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Energy balance in rats given chronic hormone treatment

2. Effects of corticosterone

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1. Sprague–Dawley rats were given corticosterone for 4 to 14 d either by subcutaneous injection (50 mg/kg body-weight per d) or as a higher dose in the diet (1 g/kg diet). Energy balance was calculated using the comparative carcass technique.

2. Corticosterone significantly suppressed growth rate by at least 50% ($P < 0.001$ in all experiments). The reduction in growth was more marked in males than in females.

3. Hormone treatment significantly reduced metabolizable intake (kJ/d) in males but not in females. Expressed relative to either metabolic body size (kg body-weight$^{0.75}$) or fat-free mass, metabolizable intake tended to be increased in the treated groups.

4. Energy expenditure, calculated as the difference between metabolizable intake and gain and expressed as kJ/d, did not differ between treated and control rats. Relative to either metabolic body size or fat-free mass, expenditure was consistently increased in treated rats. This change was statistically significant in five of the eight comparisons.

5. The corticosterone-treated rat is characterized by high energy intake and expenditure relative to its body size and growth rate. Alterations in the relative sizes of different lean tissues may contribute to these changes.

High doses of corticosteroids generally produce weight loss in animals. This is attributable to reduced lean body mass, with relatively little change in body fat (Kochakian & Robertson, 1951; Hausberger & Hausberger, 1958; Kekwick & Pawan, 1965). Less is known about the effects of corticosteroids on energy balance. Despite substantial weight loss, cortisol-treated rats show no marked reduction of food consumption (Bellamy, 1964). Such evidence might indicate increased energy expenditure, but in rats treated with corticosterone this has not been consistently found (Coyer *et al.* 1985). Other studies in mice suggest that energy expenditure is reduced by corticosteroids (Babikian, 1962; Kekwick & Pawan, 1965; Galpin *et al.* 1983).

In the present experiments we have, therefore, measured energy balance in corticosterone-treated rats using the comparative carcass technique. A preliminary account of the present work has already been published (Woodward & Emery, 1986).

MATERIALS AND METHODS

Animals

Sprague–Dawley rats weighing approximately 140 g were obtained from the College animal colony and housed singly in plastic cages with wire-mesh bases. Groups of six rats were used. The animal house was maintained at 20–25° with a 12 h light–12 h dark cycle. The rats were given, *ad. lib.*, water and a powdered semi-synthetic diet containing 210 g casein/kg and with a gross energy content of 18·5 kJ/g (Woodward & Emery, 1989). The food pots were covered by metal grids to minimize spillage. Body-weight and the amount of food removed from the pots were measured every 2–4 d.

Corticosterone (Sigma Chemical Co., Poole, Dorset) was suspended in a vehicle

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containing (g/l): sodium chloride 8, Tween 80 (polyoxyethylenesorbitan monooleate) 4, sodium carboxymethylcellulose 5, benzyl alcohol 9 (Tomas *et al.* 1979). The hormone was injected subcutaneously once daily at 15.00–17.00 hours using a dose of 50 mg/kg body-weight. Control animals were injected with vehicle alone. In experiments using dietary corticosterone, the hormone was suspended in maize oil before mixing in bulk with the diet at a level of 1 g/kg diet. On a body-weight basis, this dose was approximately double that injected. A higher dose was used in case low absorption or inactivation by the liver reduced the effectiveness of the oral route.

Experiments were initially planned to last for 12–13 d. However, since males lost weight rapidly when treated with corticosterone, studies using this sex were terminated when the treated group weighed 30% less than the controls. The following experiments were carried out.

Expt 1: male rats were injected with corticosterone for 11 d.
Expt 2: female rats were injected with corticosterone for 12 d.
Expt 3: male rats were fed on corticosterone for 6 d.
Expt 4: female rats were fed on corticosterone for 13 d.

Carcass analysis

Carcasses were analysed using oven-drying and Soxhlet extraction as previously described (Woodward & Emery, 1989). Random samples taken for nitrogen analysis using the Kjeldahl technique showed that N constituted a constant fraction of defatted dry matter (DDM) which was unaffected by corticosterone treatment, sex or age of rat. Carcass crude protein content was therefore routinely calculated as weight of DDM $\times 0{\cdot}81$.

Calculation of carcass gains and energy balance

Initial composition was estimated using carcass analyses of basal groups of appropriate sex and weight (Woodward & Emery, 1989). Gains of carcass components were then calculated by difference. Metabolizable energy intake was determined as the difference between dietary energy removed from the food pots and the pooled energy content of excreta and spillage. Urine, faeces and small amounts of spilled diet were collected together on plastic trays placed underneath the cages. After diluting and mixing in a blender, portions were freeze-dried and analysed by ballistic-bomb calorimetry.

Carcass energy content was calculated using energy densities of 39 and 19 kJ/g for fat and DDM respectively. These factors were found from ballistic-bomb calorimetry of pooled samples. Energy expenditure, calculated by subtracting carcass energy gain from metabolizable intake, was expressed (1) per whole rat, (2) relative to metabolic body size (kg body-weight$^{0{\cdot}75}$), or (3) relative to fat-free mass.

Statistical analyses

Results are expressed as means with their standard errors. Means were compared using Student's unpaired t test. Where the Fisher test indicated that variances were unequal, Cochran's approximation was used (Snedecor & Cochran, 1978).

RESULTS

Growth rate

Since the designs of Expts 1–4 were similar, the results are presented together. There were no deaths during the experiments, but polyuria was noted in the treated male rats in Expts 1 and 3.

Weight gain in the treated males in Expt 1 was 1·9 g/d, 76% less than that of the control group (Table 1). The treated females in Expt 2 gained 2·3 g/d, half the rate of their controls.

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Table 1. *Body-weight and carcass composition of rats given corticosterone by subcutaneous injection (50 mg/kg body-weight per d) or in the diet (1 g/kg diet) and their controls*

(Values are means with their standard errors for six rats per group)

Expt no.†	Sex	Treatment	Body-wt: Final (g) Mean	SE	Body-wt: Gain (g/d) Mean	SE	Gains of carcass components (g/d): Water Mean	SE	Fat Mean	SE	DDM Mean	SE	Crude protein‡ Mean	SE
1	Male	Corticosterone (injected)	150***	4·8	1·9***	0·24	0·05**	0·12	1·43	0·07	0·51***	0·09	0·41***	0·07
		Control	213	7·1	7·8	0·53	4·64	0·39	1·49	0·14	1·67	0·10	1·35	0·08
2	Female	Corticosterone (injected)	154**	4·5	2·3***	0·19	−0·06***	0·15	1·77	0·17	0·54***	0·04	0·44***	0·03
		Control	182	6·0	4·6	0·41	2·38	0·24	1·52	0·22	1·04	0·06	0·84	0·05
3	Male	Corticosterone (fed)	115***	2·3	−1·9***	0·38	−2·43***	0·27	0·66**	0·06	0·22***	0·10	0·18***	0·08
		Control	160	4·1	5·8	0·56	3·18	0·34	1·57	0·16	1·27	0·11	1·03	0·09
4	Female	Corticosterone (fed)	166*	11·1	2·9***	0·45	0·78***	0·28	1·59	0·28	0·57***	0·09	0·46***	0·07
		Control	204	8·5	5·7	0·23	3·02	0·19	1·42	0·11	1·26	0·05	1·02	0·04

DDM, defatted dry matter.
Mean values were significantly different from those of the corresponding control group: * $P < 0·05$, ** $P < 0·01$, *** $P < 0·001$.
† For details of individual experiments, see p. 446.
‡ Calculated as weight of DDM × 0·81.

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Thus, the females showed a somewhat smaller response to injected corticosterone than the males. A similar sex difference was found with dietary corticosterone: the male rats given corticosterone in Expt 3 actually showed a net weight loss, whereas the treated females in Expt 4 again grew at about half the rate of their respective controls.

Carcass composition

Gains of carcass water, fat and DDM are given in Table 1. The male rats injected with corticosterone (Expt 1) showed significantly lower gains of carcass water and DDM compared with their respective controls; the reduction in water was rather greater than that of DDM, both on an absolute and percentage basis. By contrast the accretion of fat was almost identical in the two groups. Corticosterone again caused significant reductions in water and DDM gain in Expt 2, and did not alter fat deposition.

In Expt 3, where growth suppression was most pronounced, a significant reduction in fat gain (−58%) was found in addition to the losses in water and DDM. There was a net loss of carcass water and, as in Expts 1 and 2, this component accounted for most of the loss in body-weight. In Expt 4 with females, carcass fat was not reduced in the treated group and the lower gains of water and DDM followed a similar pattern to Expts 1 and 2.

Metabolizable intake

In Expt 1, the metabolizable intake of the treated males was 303 kJ/d, 12% lower than that of the control animals (Table 2). There was no effect of corticosterone on this variable in Expt 2. A comparable sex difference was found with dietary corticosterone: in Expt 3 the treated male rats had an intake 17% less than that of their controls, but there was no difference between the groups in Expt 4. When metabolizable intake was expressed relative to metabolic body size, the treated females in Expt 2 showed an increased intake, but no significant differences were observed in the remaining three experiments. When expressed relative to fat-free mass, metabolizable intake was significantly increased in the treated groups in Expts 1 and 2.

Carcass energy gain

Corticosterone significantly attenuated the rate of carcass energy gain (kJ/d) in the two experiments with males, by 27 and 66% for injected and dietary hormone respectively. In contrast this variable was not altered in either of the experiments with females.

Energy expenditure

When expressed per rat (kJ/d), there was no significant difference of energy expenditure in any of the four experiments, and in all cases the value for the treated group was within 6% of that for the controls. Relative to metabolic body size, expenditure was consistently higher in the treated groups by 4–16%, but this difference was significant only in Expt 3. In Expts 1 and 2, the difference was of borderline significance ($P < 0{\cdot}1$). Relative to fat-free mass, energy expenditure was consistently increased in the treated groups, by 11–17%. This difference reached statistical significance in all cases.

DISCUSSION

When comparing animals of different size or composition, energy expenditure is usually expressed relative to either metabolic body size or lean body mass (Ford, 1984; Van Es, 1986). In the present study, fat-free mass has been used as an alternative to lean body mass (fat-free mass includes the non-lipid components of adipose tissue but excludes the lipids present in other tissues). Increased expenditure in the present case was most pronounced

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Table 2. *Energy balance of rats given corticosterone either by subcutaneous injection (50 mg/kg W per d) or in the diet (1 g/kg diet), and their controls*

(Values are means with their standard errors for six rats per group)

Expt no.†	Sex	Treatment	Metabolizable energy intake						Carcass gain (kJ/d)		Energy expenditure					
			kJ/d		kJ/kg $W^{0.75}$ per d		kJ/kg FFM per d				kJ/d		kJ/kg $W^{0.75}$ per d		kJ/kg FFM per d	
			Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
1	Male	Corticosterone	303*	10·5	1322	27	2445*	45	66**	3·8	238	7·7	1034	20	1918**	35
		Control	344	11·2	1298	33	2247	65	90	6·2	254	8·7	961	29	1655	52
2	Female	Corticosterone	282	17·2	1215*	52	2367*	98	79	6·5	203	13·5	873	42	1700*	72
		Control	266	9·2	1075	13	1997	28	79	5·4	187	4·5	756	7	1404	18
3	Male	Corticosterone	254*	9·0	1253	43	2255	78	30***	3·2	224	9·3	1106*	44	1983*	82
		Control	305	15·0	1325	62	2382	116	86	8·5	219	8·0	950	37	1700	70
4	Female	Corticosterone	272	17·3	1151	48	2236	125	73	10·5	199	11·7	839	23	1623*	52
		Control	289	11·6	1110	14	2013	35	79	4·5	210	9·7	806	22	1462	42

W, body-weight; FFM, fat-free mass.

Mean values were significantly different from those of the corresponding control group: * $P < 0·05$, ** $P < 0·01$, *** $P < 0·001$.

† In Expts 1 and 2, corticosterone was given by subcutaneous injection, whereas in Expts 3 and 4 it was given in the diet. For other details of individual experiments, see p. 446.

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when expressed on the basis of fat-free mass, although increases were also observed relative to metabolic body size. However, the design of the experiments was such that, in addition to body size, the treated and control groups differed in growth rate and in metabolizable energy intake.

When growth is impaired, a reduction in energy expenditure is expected because of the lower costs of tissue deposition. In addition a reduction in body size is generally considered to result in lower expenditure per whole rat, because of reduced maintenance requirements. Such effects can be quantified using factorial methods (Pullar & Webster, 1977; Webster, 1986). In the present experiments, none of the treated groups showed a reduced energy expenditure, despite differences in size and growth rate. Indeed some of the expressions showed increased expenditure in the treated groups, which is the converse of the effect predicted. Factorial methods suggest that such an increase might be attributable either to raised maintenance requirements or to a low efficiency of growth. The present results do not allow the contribution of these two processes to be distinguished. However, the known effects of corticosteroids, for example on protein turnover (Odedra *et al.* 1983) and on relative organ sizes (Fain & Czech, 1975), suggest that both maintenance and growth costs might be affected. Such metabolic changes are discussed later.

Metabolizable energy intake per rat (kJ/d) was reduced by corticosterone in males, but not in females. The presence of glycosuria in the males suggests that urinary glucose loss may have contributed to this sex difference. Metabolizable energy intake relative to metabolic body size or fat-free mass tended to be increased in the treated groups. The magnitudes of the differences were in some cases as great as those found for expenditure. It might be argued that the increased expenditures were dependent on these high intakes. This would imply that corticosterone was inducing a form of dietary-induced thermogenesis. However, there is no evidence to support such a view; indeed, it is thought that corticosteroids suppress dietary-induced thermogenesis (Rothwell & Stock, 1984).

Several biochemical mechanisms might contribute to the effects of corticosterone on energy expenditure. The possible role of increased amino acid oxidation has been discussed by Coyer *et al.* (1985). Corticosteroids also alter the relative rates of protein synthesis and degradation (Odedra *et al.* 1983; Tomas *et al.* 1979), which may raise the cost of net protein synthesis. There may also be an effect on physical activity (Beatty *et al.* 1971). Last, it is known that while corticosteroids reduce the size of skeletal muscle, visceral tissues are less affected and may even become enlarged (Hausberger & Hausberger, 1958; Fain & Czech, 1975). Metabolic variables, such as protein turnover and enzyme activity, are normal or increased in visceral tissues after corticosteroid treatment (Odedra *et al.* 1983; Woodward & Emery, 1987). Since there is evidence that visceral tissues have a higher metabolic rate than skeletal muscle (Koong *et al.* 1985; Webster, 1986), this effect would itself be expected to increase whole-body energy expenditure relative to body size.

Compared with males, female rats showed less weight loss, no obvious polyuria, and no reduction in either metabolizable energy intake or carcass energy gain after corticosterone treatment. It is likely that these differing responses were caused by the markedly higher turnover of corticosterone in female rats (Glenister & Yates, 1961; Kitay, 1961), which would accelerate the clearance of exogenous hormone from the body. This sex difference is not consistently found in other species.

A number of reports indicate a species difference between rats and mice in the response to exogenous corticosteroids. Hyperphagia, increased fat deposition and reduced energy expenditure have been reported in mice (Babikian, 1962; Kekwick & Pawan, 1965; Galpin *et al.* 1983). These effects are in some ways similar to those found in human beings (Walsh *et al.* 1984; Willcox *et al.* 1984; Horber *et al.* 1986).

Adrenalectomized rats are hypophagic, but grow normally when force-fed the same

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amount of food as control rats (Cohn *et al.* 1955). This suggests that adrenal steroids may affect appetite, but have no independent effect on energy expenditure. Endogenous corticosteroids are also thought to play a role in the genetically-obese *fa/fa* rat and *ob/ob* mouse, since many features of their obesity can be normalized by adrenalectomy (Fletcher, 1986; Warwick & Romsos, 1988).

One aim of the present study was to determine how corticosterone-treated rats lose weight despite having relatively high metabolizable energy intakes. The results show first that the tissue lost consists mainly of protein and water and, therefore, has a low energy content. At the same time the treated rats maintained a relatively high energy expenditure. Perhaps the simplest explanation for these results is that corticosterone has no independent effects on energy balance. However, the substantial loss of lean body mass in the form of skeletal muscle may cause apparent changes when energy variables are expressed relative to body size. In addition, the presence of glycosuria will influence metabolizable energy intake.

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