

## Mitochondrial energy metabolism in a model of undernutrition induced by dexamethasone

Jean-François Dumas, Gilles Simard, Damien Roussel, Olivier Douay, Françoise Foussard, Yves Malthiery and Patrick Ritz\*

Medecine B, CHU, F-49033 ANGERS Cedex 01, France

(Received 20 February 2003 – Revised 18 June 2003 – Accepted 21 July 2003)

The present investigation was undertaken to evaluate whether mitochondrial energy metabolism is altered in a model of malnutrition induced by dexamethasone (DEX) treatment (1.5 mg/kg per d for 5 d). The gastrocnemius and liver mitochondria were isolated from DEX-treated, pair-fed (PF) and control (CON) rats. Body weight was reduced significantly more in the DEX-treated group (–16%) than in the PF group (–9%). DEX treatment increased liver mass (+59% v. PF, +23% v. CON) and decreased gastrocnemius mass. Moreover, in DEX-treated rats, liver mitochondria had an increased rate of non-phosphorylative O<sub>2</sub> 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 the liver mitochondria of DEX-treated rats. In addition, DEX decreased the thermodynamic coupling and efficiency of oxidative phosphorylation. We therefore suggest that this increase in the proton leak and/or redox slip in the liver is responsible for the decrease in the thermodynamic efficiency of energy conversion. In contrast, none of the variables of energy metabolism determined in gastrocnemius mitochondria was altered by DEX treatment. Therefore, it appears that DEX specifically affects mitochondrial energy metabolism in the liver.

### Glucocorticoid: Mitochondrion: Oxidative phosphorylation: Respiratory chain complexes

Malnutrition is prevalent in many patients, especially in those who are elderly. It causes 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 O<sub>2</sub> consumption rate (referred to as increased energy requirements) can be shown (Nguyen *et al.* 1999). In contrast, most studies with human subjects and animals show that energy restriction decreases energy expenditure (Ramsey *et al.* 2000). Therefore, it appears that in these clinical states, adaptive mechanisms that lead to a reduction of energy requirements fail to operate. However, the biochemical nature of this negative energy balance phenomenon, i.e. the increased energy expenditure, is poorly understood at the present time. A wasting of energy may be a possible explanation. In other words, the mitochondrial oxidative phosphorylation yield may be less efficient, and in turn O<sub>2</sub> consumption may be increased, for a same ATP synthesis rate.

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 that 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 N balance (Odedra *et al.* 1983; Max *et al.* 1988; Bowes *et al.* 1996). Moreover, it is a model of hypercortisolism, which occurs during metabolic stress in human subjects and which is associated with increased energy expenditure (Woodward & Emery, 1989; Brillon *et al.* 1995; Tataranni *et al.* 1996). On the other hand, acute high-dose treatment (<1 week) with dexamethasone (DEX) decreases food intake in rats (Kaur *et al.* 1989; Minet-Quinard *et al.* 1999). Therefore, a negative energy balance ensues, because of both an increase in energy expenditure and a decrease in energy intake. The mechanisms leading to this increased energy expenditure are not fully understood. There are arguments to suggest that mitochondrial energy production may be affected. However, the effects of glucocorticoids on cellular energy metabolism depend on the tissues being investigated and on the duration of treatment. In the liver for example, short-term administration (<24 h) of DEX

appears to increase oxidative phosphorylation, while having no effect on non-phosphorylative respiration (Wakat & Haynes, 1977; Allan *et al.* 1983). In contrast, longer administration of glucocorticoids (<1 week) results in decreased liver oxidative phosphorylation and ATP synthesis when fuelled through complex I, remaining unchanged, however, when fuelled through complex II or IV (Kerppola 1960; Kimura & Rasmussen, 1977; Jani *et al.* 1991). In isolated skeletal muscle mitochondria, studies have shown no change, a decrease or an increase in oxidative capacity (cytochrome *c* oxidase activity) or in oxidative phosphorylation (Vignos & Greene, 1973; Koski *et al.* 1974; Capaccio *et al.* 1985; Marone *et al.* 1994; Weber *et al.* 2002).

Since liver and muscle contribute approximately 50% of body O<sub>2</sub> consumption (Rolfe & Brown, 1997), most of it being coupled with ATP synthesis, we undertook the present study to investigate the effect of 5 d of a high dose of DEX (1.5 mg/kg per d) on energy metabolism, particularly in muscle and liver mitochondria. Comparison with energy restriction helps in the understanding of the increased energy expenditure observed at the whole-body level.

## 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 (30–50 g). Animals were provided with water *ad libitum* and a standard diet (UAR A04; Ifa-credo, L'Arbresle, France) consisting of (g/kg): protein 160, fat 30, carbohydrate 600, water, fibre, vitamins and minerals 210. The metabolizable energy content was 12 kJ/g. Rats were divided into four groups of eight animals as follows: DEX-treated rats received a daily intraperitoneal injection of 1.5 mg DEX/kg for 5 d. Due to the fact that DEX treatment induces a reduced food intake, pair-fed (PF) rats were used to discriminate between the effect of a reduced food intake and the effect of DEX itself on the variables measured. PF rats were pair-fed with DEX-treated animals (rats received the same quantity of food as consumed by DEX-treated rats the previous day) and were injected daily with an isovolumetric solution of NaCl (9 g/l). Rats from the control group (CON) were healthy, received no treatment and were fed *ad libitum*. Animals in the control injected group were fed *ad libitum* and were injected with an isovolumetric solution of NaCl (9 g/l). This group was used to study the effects of NaCl injection. As results were similar between the two control groups, the control injected group was omitted in the presentation of the results. Experiments were conducted over a 5 d period. The dose and duration of the DEX treatment was chosen to induce a reproducible maximum hypercatabolic state with reference to current literature (Minet-Quinard *et al.* 2000). On the sixth day, following an overnight fast, the animals were killed by decapitation. Gastrocnemius (a mixed muscle, which consists of approximately

20% fast-twitch oxidative glycolytic fibres, 74% fast-twitch glycolytic fibres and 6% slow-twitch oxidative fibres), 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 N<sub>2</sub> and stored at –80°C in order to measure enzyme activity levels. Energy balance was estimated by the ratio body weight loss:total food intake over the 5 d experiment.

### Liver water content determination

A portion of liver (1–2 g) was weighed and placed in an oven at 85°C to dry for 24 h. Dry weights were measured, and the ratio wet weight:dry weight was calculated for the determination of liver water content.

### Mitochondrial enzyme activities

Frozen liver and gastrocnemius (10–30 mg) were thawed and homogenized with a Potter-Elvehjem homogenizer (seven strokes; Wheaton Science Products, Millville, NJ, USA) in an isolation medium consisting of 220 mM-mannitol, 75 mM-sucrose, 10 mM-Tris and 1 mM-ethylene glycol-*O,O'*-bis(2-amino-ethyl)-*N,N,N',N'*-tetraacetic acid, pH 7.2. Each homogenate was centrifuged at 600 g for 10 min and the resulting supernatant fraction was filtered through cheesecloth. All procedures were performed at 4°C. The activities of citrate synthase, succinate dehydrogenase and complexes I, III and IV were measured spectrophotometrically at 37°C in the supernatant fraction (citrate synthase activity was also determined in isolated mitochondria) via an adaptation of the method described by Malgat *et al.* (1999), and in agreement with the Mitochondrial Diseases Group of the Association Française de Myopathie. Protein concentration was determined using the bicinchoninic acid assay kit (Interchim, Montluçon, France) with bovine serum albumin used as a control.

The activity of citrate synthase was measured in a reaction medium consisting of 100 mM-Tris–HCl, 40 µg 5,5'-dithio-bis(2-nitrobenzoic acid)/ml, 1 mM-oxaloacetate, 0.3 mM-acetyl CoA and Triton X 100 (40 ml/l), pH 8.1. After 3 min of incubation, the reaction was initiated by adding the supernatant fraction (20–50 µg protein) or isolated mitochondria (9–11 µg protein) and the change in optical density at 412 nm was recorded for 3 min.

The activity of succinate dehydrogenase was measured by following the reduction of 2,6-dichlorophenolindophenol in the presence of phenazine methosulfate at 600 nm. The supernatant fraction (20–50 µg protein) was preincubated in a buffer containing 50 mM-KH<sub>2</sub>PO<sub>4</sub>, 16 mM-succinate, 1.5 mM-KCN, 100 µM-phenazine methosulfate, pH 7.5 for 5 min. The reaction began by the addition of 103 µM-2,6-dichlorophenolindophenol and the optical density was recorded for 3 min.

The activity of complex I was determined by monitoring the oxidation of NADH at 340 nm. The supernatant fraction (40–100 µg protein) was preincubated for 3 min in 820 µl distilled water. Following this, 10 mM-KH<sub>2</sub>PO<sub>4</sub>, 2 mM-KCN, 5 µg antimycin A/ml, 100 µM-decylubiquinone, 1.3 mg bovine serum albumin/ml, 5 mM-MgCl<sub>2</sub>, pH

7.5, were added. The reaction was initiated by the addition of 200  $\mu\text{M}$ -NADH and the change in the optical density was analysed for 3 min. The NADH decylubiquinone reductase activity was also measured in the presence of 12.7  $\mu\text{M}$ -rotenone. The specific activity of complex I represented the difference between NADH oxidation activity, both with and without the rotenone.

The activity of complex III was determined by monitoring the reduction of cytochrome *c* at 550 nm. The supernatant fraction (20–50  $\mu\text{g}$  protein) was incubated for 30 s in a reaction medium consisting of 35 mM-KH<sub>2</sub>PO<sub>4</sub>, 5 mM-MgCl<sub>2</sub>, 2.5 mg bovine serum albumin/ml, 1.8 mM-KCN, 125  $\mu\text{M}$ -oxidized cytochrome *c*, 12.5  $\mu\text{M}$ -rotenone and 62.5 mM-EDTA, pH 7.5. The reaction was initiated by adding 80  $\mu\text{M}$ -decylubiquinol and the optical density was measured for 3 min. The non-enzymatic reduction of cytochrome *c* was measured under identical conditions after the addition of 10  $\mu\text{g}$  antimycin A/ml. The specific activity of complex III was calculated by subtracting the activity of the non-enzymatic reaction from that of the total activity of complex III.

The activity of complex IV was measured by monitoring the oxidation of reduced cytochrome *c* at 550 nm. A 50  $\mu\text{M}$  solution of reduced cytochrome *c* (92–97% reduced using dithionite) in 10 mM-KH<sub>2</sub>PO<sub>4</sub>, pH 7.0, was preincubated for 5 min. The reaction was initiated by adding the supernatant fraction (20–50  $\mu\text{g}$  protein) and the change in optical density was measured for 1.5 min.

#### Mitochondrial isolations

Gastrocnemius muscle, in its entirety, and liver were removed, weighed and immediately placed in an ice-cold isolation medium consisting of 250 mM-sucrose, 1 mM-ethylene glycol-*O,O'*-bis(2-amino-ethyl)-*N,N,N',N'*-tetraacetic acid and 10 mM-Tris-HCl, pH 7.4. Muscle mitochondria (subsarcolemmal subpopulation) were isolated from gastrocnemius via an adaptation of the differential centrifugation procedure, as used previously by Roussel *et al.* (2000). All steps were performed at 4°C.

The muscle (2.0–2.5 g) was cut with scissors, minced using a Polytron mincer (4–5 s; Kinematica, Cincinnati, OH, USA) in an isolation medium (20 ml/g tissue) then homogenized using a Potter-Elvehjem homogenizer (seven strokes; Wheaton Science Products). The homogenate was centrifuged at 600 g for 10 min. The resulting supernatant fraction was filtered through cheesecloth and centrifuged at 10 000 g for 10 min. The pellet was resuspended in the isolation medium and centrifuged at 10 000 g for 10 min. Finally, the pellet was resuspended in a minimal volume of respiratory medium consisting of 120 mM-KCl, 5 mM-KH<sub>2</sub>PO<sub>4</sub>, 3 mM-4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid, 1 mM-ethylene glycol-*O,O'*-bis(2-amino-ethyl)-*N,N,N',N'*-tetraacetic acid, 2 mM-MgCl<sub>2</sub> and bovine serum albumin (3 g/l), pH 7.4. Liver mitochondria were isolated by using a modification of the differential centrifugation procedure of Krahenbuhl *et al.* (1994). The liver (6–7 g) was cut with scissors and homogenized using a Potter-Elvehjem homogenizer (seven strokes; Wheaton Science Products) in the isolation medium (8 ml/g tissue). The homogenate was centrifuged

at 600 g for 10 min. The resulting supernatant fraction was filtered through cheesecloth and centrifuged at 7000 g for 10 min. The pellet was resuspended in the isolation medium (10 ml/g tissue) and centrifuged at 3500 g for 10 min. The resulting pellet was resuspended in a minute volume of respiratory medium.

#### Mitochondrial respiration

O<sub>2</sub> was measured using a Clark O<sub>2</sub> electrode (oxygraph Hansatech, Cergy Pontoise, France) in a 2 ml glass cell with continuous stirring at a constant temperature of 30°C. Mitochondria (0.4–0.6 mg protein/ml) were incubated in the respiratory reaction medium as described earlier and saturated with room air. Mitochondrial respiration was conducted in two different experiments (one with glutamate, and the other for succinate and ascorbate-*N,N,N',N'*-tetramethyl-*p*-phenylenediamine (TMPD)). Substrate concentrations from liver measurements were 5 mM-glutamate, 5 mM-succinate and 5 mM-ascorbate + 0.5 mM-TMPD. With regard to gastrocnemius measurements, 5 mM-pyruvate + 5 mM-malate, 5 mM-succinate and 2 mM-ascorbate + 0.5 mM-TMPD were used. Inhibitor concentrations included 5  $\mu\text{M}$ -rotenone (to inhibit complex I of the respiratory chain) and 3 mM-myxothiazole (to inhibit complex III). The active state of respiration (state 3) was initiated by the addition of ADP (150  $\mu\text{M}$  to the liver mitochondria or 200  $\mu\text{M}$  to the gastrocnemius mitochondria). The basal non-phosphorylating respiration rate (state 4) was obtained by the addition of 3  $\mu\text{g}$  oligomycin/ml. The respiratory control ratio was the ratio O<sub>2</sub> consumed after the addition of ADP:O<sub>2</sub> consumed in the presence of oligomycin. The uncoupled state of respiration was initiated by the addition of 2  $\mu\text{M}$ -carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone. The respiratory variables measured in isolated mitochondria (state 3, state 4 and the uncoupled state of respiration) were normalized in relation to the specific activity of citrate synthase. Indeed, while the citrate synthase activity was not modified in the 600 g supernatant fraction, it was decreased in isolated liver mitochondria of DEX-treated rats. Moreover, we found a lower percentage of intact mitochondria in isolated liver mitochondria of DEX-treated rats (91 v. 96 and 94% in CON and PF groups respectively). Therefore, for the measurement of respiratory variables in isolated mitochondria preparations, we used the specific citrate synthase activity as a mitochondrial marker enzyme. The ratios of the respiratory variables:citrate synthase may reflect a change originating from the mitochondria themselves, rather than from the homogenization.

#### Calculation of thermodynamic coupling and efficiency of oxidative phosphorylation

Variables were calculated using the methodology of Cairns *et al.* (1998). The thermodynamic coupling of the energy conversion is designated by the dimensionless variable *q*, known as the degree of coupling of oxidative phosphorylation:

$$q = (1 - (J_{\text{sh}}/J_{\text{unc}}))^{1/2},$$

where  $J_{sh}$  is the net  $O_2$  consumption of state 4-oligomycin respiration in the presence of oligomycin that inhibits ATP synthase and  $J_{unc}$  is the uncoupled rate of  $O_2$  uptake induced by the addition of carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone, which dissipates the mitochondrial proton gradient and as a result ATP production becomes nil.

Kedem & Caplan (1965) have defined the efficiency of the energy conversion for oxidative phosphorylation ( $\eta$ ). Between state 4-oligomycin and state 3-uncoupled respiration, which represent two steady states, an optimal thermodynamic efficiency of the energy conversion ( $\eta_{opt}$ ) can be discerned for any value of  $q$  (Stucki, 1980):

$$\eta_{opt} = \tan^2(\alpha/2),$$

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

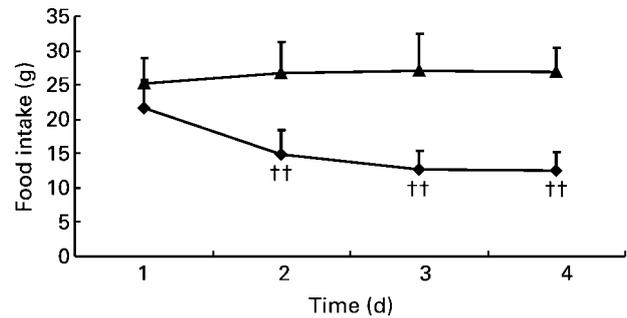
In comparison with conventional measurements (respiratory control ratio, ATP/ $O_2$ ), non-equilibrium thermodynamics analysis provides a quantitative description and a better estimation of stoichiometry and the efficiency of energy conversion.

### Statistical analysis

Results were expressed as mean values and standard deviations. Mean values were compared by ANOVA using a Fisher *post hoc* test. A  $P$  value  $< 0.05$  was considered significant in all cases. All analyses were performed using StatView, version 5.0 (SAS Institute, Cary, NC, USA).

### Results

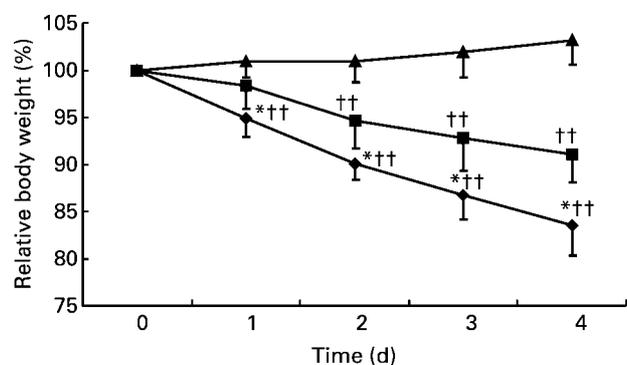
DEX treatment induced a significant reduction in food intake from day 2 (Fig. 1). Animals in the three groups did not differ in body weight at the beginning of the experimental procedure. Body weight decreased in DEX-treated rats from day 1 and in PF animals from day 2 (Fig. 2). This decrease was significantly greater ( $P < 0.01$ ) in DEX-treated rats than in PF animals, corresponding to 16% (DEX-treated) and 9% (PF) of initial body mass on the fourth day of treatment. At the same time, CON rats increased their body mass by 3.2% (Fig. 2). The body-weight loss in relation to total food intake was higher in DEX-treated than in PF rats (+57%) (Table 1).



**Fig. 1.** Effect of dexamethasone treatment on food intake per d over the last 4 d of treatment. ▲, Control; ◆, dexamethasone-treated. For details of procedures, see p. 970. Values are means with standard deviations shown by vertical bars (eight rats per group). Mean values were significantly different from those of the control group: †† $P < 0.01$ .

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

In the liver, complex I activity was significantly higher ( $P < 0.01$ ) in the DEX-treated group than in the PF group (+120%), but it was not different in comparison to the CON group (Table 2). DEX treatment significantly decreased ( $P < 0.01$ ) the specific activity of complex IV (−28% compared with PF rats), although it was not different when compared with CON. There was no significant difference in the specific activities of citrate synthase,



**Fig. 2.** Effect of dexamethasone treatment on body weight changes over the first 4 d of treatment in rats. ▲, Control; ◆, dexamethasone-treated; ■, pair-fed. For details of procedures, see p. 970. Values are means with standard deviations shown by vertical bars (eight rats per group). Mean values were significantly different from those of the pair-fed group: \* $P < 0.05$ . Mean values were significantly different from those of the control group: †† $P < 0.01$ .

**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‡  
(Mean values and standard deviations for eight rats per group)

	DEX-treated		PF		CON		Statistical significance of effect (ANOVA)
	Mean	SD	Mean	SD	Mean	SD	
Body weight (g)							
Initial	310	28	329	38	329	32	NS
At killing	242**††	19	285	37	315	33	<i>P</i> = 0.0006
Liver							
g	13.38**††	2.30	8.58†	1.12	10.84	1.47	<i>P</i> < 0.0001
g/kg body weight	54.9**††	6.6	30.4	2.8	34.4	2.4	<i>P</i> < 0.0001
Gastrocnemius							
g	3.27**††	0.21	4.04	0.50	4.25	0.27	<i>P</i> < 0.0001
g/kg body weight	13.6	0.7	14.1	0.6	13.6	0.9	NS
Brown adipose tissue							
g	0.76**††	0.20	0.35	0.10	0.40	0.14	<i>P</i> < 0.0001
g/kg body weight	3.1**††	0.8	1.2	0.4	1.2	0.4	<i>P</i> < 0.0001
Body weight change/total intake (g/g)	-0.88**††	0.31	-0.56†	0.27	0.09	0.08	<i>P</i> < 0.0001

Mean values were significantly different from those of the PF group: \*\**P* < 0.01.  
Mean values were significantly different from those of the CON group: ††*P* < 0.01.  
‡ For details of procedures, see p. 970.

succinate dehydrogenase and complex III between the DEX-treated and other groups (Table 2).

In the gastrocnemius, none of the enzymatic activities was significantly affected by glucocorticoid treatment (complex I 85–111, complex IV 166–213, complex III 363–409, succinate dehydrogenase 125–147 nmol/min per mg protein). There was no difference in the citrate synthase activity across the rat groups (538–602 nmol/min per mg protein).

In the isolated liver mitochondria, the specific citrate synthase activity was significantly lower (*P* < 0.01) in the DEX-treated group than in the CON and PF groups (Table 3). Basal non-phosphorylative, phosphorylative and uncoupled respiration rates, normalized by the specific citrate synthase activity, are shown in Table 3. With succinate and TMPD–ascorbate used as substrates, state 4-oligomycin respiration was significantly increased (+46%; *P* < 0.05) in isolated liver mitochondria of DEX-treated rats compared with other groups. In the glutamate-respiring mitochondria of the liver, DEX treatment significantly increased (+33%; *P* < 0.05) the state 4-oligomycin O<sub>2</sub> consumption in comparison with PF rats, but not when compared with CON rats. The respiratory variables in the

liver (state 3, state 4 and uncoupled state respiration) expressed per mg mitochondrial protein were similar across groups regardless of the respiratory substrate used (results not shown).

In the gastrocnemius, whatever the normalization of results (per mg mitochondria protein or by the specific citrate synthase activity), none of the respiratory variables was affected by DEX treatment whatever the substrate used (state 3 and state 4-oligomycin respiration rates were in relation to the specific activity synthase ((natonnes of O<sub>2</sub>/nmol) × 10<sup>-3</sup>): pyruvate + malate 84–121 and 7–10, succinate 106–137 and 29–40, ascorbate-TMPD 263–312 and 178–207).

In liver succinate-respiring mitochondria, *q* was significantly decreased by DEX treatment (*P* < 0.05). With glutamate, *q* was slightly (*P* = 0.12) lower in DEX-treated and PF than in CON rats (Table 4). Similar results were obtained for the determined thermodynamic optimal efficiency of oxidative phosphorylation ( $\eta_{opt}$ ).

In gastrocnemius, *q* (pyruvate + malate 0.970, succinate 0.885, ascorbate–TMPD 0.745) and  $\eta_{opt}$  (pyruvate + malate 0.619, succinate 0.384, ascorbate–TMPD 0.619) were similar in the three groups of rats.

**Table 2.** Mitochondrial enzyme activities in liver 600 g supernatant fractions from pair-fed (PF), control (CON) and dexamethasone (DEX)-treated rats‡  
(Mean values and standard deviations for eight rats per group)

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

Mean values were significantly different from those of the PF group: \**P* < 0.05, \*\**P* < 0.01.  
Mean value was significantly different from that of the CON group: ††*P* < 0.01.  
‡ For details of procedures, see p. 970.

**Table 3.** Respiratory variables and citrate synthase specific activity of liver isolated mitochondria from pair-fed (PF), control (CON) and dexamethasone (DEX)-treated rats‡  
(Mean values and standard deviations for eight rats per group)

Respiratory variables	Variables	DEX-treated		PF		CON		Statistical significant of effect (ANOVA)
		Mean	SD	Mean	SD	Mean	SD	
First experiment (glutamate)								
	Citrate synthase specific activity	264*†	45	311	37	374	41	$P < 0.01$
Glutamate	State 3§	114	51	71	28	110	44	NS
	State 4§	18*	3.5	13	2	15	3	$P < 0.05$
	RCR	6.4	3.1	5.6	3.1	7.2	3.2	NS
	Uncoupled state§	95	55	64	28	118	57	NS
Second experiment (succinate and ascorbate–TMPD)								
	Citrate synthase specific activity	306*†	11	359	46	358	36	$P < 0.05$
Succinate	State 3§	285	98	255	71	267	46	NS
	State 4§	70*†	22	46	4	50	7	$P < 0.05$
	RCR	4.1	1.4	5.6	1.4	5.4	0.8	NS
	Uncoupled state§	354	93	331	82	341	67	NS
TMPD–ascorbate	State 3§	511	168	398	49	409	91	NS
	State 4§	382*†	126	261	34	261	57	$P < 0.05$
	RCR	1.3	0.1	1.5	0.2	1.6	0.1	NS
	Uncoupled state§	645	186	525	64	529	141	NS

RCR, respiratory control ratio; TMPD, *N,N,N',N'*-tetramethyl-*p*-phenylenediamine.Mean values were significantly different from those of the PF group: \* $P < 0.05$ .Mean values were significantly different from those of the CON group: † $P < 0.05$ .

‡ For details of procedures, see p. 970.

§ States 3 and 4 and uncoupled state respiration were expressed as (natom  $O_2$ /nmol)  $\times 10^{-3}$ .

## Discussion

The present study shows that the induction of a catabolic state by DEX results in an increased liver mass and increased non-phosphorylative  $O_2$  consumption in liver mitochondria. In addition, we found a decreased thermodynamic coupling and efficiency of oxidative phosphorylation in the complex II respiratory pathway in the liver mitochondria of DEX-treated rats. In contrast, there was no change in gastrocnemius mitochondrial energy metabolism.

Of particular interest is the finding that DEX treatment significantly increased liver non-phosphorylative  $O_2$  consumption using succinate (+46%) and ascorbate as substrates (+46% *v.* CON and PF). With regard to glutamate, state 4-oligomycin respiration was not different from that of CON, but it was higher (+33%) in DEX-treated relative to PF animals. Our present results agree with results obtained from the long-term administration of

high-dose glucocorticoids, which are reported to decrease liver oxidative phosphorylation (state 3 respiration) via complex I respiratory substrates, while remaining unchanged when fuelled through complex II or IV (Kerppola, 1960; Kimura & Rasmussen 1977; Jani *et al.* 1991). Our present finding that state 4 respiration is increased is therefore complementary to those studies, since it has never been studied in DEX-treated rats, although inconsistencies were shown in corticosterone-treated rats (Jani *et al.* 1991). Therefore, it appears that there is a specific catabolic state-related increase (+42%) in the non-phosphorylative  $O_2$  consumption of liver mitochondria. Indeed, state 4-oligomycin respiration was increased in DEX-treated rats compared with CON rats, except in complex I (effect of food restriction and/or hypercatabolism), and consistent increases were found when DEX-treated animals were compared with PF animals (effect of hypercatabolism). On the other hand, food restriction appears to reduce state 4-oligomycin  $O_2$

**Table 4.** Thermodynamic degree of coupling ( $q$ ) and optimal efficiency ( $\eta_{opt}$ ) of the oxidative phosphorylation in liver isolated mitochondria from pair-fed (PF), control (CON) and dexamethasone (DEX)-treated rats‡  
(Mean values and standard deviations for eight rats per group)

Respiratory variables	Variables	DEX-treated		PF		CON		Statistical significance of effect (ANOVA)
		Mean	SD	Mean	SD	Mean	SD	
Glutamate	$q$	0.903	0.044	0.896	0.040	0.942	0.013	NS
	$\eta_{opt}$	0.413	0.092	0.396	0.080	0.500	0.039	NS
Succinate	$q$	0.896*†	0.019	0.923	0.022	0.922	0.011	$P < 0.05$
	$\eta_{opt}$	0.387*†	0.038	0.451	0.052	0.439	0.028	$P < 0.05$
Ascorbate–TMPD	$q$	0.656**††	0.028	0.708	0.024	0.706	0.028	$P < 0.005$
	$\eta_{opt}$	0.140**††	0.016	0.173	0.016	0.173	0.018	$P < 0.005$

Mean values were significantly different from those of the PF group: \* $P < 0.05$ , \*\* $P < 0.01$ .Mean values were significantly different from those of the CON group: † $P < 0.05$ , †† $P < 0.01$ .

‡ For details of procedures, see p. 970.

consumption (via complex I) in PF compared with CON rats. The PF group was chosen to discriminate between the effect of a reduced food intake and the effect of DEX itself on the variables measured, in spite of the effect of undernutrition on the thyroid hormone axis that can in turn regulate thermogenesis (Dauncey, 1990). Indeed plasma triiodothyronine concentration, the most metabolically active thyroid hormone, appears to respond to food restriction more slowly than does thyroxine concentration (Cassar-Malek *et al.* 2001). Moreover, Cassar-Malek *et al.* (2001) found that liver and muscle type I 5'-deiodinase activity was not affected by food restriction. Consequently, it is unclear whether energy expenditure would have been affected by food restriction in relation to the thyroid hormone axis. Alternatively, DEX treatment has been found to inhibit or increase the conversion of thyroxine to triiodothyronine (Chopra *et al.* 1975; Balsam & Ingbar, 1978; Kaplan & Utiger, 1978; Sato *et al.* 1984; Song & Oka, 2003).

Such a change under these non-phosphorylative conditions is not due to modifications in respiratory chain complex activities (Table 2), but it could be explained by an increased basal proton conductance (Roussel *et al.* 2003). Nevertheless, the results reported by Roussel *et al.* (2003) could also be explained by a change in the intrinsic coupling of the respiratory chain ( $H^+/2e^-$ ). Therefore, we cannot completely rule out the possibility that DEX may affect the efficiency of the respiratory chain activity. In this case, we can speculate an effect on cytochrome oxidase, as state 4-oligomycin respiration was increased by using ascorbate-TMPD as substrate. Moreover, it has been demonstrated that cytochrome oxidase  $H^+/2e^-$  stoichiometry is variable and represents a possible location for intrinsic uncoupling at the level of the respiratory chain (Capitanio *et al.* 1991; Papa *et al.* 1991). However, further experiments are necessary to confirm such possibilities: whatever the mechanism involved, proton leak and redox slipping would both represent a substantial increase in state 4 energy wastage and would in turn decrease the oxidative phosphorylation yield. This is further highlighted by the decreased value of the thermodynamic coupling of oxidative phosphorylation reported in Table 4. According to Stucki (1980), values observed (Table 4) therefore suggest that in DEX-treated rats, liver mitochondria adapt their function for maximum ATP production and also to maintain cellular phosphate potential at the expense of the energy conversion efficiency. Interesting adaptive reductions in the efficiency of oxidative phosphorylation have already been noted by Nogueira *et al.* (2001), who showed that cellular respiratory rate increases in liver mitochondria of hyperthyroid rats or rats fed on a polyunsaturated fatty acid-deficient diet.

Our present study demonstrated that a DEX-induced hypercatabolic state has no effect on mitochondrial energy metabolism in the gastrocnemius muscle. Indeed, none of the respiratory complex activities,  $O_2$  consumption rates or the thermodynamic degree of coupling of oxidative phosphorylation were altered in the mitochondria of DEX-treated rats. The experiment was conducted in the gastrocnemius subsarcolemmal mitochondria, which exhibit different biochemical and functional features than

intermyofibrillar mitochondria. However, in a recent study, Roussel *et al.* (2003) found no difference in proton conductance in DEX-treated rats whatever the mitochondrial subpopulation used. The present results regarding the gastrocnemius are in agreement with previously reported effects of glucocorticoid treatment in other muscles (Vignos & Greene, 1973; Capaccio *et al.* 1985; Marone *et al.* 1994), but differ from the observations of Weber *et al.* (2002), who found DEX treatment (6 mg/kg per d) increased cytochrome *c* oxidase activity levels in quadriceps. This might be due to the lower dose of DEX used in our present study. Collectively, these results suggest that the mitochondrial metabolism of skeletal muscle produces enough ATP to fulfil either the cellular energy requirement and/or the energy-dependent pathways induced by glucocorticoids: these include the energy-ubiquitin-dependent proteolytic pathway (Tiao *et al.* 1996; Mitch *et al.* 1999) and the energy-dependent glutamine synthase activity pathway (Max *et al.* 1988; Minet-Quinard *et al.* 1999, 2000).

The main thermogenic tissue in rats is brown adipose tissue, the weight of which is largely increased (+100%, present study) by glucocorticoid injection. Previous studies have clearly demonstrated that such an increase in the brown adipose tissue mass was due to increased lipid storage rather than an increased thermogenic capacity of this tissue (Mazzuccheli *et al.* 1960; Strack *et al.* 1995). Furthermore, glucocorticoids are known to reduce both the activity and the gene expression of uncoupling protein 1 (Tokuyama & Himms-Hagen, 1989; Moriscot *et al.* 1993; Strack *et al.* 1995). Moreover, in our study we found no effect of DEX on mitochondrial oxidative capacity (cytochrome *c* oxidase activity; results not shown). Therefore, it is unlikely that interscapular brown adipose tissue increases energy expenditure in DEX-treated rats.

A reduction in body size (16% weight loss in the present study) generally results in the lowering of energy expenditure per whole rat, because of the reduced maintenance requirement (Ramsey *et al.* 2000). In the present study, the DEX-related decrease in body weight was greater than that observed in the PF rats, highlighting an increase in whole-body energy expenditure. This is paradoxical in view of the reducing effect of food restriction on energy expenditure (Ramsey *et al.* 2000). Furthermore, in our present study, DEX treatment increased the ratio liver weight:body weight, while the relative skeletal muscle mass remained unchanged. Such an increase in liver mass could have resulted from an increase in glycogen content (Weber & Kletzien, 1982; Michaels & Cardell, 1997; Bollen *et al.* 1998). Alternatively, hepatic lipid content is increased in DEX-treated rats, but the increase is insufficient to fully explain an increase in liver mass (Kaur *et al.* 1989; Palacios *et al.* 1995; Franco-Colin *et al.* 2000). Finally, DEX treatment increases liver protein synthesis and therefore metabolic tissue (Odedra *et al.* 1983; Savary *et al.* 2001). Since liver contributes 20% to the metabolic rate in the rat (Rolfe & Brown, 1997), it is likely that the liver would effectively contribute to the 10–20% increase in glucocorticoid-related energy expenditure seen in human subjects and rats (Woodward & Emery, 1989; Brillon *et al.* 1995; Tataranni *et al.* 1996).

despite the body-weight loss (Table 1). Obviously, we cannot rule out the influence of other biochemical mechanisms or possible determinants of energy balance, which may also have accounted for the weight loss.

In conclusion, 5 d of high-dose DEX treatment induced a significant increase in liver mass, an increase in liver mitochondrial non-phosphorylative O<sub>2</sub> consumption rate from all substrates used and a decrease in the thermodynamic coupling of oxidative phosphorylation in liver respiratory pathways. We suggest, therefore, that a DEX-induced proton leak and/or redox slipping in liver mitochondria is probably responsible for the decrease in the thermodynamic efficiency of energy conversion. Thus, in turn, rats could adapt their mitochondrial energy functions to a DEX-induced hypermetabolic state by maximizing ATP production in addition to maintaining their cellular energy state, regardless of the cost. This treatment has no effect on energy metabolism in the gastrocnemius muscle. Together with a decreased food intake, the increase in energy expenditure induced by high-dose DEX results in a negative energy balance and thus in weight loss.

### Acknowledgements

The authors thank Pierre Legras, Jérôme Roux and Dominique Gilbert for animal care, Antoine Augeraud for technical assistance and Miriam Ryan for her assistance in correcting the English. This work was supported by grants from Contrat de Plan Etat Region 2002–2004.

### References

- Allan EH, Chisholm AB & Titheradge MA (1983) The stimulation of hepatic oxidative phosphorylation following dexamethasone treatment of rats. *Biochim Biophys Acta* **725**, 71–76.
- Balsam A & Ingbar SH (1978) The influence of fasting, diabetes, and several pharmacological agents on the pathways of thyroxine metabolism in rat liver. *J Clin Invest* **62**, 415–424.
- Bollen M, Keppens S & Stalmans W (1998) Specific features of glycogen metabolism in the liver. *Biochem J* **336**, 19–31.
- Bowes SB, Jackson NC, Papachristodoulou D, Umpleby AM & Sonksen PH (1996) Effect of corticosterone on protein degradation in isolated rat soleus and extensor digitorum longus muscles. *J Endocrinol* **148**, 501–507.
- Brillon DJ, Zheng B, Campbell RG & Matthews DE (1995) Effect of cortisol on energy expenditure and amino acid metabolism in humans. *Am J Physiol* **268**, E501–E513.
- Cairns CB, Walther J, Harken AH & Banerjee A (1998) Mitochondrial oxidative phosphorylation thermodynamic efficiencies reflect physiological organ roles. *Am J Physiol* **274**, R1376–R1383.
- Capaccio JA, Galassi TM & Hickson RC (1985) Unaltered aerobic power and endurance following glucocorticoid-induced muscle atrophy. *Med Sci Sports Exerc* **17**, 380–384.
- Capitanio N, Capitanio G, De Nitto E, Villani G & Papa S (1991) H<sup>+</sup>/e<sup>-</sup> stoichiometry of mitochondrial cytochrome complexes reconstituted in liposomes. Rate-dependent changes of the stoichiometry in the cytochrome c oxidase vesicles. *FEBS Lett* **288**, 179–182.
- Cassar-Malek I, Kahl S, Jurie C & Picard B (2001) Influence of feeding level during postweaning growth on circulating concentrations of thyroid hormones and extrathyroidal 5'-deiodination in steers. *J Anim Sci* **79**, 2679–2687.
- Chima CS, Barco K, Dewitt ML, Maeda M, Teran JC & Mullen KD (1997) Relationship of nutritional status to length of stay, hospital costs, and discharge status of patients hospitalized in the medicine service. *J Am Diet Assoc* **97**, 975–978, 979–980.
- Chopra IJ, Williams DE, Orgiazzi J & Solomon DH (1975) Opposite effects of dexamethasone on serum concentrations of 3,3',5'-triiodothyronine (reverse T3) and 3,3',5'-triiodothyronine (T3). *J Clin Endocrinol Metab* **41**, 911–920.
- Dauncey MJ (1990) Thyroid hormones and thermogenesis. *Proc Nutr Soc* **49**, 203–215.
- Franco-Colin M, Tellez-Lopez AM, Quevedo-Corona L & Racotta R (2000) Effects of long-term high-sucrose and dexamethasone on fat depots, liver fat, and lipid fuel fluxes through the retroperitoneal adipose tissue and splanchnic area in rats. *Metabolism* **49**, 1289–1294.
- Hausberger FX & Hausberger BC (1958) Effect of insulin and cortisone on weight gain, protein and fat content of rats. *Am J Physiol* **103**, 455–460.
- Jani MS, Telang SD & Katyare SS (1991) Effect of corticosterone treatment on energy metabolism in rat liver mitochondria. *J Steroid Biochem Mol Biol* **38**, 587–591.
- Kaplan MM & Utiger RD (1978) Iodothyronine metabolism in rat liver homogenates. *J Clin Invest* **61**, 459–471.
- Kaur N, Sharma N & Gupta AK (1989) Effects of dexamethasone on lipid metabolism in rat organs. *Indian J Biochem Biophys* **26**, 371–376.
- Kedem O & Caplan SR (1965) Degree of coupling and its relation to efficiency of energy conversion. *Trans Faraday Soc* **21**, 1897–1911.
- Kerppola W (1960) Uncoupling of the oxidative phosphorylation with cortisone in liver mitochondria. *Endocrinology* **67**, 252–263.
- Kimura S & Rasmussen H (1977) Adrenal glucocorticoids, adenine nucleotide translocation, and mitochondrial calcium accumulation. *J Biol Chem* **252**, 1217–1225.
- Kochakian CD & Robertson E (1951) Adrenal steroids and body composition. *J Biol Chem* **190**, 495–503.
- Koski CL, Rifkinberck DH & Max SR (1974) Oxidative metabolism of skeletal muscle in steroid atrophy. *Arch Neurol* **31**, 407–410.
- Krahenbuhl S, Talos C, Fischer S & Reichen J (1994) Toxicity of bile acids on the electron transport chain of isolated rat liver mitochondria. *Hepatology* **19**, 471–479.
- Landi F, Onder G, Gambassi G, Pedone C, Carbonin P & Bernabei R (2000) Body mass index and mortality among hospitalized patients. *Arch Intern Med* **160**, 2641–2644.
- Lesourd B & Mazzari L (1997) Immune responses during recovery from protein energy malnutrition. *Clin Nutr* **16**, 37–46.
- Malgat M, Durrieu G & Mazat JP (1999) Enzymatic and polarographic measurements of the respiratory chain complexes. In *Mitochondrial Diseases*, pp. 357–377 [P Lestienne, editor]. Paris: Springer Verlag.
- Marone JR, Falduto MT, Essig DA & Hickson RC (1994) Effects of glucocorticoids and endurance training on cytochrome oxidase expression in skeletal muscle. *J Appl Physiol* **77**, 1685–1690.
- Max SR, Mill J, Mearow K, *et al.* (1988) Dexamethasone regulates glutamine synthetase expression in rat skeletal muscles. *Am J Physiol* **255**, E397–E402.
- Mazzuccheli MV, Confalonieri C & Schlechter P (1960) The nervous system and lipid metabolism of adipose tissue. 2. Influence of denervation on the lipogenic activity of adrenocortical hormones. *Metabolism* **10**, 330–334.

- Michaels JE & Cardell RR (1997) Localization of glycogen phosphorylase activity in liver of fasted normal and adrenalectomized rats and in fasted adrenalectomized rats after injection of dexamethasone. *Anat Rec* **248**, 406–412.
- Minet-Quinard R, Moinard C, Villie F, *et al.* (1999) Kinetic impairment of nitrogen and muscle glutamine metabolisms in old glucocorticoid-treated rats. *Am J Physiol* **276**, E558–E564.
- Minet-Quinard R, Moinard C, Walrand S, *et al.* (2000) Induction of a catabolic state in rats by dexamethasone: dose or time dependency? *J Parenter Enteral Nutr* **24**, 30–36.
- Mitch WE, Bailey JL, Wang X, Jurkovicz C, Newby D & Price SR (1999) Evaluation of signals activating ubiquitin-proteasome proteolysis in a model of muscle wasting. *Am J Physiol* **276**, C1132–C1138.
- Moriscot A, Rabelo R & Bianco AC (1993) Corticosterone inhibits uncoupling protein gene expression in brown adipose tissue. *Am J Physiol* **265**, E81–E87.
- Nguyen LT, Bedu M, Caillaud D, *et al.* (1999) Increased resting energy expenditure is related to plasma TNF- $\alpha$  concentration in stable COPD patients. *Clin Nutr* **18**, 269–274.
- Nogueira V, Rigoulet M, Piquet MA, Devin A, Fontaine E & Leverve XM (2001) Mitochondrial respiratory chain adjustment to cellular energy demand. *J Biol Chem* **276**, 46104–46110.
- Odedra BR, Bates PC & Millward DJ (1983) Time course of the effect of catabolic doses of corticosterone on protein turnover in rat skeletal muscle and liver. *Biochem J* **214**, 617–627.
- Palacios E, Pinon-Lopez MJ, Racotta IS & Racotta R (1995) Effect of lipectomy and long-term dexamethasone on visceral fat and metabolic variables in rats. *Metabolism* **44**, 1631–1638.
- Papa S, Capitanio N, Capitanio G, De Nitto E & Minuto M (1991) The cytochrome chain of mitochondria exhibits variable H<sup>+</sup>/e<sup>-</sup> stoichiometry. *FEBS Lett* **288**, 183–186.
- Ramsey JJ, Harper ME & Weindruch R (2000) Restriction of energy intake, energy expenditure, and aging. *Free Radic Biol Med* **29**, 946–968.
- Rolfe DF & Brown GC (1997) Cellular energy utilization and molecular origin of standard metabolic rate in mammals. *Physiol Rev* **77**, 731–758.
- Roussel D, Dumas JF, Augeraud A, *et al.* (2003) Dexamethasone treatment specifically increases the basal proton conductance of rat liver mitochondria. *FEBS Lett* **541**, 75–79.
- Roussel D, Lhenry F, Ecochard L, Sempore B, Rouanet JL & Favier R (2000) Differential effects of endurance training and creatine depletion on regional mitochondrial adaptations in rat skeletal muscle. *Biochem J* **350**, 547–553.
- Sato K, Mimura H, Han DC, Tsushima T & Shizume K (1984) Ontogenesis of iodothyronine-5'-deiodinase. Induction of 5'-deiodinating activity by insulin, glucocorticoid, and thyroxine in cultured fetal mouse liver. *J Clin Invest* **74**, 2254–2262.
- Savary I, Debras E, Dardevet D, *et al.* (2001) Evidence for an alteration of plasma and liver proteins response to dexamethasone in aging rats. *Mech Ageing Dev* **122**, 105–120.
- Song S & Oka T (2003) Regulation of type II deiodinase expression by EGF and glucocorticoid in HC11 mouse mammary epithelium. *Am J Physiol* **284**, E1119–E1124.
- Strack AM, Bradbury MJ & Dallman MF (1995) Corticosterone decreases nonshivering thermogenesis and increases lipid storage in brown adipose tissue. *Am J Physiol* **268**, R183–R191.
- Stucki JW (1980) The optimal efficiency and the economic degrees of coupling of oxidative phosphorylation. *Eur J Biochem* **109**, 269–283.
- Tataranni PA, Larson DE, Snitker S, Young JB, Flatt JP & Ravussin E (1996) Effects of glucocorticoids on energy metabolism and food intake in humans. *Am J Physiol* **271**, E317–E325.
- Tiao G, Fagan J, Roegner V, *et al.* (1996) Energy-ubiquitin-dependent muscle proteolysis during sepsis in rats is regulated by glucocorticoids. *J Clin Invest* **97**, 339–348.
- Tokuyama K & Himms-Hagen J (1989) Enhanced acute response to corticosterone in genetically obese (ob/ob) mice. *Am J Physiol* **257**, E133–E138.
- Tucker HN & Miguel SG (1996) Cost containment through nutrition intervention. *Nutr Rev* **54**, 111–121.
- Vignos PJ & Greene R (1973) Oxidative respiration of skeletal muscle in experimental corticosteroid myopathy. *J Lab Clin Med* **81**, 365–378.
- Wakat DK & Haynes RC Jr (1977) Glucocorticoid-stimulated utilization of substrates in hepatic mitochondria. *Arch Biochem Biophys* **184**, 561–571.
- Wallace JI, Schwartz RS, LaCroix AZ, Uhlmann RF & Pearlman RA (1995) Involuntary weight loss in older outpatients: incidence and clinical significance. *J Am Geriatr Soc* **43**, 329–337.
- Weber CA & Kletzien RF (1982) Hormonal and nutritional factors influencing glycogen deposition in primary cultures of rat liver parenchymal cells. *J Cell Physiol* **110**, 300–303.
- Weber K, Bruck P, Mikes Z, Kupper JH, Klingenspor M & Wiesner RJ (2002) Glucocorticoid hormone stimulates mitochondrial biogenesis specifically in skeletal muscle. *Endocrinology* **143**, 177–184.
- Woodward CJ & Emery PW (1989) Energy balance in rats given chronic hormone treatment. 2. Effects of corticosterone. *Br J Nutr* **61**, 445–452.