, respectively, 89%, 74% and 42% of the mean sham-operated values (Figure 5). For Fischer rats, the relative mtDNA signal was 89%, 94% and 33% (respectively for ADX + 50 mg, + 100 mg and + 200 mg corticosterone rats) compared to sham-operated rats (Figure 5).

Discussion

Methodological considerations

In the present experiment, the rats were adrenalectomized and implanted with subcutaneous pellets with various concentrations of corticosterone, the rat natural GC. The advantage of the pellet method includes the establishment of constant corticosterone concentrations with no evidence of circadian variation (24). Therefore, in our experimental conditions, the only difference between experimental groups was plasma corticosterone concentrations. One criticism of this model may be that it induces constant 24-h plasma corticosterone concentration which does not mimic the nycthemeral rhythm of plasma corticosterone concentration. On the other hand, this absence of nycthemeral rhythm is similar to pathological conditions such as Cushing’s syndrome, or more interestingly, exogenous corticosteroid treatment.

Data of plasma corticosterone concentrations, body weight gain and thymus weight obtained in the different experimental groups, show that in Lewis rats the levels of corticosterone achieved by the 12 mg corticosterone pellets were in the low physiological range whereas the corticosterone levels obtained after the implantation of the 50 mg, 100 mg and the 200 mg corticosterone pellets were in the high physiological range. This is also true for Fischer rats,
albeit less marked for the 50 mg pellet. It is important to note that the plasma corticosterone values of the ADX + 200 mg corticosterone pellets remained in the range of plasma corticosterone observed in chronically stressed rats (9), suggesting that the range of corticosterone concentrations achieved in the present experiment represents reference values ranging from low HPA activity to high (stress-induced) HPA axis activity.

A strain difference between plasma corticosterone concentrations obtained with the same corticosterone pellets was also depicted (Table 1). To our knowledge, this is the first description of such a difference between Lewis and Fischer rats. This suggests a strain difference in absorption and/or metabolism of corticosterone.

*Tissue sensitivity to corticosterone*

*Mitochondrial metabolism:*

We evaluated the amount of functional mitochondria in cells (16, 17) through the normalization of COX activity by SDH. This allowed us to distinguish two types of adaptation mechanisms: (i) a variation in the amount of functional mitochondria (in this case, COX/SDH ratio remains constant); (ii) a direct effect on the COX kinetic parameters (modification of the COX/SDH ratio whereas SDH remains constant) (16, 17).

The present results provide evidence for the first time that corticosterone at physiological concentrations (from 25 to 210 ng/ml) is involved in the regulation of muscle mitochondrial function. Compared to ADX + 12 mg and ADX + 50 mg corticosterone rats, corticosterone concentrations in the range of chronic stress-induced levels (ADX + 200 mg corticosterone) are associated with lower amount of functional muscle mitochondria in both Lewis and Fischer rats. In addition, we also report deleterious effects of the absence of GC on muscle mitochondrial metabolism (ADX + 0 mg corticosterone group). In Fischer rats, ADX induces a huge decrease in COX and SDH activities indicating a strong dependency of mitochondrial
function on corticosterone levels, the lack of corticosterone having the same effect than high corticosterone levels on SDH levels (Fig. 4A). In Lewis rat, ADX induces a weak decrease in SDH activity with no variation in COX activity. Therefore, we postulate that corticosterone is necessary to muscle mitochondrial metabolism exerting its effects with an inverted U curve, whereby too little (Fischer only) or too much corticosterone (Fischer and Lewis) is deleterious to muscle mitochondria metabolism. These dual actions of corticosterone have also been reported for body weight gain and feeding efficiency (6) as well as for cognitive process (19).

In a previous study, we have demonstrated that chronic stress-induced (novel environment or forced exercise for eight weeks) increases in endogenous corticosterone concentrations were negatively correlated with functional muscle mitochondrial amount (9). These previous results are confirmed by the present study where the only manipulated variable was the concentration of corticosterone delivered by the pellet beared by rats of the different experimental groups. Compared to sham-operated rats, corticosterone concentrations in the range of chronic stress-induced levels (ADX + 200 mg corticosterone) are associated with lower amount of functional muscle mitochondria in both Lewis and Fischer rats. Moreover, in Lewis rats, we report a linear regression curve between high plasma corticosterone concentrations (≥ 50 ng/ml) and functional muscle mitochondria amount, the higher the corticosterone levels the lower muscle functional mitochondria amount (figures 3 and 4). Although such correlations do not necessarily indicate a direct effect of the change in corticosterone concentration on mitochondrial function, this does suggest that plasma corticosterone and functional muscle mitochondrial amount are related. These results were confirmed by the direct measure of the mitochondrial DNA by Southern Blot analysis (Figure 5).

These results are in agreement with earlier reports on the in vitro effects of high synthetic GC (dexamethasone, triamcinolone, methylprednisolone, etc.) concentrations on mitochondrial
oxidative phosphorylation (ATP production) (21, 22, 35). In vivo, defects in mitochondria function have been involved in GC-induced myopathy (21, 22, 35). With the administration of high doses of GC in rat, electron microscopic and biochemical studies have demonstrated enlarged mitochondrial volume, decreased numbers of mitochondria, decreased substrate utilization, and uncoupling of oxidative phosphorylation (21).

Our findings are in disagreement with those of Weber et al. (36) who investigated the in vivo and in vitro effects of dexamethasone on mitochondrial biogenesis in rats over a period of 3 days. They showed that high levels of GC stimulate mitochondrial biogenesis in skeletal muscle. Nevertheless, their results are difficult to compare to ours because of differences in the GC used (dexamethasone vs the natural rat’s GC: corticosterone, (36) vs present study), the amounts of GC (higher dexamethasone doses than those used in GC therapy (15) vs plasma corticosterone levels of chronically stressed rats (1)), and the duration of GC treatment (3 days vs 12 days).

One mechanism for the relationship reported in the present experiment between corticosterone and muscle mitochondrial biogenesis could be the presence of GC receptor acting via hormone response elements (HRE) in muscle mitochondria. In liver mitochondria (34) and in several cell types (31), the GC receptor (GR) has been detected and its interactions with putative mitochondrial HRE have been demonstrated (34). However, the action of the GC receptor on mitochondrial gene expression remains to be determined.

The present study also demonstrates that the absence of corticosterone (ADX + 0 mg group) also induces a deleterious effect on mitochondrial function in Fischer rats. GC have been reported to be essential for the postnatal rise in mitochondrial enzymes in the developing rat kidney (8). However, to our knowledge, there is no report on the effects of the absence of corticosterone on muscle functional mitochondria in adult rats. In Fischer rats, ADX induces an large decrease in COX and SDH activities with a further decrease in COX activity in ADX
+ 200 mg corticosterone. In Lewis rats, ADX induced no variation in COX nor SDH activities (ADX + 0 mg corticosterone group) whereas in ADX + 200 mg corticosterone group, the decrease in muscle mitochondrial content was associated with both decreased COX and SDH activities. This suggests that mitochondrial function is impaired probably by two different mechanisms: a direct action on enzymatic activity or a decreased number of functional mitochondria. A possible answer may lie in corticosterone’s dual receptors population: the two glucocorticoid receptors (GR and MR) may instigate differential mitochondrial actions, with MR-mediated effects upon low GC concentrations and GR-mediated effects upon high GC concentrations (6, 29).

**Interstrain differences**

The second purpose of this study was to examine the effects of similar concentrations of corticosterone on target tissues in Lewis and Fischer rats. We found that tissue sensitivity to GC was higher in Lewis than in Fischer rats. Indeed, identical corticosterone concentrations induce a more pronounced thymus decrease in Lewis than in Fischer rats (see figure 2B). This is the first report of such a strain difference in tissue sensitivity to corticosteroid. This point is also exemplified by the data on decrement of body weight, offered in support of the greater sensitivity of Lewis rats to corticosterone. We also report a higher muscle mitochondrial sensitivity to high GC levels between Fischer and Lewis rats (Fig.3 and 4). In sham-operated rats, SDH and COX activities were significantly higher in Lewis compared to Fischer rats (similar effects were obtained with CS activity, data not shown). By contrast, their COX/SDH ratio were similar. This suggests that the mitochondria are similar between these two strains of rats and that Lewis rats have more mitochondria in their plantaris muscle than Fischer rats. These results are in agreement with the interstrain relationship between plasma corticosterone concentration and muscle mitochondria functional amount, as in Fischer rats the range in
plasma corticosterone levels that occurs during a 24-h period is significantly higher than for the Lewis rat (12).

By contrast Karalis et al. (14) have not reported differences in tissue sensitivity to GC between Fischer and Lewis rats. Possible explanations might be differences in the age of the rats (6 weeks vs 6 months, present study vs (14)), the type of GC used (corticosterone vs dexamethasone) and its mode of administration (pellet vs intraperitoneal injection, this latter being responsible of a stress effect), and the duration of the treatment (7 days vs 12 days). Another potential consideration concerns the role of corticosterone binding globulin (CBG). In plasma, more than 90% of corticosterone is bound to CBG, and only the free fraction of the steroid represents the biologically active form of corticosterone (37). By contrast, dexamethasone is almost exclusively bound to albumin in plasma (28). It has been shown that F344 displayed higher CBG levels in plasma, thymus and spleen, resulting in less free and bioactive corticosterone (7). This could explain on the one hand the less effectiveness of similar corticosterone levels in F344 compared to Lewis rats in the present study, and moreover, the similar sensitivity to dexamethasone in Karalis’ study. Whether such a strain difference is directly linked to CBG concentrations and affinity in basal conditions and after corticosterone replacement is currently investigated in our laboratory.

The biological efficiency of corticosterone also depends on its GR-mediated effects. When compared to Fischer, Lewis rats appear to have similar GR-mediated effects of corticosterone (26). GC sensitivity is also influenced by intracellular GC availability depending on 11 β hydroxysteroid dehydrogenases activities, mediating prereceptor metabolism of corticosterone (32). Determining the magnitude of tissue sensitivity to circulating GC through the levels of expression of 11 β hydroxysteroid dehydrogenases activities in Lewis and Fischer strains may provide a plausible mechanism underlying these strong interstrain differences.
In conclusion, the major findings of this study are that tissue sensitivity to corticosterone is higher in Lewis than in Fischer rats. Moreover, we report that mitochondria is another corticosterone sensitive physiological target as physiological corticosterone concentrations from low to high levels influence muscle functional mitochondria amount. This new track has to be considered when studying the pathologies induced by GC and, notably, the molecular mechanisms linking corticosterone and the muscle functional mitochondrial amount.
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LEGENDS

**Figure 1A:** Body weight variations of Lewis (solid lines and black symbols) and Fischer rats (dotted lines and white symbols) either sham-operated or adrenalectomized and implanted with subcutaneous pellets containing 0 mg (ADX + 0 mg), 12 mg (ADX + 12 mg), 50 mg (ADX + 50 mg), 100 mg (ADX + 100 mg) or 200 mg (ADX + 200 mg) corticosterone 12 days after surgery. Results are expressed as means of 6 rats per experimental groups.

**Figure 1B:** Effects of corticosterone on total body weight variation (D12-D0) of Lewis (solid lines and black symbols) and Fischer rats (dotted lines and white symbols). For this purpose, for each experimental group (O: ADX + 12 mg, V: ADX + 50 mg, □ : ADX + 100 mg, ◇: ADX + 200 mg corticosterone), total body weight variation has been compared (subtracted) to the one obtained in condition with no corticosterone (ADX + 0 mg group).

**Figure 2A:** Thymus weight of Lewis and Fischer rats either sham-operated or adrenalectomized and implanted with subcutaneous pellets containing 0 mg, 12 mg, 50 mg, 100 mg or 200 mg corticosterone 12 days after surgery. Results are expressed as means ± SE of 6 rats per experimental group. *P<0.05 with sham-operated rats; #P<0.05 with Lewis rats from the same experimental group.

**Figure 2B:** Effects of corticosterone on thymus weight of Lewis (solid lines and black symbols) and Fischer rats (dotted lines and white symbols). For this purpose, for each experimental group (O: ADX + 12 mg, V: ADX + 50 mg, □ : ADX + 100 mg, ◇: ADX + 200 mg corticosterone), thymus weight has been compared (subtracted) to the one obtained in condition with no corticosterone (ADX + 0 mg group).
Figure 3: Relationship between plasma corticosterone levels and cytochrome c oxidase activity in Fischer rats (A) and Lewis rats (B) (●: ADX + 0 mg; ○: ADX + 12 mg, ■: ADX + 50 mg, ▲: ADX + 100 mg, ◆: ADX + 200 mg corticosterone).

Figure 4: Relationship between plasma corticosterone and succinate dehydrogenase activity in Fischer rats (top) and Lewis rats (bottom) (●: ADX + 0 mg; ○: ADX + 12 mg, ■: ADX + 50 mg, ▲: ADX + 100 mg, ◆: ADX + 200 mg corticosterone).

Figure 5: Southern blot showing hybridization results for the mitochondrial DNA and 18S nuclear DNA used for normalization in sham-operated (sham), ADX + 50 mg (50), ADX + 100 mg (100) or ADX + 200 mg (200) corticosterone in Lewis and Fischer rats. The values in the bottom of the figure are the means ± SEM of mtDNA/nuclear DNA (18S) ratio in sham-operated, ADX + 50 mg, ADX + 100 mg and ADX + 200 mg corticosterone in Lewis and Fischer rats. This figure represents the data of three independent experiments with similar results.
Table 1: Plasma corticosterone, cytochrome-c-oxidase (COX), succinate dehydrogenase (SDH) activities and COX / SDH ratio in sham-operated (Sham), ADX + 0 mg, + 12 mg, + 50 mg, + 100 mg and + 200 mg Lewis and Fischer rats. ND: non detectable.

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<th>Plasma Corticosterone (ng/ml)</th>
<th>Cytochrome-c-oxidase activity (nmol/min/mg prot)</th>
<th>Succinate dehydrogenase activity (nmol/min/mg prot)</th>
<th>Cytochrome-c-oxidase/Succinate dehydrogenase</th>
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<tr>
<td>Sham Lewis (n=6)</td>
<td>19.29 ± 3.67</td>
<td>481.65 ± 38.02</td>
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<td>17.11 ± 2.97</td>
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<td>ADX+0 mg Lewis (n=5)</td>
<td>ND</td>
<td>388.82 ± 20.07</td>
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<td>ADX+0 mg Fischer (n=5)</td>
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<td>164.76 ± 5.66 #+oΔ</td>
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<td>ADX+12 mg Lewis (n=7)</td>
<td>25.47 ± 2.65  ΩΔ</td>
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<td>ADX+12 mg Fischer (n=6)</td>
<td>41.70 ± 6.49%ΩΔ</td>
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<td>ADX+50 mg Lewis (n=6)</td>
<td>62.29 ± 3.03+ΔΑ</td>
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<td>ADX+50 mg Fischer (n=6)</td>
<td>72.87 ± 5.87+ΔΑ</td>
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<td>ADX+100 mg Fischer (n=5)</td>
<td>129.49 ± 6.27 ++οΔ</td>
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<td>ADX+200 mg Lewis (n=5)</td>
<td>215.74 ± 22.76+οΔ</td>
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Values are expressed as means ± SE of n = 6; * P<0.05 with ADX + 0 mg rats of the same strain; + P<0.05 with ADX + 12 mg rats of the same strain; o P<0.05 with ADX + 50 mg rats of the same strain; Δ P<0.05 with ADX + 100 mg rats of the same strain; ∇ P<0.05 with ADX + 200mg rats of the same strain; # P<0.05 with Lewis rats (same group).
Figure 1A

Figure 1B
Figure 3
Figure 4
Fischer

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Lewis

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