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Acute effects of corticosterone on tissue protein synthesis and insulin-sensitivity in rats *in vivo*

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The effect of corticosterone treatment on the sensitivity of muscle protein synthesis to insulin infusion was assessed in post-absorptive young rats. To select the optimal time period for corticosterone treatment, protein synthesis was measured by injection of L-[2,6-^{3}H]phenylalanine (1.5 mmol/kg body weight) 1, 4, 12 or 24 h after injection of corticosterone (5 mg/kg body wt.). Muscle protein synthesis was significantly decreased at 4 h and the effect was maximal by 12 h; liver protein synthesis was elevated at 12 h and 24 h. The dose–response of muscle protein synthesis to a 30 min infusion with 0–150 munits of insulin/h was then compared in rats pretreated with corticosterone (10 mg/100 g body wt.) or vehicle alone. When no insulin was infused, corticosterone inhibited protein synthesis in gastrocnemius muscle. High doses of insulin stimulated protein synthesis, but the inhibition by corticosterone was similar to that in the absence of insulin. At intermediate doses of insulin there was an increased requirement for insulin to elicit an equivalent response in muscle protein synthesis. Plantaris muscle responded in a manner similar to that of gastrocnemius, but neither soleus muscle nor liver responded significantly to insulin. These data suggest that corticosterone has two modes of action; one which is independent from and opposite to that of insulin, and a second which causes insulin-resistance through a decrease in sensitivity rather than a change in responsiveness.

INTRODUCTION

Glucocorticoid hormones are important in the response of animals to stress (Munck *et al.*, 1984), probably by facilitating provision of substrates for essential functions such as gluconeogenesis. The physiologically active glucocorticoid in the rat is corticosterone, and it is well documented that injection of this hormone into rats over a period of days will result in muscle wasting and reduced growth rate (Santidrian *et al.*, 1981; Odedra *et al.*, 1983). Injection of dexamethasone, a synthetic steroid, induces similar changes (Kelly & Goldspink, 1982). The glucocorticoid hormones are usually considered to be long-term regulators of metabolism and therefore most studies have concentrated on their effects after prolonged treatment. However, changes in muscle protein synthesis may be demonstrated within 4 h of corticosterone injection (Garlick *et al.*, 1987) *in vivo* and within 3–4 h of treatment *in vitro* (McGrath & Goldspink, 1982; Reeds & Palmer, 1984).

The action of insulin on protein synthesis is opposite to that of the glucocorticoids, causing increased muscle protein synthesis when infused into young post-absorptive rats (Garlick *et al.*, 1983). Interactions between the effects of insulin and corticosterone on carbohydrate metabolism are well documented (Leighton *et al.*, 1987). Daily injection of corticosterone causes an apparent insulin-resistance, characterized by elevated plasma insulin and glucose concentrations (Odedra *et al.*, 1983). Data presented by Odedra *et al.* (1982) suggested that excess corticosterone also caused insulin-resistance with respect to protein synthesis. However, a full dose–response to insulin, which is necessary for adequate characterization of the interactions between these two hormones, was not performed in their study. The objective of these experiments was therefore to investigate the interactions between insulin and corticosterone in the young post-absorptive rat by observing the dose–response relationship of insulin action on the rate of muscle protein synthesis in the presence and absence of exogenous corticosterone.

MATERIALS AND METHODS

Animals

Male hooded Lister rats of the Rowett strain were individually housed from 19 days of age. They were exposed to a 12 h light/dark schedule and provided with a commercial pelleted diet (Labsure, Manea, Cambs., U.K.) and water *ad libitum*. Experiments were performed when the animals had reached approx. 100 g body weight (14 days post-weaning). Protein synthesis measurements were made after an overnight fast (12 h), and 60 rats were used per trial.

Corticosterone was suspended in a vehicle similar to that used by Santidrian *et al.* (1981), except that the benzyl alcohol (0.9%) was replaced by 25% (v/v) ethanol. The suspension was injected subcutaneously (0.5 ml/rat), and control animals were injected with the vehicle alone.

Experiment 1

This experiment was performed to determine the persistence of the effects of a single dose of corticosterone. Rats were injected with corticosterone (5 mg/rat) and left for 1, 4, 12 or 24 h. Animals were killed between 10:00 h and 13:00 h. Food was withdrawn 12 h before death in all groups, and protein synthesis was measured by the injection of a flooding dose of L-[2,6-^{3}H]phenylalanine into a lateral tail vein precisely 10 min before each animal was killed (Garlick *et al.*, 1980). A small blood sample was collected from the tail vein before phenylalanine injection for the measurement of the plasma corticosterone concentration, as phenylalanine injection has been previously demonstrated to cause an elevated level of corticosterone within 10 min of injection (Preedy & Garlick, 1986). Tissues sampled were the gastrocnemius, soleus and plantaris muscles and a single lobe of the liver. Tissues were collected, stored and subsequently analysed for protein-bound and free phenylalanine specific radioactivities as described previously (Garlick *et al.*, 1980). RNA content was determined by measuring the absorbance at

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260 and 232 nm of tissue extracts using the relationship presented by Ashford & Pain (1986). Protein content of tissue extracts was determined by an automated Lowry method (Gaunce & D'Iorio, 1970).

Experiment 2

This experiment was conducted to assess the effect of corticosterone pretreatment on the dose–response of protein synthesis to insulin. Corticosterone (10 mg/rat) or injection vehicle was administered subcutaneously at 23:00 h, at which time food was withdrawn. The following morning, the rats were immobilized by wrapping them in perforated towels and infused for 30 min with insulin (Neutral Insulin Injection; Wellcome) at doses ranging from 0 to 150 munits/h. Tissues were sampled and protein synthesis was measured as in Experiment 1, but plasma corticosterone was not measured.

Plasma hormones and glucose

The plasma insulin concentration in Experiment 1 was measured by a double antibody radioimmunoassay, with rat standards (Novo Biolabs, Cambridge, U.K.). Samples from Experiment 2 were assayed for insulin using pig standards (Sigma, Poole, Dorset, U.K.) because most of the insulin present in the samples would be of pig origin, and pig insulin does not respond linearly on the rat standard curve. Sheep anti-(bovine insulin) serum (1:75000 dilution) was obtained from Miles Scientific (Slough, Bucks., U.K.) and guinea pig anti-sheep serum was obtained from the Scottish Antibody Production Unit (Carluke, Lanarkshire, Scotland, U.K.). The interassay coefficient of variation was 10% and the intra-assay coefficient of variation was 6.5%. Plasma corticosterone was measured using the method of Gwosdow-Cohen *et al.* (1982). Plasma glucose was measured by the automated method of Trinder (1969).

Calculations and statistics

Protein synthesis rates were calculated from the ratio of protein-bound to free phenylalanine specific radioactivities in the tissues, as described by Garlick *et al.* (1983). All data are presented as mean values ± S.E.M. The significance of differences between treatment groups was assessed by two-tailed *t* tests.

RESULTS

Experiment 1

The injection of corticosterone into fasted rats caused a large increase ($P < 0.001$) in the plasma concentration of corticosterone at 1 h after injection (Table 1). Elevated concentrations remained apparent until at least 12 h post-injection ($P < 0.05$), but by 24 h there was no significant difference in plasma corticosterone concentration between the two groups. There was a trend towards increased plasma insulin concentration in the corticosterone-treated animals at 12 and 24 h after injection (Table 1), but this effect failed to reach statistical significance. Plasma glucose concentration (Table 1) was higher in the corticosterone-treated animals at all time points, attaining statistical significance at 4 h post-injection.

There was no effect of corticosterone on gastrocnemius muscle protein synthesis rates at 1 h (Table 1), but by 4 h post-injection corticosterone had caused a decrease in the fractional synthesis rate of 1.8% day (−20%). The difference was increased to 29% at 12 h, but by 24 h post-injection the protein synthesis rates were again not different. The rate of liver protein synthesis was increased by corticosterone (Table 1); this difference persisted from 1 h ($P < 0.05$) to 24 h ($P < 0.001$).

Protein synthesis has also been expressed relative to the RNA content of the tissue (k_{RNA}) for both liver and muscle. The results (Table 1) show that when synthesis is expressed in this way, trends similar to those of the data on fractional rates of protein synthesis were observed in both tissues.

Experiment 2

Corticosterone treatment for 12 h raised the plasma glucose concentration in comparison with that of the control rats, whereas infusion of insulin into both corticosterone-treated and control rats caused a decrease in plasma glucose concentration (Table 2) which approached a plateau at the higher insulin levels. In the corticosterone-injected rats the glucose concentration was consistently significantly higher than in the control rats, so that the total drop in plasma glucose concentration resulting from insulin infusion was approximately the same in corticosterone-treated and control animals.

Fractional rates of protein synthesis in the gastrocnemius

Table 1. Time course of changes in plasma corticosterone, insulin and glucose concentrations and rates of protein synthesis in gastrocnemius muscle and liver after injection of 5 mg of corticosterone/100 g body wt.

Corticosterone (+) or vehicle (−) was injected subcutaneously into rats for 1, 4, 12 or 24 h. All animals were fasted for 12 h prior to death, regardless of the timing of the corticosterone treatment. Protein synthesis was measured between 10:00 h and 13:00 h by [^{3}H]phenylalanine injection; blood was sampled prior to phenylalanine injection for the determination of corticosterone and at the time of death (10 min later) for plasma insulin and glucose determinations. Values are means ± S.E.M. for six replicates. Statistical significance of differences: $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ versus corresponding control group (vehicle only).

Time (h)	Corticosterone (ng/ml)	Insulin (ng/ml)	Glucose (mM)	Fractional synthesis rate (%/day)		Protein synthesis (mg/day per mg of RNA)	
				Gastrocnemius	Liver	Gastrocnemius	Liver
1 (−)	390 ± 60	0.12 ± 0.06	5.7 ± 0.2	7.5 ± 0.6	96.9 ± 7.4	7.1 ± 0.5	18.5 ± 2.5
1 (+)	1320 ± 130***	0.11 ± 0.05	6.2 ± 0.1	8.0 ± 0.3	116.3 ± 3.9*	7.4 ± 0.3	21.3 ± 1.7
4 (−)	280 ± 60	0.20 ± 0.06	6.8 ± 0.4	8.9 ± 0.5	115.3 ± 8.0	8.6 ± 0.4	20.0 ± 2.0
4 (+)	730 ± 70***	0.21 ± 0.05	8.0 ± 0.2*	7.2 ± 0.5*	127.3 ± 6.5	6.8 ± 0.3**	23.4 ± 1.4
12 (−)	230 ± 30	0.29 ± 0.15	6.4 ± 0.2	8.6 ± 0.3	99.9 ± 5.6	8.9 ± 0.3	16.8 ± 1.2
12 (+)	420 ± 30*	0.47 ± 0.09	7.4 ± 0.3*	6.1 ± 0.5**	148.3 ± 5.7***	6.2 ± 0.4***	25.2 ± 1.0***
24 (−)	170 ± 60	0.21 ± 0.13	5.9 ± 0.4	7.9 ± 0.6	89.5 ± 8.5	7.2 ± 0.5	14.2 ± 1.1
24 (+)	100 ± 20	0.53 ± 0.27	7.2 ± 0.6**	8.2 ± 0.6	126.5 ± 5.9***	8.0 ± 0.5	21.1 ± 1.4**

Table 2. Concentrations of insulin and glucose in plasma and fractional rates of protein synthesis in skeletal muscles of post-absorptive rats treated with corticosterone and insulin

Rats were injected with 10 mg of corticosterone/100 g body wt. (+) or vehicle alone (−) at the time of food withdrawal (23:00 h). Approx. 12 h later, insulin at various concentrations was infused for 30 min. Protein synthesis was measured during the final 10 min of the insulin infusion by injection of [^{3}H]phenylalanine and blood was sampled at the time of death. Statistical significance of differences: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ versus corresponding control group (no insulin); †$P < 0.05$; ††$P < 0.01$; †††$P < 0.001$ versus corresponding group without corticosterone; $n = 6$ unless otherwise indicated in parentheses.

Insulin dose (munits/h)	Plasma insulin (μunits/ml)	Plasma glucose (mM)	Protein synthesis (%/day)		
			Gastrocnemius	Plantaris	Soleus
0 (−)	7.5±0.7	6.0±0.2	10.7±1.0	11.3±1.0	20.7±1.4 (5)
0 (+)	16.0±1.6	7.9±0.2†††	7.0±0.5†††	8.4±0.5††	18.1±0.4†
25 (−)	10.4±1.2	4.9±0.2***	12.1±0.8	13.0±0.6	21.5±0.6
25 (+)	18.4±1.2	7.0±0.4**†††	7.2±0.3†††	8.4±0.3†††	18.5±0.4††
50 (−)	29.4±5.1	3.0±0.1***	12.5±0.6*	13.7±0.4**	20.5±0.3
50 (+)	27.7±5.1	5.8±0.1***†††	6.9±0.3†††	8.9±0.4†††	19.3±0.8
100 (−)	67.1±14.1	2.5±0.2***	14.0±0.6***	14.8±0.7***	21.4±0.9
100 (+)	84.2±8.8	3.9±0.2***†††	9.2±0.7*†††	10.7±0.8*†††	19.9±0.6
150 (−)	154.1±26.9	2.2±0.1***	13.3±0.4**	14.9±0.6***	20.6±0.3
150 (+) (5)	122.0±12.1	3.6±0.1***†††	9.2±0.4*†††	10.9±0.9*†††	19.4±0.6

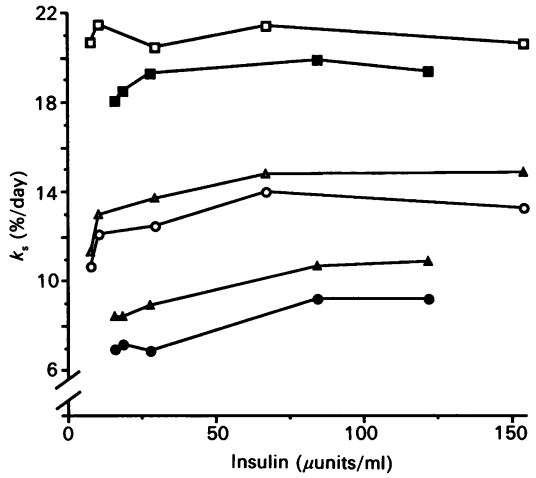

Fig. 1. Response of muscle protein synthesis to increasing concentrations of plasma insulin in the presence or absence of corticosterone

Fractional rates of protein synthesis (k_s, %/day) in gastrocnemius (○, ●), plantaris (△, ▲) and soleus (□, ■) muscles of rats injected with 10 mg of corticosterone/100 g body wt. (●, ▲, ■) or with vehicle only (○, △, □). Data are taken from Table 2.

muscle in response to corticosterone injection and insulin infusion are shown in Table 2. Insulin infusion into control rats caused a dose-dependent increase in protein synthesis, with a maximal stimulation of 31% at 100 munits/h. Corticosterone treatment for 12 h caused a decrease in protein synthesis of 3.7%/day (−35%) in the absence of infused insulin. Prior treatment with corticosterone also diminished the protein synthesis response of the gastrocnemius muscle to insulin at low doses. Whereas gastrocnemius protein synthesis in corticosterone-treated rats responded significantly ($P < 0.05$) to a plasma concentration of 84 μunits of insulin/ml, the control rats responded at 29 μunits of insulin/ml (Fig. 1). There was no effect of corticosterone on the total response to insulin, as the difference between the insulin dose–response curves of control and corticosterone-treated animals (Fig. 1) was approximately the same at the lowest and highest doses.

Protein synthesis in the plantaris muscle responded to insulin and corticosterone treatment in a similar manner to that of gastrocnemius muscle (Table 2). However, soleus muscle was less responsive to insulin than the other skeletal muscles tested, and although corticosterone caused a decrease in protein synthesis in this muscle, it did not appear to affect the response to insulin (Table 2).

DISCUSSION

Time course of effects on protein synthesis

The first objective of these experiments was to determine if injection of exogenous corticosterone into non-adrenalectomized rats could elevate the plasma concentration of corticosterone and produce metabolic effects, relative to the control animals, which have the capacity to produce some endogenous corticosterone in response to the injection of vehicle. Many of the effects of injected corticosterone have only been demonstrated previously in adrenalectomized animals (e.g. Odedra & Millward, 1982), where interpretation of the results is complicated for two reasons. First, the animals lack both corticosterone and aldosterone, and secondly, they are probably still exhibiting altered metabolism due to the stress of surgery. The data on the plasma concentration of corticosterone in Experiment 1 show that it is possible to elevate this concentration in a non-adrenalectomized animal, thus eliminating the confounding factors. However, the hyperinsulinaemia normally associated with elevated plasma glucocorticoid concentrations was not consistently shown in this experiment. It is possible that the development of a sustained high plasma insulin concentration requires long-term treatment with corticosterone, such as in the study of Odedra *et al.* (1983).

This is the first study which has documented the response to corticosterone injection throughout the first day of treatment in normal non-adrenalectomized animals in a single experiment. Garlick *et al.* (1987) studied the effect of corticosterone on muscle protein synthesis during the first 4 h after injection, while Odedra *et al.* (1983) measured protein synthesis after 24 h. The results of the present study showed that a single injection of corticosterone into fasted rats inhibited protein synthesis in gastrocnemius muscle within 4 h. This effect persisted for 12 h but not for 24 h. Rates of protein synthesis per unit of muscle RNA followed a similar time course, suggesting that changes in

RNA activity are important in the early response to glucocorticoid treatment. The RNA/protein ratio in the muscle was not changed within the first 24 h after corticosterone injection. Odedra *et al.* (1983) also demonstrated that protein synthesis expressed per g of RNA in the gastrocnemius muscle was decreased after 1 day of treatment (i.e. 24 h after the first injection). No additional effect was observed up to 12 days of treatment, the further decrease in protein synthesis over this period being principally due to a loss of RNA. Similarly, Kayali *et al.* (1987) showed that the decrease in muscle protein synthesis in perfused hindlimbs from rats treated with corticosterone for 2–8 days was due to a decrease in the muscle RNA content, not the RNA activity. Thus, although changes in the total tissue RNA content are important in the maintenance of lowered protein synthesis during chronic treatment, changes in RNA activity are more important in the initial effect of glucocorticoid treatment on muscle protein synthesis.

The results of Experiment 1 confirm the response of gastrocnemius protein synthesis observed at 4 h after injection by Garlick *et al.* (1987) in similar rats under similar conditions, and also show that the effect persists for 12 h but not for 24 h. The data do not, therefore, corroborate those of Odedra *et al.* (1983), who found a decrease in gastrocnemius protein synthesis at 24 h after injection of corticosterone. However, the rats used by Odedra *et al.* (1983) were fed *ad libitum* until the time of protein synthesis measurement, while those in the current experiment were fasted for 12 h. Although Garlick *et al.* (1987) showed that the response to corticosterone was unaltered by an overnight fast, it is possible that fasting may decrease the persistence of the effects of a single dose of corticosterone. In a similar study *in vitro*, Reeds & Palmer (1984) found that the first hour of treatment of isolated rabbit muscle with dexamethasone was associated with an increase in protein synthesis, and the rate decreased at later times. This increase in the first hour of treatment has not been observed *in vivo* and may be related to the absence from the culture medium of hormones or other factors in serum which influence the response to the glucocorticoid *in vivo*.

The protein synthesis rate in the livers of animals treated with corticosterone has been shown to increase transiently after the first day of treatment (Odedra *et al.*, 1983). Using a lower dose of corticosterone, Garlick *et al.* (1987) showed no effect of corticosterone treatment on liver protein synthesis within 4 h of injection. The results of Experiment 1 are in agreement with these previous observations, showing the most pronounced effects on liver protein synthesis at 12 h and 24 h post-injection.

One purpose of Experiment 1 was to determine an appropriate time point to use for the insulin dose–response experiments, and 12 h post-injection was chosen for three reasons. First, the effect of corticosterone injection on muscle protein synthesis was maximal at this point. Secondly, this may be a short enough period for the observed effects to be due directly to the action of the glucocorticoid and not to be complicated by secondary consequences of changes in tissue composition (e.g. altered RNA/protein ratio). Finally, 12 h was a very convenient time to use because the rats were fasted from this point. The amount of handling, and therefore stress, was minimized.

Insulin-sensitivity

Kahn (1978) suggested that resistance to the normal physiological response to insulin exists when a normal concentration of insulin produces a less than normal biological response. Resistance may be partitioned into two components; responsiveness and sensitivity. A decreased responsiveness exists when the maximum response to the hormone is decreased, whereas decrease in sensitivity occurs when the normal maximum response may be achieved, but only in the presence of a higher concentration of the hormone. Decreases in sensitivity and responsiveness may occur in the same model; equally, it is possible that the two hormones simply have independent and opposite effects.

Experiment 2 was designed to investigate glucocorticoid-induced changes in sensitivity and responsiveness to insulin at a range of concentrations, because the level of insulin chosen may greatly affect the interpretation of the results. The results suggest firstly that corticosterone has effects on protein synthesis in gastrocnemius, plantaris and soleus muscles which are opposite to and independent of the effects of insulin. Secondly, there is evidence for a decrease in insulin-sensitivity in corticosterone-treated animals. Thus in the absence of corticosterone both plantaris and gastrocnemius muscles exhibited an increase in protein synthesis in response to 29 μunits of insulin/ml of plasma, whereas in the presence of corticosterone a higher concentration of insulin, 84 μunits/ml, was required to stimulate protein synthesis. Thirdly, there did not appear to be any effect of corticosterone on the responsiveness of protein synthesis to insulin, as the maximal response to insulin treatment was similar in control and corticosterone-treated animals. However, the effect of insulin infusion was not the same in all the tissues studied. The liver was not affected at all by insulin (results not shown), as has been shown previously (Reeds *et al.*, 1985). The response of the tonic soleus muscle to corticosterone was small, i.e. a 13% reduction in the rate of protein synthesis at the lower insulin concentrations. Soleus muscle has been shown to be less responsive to insulin and to experimentally induced diabetes than the phasic muscles (Pain *et al.*, 1974), and therefore the failure of this muscle to respond to insulin in the absence of corticosterone was not unexpected. Even when corticosterone was present, the increase in protein synthesis of 10% did not attain statistical significance at any insulin concentration. The plantaris muscle, however, was very similar to the gastrocnemius. Both muscles responded more than the soleus to corticosterone, by 26% and 35% respectively, and this effect appeared to be independent of and opposite to that of insulin. The increase in the fractional rate of protein synthesis in response to insulin was 3.6%/day (+32%) in the plantaris and 3.3%/day (+31%) in the gastrocnemius. In the presence of corticosterone the maximal effect of insulin was smaller in absolute terms: 2.2%/day in the gastrocnemius and 2.5%/day in the plantaris. However, these values should not be interpreted as indicating a decrease in responsiveness to insulin since, relative to the corresponding controls, they represent an effect of insulin which was proportionately similar to that observed in the absence of corticosterone, i.e. increases of 31% and 30% respectively.

Acute effects of insulin and glucocorticoid hormones have only been investigated previously *in vitro*. Palmer (1987) studied rates of protein synthesis in rabbit muscles treated with dexamethasone and insulin and found a similar stimulation by insulin in control and dexamethasone-treated muscles. The 'parallel' response is similar to the results of Experiment 2, but the dose of insulin (100 μunits/ml) used was high, causing maximal stimulation of protein synthesis, and intermediate concentrations were not used. Studies reporting insulin–glucocorticoid interactions *in vivo* have all used chronic hormone treatment. They have suggested that protein synthesis is insulin-resistant after glucocorticoid treatment, but none has examined a full dose–response with insulin. For example, Odedra & Millward (1982) showed a decreased response to chronic insulin infusion at two doses in diabetic rats treated with corticosterone over 6 days. Also, Odedra *et al.* (1982) showed that the resistance to acute insulin treatment in diabetic rats probably results from the elevated plasma corticosterone concentrations induced by the

stress of diabetes, because diabetic and adrenalectomized animals were more sensitive to insulin. Similarly, Tomas *et al.* (1984) demonstrated that treatment of diabetic rats with corticosterone and insulin did not restore protein synthesis rates to normal levels, whereas larger doses of insulin were capable of partially restoring growth rate. However, as these experiments were all based on chronic treatments with the two hormones, it was not possible to assess the effects of subtle and short-term fluctuations which could be of importance to the maintenance of the animals on an hourly basis. None of these studies has examined the acute effects of a glucocorticoid hormone on the responsiveness or sensitivity of protein synthesis to stimulation by insulin. The data presented here suggest that glucocorticoids do not alter the responsiveness of muscle protein synthesis to insulin, but induce a decrease in insulin-sensitivity.

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