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Modification of glucocorticoid-induced changes in myofibrillar protein turnover in rats by protein and energy deficiency as assessed by urinary excretion of N^{τ}-methylhistidine

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1. The effects of differing degrees of experimental protein–energy malnutrition on the response of myofibrillar protein turnover rates to administration of corticosteroid has been studied in two experiments on rats. The basal control diet, offered *ad lib.* in each case, contained 40 g protein/kg, and other groups received diets containing 62·5, 95 or 220 g protein/kg at 0·67, 1 or 1·5 times the level of the control energy intake.

2. Daily administration of 25 or 30 mg corticosterone/kg body-weight after 18 d pre-feeding caused an increase in plasma protein, glucose and insulin concentrations, but a decrease in the corticosterone:insulin values. Liver size and protein content increased, as did the fractional excretion of dietary nitrogen as urea-N in all treated groups. However, whereas a fall in food intake and body-weight occurred in one experiment the reverse occurred in the other.

3. N^{τ}-Methylhistidine excretion was 12% lower for rats receiving 40 *v.* 220 g protein/kg diet and excretion was increased by only 57 *v.* 90% respectively, when the two groups of rats were given 30 mg corticosterone/kg per d. Rats which received 25 mg corticosterone/kg per d and up to 95 g protein/kg diet increased excretion of N^{τ}-methylhistidine by an average 35%.

4. The fractional degradation rate of myofibrillar protein (k_d) was reduced by about 10% by the low-protein diet from 3·1 to 2·8%/d. During corticosterone treatment the increment in k_d for rats on this diet was only 60% of that for rats receiving the 220 g protein/kg diet, i.e. an increase of 1·8 *v.* 3·0%/d. Energy restriction further reduced k_d during low-protein intake but did not affect the response to the corticosterone. Variations in dietary protein from 40 to 95 g/kg had little effect on the increase in k_d during steroid treatment. The effect of corticosterone on calculated synthesis rates (k_s) differed markedly between experiments. While k_s fell by 50–65% in rats which lost weight on treatment, it rose by up to 60% in rats where carcass non-collagen-protein accretion remained unchanged or increased, despite an increase in k_d.

5. Protein deficiency decreases the catabolic response to glucocorticoid, but the net metabolic response appears crucially dependent on changes in food intake or the stage of growth of the rat or both. A net anabolic response with increased fractional rates of myofibrillar protein breakdown, synthesis and accretion was observed in growing rats fed on relatively-low-protein diets and given 25 mg corticosterone/kg per d. This novel finding indicates that a particular role for cortisol in the adaptation to protein–energy malnutrition by humans should be ascribed only with caution.

The role of glucocorticoids in the aetiology of protein–energy malnutrition remains unclear. There is evidence in humans that the spectrum of sequelae from marasmus to kwashiorkor may be correlated with a range of adrenal corticosteroid secretory activities, and that the development of kwashiorkor is due to failure of the adrenals to increase secretion. Thus the body does not adapt by losing muscle mass in order to maintain plasma protein levels and thereby prevent oedema (Jaya Rao, 1974). While this concept is now regarded as too simplistic (Coward & Lunn, 1981; Golden, 1982) and it is accepted that the pathogenesis of protein–energy malnutrition sequelae is multifactorial, sufficient evidence exists to suggest that maintenance of relatively low corticosteroid levels may play an important role in the development of kwashiorkor. For example, Whitehead & Lunn (1979) and Smith *et al.* (1981) have reported results which show that kwashiorkor is associated with lower levels of plasma cortisol than is marasmus.

Experimentally, administration of cortisone to protein-deficient rats leads to an increase in plasma albumin levels and liver protein content, coincidentally with a loss of muscle

protein (Lunn *et al.* 1976). Goodlad & Munro (1959) have shown earlier that administration of cortisone to rats receiving different levels of protein and energy increases liver protein content but also increases the net loss of nitrogen from the body. The catabolic effect of the hormone appeared independent of the nutritional state of the animal, but the animals were not given the deficient diets before treatment. Also the doses administered were relatively high and the comparative changes in muscle protein synthesis and breakdown rates were not determined. Recently, Santidrian *et al.* (1981), using a similar experimental regimen to that of Goodlad & Munro (1959), found that rats fed on energy-deficient diets are more sensitive than protein-deficient rats to simultaneous treatment with glucocorticoid as assessed by N^{τ}-methylhistidine excretion.

Both malnutrition (protein deficiency) and exogenously administered glucocorticoids are known to cause a reduction in the rate of protein synthesis and the RNA content of muscle (Waterlow *et al.* 1978). However, whether the reduced protein synthesis in malnutrition is partially mediated by a secondary rise in corticosterone levels (or corticosterone:insulin value) is not clear. The muscle is possibly less sensitive to glucocorticoids during malnutrition, and this may be expressed as a relative resistance to increased muscle protein breakdown at a particular level of hormone.

We have tested the effect of protein and energy deficiency states on the catabolic effects of corticosterone, especially on the changes in the rate of protein breakdown as monitored by the excretion of N^{τ}-methylhistidine. The results indicate that the nutritional state can affect the response to exogenous glucocorticoid administration. Malnutrition decreases the efficacy of this hormone in respect of enhancement of myofibrillar protein breakdown, and increases the efficiency of re-utilization of that N which is released from muscle.

METHODS

Expt 1

Thirty male hooded rats of 140–150 g body-weight were used in the experiment. The rats were housed individually in metabolism cages to enable control of the food intake and collection of urine and faeces. Initially twenty rats were placed in metabolism cages and given a diet containing 220 g casein/kg. After 4 d of adaptation to the diet, ten rats were given *ad lib.* a diet containing 40 g casein/kg while the remaining rats received matched intakes of the 220 g protein/kg diet at 10.00 hours daily. Details of the diets are given in Table 1.

After 14 d of giving the low-protein diet, daily urine collection was begun. After a further 3 d, five rats from each diet group were injected subcutaneously with 30 mg corticosterone/kg body-weight each day for 7 d just before feeding. From previous experience this dose was expected to produce growth stasis. This point of balance between anabolic and catabolic influences was felt to be a suitable and reproducible physiological state to investigate nutritional modification of glucocorticoid effects. The remaining rats received injection of vehicle only, as described previously (Tomas, 1982).

Urine collections (24 h) were made for 3 d before injections to the end of the experiment. Thymol was used as a preservative and the collection funnels were carefully rinsed at the end of each day. Trunk blood was collected at the end of the experiment on decapitation of the animals, after which the liver and gastrocnemius muscles were removed, weighed and stored at $-20°$.

At a later date, two groups of five rats of 140–150 g body-weight were placed in metabolic cages and given either one of the experimental diets in quantities exactly matched to the daily intakes of the experimental groups described previously, during the period before corticosterone injection. They were then killed and the carcass prepared as described in Expt 2 for analysis of body composition.

Table 1. *Composition of diets given to rats (g/kg)*

Constituent	Expt 1		Expt 2		
Casein (protein)	40	220	40	62·5	95
DL-Methionine	0·56	3·0	0·56	0·87	1·33
Starch	660	500	660	638	605
Sucrose	197	175	197	197	197
Peanut oil	20	20	50	50	50
Cod-liver oil*	30	30	—	—	—
Halibut oil†	—	—	0·4	0·4	0·4
Mineral mixture‡	50	50	50	50	50
Vitamin mixture§	1	1	1	1	1
Choline chloride	1	1	1	1	1

* Provides not less than 65 μg cholecalciferol and 6 mg retinol/kg diet.
† Provides 30 μg cholecalciferol and 3·6 mg retinol/kg diet.
‡ Rogers & Harper (1965).
§ Provides (mg/kg diet): thiamin hydrochloride 70, riboflavin 30, nicotinamide 50, calcium pantothenate 150, pyridoxal hydrochloride 15, inositol 400, *p*-aminobenzoic acid 50, folic acid 10, biotin 0·4, cyanocobalamin 0·02.

N^{τ}-methylhistidine concentrations in urine were determined on an amino acid analyser (JEOL JA6) after preparation of the samples as described earlier (Tomas *et al.* 1979). Urinary creatinine and urea concentrations were determined on daily samples by using Technicon AutoAnalyzer methods (Technicon Instruments Co. Ltd, 1965, 1967 respectively). Plasma albumin was assayed by a dye-binding method using bromcresol green (Webster, 1977). Protein determinations on plasma and powdered liver samples were made by the method of Lowry *et al.* (1951) adapted for autoanalysis, and on powdered muscle and carcass samples by the heated Biuret method of Dorsey *et al.* (1977) with crystalline bovine albumin as the standard. Glucose concentrations in plasma were assayed with a hexokinase (*EC* 2.7.1.1) method ('Glucoquant'; Boehringer, Mannheim). Plasma insulin was measured with a radioimmunoassay kit (Amersham IM78; Amersham International, Bucks) and plasma corticosterone was measured by radioimmunoassay with an antibody to corticosterone obtained from Endocrine Sciences (Tarzana, California).

Expt 2

Fifty-two male hooded rats of 70–80 g body-weight were used. Twenty rats were placed in metabolism cages and given a diet containing 95 g protein/kg for 3 d, after which groups of four animals were given the following diets for 3 weeks: 40 g protein/kg, *ad lib.* (control group); 62·5 g protein/kg, restricted to maintain isonitrogenous intake; 62·5 g protein/kg, restricted to maintain isoenergetic intake; 95 g protein/kg, restricted to maintain energy and N intake levels of the isonitrogenous and isoenergetic diets respectively and 62·5 g protein/kg, fed to allow 50% greater energy intake. The relative protein:energy values of these dietary regimens were 1:1, 1:0·7, 1·6:1, 1·6:0·7 and 2·3:1·5 respectively. A further sixteen rats were held concurrently in individual cages. Of these animals, eight received the 40 g protein/kg diet *ad lib.* and eight the 62·5 g protein/kg diet at isonitrogenous intake. The remaining sixteen rats were placed in the metabolism cages after completion of observations on the other rats, and groups of four were given the experimental diets (except the 62·5 g protein/kg at isonitrogenous intake) in quantities exactly matched to those of the appropriate experimental group during the previous pre-feeding period.

After the 3 weeks pre-feeding period, these thirty-two control rats were killed to provide baseline values for the five experimental groups which remained in metabolism cages and

received a daily subcutaneous injection of 25 mg corticosterone/kg body-weight for 7 d. As in Expt 1, this dose was expected to produce growth stasis in these particular rats. Urine was collected before and during treatment. At the completion of the experiment the rats were decapitated, trunk blood collected and the liver removed as in Expt 1. The carcass was prepared by removal of the skin, viscera, feet, head and most of the tail. Dissection and analysis of rats in our laboratory showed this carcass preparation to contain 85·0% (SE 0·5, *n* 6) of total skeletal muscle and 88·9% of total non-collagen-protein. The carcass was weighed and stored at −20°. Samples were analysed as in Expt 1, except that urinary N^{τ}-methylhistidine concentrations were determined with an AutoAnalyzer (Murray *et al.* 1982). Carcass protein concentrations were assayed after the whole carcass was powdered in solid carbon dioxide as described by Benville & Tindle (1970). The sarcoplasmic, myofibrillar and collagen fractions were those sequentially extracted by 0·05 M-phosphate buffer (pH 7·4), 0·05 M-sodium hydroxide (Pennington & Robinson, 1968) and 0·5 M-NaOH (80°, 3 h) respectively. A separate analysis of total non-collagen-protein (total soluble in 0·05 M-NaOH) was also done, with all carcasses being analysed in the same manner and at the same time to avoid systematic differences between corticosterone-treated and untreated rats.

Calculations

The calculation of myofibrillar protein breakdown from the rate of excretion of N^{τ}-methylhistidine was done as described earlier (Tomas, 1982). Briefly, it is assumed that 75% of the excreted N^{τ}-methylhistidine arises from the carcass, that this proportion does not decrease on corticosterone treatment and that the N^{τ}-methylhistidine content of the carcass myofibrillar protein remains virtually constant. Thus the average fractional rate of myofibrillar protein breakdown can be calculated as follows:

$$\frac{N^{\tau}\text{-Methis} \times 0{\cdot}75}{\text{NCP} \times 3{\cdot}50}/\text{d},$$

where N^{τ}-Methis is the average excretion rate of N^{τ}-methylhistidine (μmol/d) over the observation period, NCP is the average non-collagen-protein mass (g) and 3·50 is the concentration of N^{τ}-methylhistidine in the carcass non-collagen-protein fraction (μmol/g protein). Although based on the total non-collagen-protein fraction, the calculated turnover rates more accurately reflect those of the N^{τ}-methylhistidine-containing myofibrillar proteins and not the sarcoplasmic fraction. Since we have found the ratio, sarcoplasmic: myofibrillar protein remains remarkably constant under a range of dietary and hormonal treatments (sarcoplasmic protein 35·6 (SE 0·3)% of non-collagen-protein), the myofibrillar protein pool parallels that of the total non-collagen-protein pool and thus the protein turnover values are directly applicable to the myofibrillar protein fraction. For Expt 1, the initial carcass non-collagen-protein content was estimated from the body-weight using values from the extra control groups. Thus, carcass non-collagen-protein was 7·28 and 6·96% of body-weight for high- and low-protein groups respectively (Table 2, see p. 328). To obtain the final carcass non-collagen-protein content, carcass weight was estimated from its relationship to the gastrocnemius weight (carcass weight = (33·97 × gastrocnemius weight) + 0·71; $SE_{\bar{Y}_X}$ 0·81, *r* 0·98, *n* 14) established in rats of 150–220 g body-weight which had been fed on high- and low-protein diets as in the experiment. Since the gastrocnemius protein content was unchanged by corticosterone treatment (Table 2), carcass non-collagen-protein concentration was assumed to be the same as determined in the extra control groups. To compare these methods, estimates can be made of final non-collagen-protein content of the carcasses from the two experimental groups which did not receive corticosterone. These estimates, based either on body-weight (as for initial carcass

non-collagen-protein) or gastrocnemius weight, were 14·55 (SE 0·13) and 14·39 (SE 0·09) g for the high-protein group and 10·60 (SE 0·12) and 10·66 (SE 0·11) g for the low-protein group respectively. For Expt 2, final carcass non-collagen-protein was measured directly and initial carcass protein was extrapolated on a body-weight basis from measurements made on the control animals. In both experiments, carcass protein accretion rates before corticosterone treatment were calculated on the assumption that the non-collagen-protein/kg body-weight was constant during the 3 d before treatment.

Statistical analyses

The results were analysed statistically by standard procedures for deriving Student's t values and by analysis of variance. Least significant differences were calculated for $P = 0{\cdot}05$ from the pooled standard error of difference when analysis of variance showed a significant treatment effect. Due to the number of assumptions and cumulative errors in the calculation of protein synthesis rates, these values were not analysed statistically. Rather, they were compared on the basis of their qualitative changes.

RESULTS

Food intake, body- and organ-weights and composition

Food intakes are shown in Table 2. Match feeding was based on initial body-weight and consequently as group mean weights changed so also did the intake per unit body-weight. All food intakes were within the intended limits except for the high-intake group of Expt 2 which, instead of eating 50% more than controls, ate only 38 and 12% more before and during corticosterone treatment respectively. Thus this group ate the 62·5 g protein/kg diet essentially *ad lib.* and the protein:energy intake values relative to controls were 2·2:1·4 and 1·8:1·1 before and after treatment respectively. The control rats for Expts 1 and 2 receiving the 40 g protein/kg diet *ad lib.* ate 83 and 100 g food/kg body-weight respectively before treatment, and the difference is consistent with the smaller body-weight of the rats used in Expt 2. Diets containing 62·5 g protein/kg promoted a 38% higher intake, indicating a limit to voluntary intake set by the lower protein intake.

Those groups which lost weight on the experimental diets showed the greatest decline in the first 7–10 d. Thereafter the 40 g protein/kg groups either maintained or only slowly lost weight while the energy-restricted groups, which lost more weight initially, slowly gained in body-weight over the final 7–10 d period before corticosterone treatment. This explains the positive accretion rates in these groups at this time (Table 5, see p. 332).

Corticosterone treatment caused a fall in food intake in Expt 1 but an increase in Expt 2, and this may largely account for the differing responses in body-weight changes to the glucocorticoid in the low-protein groups. Rats receiving 220 g protein/kg diet lost weight at four times the rate of those receiving 40 g protein/kg following corticosterone injections of 30 mg/kg body-weight, but the fall in the gastrocnemius muscle mass relative to body-weight was similar. Both the weight and protein content of the liver were increased to a greater extent in the low-protein group by the corticosterone treatment (Table 2). Similarly, in Expt 2, an increase in the protein intake alone significantly diminished the rise in protein content of the liver in response to corticosterone injection, whereas variation in energy intake alone (constant protein) had little effect on this response. Diet appeared to have little influence on the final carcass weight relative to body-weight (Expt 2) except for a 5% reduction for the control group and a similar increase in the 95 g protein/kg group (Table 2). However, the carcass protein concentration was less affected and thus the carcass non-collagen-protein mass reflected the carcass weights. Corticosterone treatment

Table 2. *Details of food intake and body composition of rats fed on diets with differing protein:energy values and treated with corticosterone for 7 d*

(Values are means of either five (Expt 1) or four (Expt 2) rats in each group. Values in parentheses are means from separate control groups matched for weight and food intake and killed at the end of the pre-feeding period before corticosterone treatment)

	Expt 1					Expt 2					
Dietary protein (g/kg)...	220	220	40	40		40	62	62	62	95	
Relative protein:energy...	5·5:1	5·5:1	1:1	1:1		1:1	1:0·7	1·6:1	2·2:1·4*	1·6:0·7	
Corticosterone (mg/kg per d)...	0	30	0	30	LSD ($P = 0·05$)	25	25	25	25	25	LSD ($P = 0·05$)
Body-wt (g):											
Start on diet	151	147	150	146	NS	79·0	79·8	78·0	80·0	81·5	NS
	(148)		(150)			(75·8)	(85·2)	(74·8)	(76·1)	(78·3)	(3·4)
Before treatment	194	190	152	148	7·2	72·0	66·2	82·8	104·0	67·5	7·8
	(195)		(147)			(75·4)	(75·0)	(82·4)	(102·9)	(67·9)	(4·0)
Final	200	162	153	142	6·1	77·5	69·5	90·0	107·5	71·5	7·0
Treatment change	+6	−28	+1	−6	4·0	+5·5†	+3·2†	+7·2†	+3·5	+4·0†	NS
Food intake (g/d):											
Before treatment	12·5	12·5	12·4	12·4		7·2	4·8	7·2	9·9	4·8	
During treatment	10·7	10·7	10·8	10·9		9·0	6·0	9·0	10·1	6·0	
Final tissue wt (g/kg body-wt):											
Liver	37	49	39	59	4	75	67	64	64	57	8
Liver protein	7·8	10·1	6·7	10·8	0·8	9·8	10·4	8·8	9·1	8·4	0·9
Gastrocnemius	13·0	10·9	12·6	10·8	0·5	—	—	—	—	—	—
Carcass	—	—	—	—		376	402	405	392	413	15
	(442)		(435)			(404)‡	(423)‡	(428)‡	(414)‡	(448)‡	(13)
Carcass NCP	—	—	—	—		60·4	65·7	68·4	67·5	68·4	3·9
	(72·8)		(69·6)			(63·9)‡	(68·0)	(67·8)	(64·7)	(73·5)‡	(3·2)
Final tissue NCP (mg/g):											
Carcass	—	—	—	—		161	163	169	172	166	6·3
	(163)		(160)			(158)	(161)	(158)‡	(157)‡	(164)	(4·9)
Gastrocnemius	205	208	198	207	NS	—	—	—	—	—	

NCP, non-collagen-protein; NS, not significant; LSD, least significant difference.

* Ratio fell during corticosterone treatment to 1·8:1·1 (see p. 327).

† Significant change (paired t test): $P < 0·05$.

‡ Values were significantly different from those for corticosterone-treated group (analysis of variance): $P < 0·01$.

Table 3. *Concentrations of hormones and proteins in plasma from rats fed on diets with differing protein:energy values and treated with corticosterone for 7 d*

(Values are means for either five (Expt 1) or four (Expt 2) rats in each group. Values in parentheses are from a preliminary experiment (see p. 300) and are means for four rats/group which received identical diets for 18 d before blood sampling, but did not receive corticosterone treatment)

	Expt 1					Expt 2					
Dietary protein (g/kg)...	220	220	40	40		40	62	62	62	95	
Relative protein:energy...	5·5:1	5·5:1	1:1	1:1		1:1	1:0·7	1·6:1	2·2:1·4†	1·6:0·7	
Corticosterone (mg/kg per d)...	0	30	0	30	LSD ($P = 0·05$)	25	25	25	25	25	LSD ($P = 0·05$)
Corticosterone (ng/ml)	179	331	324	317	70**	417 (377)	332 (254)	331	402	361	52**
Insulin (ng/ml)	0·9	3·0	0·7	6·0	2·8**	1·2 (0·8)	2·8 (0·8)	4·0 (1·0)	6·1 (0·8)	2·7 (0·8)	1·7**
Corticosterone:insulin	243	121	509	77	145**	323	130	98	70	161	81**
Glucose (mM)	7·0	7·5	7·2	9·2	0·6***	6·9	7·6	7·9	7·9	6·9	0·7*
Protein (g/l)	56·6	67·0	50·0	70·6	3·9***	65·8 (49·8)	75·0 (53·3)	74·5 (54·3)	78·3 (51·3)	76·0 (57·0)	7·3*
Albumin (g/l)	33·1	38·7	26·8	41·3	1·7***	32·0 (25·8)	37·0 (30·9)	39·0 (29·8)	40·4 (31·2)	37·0 (34·0)	2·9***
Albumin:protein	0·59	0·58	0·54	0·59	0·04*	0·48 (0·52)	0·50 (0·58)	0·52 (0·55)	0·52 (0·55)	0·49 (0·56)	NS

NS, not significant; LSD, least significant difference.
Treatment effects were statistically significant (analysis of variance): * $P < 0·05$, ** $P < 0·01$, *** $P < 0·001$.
† Ratio fell during corticosterone treatment to 1·8:1·1 (see p. 327).

significantly decreased relative carcass weights but, as carcass protein concentrations were generally increased, there was little over-all change in carcass protein/kg body-weight.

Hormone and protein concentrations in plasma

Values for plasma measurements are shown in Table 3. For purposes of comparison, values from a preliminary experiment virtually identical to Expt 2 are provided to indicate baseline values just before corticosterone treatment. Corticosterone concentrations in plasma were significantly increased by protein deficiency in untreated rats (Expt 1). Following corticosterone treatment there was no consistent effect of protein or energy intake on the corticosterone levels. Plasma insulin levels before treatment were similar in animals receiving either low- or high-protein diets, and corticosterone treatment caused a substantial rise of 2–8-fold in all diet groups. Although there were significant effects of diet within experiments, there was no consistent pattern of response between the experiments. The corticosterone:insulin values varied between 70 and 509 and inversely reflected the relative changes in plasma insulin concentrations.

Plasma albumin concentrations, and its proportion in total plasma protein, were significantly reduced in protein deficiency alone (Expt 1), but treatment with corticosterone markedly increased albumin concentrations in both deficient- and adequate-protein groups with little influence of the protein–energy content on the extent of the response.

Urinary excretion of metabolites

Daily urinary excretion of urea-N, creatinine and N^{τ}-methylhistidine before and after treatment of the rats with corticosterone are shown in Table 4. Corticosterone treatment significantly increased the loss of N as urea-N and the response was little modified by diet. For example, in Expt 1 the increase in urea-N was about 50% of ingested N whether rats received the 220 or 40 g protein/kg diets, although the percentage change indicated a differential response. For Expt 2, where a lower dose of corticosterone was administered, the average increase in urea-N excretion was much less, generally less than 10% of ingested N. However, the group which ate *ad lib.* the 62·5 g protein/kg diet and which reduced its food intake during corticosterone treatment showed a marked increase in the excretion of urea-N. This increase was 2·5–30-fold greater than that for the other groups relative to N intake. Creatinine excretion was largely unaffected by corticosterone treatment in either experiment but was significantly increased by 11·6% in rats receiving a high-protein diet (Expt 1) and by 5·8 and 9·0% in rats receiving a restricted energy intake (Expt 2).

N^{τ}-Methylhistidine excretion relative to body-weight was decreased by 12% in rats receiving a low-protein diet and showing growth retardation (Expt 1). However, N^{τ}-methylhistidine excretion was substantially increased by corticosterone treatment in all groups of rats and was directly related to the dose. Protein restriction reduced the increase from 90·5 to 56·8% in Expt 1, but the dietary perturbations in Expt 2 caused no significant difference in the increment in N^{τ}-methylhistidine excretion during corticosterone treatment, albeit the energy-restricted rats showed a greater average increment.

Myofibrillar protein turnover

The average turnover rates of myofibrillar protein calculated from the average daily N^{τ}-methylhistidine excretion rates and the changes in muscle non-collagen-protein mass are shown in Table 5. Due to some rates changing from positive to negative values following treatment, absolute rather than percentage changes are shown. As expected, the growth rate before glucocorticoid treatment was directly related to the dietary protein and energy intakes and ranged from near zero to 1·5%/d. The response to glucocorticoid treatment differed markedly between experiments. Rats receiving 220 and 40 g protein/kg diet and

Table 4. *Urinary excretion of urea, creatinine and* N^{τ}*-methylhistidine by rats fed on diets with differing protein:energy values before and during treatment with corticosterone for 7 d*

(Values are means for either five (Expt 1) or four (Expt 2) rats in each group over 3 d before and 7 d after commencement of corticosterone treatment)

	Expt 1					Expt 2					
Dietary protein (g/kg)...	220	220	40	40		40	62	62	62	95	
Relative protein:energy...	5·5:1	5·5:1	1:1	1:1		1:1	1:0·7	1·6:1	2·2:1·4†	1·6:0·7	
Corticosterone (mg/kg per d)...	0	30	0	30	LSD ($P = 0·05$)	25	25	25	25	25	LSD ($P = 0·05$)
Urea-nitrogen (mol/mol intake):											
Before	0·51	0·48	0·30	0·28	0·05***	0·33	0·50	0·30	0·18	0·56	0·05***
During	0·57	0·97	0·33	0·79	0·09***	0·42	0·51	0·36	0·45	0·60	0·09**
Δ%	12·9	101	12·1	193	51***	28·2	3·0	20·9	152	8·4	51*
Creatinine (μmol/kg per d):											
Before	315	311	321	320	NS	268	255	276	289	258	19·5*
During	326	347	320	324	15·4**	256	270	281	282	280	16·0*
Δ%	3·5	11·6	−0·4	1·9	4·4***	−4·2	5·8	−0·4	−2·3	9·0	5·9**
N^{τ}-Methylhistidine (μmol/kg per d):											
Before	10·46	10·02	8·86	9·14	0·60**	7·12	6·49	7·04	7·42	6·68	NS
During	10·24	19·06	8·62	14·34	1·36***	8·91	9·35	8·68	10·40	8·99	1·03*
Δ%	−1·8	90·5	−2·7	56·8	13·3***	25·5	55·0	15·2	40·0	35·0	NS

Δ%, Percentage change due to treatment with corticosterone; NS, not significant; LSD, least significant difference. Treatment effects were statistically significant (analysis of variance): * $P<0·05$, ** $P<0·01$, *** $P<0·001$.

† Ratio fell during corticosterone treatment to 1·8:1·1 (see p. 327).

Table 5. *Rates of myofibrillar protein accretion, degradation and synthesis (% /d) in rats fed on diets with differing protein:energy values before and during treatment with corticosterone for 7 d.*

(Values are means for either five (Expt 1) or four (Expt 2) rats in each group over 3 d before and 7 d after commencement of corticosterone treatment)

		Expt 1					Expt 2					
Dietary protein (g/kg)...		220	220	40	40		40	62	62	62	95	
Relative protein:energy...		5·5:1	5·5:1	1:1	1:1		1:1	1:0·7	1·6:1	2·2:1·4†	1·6:0·7	
Corticosterone (mg/kg per d)...		0	30	0	30	LSD ($P = 0·05$)	25	25	25	25	25	LSD ($P = 0·05$)
k_a	Before	1·48	1·63	0·53	0·33	0·50***	−0·26	0·15	0·94	1·24	0·53	0·39***
	During	0·48	−4·35	0	−2·96	0·43***	0·26	0·38	1·58	1·03	−0·01	0·83**
	Δ	−1·00‡	−5·98‡	−0·53	−3·30‡	0·75***	0·52‡	0·23	0·63‡	−0·21	−0·54	0·86*
k_d	Before	3·09	2·96	2·75	2·84	0·17**	2·40	2·20	2·28	2·59	2·07	0·26**
	During	3·09	5·91	2·67	4·67	0·45***	3·17	3·00	2·72	3·37	2·64	0·36**
	Δ	0·00	2·95‡	−0·09	1·83‡	0·38***	0·77‡	0·80‡	0·45	0·78‡	0·58‡	NS
k_s§	Before	4·6	4·6	3·3	3·2		2·1	2·4	3·2	3·8	2·6	
	During	3·6	1·6	2·7	1·7		3·4	3·4	4·3	4·4	2·6	
	Δ	−1·0	−3·0	−0·6	−1·5		1·3	1·0	1·1	0·6	0·0	

k_a, k_d, k_s, fractional rates of myofibrillar protein accretion, degradation and synthesis respectively; Δ, change in k_a, k_d or k_s due to treatment with corticosterone; NS, not significant; LSD, least significant difference.

Treatment effects were statistically significant (analysis of variance): * $P < 0·05$, ** $P < 0·01$, *** $P < 0·001$.

† Ratio fell during corticosterone treatment to 1·8:1·1 (see p. 327).

‡ The change for each group was statistically significant (paired t test): $P < 0·05$.

§ Values were not analysed statistically due to cumulative errors in their derivation. Values were included to allow qualitative comparisons to be made.

injected with 30 mg corticosterone/kg body-weight lost 4·4 and 3·0% non-collagen-protein/d respectively, a highly significant change from the positive rates before treatment. Also these two rates of protein loss between dietary groups were significantly different. In contrast, administration of 25 mg corticosterone/kg led to either no significant change or a significantly increased protein accretion rate for different dietary groups in Expt 2. Accretion rates did not change in those groups restricted in intake nor where intake fell during corticosterone treatment.

The fractional rates of myofibrillar protein breakdown (k_d) were reduced by 10% with restriction of protein intake (Expt 1). Even lower rates were observed in Expt 2, where all protein intakes were relatively low, but the significant differences between dietary groups were due rather to the dietary energy intake than to protein intake. Protein breakdown rates were depressed by a decrease in energy intake. Treatment with corticosterone increased k_d in all groups, the increase being three to four times greater for rats receiving 30 mg steroid/kg body-weight rather than 25 mg steroid/kg body-weight. However, although the low-protein diet reduced the effect of the glucocorticoid on k_d in Expt 1 (+1·83 *v.* +2·95%/d for low-and high-protein diets respectively), there was no significant effect of the diet on the change in k_d in Expt 2, where the average increment following treatment was 0·7%/d. Fractional synthesis rates (k_s), calculated as the difference between accretion and breakdown, appeared to be influenced by both diet and glucocorticoid treatment. Synthesis rates generally were reduced by 30% or more by diets deficient in protein or energy, or both, compared with adequate diets. In Expt 2, synthesis rates were about 40–50% higher in the two groups receiving the highest levels of protein and energy (62·5 g protein/kg at 1 and 1·5 times isoenergetic levels) compared with other groups both before and after corticosterone treatment. Corticosterone substantially reduced calculated synthesis rates in Expt 1, regardless of protein intake, but the average k_s values were increased in all groups in Expt 2. This apparent increase in k_s was due to protein accretion rates being maintained or increased despite the increase in k_d following hormone treatment.

DISCUSSION

These results show that rats accustomed to a protein-deficient diet are less affected in respect of carcass protein catabolism than are adequately fed animals by the administration of exogenous corticosterone. This is at variance with the observations of Goodlad & Munro (1959) and Santidrian *et al.* (1981). Although the weight losss and increase in N^{τ}-methylhistidine excretion caused by the treatment were significantly less for protein-deficient animals, the changes in organ weights and plasma proteins were similar for each dietary group. In addition, the proportion of N intake excreted as urea was lower in rats receiving the low-protein diet than in rats receiving the high-protein diet, both before and during corticosterone treatment (Table 4). Thus, although the protein-deficient animals mobilized less muscle protein in response to the glucocorticoid treatment, they appeared to utilize more efficiently those extra amino acids made available from muscle for the synthesis of other proteins. This may in part be due to the relatively higher plasma insulin concentrations which were induced by the corticosterone treatment in this group (Table 3), since it has been shown that this secondary rise in insulin counteracts the catabolic effect of the steroid on muscle (Tomas, 1982). Replenishment of liver and plasma protein in protein-deficient rats could also play a role. While total liver protein remained virtually unchanged in the high-protein group (+75 mg, Expt 1), 500 mg protein was accumulated in the livers of the low-protein group during corticosterone treatment compared with untreated controls (Table 2) and represented about 20% of the net loss of protein from muscle.

Despite the benefits of corticosterone treatment such as the improvement in plasma

protein levels, the mobilization of muscle protein in Expt 1 caused a net loss of N and hence, presumably, decreased the animals' viability. Similar results were reported by Lunn *et al.* (1976), who used smaller rats and a lower dose of glucocorticoid. Thus, in the longer term, survival of the animals probably would not be enhanced by this level of corticosteroid treatment.

In the second experiment, the control rats receiving 40 g protein/kg diet responded to glucocorticoid treatment with an increase in *ad lib.* intake of food, not a decrease as in Expt 1. There is no obvious reason for this difference, particularly as the animals already had a higher intake relative to body-weight. The differing age, stage of growth of the rats or dose rate of corticosterone may be the basis for the difference. However, the liver and plasma proteins showed the usual response to corticosterone and were elevated above normal. The unusual and unexpected increase in growth rate (protein accretion) and the calculated synthesis rates which were observed, despite an increase in the myofibrillar protein degradation rate, may have stemmed from the increase in food intake. Such a proposal was also made by Odedra & Millward (1982) to explain differences between their results using *ad lib.*-fed rats and those of Tomas *et al.* (1979). Interestingly, the groups which did not show a significant increase in protein accretion rates were those receiving restricted energy intake and the group which did not show a sustained increase in intake (62·5 g protein/kg, *ad lib.*) following treatment. Thus, although analysis of variance shows only one of the changes in accretion rate to be different from the others (95 g protein/kg group) these results are at variance with the conclusions of Goodlad & Munro (1959) that dietary energy and protein levels do not influence the catabolic effects of glucocorticoids given at moderate dose levels.

The apparent increase in synthesis rates during corticosterone treatment in Expt 2 is difficult to explain. Almost all reported studies on the effect of glucocorticoids on muscle protein turnover have reported a decrease in muscle protein synthesis rates, and that the synthetic processes are more sensitive to glucocorticoids than are those for the breakdown of muscle proteins (Millward *et al.* 1976*a*, *b*; Shoji & Pennington, 1977; Rannels & Jefferson, 1980; Odedra & Millward, 1982). There is little basis to doubt the validity of our carcass non-collagen-protein accretion measurements, albeit initial values are based (unavoidably) on an extrapolation from identically fed untreated controls. Certainly all the animals gained body-weight during treatment. A similar increase in apparent synthesis rates was also observed in a preliminary experiment with virtually identical diets and treatments. One potential source of error may, however, arise from alteration in the proportions of N^{τ}-methylhistidine arising from skeletal muscle or from other sources (Bates & Millward, 1981), but this seems unlikely. If there were no change in myofibrillar protein degradation rate with glucocorticoid treatment (i.e. in line with the results of Rannels & Jefferson (1980), Kelly & Goldspink (1982), McGrath & Goldspink (1982)) in Expt 2, then for the low-protein control group the extra 1·8 μmol N^{τ}-methylhistidine/kg body-weight per d would need to arise from non-skeletal muscle sources. The gut serosa would be a likely source (Wassner & Li, 1982). Kelly & Goldspink (1982) found no change in the fractional synthesis rate of proteins in the intestinal serosa following treatment of rats with dexamethasone at a dose equivalent to 100 mg corticosterone/kg (four times the dose rate used in the experiment reported here), but the protein mass declined by 9·5%/d. Thus, the extra N^{τ}-methylhistidine released could be as much as 10% of the gut pool/d, or about 0·1 μmol/d for a 100 g rat (Millward *et al.* 1980), about half the observed increase. Furthermore, in a recent experiment using a similar regimen to that reported here, we did not find any change in gut serosal protein content following corticosterone treatment (values not shown). As the skin appears to contribute little to N^{τ}-methylhistidine excretion (Wassner & Li, 1982), myofibrillar protein breakdown in skeletal muscles of our rats seems more likely to have increased

than to have declined or remained constant. Thus, corticosterone treatment caused an apparent anabolic response by muscle in this experiment with over-all average increases in accretion, synthesis and breakdown. The reasons for this are obscure, but may be related to the change in either intake or corticosterone:insulin values (Odedra & Millward, 1982; Tomas, 1982). The differential effects of the energy-restricted groups on protein accretion rates argue that the change in food intake was an important factor.

The metabolism of nutrients by a tissue is determined by a number of mechanisms, such as nutrient supply, organ function and the balance of hormonal influences. Of the latter, the balance of glucocorticoids and insulin, the principal catabolic and anabolic hormones, is of major importance (e.g. Tischler, 1981). One weakness in the design of experiments using exogenous glucocorticoid administration is the accompanying increase in circulating insulin concentrations in plasma (Lunn *et al.* 1976; Millward *et al.* 1976*a*, *b*; Tomas *et al.* 1979; Tomas, 1982) which is evident in the experiments reported here (Table 3). While it has been postulated that corticosterone:insulin values, rather than absolute concentrations, may be important in determining the direction of metabolism (e.g. Tischler, 1981), others have found that insulin is unable to reverse the glucocorticoid inhibition of muscle protein synthesis (Odedra & Millward, 1982) and can only partially buffer the increase in myofibrillar protein breakdown (Tomas, 1982). Analysis of the results presented by Lunn *et al.* (1976) shows that from day 4 to day 15 of either glucocorticoid or vehicle injection to protein-deficient rats, the corticosterone:insulin values for each group were essentially the same (mass ratio approximately 470). In contrast, the ratios observed in this experiment varied widely between treatment groups and, furthermore, appear to be related to the changes in myofibrillar protein turnover. The two groups showing the highest accretion and synthesis rates during corticosterone treatment had significantly higher insulin and lower corticosterone:insulin values in the plasma. Such a relationship was also seen in Expt 1, but with insulin in an anti-catabolic role. However, a single daily plasma sample may be inadequate to determine with confidence whether such a relationship exists since the 24 h pattern may differ between treatment groups. This could arise from the differing extents of food restriction which are superimposed on the nutritional status (Gallo & Weinberg, 1981). Also, an apparent relationship may not indicate metabolic modulation, since the energy status of the rat at a particular time may determine whether the prevailing corticosterone and insulin levels are anabolic or catabolic in their effects (Griffin & Wildenthal, 1978; Goldberg *et al.* 1980). None the less, the results do indicate that the circulating insulin levels may be determining the response to some extent.

In our calculations we have assumed that 75% of the excreted N^{τ}-methylhistidine originates from skeletal muscle proteins and that this percentage does not change with glucocorticoid treatment. The reasons for these assumptions have been discussed previously (Tomas, 1982) and, in view of the apparent sensitivity of the skeletal muscle actin breakdown rate to glucocorticoids (Odedra *et al.* 1980), the assumption that the percentage arising from muscle is at least not decreased by treatment seems reasonable. As discussed previously, the possibility that gut serosal protein turnover may be increased by the treatments used here cannot be ruled out, particularly in view of the apparent dependence of the protein synthesis rates on the dietary protein supplies (McNurlan & Garlick, 1981). Because of these, and the several other assumptions involved in our calculations such as estimation of body composition, more emphasis should be placed on the comparative rather than the quantitative aspects of the calculated protein turnover values, especially with regard to the fractional synthesis rates of myofibrillar protein. This is consistent with the approach used by others to similar findings (Burini *et al.* 1981; Santidrian *et al.* 1981; Dunn *et al.* 1982).

The rat is regarded as a poor model for protein-deficiency studies in humans (Whitehead, 1980), due in part to its restriction of food intake and raised plasma 3,5,3′-triiodothyronine

levels when fed on low-protein diets (e.g. Edozien *et al.* 1978). In our studies protein deficiency did, however, lead to lowered plasma albumin and insulin concentrations, decreased liver protein and elevated plasma corticosterone levels, features common to the marasmic sequelae of malnutrition (Coward & Lunn, 1981). Muscle protein turnover was also depressed, as found by others in both humans and rats (Nagabhushan & Narasinga Rao, 1978; Millward, 1979). Administration of corticosterone reversed these features, but the cost was a reduction in the utilization of the dietary N, most marked in Expt 1, where weight loss and relative muscle wasting occurred. The findings from the two experiments indicate, first, that protein deficiency decreases the catabolic effects of glucocorticoids and second, depending on nutritional state and hormone dosage level, that glucocorticoids may also be 'anabolic' in muscle of protein-deficient animals. Thus, caution should be used when particular metabolic consequences are ascribed on the basis of circulating hormone levels, particularly in malnutrition states.

In contrast to our results, Santidrian *et al.* (1981) found energy and protein–energy-deficient rats to be more sensitive to glucocorticoids than were protein-deficient or control rats. However, their experiment was similar to that reported by Goodlad & Munro (1959), and their rats were not pre-fed on the experimental diets so that the glucocorticoid treatment coincided with a sudden severe reduction in energy intake. Thus their experiments were not comparable with ours except that they emphasize the apparent importance of a fall in energy intake, such as occurred in Expt 1, on the response to glucocorticoid treatment. In addition, well-fed growing rats may have a different proportion of 'red' and 'white' muscle fibres in the carcass, compared with rats adapted to deficient diets and in growth stasis. Since different muscle types respond differently to glucocorticoids (Kelly & Goldspink, 1982), this may also account for some of the apparent discrepancies between experiments. In fact, a possible explanation for the decreased sensitivity of muscle to glucocorticoids in protein–energy-deficient states is a preferential wasting of the more sensitive white muscle fibres before treatment.

Although glucocorticoids can be shown to cause protein loss from muscle and protein gain by the liver (Expt 1; Lunn *et al.* 1976) in protein-deficient animals, thus enhancing the typical marasmic pattern of metabolism, the net result is an accelerated weight loss, at least where food intake falls or remains restricted. The decreased effectiveness of glucocorticoids to increase muscle protein breakdown and net muscle loss in protein-deficient animals, where muscle protein turnover is already depressed (e.g. Millward, 1979; Rikimaru *et al.* 1980; present results, Table 5), indicates that care should be taken in interpreting the physiological significance of the elevated cortisol levels found in human infants suffering from protein–energy malnutrition (Lunn *et al.* 1973; Olusi *et al.* 1977; Whitehead & Lunn, 1979). Our finding, that administered corticosterone can lead to an anabolic response by rats restricted in protein and energy intake, in spite of an apparent increase in myofibrillar protein degradation rate, is a novel one and raises further questions concerning the interactions between nutrient supply and hormonal effects on metabolic processes. The mechanisms for this apparent anomalous response deserve further study.

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