Effect of corticosterone on myofibrillar protein turnover in diabetic rats as assessed by N^{τ} -methylhistidine excretion

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(Received 10 May 1982/Accepted 9 September 1982)

The effect of corticosterone on myofibrillar protein breakdown in diabetic rats was investigated in order to assess the possible counteracting effects of the secondary rise in plasma insulin concentrations which normally accompanies such treatment. N^{τ} -Methylhistidine excretion, an index of myofibrillar protein breakdown, was compared before and after corticosterone treatment (4.0 mg/100 g body wt. per day) of normal control, adrenalectomized, 10-day-streptozotocin-diabetic and adrenalectomized diabetic rats. Diabetic rats received 1.5 units of insulin/100 g body wt. per day throughout the experiment and showed marked hyperglycaemia and glucosuria during corticosterone treatment, whereas non-diabetic rats had only mild hyperglycaemia but elevated insulin concentrations. Corticosterone treatment increased the average rate of myofibrillar protein breakdown by 68% and 95% respectively in non-diabetic and diabetic rats. Net loss of muscle non-collagen protein for the same 7-day period was greater in diabetic than in non-diabetic animals (4.15 versus 2.84% per day), and the calculated average synthesis rates were lowest in diabetic rats. Adrenalectomy had little effect except to decrease slightly the rate of muscle protein breakdown. These results show that the rise in plasma insulin concentrations that accompanies exogenous corticosterone administration to non-diabetic rats diminishes the catabolic effect of this glucocorticoid on muscle. Insulin appears to antagonize the effects of the glucocorticoid by attenuating the increased rates of myofibrillar protein breakdown and, to a lesser extent, by limiting the decrease in synthesis rates.

High circulating concentrations of glucocorticoid hormones from either endogenous or exogenous sources lead to loss of muscle protein in man and other animals (Goldberg & Goodman, 1969; Steele, 1975). An inhibition of muscle protein synthesis under these conditions has been established (Goldberg, 1969; Millward *et al.*, 1975; Shoji & Pennington, 1977), but effects on protein breakdown remain equivocal (Waterlow *et al.*, 1978; Rannels & Jefferson, 1980). An earlier study on adrenalectomized rats has shown that, when sufficient corticosterone is administered to raise plasma concentrations of the steroid to those induced by stress, there is a dose-related increase in myofibrillar protein breakdown as assessed by N^{τ} -methylhistidine excretion (Tomas *et al.*, 1979). These findings have been confirmed (Odedra *et al.*, 1980). However, the glucocorticoid administration led to a concomitant increase in plasma insulin, a response also observed by others (Steele, 1975; Millward *et al.*, 1975). Such an increase does not

usually occur when plasma glucocorticoid concentrations are raised endogenously after stress (Batstone *et al.*, 1975).

Insulin may be considered an antagonist of glucocorticoids in its metabolic effects, and it seems reasonable to expect that the insulin increase would diminish the magnitude and/or duration of the effects of the glucocorticoid (Goldberg *et al.*, 1980; Tischler, 1981). Preliminary evidence suggests that this does occur (Tomas & Ballard, 1979), but it has not been fully established that the increased insulin concentrations do in fact modify the glucocorticoid response. Apart from increasing glucose production by the liver, glucocorticoids also induce a resistance to the actions of insulin in peripheral tissues and decrease glucose utilization (Reaven & Olefsky, 1978). Whether the inhibition of muscle-protein breakdown rates by insulin is similarly prevented by glucocorticoids is not clear (Tischler, 1981).

The present investigation was undertaken to determine whether diabetic rats which are unable to

increase plasma insulin when given corticosterone show rates of *N*^ε-methylhistidine excretion different from those of non-diabetic rats given the same glucocorticoid treatment. The data show that the insulin status does influence the myofibrillar-protein breakdown response to glucocorticoid treatment.

Materials and methods

Experimental procedures

Corticosterone and streptozotocin were purchased from Sigma Chemical Co., St. Louis, MO, U.S.A. The corticosterone was finely ground and suspended in a vehicle which contained 0.5% soluble CM-cellulose of medium viscosity (Tomas *et al.*, 1979). The streptozotocin was dissolved in 20 mM-citrate buffer, pH 4.35, immediately before administration to the animals. Protamine zinc insulin [Isophane insulin (N.P.H.), 80 units/ml; Commonwealth Serum Laboratories, Melbourne, Australia] was obtained from a pharmacy. Where necessary, the insulin was diluted before injection in a vehicle consisting of 0.2% Na₂HPO₄ and 1.6% glycerol (pH 7.4).

Male hooded rats (28; body wt. 130 g) were obtained from the laboratory's own colony; 14 rats were bilaterally adrenalectomized and subsequently maintained with daily subcutaneous injections of 0.4 mg of corticosterone/100 g body wt. and given 0.9% NaCl to drink. After 7 days, eight adrenalectomized and eight intact rats were injected intraperitoneally with 95 mg of streptozotocin/kg body wt. All streptozotocin-treated rats showed glucosuria, polyuria and polydipsia and subsequently received daily subcutaneous injections of 1.5 units of insulin/100 g body wt. This dose decreased glucosuria to less than 2 mmol of glucose/100 g body wt. per day and maintained normal growth rate. After a further 4 days, five rats from each of the four groups were placed in individual metabolism cages. The four groups were (i) intact control, (ii) adrenalectomized, (iii) intact diabetic and (iv) adrenalectomized diabetic rats. All rats were given the same diet, which contained 21% (w/w) protein and was free of *N*^ε-methylhistidine and creatine. The food offered to adrenalectomized and diabetic groups was matched to the intake of the intact control animals for the previous day.

After 3 days of urine collection, during which the intact control rats received injections of corticosterone vehicle, all rats were injected subcutaneously each day with 4 mg of corticosterone/100 g body wt. for 7 days. Insulin injections to diabetic rats were continued without change.

Food intake was recorded and rats were weighed each day. Daily insulin and corticosterone injections were given just before feeding, at about 10:00 h. Urine was collected into containers with thymol as a

preservative and stored at -20°C. Blood samples were taken on the last day of corticosterone treatment at 5 and 24 h after feeding (T₅ and T₂₄). At the end of the experiment the rats were killed by decapitation, trunk blood was collected and the liver was removed and weighed. The skin, viscera, visible fat, feet and tail were then removed, and the resulting carcass (trunk muscles and skeleton) was weighed. Gastrocnemius muscles were then removed and weighed separately.

Analytical procedures

Urine *N*^ε-methylhistidine concentrations were determined by an Auto-Analyser method (Murray *et al.*, 1982). Daily samples were prepared as described previously (Tomas *et al.*, 1979), except that 1.0 M-NH₃ was used to elute *N*^ε-methylhistidine from the columns of ion-exchange resin. This prevents co-elution of *N*^ε-methylhistamine, which contributes to net fluorescence in the analytical system. In addition, samples of daily urine collections were pooled for the control period and for days 1-3 and 4-7 of corticosterone treatment for each rat, and the *N*^ε-methylhistidine concentrations determined as described previously (Tomas *et al.*, 1979) with an LKB 4103 amino acid analyser. Urinary creatinine and urea were determined on daily samples by using Technicon Auto-Analyser methods N11b and N1c respectively. Glucose concentrations in plasma and urine were assayed with a hexokinase method (Boehringer Mannheim, 'Glucoquant'). Plasma insulin was measured with a radio-immunoassay kit (Amersham, IM78) and plasma corticosterone was measured by a competitive protein-binding assay based on the procedures of Murphy (1967), with plasma from adrenalectomized rats as the source of binding protein. Carcass non-collagen protein content was assayed as described by Pennington & Robinson (1968) after the whole carcass was powdered in solid CO₂ as described by Benville & Tindle (1970).

Calculation of myofibrillar protein breakdown and synthesis rates

Dissections of rats in our laboratory have shown that the carcass contains 86% of total skeletal muscle (including tongue), and the protein turnover calculations are based on the muscle non-collagen protein contained in the carcass. The carcass non-collagen protein content before corticosterone treatment is obtained from an estimate of carcass weight based on body weight [$38.5 \pm 0.33\%$ (S.E.M.) for 12 animals of similar weight] multiplied by the non-collagen protein content of carcass from untreated rats ($15.8 \pm 0.27\%$). For the calculation of myofibrillar protein breakdown rates, 75% of the excreted *N*^ε-methylhistidine is assumed to arise from

carcass myofibrillar protein. The N^{α} -methylhistidine content of rat muscle was taken as $3.92 \mu\text{mol/g}$ of muscle protein (F. M. Tomas & L. M. Pope, unpublished work). Protein synthesis rates are the sum of protein accretion and degradation rates.

Essentially, the fractional rate of release of N^{α} -methylhistidine from the muscle pool was determined and the fractional rates of myofibrillar protein turnover were then calculated on the assumptions that the N^{α} -methylhistidine concentration in non-collagen protein and the ratios of myofibrillar to sarcoplasmic protein mass and turnover remains constant throughout the experiment.

Statistical analysis

The results were analysed statistically by using standard methods for determination of Student's t values, factorial analysis and analysis of variance (Snedecor & Cochran, 1967).

Results

Food intake, body and organ weights

The *ad libitum* food intake of non-diabetic rats fell abruptly during corticosterone treatment (Table 1). The 24 h delay in the matching of food intakes resulted in the diabetic rats receiving a slightly higher average food intake during the first few days of corticosterone treatment. All groups lost weight in the treatment period, but the diabetic rats lost significantly more, particularly those not adrenalectomized. This body-weight loss is reflected in the final gastrocnemius muscle weights (Table 1), which shows that the intact diabetic group lost relatively more muscle per unit body weight than did the other rats. In addition, the liver weights for this group were significantly higher than for the others.

Plasma glucose, insulin and corticosterone

The trauma associated with blood sampling can be poorly tolerated by adrenalectomized and/or diabetic rats. Since this may have influenced the outcome of the experiment, blood was not sampled before corticosterone treatment. Samples obtained from other groups of similar untreated normal rats just before feeding contained 0.95 ± 0.38 (s.d.) ng of insulin/ml, 6.3 ± 1.1 (s.d.) mM-glucose and 238 ± 102 (s.d.) ng of corticosterone/ml, which represent the peak and nadir concentrations of corticosterone and insulin respectively (F. M. Tomas & L. M. Pope, unpublished work). Corresponding values for diabetic rats receiving the same maintenance dose of insulin (1.5 units/100 g body wt. per day) were 0.67 ± 0.30 (s.d.) ng/ml, 16.0 ± 6.7 (s.d.) mM and 312 ± 105 ng/ml. Similarly plasma from adrenalectomized rats maintained on 0.5 mg of corticosterone/day contained 16 ± 15 (s.d.) ng of corticosterone/ml (178 ± 44 ng/ml 5 h after injection).

Data obtained from this experiment from samples taken on the last day of corticosterone treatment are shown in Table 2. The corticosterone treatment increased and maintained plasma corticosterone concentrations to the same extent in each group. The concentrations ranged from an average of 485 to 435 ng of corticosterone/ml at 5 and 24 h after injection respectively. Plasma glucose concentrations, however, were 2–3 times higher in diabetic rats than in non-diabetic rats at both 5 and 24 h after hormone injection and feeding. In addition, corticosterone treatment increased the plasma glucose concentrations of all groups relative to their respective untreated controls referred to above.

Despite the high plasma glucose concentrations in diabetic rats, plasma insulin concentrations in these

Table 1. Effect of diabetes and adrenalectomy (ADX) on feed intake, live body weight, liver, carcass and muscle weights and carcass non-collagen protein content in rats receiving corticosterone treatment (4 mg/100 g body wt. per day) for 7 days

Values are means $\pm$ S.E.M. for five rats in each group. Diabetic rats received 1.5 units of NPH insulin/100 g body wt. per day. Abbreviations: LSD, least significant difference at $P = 0.05$; NS, not significant on analysis of variance.

	Non-diabetic		Diabetic		LSD
	Intact	ADX	Intact	ADX	
Daily food intake (g/100 g body wt.)					
Before treatment	7.18 ± 0.17	7.67 ± 0.20	7.93 ± 0.15	7.78 ± 0.16	0.55
During treatment	5.27 ± 0.32	5.56 ± 0.40	6.32 ± 0.46	5.94 ± 0.49	NS
Body weight (g)					
Before treatment	231 ± 2.8	225 ± 5.9	224 ± 2.0	228 ± 3.9	NS
After treatment	197 ± 4.2	196 ± 3.8	173 ± 3.4	192 ± 2.9	10.8
Final liver weight (g/100 g body wt.)	5.60 ± 0.30	5.58 ± 0.22	6.66 ± 0.22	5.70 ± 0.31	0.79
Final gastrocnemius weight (mg/100 g body wt.)	1094 ± 16	1058 ± 19	1011 ± 24	1042 ± 16	57
Final carcass weight (g/100 g body wt.)	38.8 ± 0.55	37.1 ± 0.28	37.3 ± 0.52	36.5 ± 0.34	1.3
Carcass non-collagen protein (mg/g)	150 ± 1.3	155 ± 1.5	151 ± 1.9	147 ± 1.7	4.9

Table 2. *Effect of diabetes and adrenalectomy (ADX) on plasma glucose, insulin and corticosterone in response to daily injection of corticosterone (4 mg/100 g body wt.) for 7 days*

Values are means $\pm$ S.E.M. for five rats in each group. Values in parentheses are means $\pm$ S.D. for normal control animals at T₂₄, where T₅ and T₂₄ are 5 and 24 h after hormone injections and feeding. Diabetic rats received 1.5 units of NPH insulin/100 g body wt. per day. Abbreviations: LSD, least significant difference for $P = 0.05$; NS, not significant by analysis of variance.

		Non-diabetic		Diabetic		LSD
		Intact	ADX	Intact	ADX	
Glucose (mm)	T ₅	10.0 $\pm$ 1.5	8.9 $\pm$ 0.9	22.0 $\pm$ 0.8	16.9 $\pm$ 1.2	3.5
	T ₂₄	8.2 $\pm$ 0.5 (6.3 $\pm$ 1.1)	8.3 $\pm$ 0.6	28.5 $\pm$ 0.7 (16.0 $\pm$ 6.7)	19.4 $\pm$ 0.8	2.0
Insulin (ng/ml)	T ₅	6.0 $\pm$ 1.0	6.0 $\pm$ 1.2	3.3 $\pm$ 0.7	8.3 $\pm$ 2.9	NS
	T ₂₄	6.7 $\pm$ 0.5 (1.0 $\pm$ 0.4)	3.5 $\pm$ 0.4	1.1 $\pm$ 0.04 (0.7 $\pm$ 0.3)	1.4 $\pm$ 0.4	1.1
Corticosterone (ng/ml)	T ₅	494 $\pm$ 21	451 $\pm$ 40	523 $\pm$ 42	474 $\pm$ 9	NS
	T ₂₄	451 $\pm$ 22 (238 $\pm$ 100)	410 $\pm$ 18 (16 $\pm$ 15)	419 $\pm$ 20 (312 $\pm$ 105)	458 $\pm$ 18	NS

Fig. 1. Urinary excretion of glucose (a), urea nitrogen (b), creatinine (c) and N^{ϵ} -methylhistidine (d) by intact (●), adrenalectomized (▲), diabetic (○) and adrenalectomized diabetic (△) rats before and during treatment with 4.0 mg of corticosterone/100 g body wt. per day

Each point is the mean for five rats. Vertical bars indicate $\pm$ S.E.M.

animals were lower than in non-diabetic animals 24 h after the last injection, and in the normal range. Plasma insulin concentrations were raised above normal in all non-diabetic rats in response to corticosterone treatment, but the intact control rats

showed a more sustained increase in plasma insulin than did adrenalectomized rats. No obvious difference in their feeding pattern that may account for this was noticed. As expected, the diabetic rats showed higher and variable insulin concentrations

shortly after injection (T_5 samples), and these values were not different from those of the non-diabetic rats at this time.

Urinary excretion of metabolites

Daily urinary excretion of glucose, creatinine, urea and N^{α} -methylhistidine before and during corticosterone treatment are shown in Fig. 1. All were increased after corticosterone treatment, and in each case the increase was greater for diabetic animals. In addition, the intact diabetic animals had consistently higher urinary creatinine excretion than did the adrenalectomized diabetic group. Urinary glucose excretion was negligible for non-diabetic animals. A summary of the N^{α} -methylhistidine excretion data based on analysis of pooled samples is presented in Table 3. Before corticosterone treatment, diabetic animals showed a significantly higher rate of N^{α} -methylhistidine excretion compared with non-diabetic rats, and this difference was accentuated by corticosterone treatment. Thus the average N^{α} -methylhistidine excretion rate increased by 0.54 and 0.92 $\mu\text{mol}/100\text{g}$ body wt. per day in non-diabetic and diabetic rats respectively. Con-

versely, adrenalectomy caused a decrease of 5–10% in the rate of N^{α} -methylhistidine excretion.

Myofibrillar protein turnover

The average myofibrillar protein turnover rates for the control and 7-day treatment periods as well as for the last 4 days of treatment are shown in Table 4. There was no significant difference in protein accretion rates between groups in the pre-treatment control period, but during corticosterone administration diabetic rats lost more non-collagen protein from the carcass than did non-diabetic rats. Adrenalectomy did not affect the protein loss by corticosterone treatment.

During the control period the fractional rates of carcass myofibrillar protein degradation were increased by diabetes and decreased by adrenalectomy (Table 4). These differences were relatively small.

Corticosterone treatment increased the average fractional rates of protein breakdown over the 7 days by 68% and 96% for non-diabetic and diabetic rats respectively (Table 4). Thus the disparity that existed between these groups before treatment was

Table 3. Effect of diabetes and adrenalectomy (ADX) on urinary excretion of N^{α} -methylhistidine for 3 days before (control) and 7 days during treatment with daily injection of corticosterone (4 mg/100 g body wt.)

Values are means $\pm$ S.E.M. ($\mu\text{mol}/100\text{g}$ body wt. per day) from pooled daily samples for five rats in each group. Diabetic rats received 1.5 units of NPH insulin/100 g body wt. per day. Abbreviation: LSD, least significant difference for $P = 0.05$.

	Non-diabetic		Diabetic		LSD	Effect of†	
	Intact	ADX	Intact	ADX		Diabetes	ADX
Control	1.03 $\pm$ 0.03	0.98 $\pm$ 0.02	1.10 $\pm$ 0.03	1.03 $\pm$ 0.03	0.08	*	*
Corticosterone, days 1–3	1.34 $\pm$ 0.04	1.21 $\pm$ 0.02	1.68 $\pm$ 0.05	1.45 $\pm$ 0.06	0.13	***	**
Corticosterone, days 4–7	1.97 $\pm$ 0.03	1.84 $\pm$ 0.06	2.30 $\pm$ 0.05	2.33 $\pm$ 0.04	0.15	***	NS

† Factorial analysis; *, **, *** indicate $P < 0.05$, 0.01 and 0.001, respectively. NS, not significant.

Table 4. Effects of diabetes and adrenalectomy (ADX) on carcass myofibrillar protein turnover in rats before (control) and during treatment with daily injection of corticosterone (4 mg/100 g body wt.)

Values are means $\pm$ S.E.M. for five rats in each group. Diabetic rats received 1.5 units of NPH insulin/100 g body wt. per day. k_a and k_d are fractional accumulation and degradation rates of myofibrillar protein (%/day). Abbreviations: LSD, least significant difference for $P = 0.05$; NS, no significant effect of treatment by analysis of variance.

		Non-diabetic		Diabetic		LSD	Effect of†	
		Intact	ADX	Intact	ADX		Diabetes	ADX
Control period	k_a	1.94 $\pm$ 0.12	2.18 $\pm$ 0.21	1.95 $\pm$ 0.18	2.06 $\pm$ 0.15	NS	NS	NS
	k_d	3.20 $\pm$ 0.10	3.02 $\pm$ 0.08	3.43 $\pm$ 0.09	3.20 $\pm$ 0.07	0.26	*	*
Corticosterone treatment	k_a	-2.92 $\pm$ 0.22	-2.76 $\pm$ 0.20	-4.18 $\pm$ 0.35	-4.31 $\pm$ 0.20	0.76	***	NS
	k_d	5.38 $\pm$ 0.06	5.04 $\pm$ 0.12	6.54 $\pm$ 0.14	6.40 $\pm$ 0.04	0.30	***	*
Percentage change from control	k_d	68.4 $\pm$ 4.5	67.5 $\pm$ 6.5	91.1 $\pm$ 3.6	100.7 $\pm$ 5.4	15.2	***	NS

† Factorial analysis; *, *** indicate $P < 0.05$ and 0.001 respectively.

substantially increased. Adrenalectomy had little effect on the response to corticosterone administration.

Discussion

The increase in glucosuria noted for the diabetic rats during corticosterone treatment indicates that the rise in insulin secretion after similar treatment of non-diabetics (Goldman & Frohman, 1974; Millward *et al.*, 1975; Tomas *et al.*, 1979) is secondary to increased glucose production by the liver, decreased glucose utilization by the peripheral tissues, or both, rather than a specific effect of glucocorticoids on the pancreatic production of insulin. Indeed, the available evidence suggests that corticosterone attenuates the pancreatic secretion of insulin in response to elevated glucose concentrations (Billaudel & Sutter, 1979). Plasma glucose concentrations are nearly always increased after corticosterone treatment in rats (Steele, 1975; Millward *et al.*, 1975), and such is the case in the present experiment (Table 2). Thus it seems likely that the plasma insulin concentration of non-diabetic rats rises primarily in response to the relative hyperglycaemia resulting from the imbalance in hepatic production and peripheral tissue utilization of glucose (Reaven & Olefsky, 1978).

In contrast with the non-diabetic rats, the rats with streptozotocin-induced diabetes did not show an increase above normal in plasma insulin concentrations at 24 h after injection in response to glucocorticoid administration, despite a marked elevation in plasma glucose concentrations. This indicates that β -cell function had been largely destroyed. Thus the experiment allows an assessment of the specific counteracting effect of the secondary rise in insulin concentration in non-diabetic rats on the metabolic effects of corticosterone, although it is recognized that the results may be confounded to some extent by concomitant hormonal changes in streptozotocin-diabetes, such as elevation in glucagon and somatostatin concentrations (Patel *et al.*, 1978; Soman & Felig, 1978). The loss of catecholamines in the adrenalectomized rats does not affect interpretation of the insulin effect, but may have had some bearing on the outcome of the experiment. For example, the steroid-induced hyperglycaemia, creatinuria and weight loss were less pronounced in diabetic rats without adrenals. One possible explanation apart from a catecholamine enhancement of glucocorticoid action is that β -cells in adrenalectomized rats may be less sensitive to streptozotocin, and hence these rats become less diabetic.

All groups of rats lost weight with corticosterone treatment, and this was exacerbated by the fall in food intake by the non-diabetic groups. These

groups ate according to their appetite, whereas the diabetic rats were restricted relative to their appetite and thus were essentially meal-fed. Hence the diabetic rats received corticosterone and insulin injections juxtaposed to a meal usually consumed within 1 h. Thus, since the peak corticosterone concentrations in the circulation would have coincided with the peak insulin concentrations and absorptive period for food, which favour anabolism, any increase in the catabolic effect of the corticosterone relative to non-diabetic rats would be conservatively estimated.

The increased rate of urea excretion after treatment was expected, since glucocorticoids mobilize muscle protein and increase the activity of gluconeogenic and urea-cycle enzymes in the liver (see Baxter, 1976, for review). The slightly higher fractional excretion of food nitrogen as urea in diabetic rats indicates a lower efficiency of nitrogen utilization by these animals. Nakhlooda *et al.* (1980) noted a 2–3-fold rise in urea excretion with the onset of diabetes in the spontaneously diabetic rat, but any change in nitrogen intake was not reported. The urea-excretion data suggest that the insulin replacement regime used here was suboptimal. However, the difference in daily urea excretion was small, which is consistent with the relatively low glucosuria in the control period. The coincidence of insulin administration and feeding would contribute to this degree of integrated control of the diabetes during each day.

Millward *et al.* (1980) and Bates & Millward (1981) report data which they contend show that most of the N^{α} -methylhistidine excreted by rats originates from non-muscle sources. Their conclusions have been disputed, however (Harris, 1981; Burini *et al.*, 1981), and other workers have shown at least 75% of urinary N^{α} -methylhistidine to originate from muscle (Nagasawa & Funabiki, 1981). The skin does not appear to contribute to N^{α} -methylhistidine release from perfused hind-quarters of rats (Hillgartner *et al.*, 1982), but no definitive determination of the relative excretion rates of N^{α} -methylhistidine from muscle and non-muscle sources in the whole animal has been made. Odedra *et al.* (1980) have shown the free N^{α} -methylhistidine concentration in muscle of rats to rise relative to plasma after treatment of the animal with corticosterone. Where net muscle loss (wasting) occurred, this apparent increase in myofibrillar protein breakdown rate coincided with increased average degradation rates of total muscle protein determined by difference after measurement of synthesis by radioisotope methods (Odedra *et al.*, 1980). In the study reported here all rats lost muscle protein after corticosterone treatment, and N^{α} -methylhistidine excretion was assumed to reflect both myofibrillar and total muscle protein breakdown. In

line with the findings of Nagasawa & Funabiki (1981), 75% of the excreted N^3 -methylhistidine was assumed to arise from the carcass muscle. A decrease in the proportion of total N^3 -methylhistidine excretion contributed by muscle after corticosterone treatment seems unlikely, since protein synthesis (and turnover) is depressed in most tissues (Odedra *et al.*, 1980; Thompson & Lippman, 1974; Steele, 1975). Thus the changes in carcass myofibrillar protein turnover after corticosterone treatment which are reported here are likely to be conservative, since the fractional contribution from this source is likely to increase. However, the calculated protein breakdown is an average value for all muscles, and it must be remembered that different muscle types show differing degrees of sensitivity to glucocorticoids (McGrath & Goldspink, 1980).

The comparatively greater increase in N^3 -methylhistidine excretion and calculated myofibrillar protein breakdown rate in the diabetic rats after corticosterone treatment is clear evidence that the accompanying rise in plasma insulin concentrations of normal rats diminishes the effect of the steroid. Thus these data confirm and extend those reported previously (Tomas & Ballard, 1979) and support the hypothesis that the secondary rise in insulin concentrations attenuates the effects of exogenous corticosterone on muscle protein breakdown (Millward *et al.*, 1975; Waterlow *et al.*, 1978; Tischler, 1981). Of note is the fact that muscle loss occurred even though plasma corticosterone/insulin ratios were considerably lower than those from endogenous sources found by Coward *et al.* (1977) to be associated with muscle loss. It appears that absolute concentrations of steroid, as well as the balance of counteracting hormones, is also important in determining the final response. Further evidence for this was obtained in another experiment (F. M. Tomas, unpublished work) where the administration of up to 5 units of insulin/100 g body wt. did not fully reverse the effects of administered corticosterone on muscle.

The apparent rates of myofibrillar protein synthesis can be calculated as the difference between the estimates of degradation and accretion rates, but this is subject to cumulative errors arising from the assumptions contained in the derivation of each of these rates. For example, initial body composition of the experimental animals cannot be directly measured, the proportion of excreted N^3 -methylhistidine arising from muscle may not be constant and the rate of turnover of the different protein fractions would differ. In addition the change in protein mass may not be linear over time, and the changes in muscle protein synthesis and degradation may not follow the same time course. Obviously day-to-day variations would have occurred, principally during the first 3 days, when N^3 -methyl-

histidine output is approaching a plateau (Fig. 1d). For example, changes in ribosomal content would occur during this period (Waterlow *et al.*, 1978). However, the same methods were applied to all treatment groups, so that the calculated synthesis rates would reflect, at least qualitatively, the relative average changes in synthesis rates integrated over the periods of measurement before and during corticosterone treatment. The calculated synthesis rates (%/day) for the four groups during the periods before and during corticosterone treatment, respectively, were as follows: non-diabetic, intact: 5.14 ± 0.20 , 2.46 ± 0.21 ; non-diabetic, adrenalectomized: 5.20 ± 0.24 , 2.28 ± 0.16 ; diabetic, intact: 5.38 ± 0.09 , 1.74 ± 0.26 ; diabetic, adrenalectomized: 5.25 ± 0.20 , 2.09 ± 0.19 . The calculated synthesis rates were decreased to a significantly greater extent in the diabetic animals. The degree of change in apparent synthesis rates are similar to those reported by Odedra *et al.* (1980, 1982) measured by radioisotope methods, but the actual rates are lower, probably owing to being an average of whole muscle mass rather than the gastrocnemius alone, as well as possible differences owing to rat age and differential turnover rates of myofibrillar and sarcoplasmic proteins (Waterlow *et al.*, 1978). We also have measured the synthesis rates directly in an experiment almost identical with that reported here with diabetic rats (125 g body wt.), and found gastrocnemius muscle protein synthesis rates to be halved after 4 days of corticosterone treatment (A. J. Murray & F. M. Tomas, unpublished work). These results suggest that the block in muscle protein synthesis is maximal at this dose rate. Further, the protein synthesis process seems more sensitive to glucocorticoids than is the degradative process (Table 4), as it is less readily restored by compensatory increases in plasma insulin. This agrees with the data of Odedra *et al.* (1980, 1982) in respect of the failure of insulin to activate synthesis in the presence of high concentrations of glucocorticoids. A paper by Odedra & Millward (1982), which appeared after the present work was submitted for publication, reported data from experiments which examined the effect of corticosterone on muscle protein synthesis in adrenalectomized and diabetic rats. Their findings corroborate the results and conclusions presented here.

Although the marked rise in protein degradation induced by corticosterone agrees with earlier work (Tomas *et al.*, 1979) and that of others who also performed measurements *in vivo* (Odedra *et al.*, 1980; Santidrian *et al.*, 1981), results from measurements *in vitro* of isolated muscle (McGrath & Goldspink, 1980; Shoji & Pennington, 1977) and perfused hemicorpus (Rannels & Jefferson, 1980; Hillgartner *et al.*, 1982) from pretreated rats is less clear. Only Hillgartner *et al.* (1982), who measured

N^ε-methylhistidine released from perfused hemi-corpus, reported an increase in protein breakdown rate after glucocorticoid treatment. It is likely that incubated or perfused tissue may be in a state of maximal protein breakdown, since 'control' preparations are also in net negative nitrogen balance. For example, calculations from the data of Rannels & Jefferson (1980) show that the degradation rate of muscle protein from the hemi-corpus of control animals was about 8–10% per day, which is high for rats weighing more than 200 g (Waterlow *et al.*, 1978). Omission of insulin from the perfusate may have masked any effect of corticosterone, but differential effects on specific protein fractions may not be detected by some methods (Hillgartner *et al.*, 1982). There is evidence that actomyosin degradation is increased to a greater extent than that of short-half-life proteins during catabolism (Li *et al.*, 1981; Odedra & Millward, 1982), and thus *N*^ε-methylhistidine excretion would be a sensitive indicator of catabolism. Thus the data presented here may exaggerate the actual effect of the corticosterone on the total non-collagen protein turnover.

Santidrian *et al.* (1981) have reported a transient elevation of *N*^ε-methylhistidine excretion by energy-deficient or protein/energy-deficient rats receiving a corticosterone dose that caused no such response in protein-deficient or control rats. They attributed the different responses to a lower rate of corticosterone degradation by the relatively smaller liver of energy-deficient rats. Our results and those of others (Tischler, 1981; Coward *et al.*, 1977) suggest that relatively depressed insulin concentrations and consequently wider corticosterone/insulin ratios in the energy-deficient rats offer a more likely explanation for the effects that they found.

The results presented here clearly show that the increased muscle protein breakdown induced by glucocorticoids is diminished by the secondary rise in plasma insulin concentration. Insulin-resistant impairment of glucose utilization by glucocorticoids is largely confined to adipose tissue and does not occur in muscle (Leung & Munck, 1975; Goldman & Frohman, 1974; Thompson & Lippman, 1974). The data on protein breakdown presented here support and extend the earlier findings in that muscle of glucocorticoid-treated rats retains sensitivity to insulin. However, the dosage at which the effect of corticosterone becomes dominant (i.e. only partially reversible by insulin) and the point(s) in the protein degradative process at which the two hormones interact remain to be defined.

Dr. A. J. Murray adapted the amino acid analyser to allow more rapid analysis of *N*^ε-methylhistidine in rat urine, and Miss Lorraine Pope gave skilled technical assistance during all phases of the work. I thank them for their valuable contributions.

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