Interactive effects of insulin and corticosterone on myofibrillar protein turnover in rats as determined by N^{τ} -methylhistidine excretion

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The effects of graded doses of insulin and corticosterone on myofibrillar protein turnover were investigated in growing diabetic rats in order to assess their counteractive roles in the control of protein accretion. N^{τ} -Methylhistidine excretion and carcass protein accretion were measured over 6 days in streptozotocin-diabetic rats receiving either a constant catabolic dose of corticosterone accompanied by graded doses of insulin or a constant dose of insulin accompanied by graded doses of corticosterone. The high corticosterone dose decreased the rate of protein accretion by both increasing the rate of degradation and decreasing the rate of synthesis. Increasing insulin dosage counteracted these effects, but could not restore positive accretion rates. Direct measurement of protein-synthesis rates gave results comparable with those obtained from use of N^{τ} -methylhistidine excretion. At constant insulin dosage, increased corticosterone to 45 mg/kg body wt. per day caused a dose-related linear decrease in protein accretion rates from +4.5 to -3.2% per day. Growth ceased at 28 mg of corticosterone/kg body wt. per day, largely owing to a fall in synthesis rates (-3.5%/day) rather than the increase in degradation rates (+1.0%/day). However, at steroid doses greater than 30 mg/kg body wt. per day the degradation rate increased markedly and accounted for most of the additional fall in accretion. These results show that insulin antagonizes the action of glucocorticoids on both the synthesis and degradative pathways of myofibrillar protein turnover. The changes in fractional degradation rates appear relatively more attenuated by insulin than are those of synthesis.

There is considerable evidence that insulin normally exerts a tonic suppressive effect on the rate of muscle protein degradation, since these rates are increased in diabetes and returned to normal with insulin treatment (Dahlman *et al.*, 1979; Tomas & Ballard, 1979; Tischler, 1981). Further, insulin treatment of diabetic rats receiving a catabolic dose of corticosterone diminishes the rate of muscle protein breakdown, as assessed by N^{τ} -methylhistidine excretion (Tomas & Ballard, 1979). The secondary rise in plasma insulin concentrations after corticosterone treatment of intact rats has also been shown to decrease the catabolic response (Tomas, 1982). Insulin and corticosterone also have opposing effects on the rates of muscle protein synthesis. Both glucocorticoid treatment and insulin deficiency lead to a decrease in the amount of rRNA available for translation (synthetic capacity) and the rate of protein synthesis per ribosomal unit (RNA activity) (Millward *et al.*, 1976; Rannels & Jefferson, 1980; Odedra *et al.*, 1982). However, Odedra & Millward

(1982) showed that the corticosterone-induced inhibition of protein synthesis, unlike the enhancement of breakdown, was not reversible by insulin.

Muscle protein synthesis appears to be more affected by glucocorticoids than is the rate of degradation (Odedra & Millward, 1982; Tomas, 1982), but the relative contributions of the changes in synthesis and degradation rates to muscle protein catabolism after glucocorticoid treatment at a range of doses under controlled insulin regimes have not been studied, despite the view that the corticosterone/insulin concentration ratio is the critical determinant of the metabolic response. Thus a high energy intake when dietary protein is inadequate is thought to exacerbate the outcome of the protein deficiency, because the consequent relatively high insulin secretion would suppress glucocorticoid-induced muscle protein breakdown and decrease the availability of certain amino acids for synthesis of export proteins by the liver (Coward & Lunn, 1981). Insulin therapy has also been suggested as a means of countering the cata-

bolic state following trauma, which is associated with elevated glucocorticoid (and catecholamine) concentrations (Batstone *et al.*, 1975; O'Keefe *et al.*, 1981). However, the degree to which insulin can decrease the catabolic effects of glucocorticoids on muscle protein, particularly via decreasing the degradation rate, is unknown. In this study we have examined the effects of graded doses of insulin and corticosterone administration on the rates of *N*¹⁵-methylhistidine excretion and carcass protein accretion and turnover in diabetic rats. Insulin decreases the effect of glucocorticoids on both breakdown and synthesis of muscle myofibrillar protein, but to a limited extent.

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; Tomas, 1982). The streptozotocin was dissolved in 20 mM-sodium 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). L-[ring-2-¹⁴C]Histidine (55 Ci/mol) was obtained from The Radiochemical Centre, Amersham, Bucks., U.K.

Male hooded rats [133; body wt. 100 ± 0.7 (S.E.M.)g] were obtained from the Divisional colony and injected intraperitoneally with 100 mg of streptozotocin/kg body wt. Only rats which showed polyuria, polydipsia and glucosuria were used in the experiments, and these received daily subcutaneous injections of 15 units of insulin/kg body wt.

After 5–7 days the rats were transferred to individual metabolism cages and offered a diet which contained 21% (w/w) protein and was free of *N*¹⁵-methylhistidine. After a further 3–4 days, daily urine was collected over a 2–3-day pretreatment period (zero-time controls) just before experimental treatments began.

*N*¹⁵-Methylhistidine-excretion experiments

Two experiments were performed. For Expt. 1, rats were divided into ten groups of four rats. One group served as the untreated age control, and the others received a daily subcutaneous injection of 45 mg of corticosterone/kg body wt. in conjunction with either 0, 10, 20 (two groups), 25, 30 (two

groups), 40 or 50 units of insulin/kg body wt. for 6 days. In Expt. 2, there were four groups of 10 rats which were injected daily with either 0 (age control), 15, 25 or 35 mg of corticosterone/kg body wt. for 6 days. Five rats in each group received 20 and the remainder 30 units of insulin/kg body wt.

Food intake was recorded and rats were weighed daily. For each experiment, food intake for all groups was matched to that of the corticosterone-treated group that had the lowest average consumption. 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. In Expt. 1, gastrocnemius muscles were then removed and weighed separately. For Expt. 2, the head was included in the carcass after removal of skin, brain (by suction) eyes and salivary gland tissue. The head portion contributed 13.6 ± 0.18 (S.E.M.)% to the total carcass weight. For each experiment an extra group of diabetic control rats was killed before commencement of the experimental treatments in order to obtain estimates of initial carcass composition (zero-time control).

Protein-synthesis experiment

In Expt. 3, three groups of five rats were used in a similar protocol to Expt. 1. The treatments were daily injections of 0, 45 and 45 mg of corticosterone/kg body wt., accompanied by injections of 15, 15 and 30 units of insulin/kg body wt., respectively. After 4 days of treatment the animals were lightly wrapped in a towel and given a constant infusion of L-[ring-2-¹⁴C]histidine (55 nmol/ml; 3.0 μCi/ml) at 0.45 ml/h for 6 h via a tail vein. The infusion commenced at around 09:00 h, about 24 h after the last hormone injections. On completion of the infusion, the animals were killed, blood was sampled and the gastrocnemius, soleus and extensor digitorum longus muscles were excised and quickly frozen in liquid N₂. Urine was not collected from these animals during treatment.

Analytical procedures

Urine *N*¹⁵-methylhistidine concentrations were determined either by a JEOL 6AH amino acid analyser (Expt. 1) as described previously (Tomas *et al.*, 1979) or by an Auto-Analyser method (Murray *et al.*, 1982). Blank values in the latter method were minimized by hydrolysis of 5 ml of

urine in 10% (v/v) HCl in a boiling-water bath for 1 h before loading on to columns containing 4 ml of ion-exchange resin (Tomas *et al.*, 1979). The resin was then washed sequentially with 50 ml of 0.15 M-citrate/phosphate buffer (pH 4.5) and 50 ml of glass-distilled water. The *N*^ε-methylhistidine (but not *N*^ε-methylhistamine) was then eluted with 9 ml of 0.2 M-sodium phosphate buffer, pH 8.3.

Urinary urea was determined on daily samples by using Technicon Auto-Analyser method N1c. Glucose concentrations in plasma and urine were assayed with a hexokinase method ('Glucoquant'; Boehringer Mannheim). Plasma insulin was measured with a radioimmunoassay kit (Amersham, IM78) and total plasma corticosterone was measured by a competitive protein-binding assay based on the procedures of Murphy (1967), with charcoal-treated rat plasma as the source of binding protein. For Expt. 1, carcass fat was determined by the method of Rothwell & Stock (1979). The non-collagen-protein content of the fat-free carcass was determined to be 159 ± 2 ($n = 9$) mg/g. The water content of the fat-free carcass was not affected by treatment. For Expt. 2, carcass non-collagen protein was determined directly as described previously (Tomas, 1982), except that the sarcoplasmic protein fraction was extracted with 0.05 M-potassium phosphate buffer, pH 7.4, before dilute alkali extraction. Non-collagen protein was obtained as the sum of myofibrillar and sarcoplasmic fractions.

For Expt. 3, tissue bound and free concentrations of histidine were determined after decarboxylation and subsequent fluorimetric determination of histamine by an automated adaptation of the method of Håkanson *et al.* (1972). Radioactivity was counted on a Searle 6892 counter with Picofluor-30 (Packard) as the scintillant.

Calculation of myofibrillar protein breakdown and synthesis rates

The calculation of myofibrillar protein breakdown and synthesis rates from the rates of urinary *N*^ε-methylhistidine excretion and mean carcass non-collagen-protein content was essentially as described by Tomas (1982), with 75% of excreted *N*^ε-methylhistidine assumed to arise from skeletal muscle. The initial non-collagen-protein content of the carcass was based on the carcass composition of the relevant group killed before treatment. The body composition of these diabetic rats, relative to body weight, remained constant in the body-weight range 125–150 g. It was necessary to use weight-adjusted initial control data because of the difficulty of achieving uniform weight for age groups with diabetic rats. The *N*^ε-methylhistidine content of total carcass non-collagen protein (as distinct from hind-leg muscle) in seven samples

pooled from 28 rats was determined to be $3.50 \pm 0.06 \mu\text{mol/g}$. Thus the fractional degradation rate of carcass non-collagen protein (%/day) is calculated as follows:

$$\frac{N^{\epsilon}\text{-Methylhistidine } (\mu\text{mol/day}) \times 0.75 \times 100}{\text{non-collagen-protein (g)} \times 3.50 (\mu\text{mol/g})}$$

The myofibrillar fraction of the non-collagen protein was $70.8 \pm 0.4\%$ ($n = 18$) and was not affected by corticosterone treatment, at least up to 35 mg/kg body wt. per day (Expt. 2). Thus non-collagen-protein turnover rates as determined here are the same as for the myofibrillar protein, from which the *N*^ε-methylhistidine excretion largely derives. The accretion and degradation rates calculated from *N*^ε-methylhistidine excretion are the integrated average over 6 days of collection and do not show between- and within-day variations. Thus the calculated synthesis rates are also an integrated 6-day average, and changes caused by treatment may follow a time course different from the other components of protein turnover.

In Expt. 3, protein synthesis rates for gastrocnemius, soleus and extensor digitorum longus muscles were calculated from the specific-radioactivity ratio of protein-bound to free intracellular histidine (Garlick *et al.*, 1973) by using factors in the equation appropriate to histidine. Protein accretion was determined from the average change in muscle protein content over the 4 days of treatment and the average rate of protein breakdown determined by difference.

Statistical analysis

All results are presented as means $\pm$ S.E.M., with numbers of observations in parentheses. The results were analysed statistically by using standard methods for determination of Student's *t* values and analysis of variance (Snedecor & Cochran, 1967). Simultaneous multiple comparisons were made by using modified *t*-statistics according to the Bonferroni method (Wallenstein *et al.*, 1980). Because of the cumulative assumptions used in deriving either synthesis (Expts. 1 and 2) or degradation (Expt. 3) rates by difference, the average values shown are considered to be largely qualitative, and no statistical analysis was made on these data.

Results

N^ε-Methylhistidine experiments

Food intake, body and organ weights. The effect on body and organ weights of increasing insulin administration to diabetic rats receiving 45 mg of corticosterone/kg per day is shown in Table 1. The group without insulin supplementation failed to survive beyond 3 days of corticosterone treatment,

Table 2. *Effect of corticosterone dose on food intake and body composition of diabetic rats receiving either 20 or 30 units of insulin/kg body wt. per day*
Rats were treated for 6 days. Initial controls were killed at the midpoint of the initial urine collection, 24h before commencement of treatments (see the text). Age controls are groups not receiving corticosterone and killed at the end of the experiment. Values are means $\pm$ s.e.m. for the numbers of animals shown in parentheses. Different superscripts indicate significant difference between corticosterone-treatment groups, with insulin-treatment subgroups pooled.

	Daily corticosterone dose (mg/kg body wt.)									
	0†		15		25		35			
	20 (4)	30 (4)	20 (4)	30 (4)	20 (5)	30 (5)	20 (5)	30 (5)		
Daily food intake (g/100g body wt.)	10.3 $\pm$ 0.34	11.3 $\pm$ 0.31	11.0 $\pm$ 0.30	11.1 $\pm$ 0.24	10.9 $\pm$ 0.19	10.8 $\pm$ 0.25	10.8 $\pm$ 0.27	10.8 $\pm$ 0.26		
Initial body wt. (g)	141 $\pm$ 2.1	133 $\pm$ 4.8 ^a	131 $\pm$ 2.9	135 $\pm$ 4.7	146 $\pm$ 3.4	147 $\pm$ 4.2 ^b	148 $\pm$ 2.8	146 $\pm$ 3.8 ^b		
Final body wt. (g)	182 $\pm$ 3.7	172 $\pm$ 8.2 ^a	143 $\pm$ 3.2	161 $\pm$ 8.0 ^b	150 $\pm$ 4.0	153 $\pm$ 9.0 ^b	140 $\pm$ 5.6	145 $\pm$ 4.7 ^b		
Body-weight change (g/6 days)	41 $\pm$ 2.5	38 $\pm$ 4.0 ^a	12 $\pm$ 3.3	25 $\pm$ 3.8 ^b	4 $\pm$ 2.0	7 $\pm$ 6.5 ^c	-8 $\pm$ 4.4	-1 $\pm$ 2.1 ^d		
Final liver wt. (g/100g body wt.)	5.5 $\pm$ 0.5	4.9 $\pm$ 0.1 ^a	6.5 $\pm$ 0.2	6.2 $\pm$ 0.4 ^b	6.6 $\pm$ 0.3	6.8 $\pm$ 0.3 ^c	6.7 $\pm$ 0.2	6.7 $\pm$ 0.1 ^c		
Final carcass non-collagen protein (g/100g body wt.)	6.54 $\pm$ 0.12	6.64 $\pm$ 0.12	6.51 $\pm$ 0.12	6.78 $\pm$ 0.08	6.46 $\pm$ 0.18	6.46 $\pm$ 0.20	6.66 $\pm$ 0.09	6.26 $\pm$ 0.11		

* There was no significant effect of insulin dose.
† Initial control.

Fig. 1. *Plasma corticosterone, insulin and glucose concentrations at killing in diabetic rats which received an injection of 45mg of corticosterone/kg per day for 6 days simultaneously with insulin doses ranging from 10 to 50 units/kg (Expt. 1)*
Rats were killed 24h after the last injections. Values are means for three to eight rats per group. Vertical bars show s.e.m. Lines for insulin and glucose data are fitted by least-squares analysis.

as can be verified by comparison with data in Fig. 2. Because of limited facilities for urine collection, the experiment was done in two stages, and this would have contributed to the variability between groups.

At constant insulin dosage, corticosterone treatment increased plasma glucose and corticosterone in a dose-related manner (Fig. 2). This relationship was more pronounced in the plasma samples taken 5h rather than 24h after injection, when concentrations of corticosterone and insulin were substantially higher. Glucose concentrations at each corticosterone dosage rate were substantially lower 5h after injection and reflected the boosted insulin concentrations at this time. However, all rats, including the control group not receiving corticosterone, were hyperglycaemic 24h after insulin injection. Initial control values in diabetic rats receiving 15 units of insulin/kg per day were 171 $\pm$ 30ng/ml, 0.82 $\pm$ 0.2ng/ml and 28.5 $\pm$ 1.2mm for corticosterone, insulin and glucose, respectively. These concentrations are similar to those of the age controls in Expt. 1 which received the same dose of insulin, but differ from those of the age

Fig. 2. Plasma corticosterone, insulin and glucose concentrations in diabetic rats which received either 20 or 30 units of insulin/kg per day for 6 days in conjunction with corticosterone doses ranging from 0 to 35 mg/kg per day (Expt. 2)

Data from Expt. 1 for identical doses of insulin and 45 mg of corticosterone/kg per day are also included. Values are means for four to eight rats per group for samples taken 5 h (solid symbols) or 24 h (open symbols) after last injections. Vertical bars show S.E.M. Insulin concentrations for 5 h samples were measured on a pooled sample from each treatment group, and variability is not known.

controls in Expt. 2 (Fig. 2), where insulin dosage was 20–30 units/kg per day.

Urinary excretion of metabolites. Daily urinary excretion of N^{α} -methylhistidine and urea N are shown in Fig. 3. Corticosterone treatment increased N^{α} -methylhistidine excretion per unit body weight in a dose-related manner (Fig. 3b), but the extent of the rise was inversely proportional to the dose of insulin (Fig. 3a). Thus for rats receiving 45 mg of corticosterone/kg for 6 days, total N^{α} -methylhistidine excretion increased by 75% above control rates when 10 units of insulin/kg was given, but only by about 25% when 40–50 units of insulin/kg was given. There is no apparent reason for the difference in the control values between the two experiments, but insulin dosage and the degree of induced diabetes are obvious considerations.

Urea-N excretion, expressed as a proportion of nitrogen intake, was increased progressively by corticosterone treatment (Fig. 3b). However, even at the highest insulin dosage in Expt. 1 the rate of urea-N excretion remained 80% above control values and was not significantly decreased in comparison with the lower-insulin treatments.

Myofibrillar protein turnover. The effect of insulin dose rate on the changes induced in myofibrillar protein turnover by a catabolic dosage of corticosterone is shown in Fig. 4. At the lowest insulin dose rate, corticosterone treatment decreased the protein accretion rate from +4% per day to -5.2% per day, increased the degradation rate from 4.3% per day to 7.6% per day (based on N^{α} -methylhistidine excretion) and decreased apparent synthesis rates (calculated by difference) from 8.3% per day to 2.4% per day. Each of these measures was progressively returned towards normal with increasing insulin dosage to about 30 units/kg body wt. per day, with no further reversal at higher doses, and all remained significantly different from both initial and age-control values. Although the increase in the rate of breakdown was decreased to almost half the observed maximum by added insulin, the accretion and synthesis rates recovered only one-third and one-quarter of their respective decline. In terms of fractional turnover, increasing insulin dosage from 10 to 30 or more units/kg body wt. per day decreased the breakdown rate by 1.5% per day and increased accretion and synthesis rates by 3.0 and 1.5% per day respectively. Thus the improvement in accretion rate arose about equally from changes in synthesis and degradation rates.

The effect of varying the corticosterone dosage while holding insulin constant at either 20 or 30 units/kg per day is shown in Fig. 5, which contains data from both Expts. 1 and 2. The carcass non-collagen-protein accretion rate was inversely related to the corticosterone dose. The relationship was virtually linear ($y = 0.172x + 4.79$, $S_{F_1} = 0.17$; $r = -0.91$, $n = 52$) where y is the accretion rate (% per day) and x is the dose rate of corticosterone (mg/kg body wt. per day) with zero accretion (growth stasis) occurring at a dose rate of about 28 mg of corticosterone/kg body wt. per day.

Degradation and synthesis rates were each affected by corticosterone treatment, but in opposing directions and with a different dose-response pattern. Whereas the rate of degradation increased by only about 1.0% per day (i.e. an increment of about one-third of control) at doses which induced growth stasis, the calculated rate of synthesis fell by 3.5% per day (i.e. a decrease of about 45% of control rates). At higher dose rates, which caused protein loss in excess of 3% per day, the rate of protein degradation increased more than the con-

Fig. 3. Excretion of urea N (Δ, ▲) and *N*⁺-methylhistidine (○, ●) in diabetic rats receiving either 45 mg of corticosterone and 10–50 units of insulin/kg per day (a, Expt. 1) or 20–30 units of insulin/kg per day and 0–35 mg of corticosterone/kg per day (b, Expt. 2). Data from Expt. 1 from rats receiving 20 and 30 units of insulin/kg per day are also shown in (b). Values are means for three to eight rats per group, and vertical bars indicate s.e.m. Open symbols are initial control values obtained immediately before corticosterone treatment while rats received 15 units of insulin/kg per day. In (a) these control values are significantly lower than for all treated groups. Symbols ● and ▲ (a) show values for untreated age controls. In (b) age controls are the groups untreated with corticosterone. Urea excretion was significantly increased by each increment in corticosterone (b) whereas insulin had no significant effect on this response. *N*⁺-Methylhistidine excretion was significantly affected by insulin dosage (a) (excretion at 10 units/kg per day > at 30, 40, 50, and at 20, 25 > at 40, 50 units/kg per day) and by corticosterone dosage (b) (excretion at 0, 15 mg/kg per day < at 25 < at 35 < at 45 mg/kg per day) ($P < 0.05$).

current fall in synthesis rate. Thus, as the dose of corticosterone was increased from that causing growth stasis to that which induced a rapid loss of protein of 3.3% per day, breakdown increased by 2.5% per day and synthesis decreased by 0.8% per day. At all dose rates the increase in degradation rate accounted for less than half of the fall in protein accretion rate. However, this fraction increased with increasing dose, representing 10, 22, 28 and 46% of the fall in accretion rate at 15, 25, 35 and 45 mg of corticosterone/kg body wt. per day respectively.

Protein-synthesis experiment (Expt. 3)

At commencement of corticosterone treatment, the average body weight of the rats was 143 ± 2.7 g. During the 4 days of treatment, the control group

gained 19.4 g body wt., whereas the corticosterone-treated groups receiving 15 and 30 units of insulin/kg body wt. per day lost 12.8 and 2.4 g respectively. Final muscle weights and protein-turnover data are shown in Table 3. Corticosterone decreased protein synthesis and accretion rates in all muscles relative to the control. Additional insulin decreased this effect of corticosterone treatment in all cases. Calculated rates of protein breakdown were increased in all muscles by corticosterone at the lower insulin dose, but only in the gastrocnemius at the higher insulin dose. The soleus muscle had higher rates of protein synthesis and turnover and appeared less affected by treatments compared with the other muscles. The measurements for gastrocnemius muscle protein turnover are of the same order as those for carcass myo-

Fig. 4. Fractional rates of degradation (k_d , Δ , $\blacktriangle$), accretion (k_a , $\circ$, $\bullet$) and synthesis (k_s , $\square$, $\blacksquare$) of myofibrillar protein in the carcass of diabetic rats before (Δ , $\circ$, $\square$) and after ($\blacktriangle$, $\bullet$, $\blacksquare$) treatment with 45 mg of corticosterone/kg per day in conjunction with 10–50 units of insulin/kg per day.

Symbols $\square$, $\bullet$ and $\blacktriangle$ are untreated age controls. Estimates of k_a just before treatment are based on body-weight gain during the 3-day control collection period when all rats ($n = 33$) received 15 units of insulin/kg per day. Values are means for three to eight rats per group (Expt. 1). Vertical bars show S.E.M., and lines are fitted by eye. All values for k_d and k_a after corticosterone treatment are significantly different from controls ($P < 0.05$ at least). Significant insulin effects are: k_a , at 20 units/kg per day $<$ at 40, 50; k_d , at 10 $>$ at 40, and at 20 $>$ at 40, 50 units/kg per day. Because of the assumptions used in calculating k_s , only the average values are shown for qualitative comparison, and no statistical analysis was attempted.

fibrillar protein determined in Expt. 1 from N^2 -methylhistidine excretion.

Discussion

Our data clearly show that glucocorticoid administration to growing diabetic rats led to a marked loss of muscle protein, owing to reciprocal changes in the rates of myofibrillar protein synthesis and degradation (Expts. 1 and 3). In agreement with the findings of other workers (Odedra & Millward, 1982; Odedra *et al.*, 1983), gluco-

Fig. 5. Fractional rates of degradation (k_d , Δ , $\blacktriangle$), accretion (k_a , $\circ$, $\bullet$) and synthesis (k_s , $\square$, $\blacksquare$) of myofibrillar protein in the carcass of diabetic rats before (Δ , $\circ$, $\square$) and after ($\blacktriangle$, $\bullet$, $\blacksquare$) receiving either 20 or 30 units of insulin/kg per day in conjunction with 0–35 mg of corticosterone/kg per day (Expt. 2).

Open symbols are control values obtained just before treatment, with k_a based on the average weight gain over the 2-day control collection period, when all rats ($n = 36$) received 15 units of insulin/kg per day. Groups not receiving corticosterone serve as internal age controls. Data from Expt. 1 for rats receiving 20 or 30 units of insulin and 45 mg of corticosterone/kg per day are also shown. Values are means for four to eight rats per group, with vertical bars indicating S.E.M. All values for k_d and k_a differ significantly, except the following: k_d , 0 versus 15 mg/kg per day. Values for k_s are treated as in Fig. 4, for qualitative comparison.

corticoid treatment appeared to alter synthesis rates more than degradation rates. Only about 35% of the change in accretion was accounted for by the change in degradation. However, the contribution from increased degradation rates rose with higher dose rates (Fig. 5; Odedra *et al.*, 1983).

Experiments on incubated muscle and perfused hindquarter indicate that effects on muscle protein degradation are of little, if any, importance in the response to glucocorticoids (Shoji & Pennington, 1977; McGrath & Goldspink, 1980; Rannels & Jefferson, 1980; Kelly & Goldspink, 1982). However, these observations were usually made at only a single rate of administration of glucocorticoid, and no attempt was made to examine the possibility of a different dose effect on synthesis and

Table 3. Rates of protein turnover in hindlimb muscles of diabetic rats treated with corticosterone

Rats received either 0 or 45 mg of corticosterone daily for 4 days in conjunction with 15 or 30 units of insulin/kg body wt. The rate of protein synthesis (k_s) was measured 24 h after the last injection, and average rates of accretion (k_a) and degradation (k_d) were determined as described in the text. Values are mean $\pm$ S.E.M. for five rats, except for k_s in soleus and extensor digitorum longus muscles, which were pooled within treatment groups. Different superscripts within each muscle show significant treatment effect ($P < 0.01$).

Insulin dose (units/kg body wt. per day) ...	Gastrocnemius			Soleus			Extensor digitorum longus		
	15	15	30	15	15	30	15	15	30
Corticosterone dose (mg/kg body wt. per day) ...	0	45	45	0	45	45	0	45	45
Final muscle wt. (mg/100 g body wt.)	1039 $\pm$ 62	941 $\pm$ 49	1026 $\pm$ 61	73 $\pm$ 2	69 $\pm$ 3	78 $\pm$ 4	73 $\pm$ 2	68 $\pm$ 6	74 $\pm$ 2
k_s (%/day)	8.5 $\pm$ 0.5 ^a	4.4 $\pm$ 0.5 ^b	5.2 $\pm$ 0.4 ^b	11.9	6.8	7.5	7.6	2.5	3.3
k_a (%/day)	3.2 $\pm$ 0.3 ^a	-5.4 $\pm$ 0.5 ^b	-1.7 $\pm$ 0.4 ^c	3.2 $\pm$ 0.2 ^a	-2.7 $\pm$ 1.2 ^b	1.5 $\pm$ 0.4 ^a	3.3 $\pm$ 0.5 ^a	-3.1 $\pm$ 0.6 ^b	0.3 $\pm$ 0.1 ^c
k_d (%/day)	5.4	9.8	6.9	8.7	9.5	6.0	4.3	5.6	3.0

degradation. The experiments *in vivo* by Odedra & Millward (1982) and Odedra *et al.* (1980) extended the earlier findings of Tomas *et al.* (1979) and showed that the relative effects of glucocorticoid on synthesis and degradation processes were indeed dose-dependent. Synthesis rates were slowed at relatively low dosage, but the breakdown rate (determined by difference from synthesis and accretion rates) increased only at higher doses of glucocorticoid. This agrees with the *N*^ε-methylhistidine-excretion data presented here and previously (Tomas *et al.*, 1979; Santidrian *et al.*, 1981).

The differing sensitivity of the synthesis and degradative processes in the muscle protein is clearly seen in the results of Expt. 2. The fall in the protein accretion rate from 4.5 to around 0.0% per day after corticosterone dosage was accomplished almost entirely by an apparent decrease in synthesis rates. However, when glucocorticoid dosage caused loss of non-collagen-protein mass, there was a marked increase in the rate of breakdown which contributed most to the change in accretion at negative growth rates. Thus it appears that only where net protein loss occurs in response to corticosterone treatment is there a marked increase in muscle protein breakdown. This agrees with the findings of others, where degradation was determined by the difference between measured rates of synthesis and accretion (Odedra *et al.*, 1980, 1983; Odedra & Millward, 1982) or by *N*^ε-methylhistidine excretion (Tomas *et al.*, 1979). On the other hand, these data indicate that protein-synthesis rates are most affected within the range of corticosterone dosage required to produce growth stasis and illustrate how protein accretion can be sensitively regulated, at least in rapidly growing animals. This has been pointed out by other workers (Rannels & Jefferson, 1980; Odedra *et al.*, 1982), but it must be recognized that the relationships shown here need not apply in mature animals, where growth is not present.

Our finding that insulin can reverse the effects of corticosterone on muscle protein turnover (Fig. 4; Table 3), albeit only partially, supports our earlier results (Tomas, 1982) but does not agree with the findings of others. Odedra & Millward (1982) injected diabetic rats with 50 mg of corticosterone/kg body wt. per day, but could not demonstrate any reversal by insulin of glucocorticoid inhibition of muscle protein synthesis. However, their insulin doses ranged only from 0.6 to 1.2 units/day (about 4–10 units/kg body wt. per day, delivered continuously), which were at the lower end of the range used here. Indeed, the circulating concentrations of insulin maintained by the rats reported by Odedra & Millward (1982) were only about one-third of the concentration found in non-diabetic rats injected with the same amount of cortico-

sterone (Odedra & Millward, 1982; Tomas, 1982). It could be argued that unless circulating insulin concentrations reached at least the high values that occur in response to glucocorticoid injection (Millward *et al.*, 1975; Tomas, 1982), and which presumably reflect insulin resistance (possibly owing to receptor down-regulation), then little if any reversal of the glucocorticoid action could be expected. In Expt. 1 plasma samples were obtained 24 h after injection (Fig. 1). On the basis of the samples taken 5 h after injection in Expt. 2 (Fig. 2), substantially higher insulin concentrations of 5–20 ng/ml would probably have been present during a large part of the day.

Nitrogen balances were not measured in these experiments, but the urea-N excretion, expressed relative to dietary N intake, provides an index of net protein utilization and balance. Increased insulin administration did not significantly decrease the loss of dietary N as urea, even though there was a substantial decrease in N^{ϵ} -methylhistidine excretion (Fig. 3a). This probably reflects the relatively small contribution of muscle protein breakdown to the total N flux in the rat. A 1% increment in muscle protein breakdown is only equivalent to about 3% of protein intake. On the other hand, urea excretion is stimulated to a greater extent than N^{ϵ} -methylhistidine excretion at the lower dose rates of corticosterone (Fig. 3b). Thus urea-N excretion was increased by 27, 40, 52 and 72% above baseline by 15, 25, 35 and 45 mg of corticosterone/kg body wt. per day, compared with 6, 27, 44 and 87%, respectively, for N^{ϵ} -methylhistidine excretion. This supports the notion that an increase in the rate of muscle protein breakdown (as distinct from muscle protein loss) is at first a relatively minor component of the overall catabolic response to increasing circulating concentrations of glucocorticoids.

The validity of the use of N^{ϵ} -methylhistidine as a measure of myofibrillar protein degradation remains controversial (Millward *et al.*, 1980; Bates & Millward, 1981; Cotellessa *et al.*, 1983). We have discussed previously the various considerations and assumptions used in this paper (Tomas, 1982; Tomas *et al.*, 1984). The most likely source of error would appear to be the contribution to N^{ϵ} -methylhistidine excretion by gut serosal protein turnover (Wassner & Li, 1982; Cotellessa *et al.*, 1983; Millward & Bates, 1983). Kelly & Goldspink (1982) found no change in the fractional synthesis rate of proteins in the intestinal serosa after treatment of rats with dexamethasone at a dose equivalent to 100 mg of corticosterone/kg body wt. (more than twice the highest dose rate used in our experiments), but the protein mass declined on average by 9.5% per day over 5 days, with a fall of 15% on the first day. Thus, at worst, the extra N^{ϵ} -

methylhistidine released could be as much as 10% of gut pool per day (on average), or about 0.1 μ mol/day for a 100 g rat (Millward *et al.*, 1980; Wassner & Li, 1982), much less than the observed increment reported here. The data of Hillgartner *et al.* (1982) provide empirical evidence that N^{ϵ} -methylhistidine excretion reflects myofibrillar protein breakdown in the perfused hemicorpus, since the difference between the rate of degradation determined from N^{ϵ} -methylhistidine excretion and the rate of synthesis determined from phenylalanine incorporation was close to the fractional growth rate of the rat. Our direct measurements of protein synthesis reported here (Expt. 3, Table 3) manifestly support and agree with the findings based on the N^{ϵ} -methylhistidine data. In other experiments we have found the average rate of degradation determined from N^{ϵ} -methylhistidine excretion and carcass analysis to correlate well with those calculated by difference after measurement of the rate of synthesis (Garlick *et al.*, 1980) in the total carcass muscle of the same groups of rats ($r = 0.98$ for group means, $n = 4$). Furthermore, average rates of synthesis calculated from the N^{ϵ} -methylhistidine data correlated strongly with myofibrillar protein synthesis rates determined directly as above ($r = 0.95$, $n = 32$) (F. M. Tomas & L. M. Jones, unpublished work). Importantly, we have not yet found the N^{ϵ} -methylhistidine excretion method to overestimate the average breakdown rate of myofibrillar protein calculated indirectly from protein synthesis measurement, and variability is much lower. Thus we consider that the calculated rates of muscle protein turnover based on our measurements adequately reflect any changes induced by the experimental treatments. This view is supported by the data of Odedra *et al.* (1983), who found N^{ϵ} -methylhistidine excretion by glucocorticoid-treated rats to reflect calculated rates of protein degradation in muscle.

Neither method can adequately resolve the temporal changes in synthesis and breakdown rates in response to treatment (Odedra *et al.*, 1983), but at least the rate of N^{ϵ} -methylhistidine excretion allows an estimate of the overall integrated average rates of turnover. However, in view of the several assumptions involved in the calculations and the difficulties inherent in the estimation of changes in body composition, more emphasis should be placed on the comparative rather than quantitative aspects of the calculated protein-turnover data, especially in regard to the fractional synthesis rates of myofibrillar protein. This is consistent with the approach used previously by us and others to similar data (Santidrian *et al.*, 1981; Burini *et al.*, 1981; Dunn *et al.*, 1982; Tomas *et al.*, 1984) and also allows for the fact that different muscles are affected differently by glucocorticoids

(Tomas *et al.*, 1979; Kelly & Goldspink, 1982; Table 3 of this paper).

These experiments show that myofibrillar protein synthesis is more sensitive to glucocorticoids than is degradation. It follows that the latter process would be proportionately more readily reversible by insulin, and this seems to be the case. However, the insulin effects appear to depend on the dose of glucocorticoids. Other factors, such as nutrient supply, growth potential and type of muscle, also influence the nature of the interaction (Griffin & Wildenthal, 1978; Goldberg *et al.*, 1980; Tischler, 1981) and indicate that the importance of the corticosterone/insulin ratio as a regulator of protein accumulation may diminish with increasing concentrations of corticosterone.

The antagonistic effects of insulin to glucocorticoid action shown here lends further support for the traditional view of the development of kwashiorkor. In children who consume diets providing adequate energy, but insufficient protein (Coward & Lunn, 1981; Lunn & Austin, 1983), the consequent higher rates of insulin secretion are believed to limit the availability of essential amino acids for synthesis of export proteins by the liver, thus leading to hypoalbuminaemia. Our data show that this general response may still occur despite an elevation in circulating glucocorticoid concentrations. However, more studies are needed to define the interactions at relatively low hormone concentrations and with different diets.

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