Biochem. J. (1983) **216**, 583–587
Printed in Great Britain

The role of insulin, corticosterone and other factors in the acute recovery of muscle protein synthesis on refeeding food-deprived rats

David J. MILLWARD, Bhanu ODEDRA and Peter C. BATES
Clinical Nutrition and Metabolism Unit, Department of Human Nutrition, London School of Hygiene and Tropical Medicine, 4 St. Pancras Way, London NW1 2PE, U.K.

(*Received 21 July 1983/Accepted 6 October 1983*)

Measurements of changes in muscle protein synthesis, insulin and corticosterone *in vivo* in refed food-deprived rats, some after pretreatment with anti-insulin serum or corticosterone, indicate that the acute increase in protein synthesis (20–40 min) requires (*a*) insulin, (*b*) a fall in corticosterone, since corticosterone acts at least in part by blocking insulin action, and (*c*) at least one other independent anabolic factor.

Protein synthesis in muscle responds markedly and rapidly to an alteration in nutritional state in rats (Millward & Waterlow, 1978; Waterlow *et al.*, 1978) and in man (Rennie *et al.*, 1982). Garlick *et al.* (1983) have shown that the reduced rate of protein synthesis per unit of RNA in post-absorptive rats can be restored completely in 60 min by refeeding. Several factors can be expected to participate in this response to refeeding. The first is insulin, which varies *in vivo* in parallel with changes in protein synthesis (Garlick *et al.*, 1973; Millward *et al.*, 1974), and which is known to be obligatory for optimal protein synthesis in muscle *in vitro* (Goldberg, 1979; Jefferson, 1980) and *in vivo* (Pain & Garlick, 1974; Odedra *et al.*, 1982). However, Garlick *et al.* (1983) argue that since protein synthesis increases on refeeding at lower levels of plasma insulin than those necessary to induce an increase when insulin is infused, other factors are likely to be responsible for the increase in protein synthesis.

Other factors may include other anabolic hormones as well as catabolic hormones, levels of which fall on refeeding, such as corticosteroids. Corticosterone in the rat depresses muscle protein synthesis even when insulin levels are very high (Odedra & Millward, 1982; Odedra *et al.*, 1982, 1983).

We report here studies in which we have investigated the extent to which changes in insulin and corticosterone can account for the acute response of muscle protein synthesis to refeeding in 4-days-food-deprived rats. We have measured the time course of changes in muscle protein synthesis, insulin and corticosterone in untreated rats and in rats in which the increase in insulin or the fall in corticosterone is blocked by pretreatment with either anti-insulin serum or corticosterone.

Materials and methods

Male 100g CD:COBS rats (Charles River, Margate, Kent, U.K.) were either fed a 20%-protein purified diet (Millward *et al.*, 1974) *ad libitum* or were deprived of food for 4 days. Muscle protein synthesis was then measured *in vivo*, using the large-dose method (Garlick *et al.*, 1980, 1983), in fed, food-deprived, and food-deprived rats after refeeding for a variety of times ranging from 20 to 360 min. Refeeding involved offering each rat a weighed amount of the 20%-protein purified diet, which was usually immediately consumed. Actual consumption was noted by weighing the remaining food after death. After 10, 30, 50, 170 and 350 min, (i.e. 10 min before death) groups of rats were injected intravenously with 1 ml of 150 mM-L-[4-^{3}H]phenylalanine (50 μCi/ml; Amersham International, Amersham, Bucks., U.K.)/100g body wt. The combined results of several identical experiments are shown in Table 1. At 10 min after injection, rats were killed by decapitation, with collection of blood, followed by rapid removal and cooling of gastrocnemius muscle in liquid N_2. The specific radioactivity of [^{3}H]phenylalanine in the free and protein-bound form in each tissue and the rate of protein synthesis (k_s) was determined by the method of Garlick *et al.* (1980, 1983). Protein and RNA were determined (Millward *et al.*, 1974) so that the rate of protein synthesis could be expressed as the RNA activity (k_s divided by the RNA/protein ratio; Millward *et al.*, 1973). Serum concentrations of insulin and

Table 1. *Effect of refeeding 4-day-food-deprived rats on muscle protein synthesis, plasma insulin and corticosterone in untreated rats and in rats treated with corticosterone or anti-insulin serum*

Protein synthesis, plasma insulin and corticosterone were measured as described in the text. Corticosterone (10mg/100g body weight) was injected subcutaneously 2h prior to refeeding. Anti-insulin serum (sufficient to bind 25 International Units in 0.35ml) was injected intravenously immediately prior to refeeding. All values are means ± 1 S.E.M. Significances of the differences in the RNA activities are: (*b*)1 versus(v.) (*b*)2 $P<0.001$; (*b*)1 v. (*b*)3 $P<0.002$; (*b*)2 v. (*4*) $P<0.05$; (*b*)2 v. (*b*)3 $P<0.002$; (*b*)3 v. (*b*)4 $P<0.1$; (*c*)1 v. (*c*)2 $P<0.001$; (*c*)1 v. (*c*)3 $P<0.002$; (*c*)2 v. (*c*)3 $P<0.02$.

	Treatment	*n*	Protein synthesis (%·day^{-1})	Protein synthesis (g/day per g of RNA)	[Insulin] (μ-units/ml)	[Corticosterone] (ng/ml)
(*a*)	1 Fed	15	15.3 (0.43)	13.7 (0.70)	30.7 (2.6)	285 (20)
	2 Food-deprived	24	2.82 (0.27)	4.42 (0.33)	5.2 (0.7)	593 (39)
	3 Refed 20min	7	3.09 (0.63)	4.14 (0.80)	8.8 (2.3)	478 (67)
	4 Refed 40min	6	4.60 (0.75)	6.70 (1.54)	11.7 (3.2)	231 (51)
	5 Refed 60min	19	5.95 (0.30)	9.20 (0.50)	17.8 (1.9)	134 (23)
	6 Refed 180min	7	7.30 (0.38)	9.73 (0.83)	28.4 (6.9)	120 (41)
	7 Refed 360min	3	7.10 (0.19)	8.59 (0.42)	42.4 (6.9)	189 (72)
(*b*)	1 Fed	6	14.97 (0.37)	14.80 (0.47)	38.3 (5.2)	244 (38)
	2 Food-deprived	6	4.21 (0.40)	6.74 (0.55)	4.5 (0.8)	621 (32)
	3 Refed 60min	6	7.69 (0.27)	11.73 (0.49)	25.0 (3.6)	28 (4)
	4 Refed 60min + corticosterone	6	5.84 (0.41)	9.37 (0.44)	12.3 (1.6)	866 (42)
	5 Refed 180min	4	7.56 (0.7)	11.8 (0.7)	21.0 (3.4)	189 (75)
	6 Refed 180min + corticosterone	6	5.84 (0.23)	9.88 (0.49)	26.1 (0.3)	807 (63)
(*c*)	1 Food-deprived	7	2.19 (0.25)	4.22 (0.43)	1.6 (0.3)	716 (76)
	2 Refed 60min	7	4.86 (0.49)	8.44 (0.85)	19.7 (0.4)	151 (39)
	3 Refed 60min + anti-insulin serum					
	All	7	3.48 (0.10)	6.19 (0.30)	–	374 (112)
	High corticosterone	4	3.39 (0.20)	5.99 (0.64)	–	665 (37)
	Low corticosterone	3	3.54 (0.09)	6.20 (0.17)	–	80 (37)

corticosterone were measured as described previously (Odedra *et al.*, 1982).

Results and discussion

Response of protein synthesis, insulin and corticosterone to refeeding

Rats which did not eat the food when offered were excluded from the study. The response to food intake was rapid in all three parameters measured: protein synthesis increased, insulin increased, and corticosterone fell within the first hour (Table 1). The changes at 20 and 40min in individual rats are shown in Fig. 1. At 20min, three out of seven, and at 40min, four out of six rats, appeared to have responded, with highly correlated increased rates of synthesis, increased insulin (Fig. 1*a*) and reduced corticosterone (Fig. 1*b*). However, there was no obvious difference between the correlation of each hormone with protein synthesis, so that although the results are highly suggestive that the increase in insulin and the fall in corticosterone are both components of the response to refeeding, which activates protein synthesis, it is not possible to determine the relative importance of each hormone.

At 60min the RNA activity had doubled, and although this value was only 67% of the value for well-fed rats (*a*5 cf. *a*1, Table 1), no further increase was observed over the following 5h. No further fall in corticosterone was observed after 60min, but insulin concentrations continued to increase, reaching values at 6h (42.4μ-units/ml), which were twice those observed at 60min and higher than in the well-fed rats (*a*1, Table 1).

These results confirm the report by Garlick *et al.* (1983) that muscle protein synthesis was increased within 20min of insulin infusion and within 60min of refeeding. Since no significant increase in the decreased RNA concentration was observed over the 6h period, the overall rate only rose to 50% of the rate in well-fed rats. This is consistent with the results of our previous studies, which showed that, although cyclic changes in muscle RNA were observed in meal-fed rats, the increase in muscle RNA was only apparent 12h after the feeding period (Millward *et al.*, 1974).

Effect of corticosterone pretreatment

The purpose of the treatment with corticosterone prior to refeeding was to maintain the 'food-

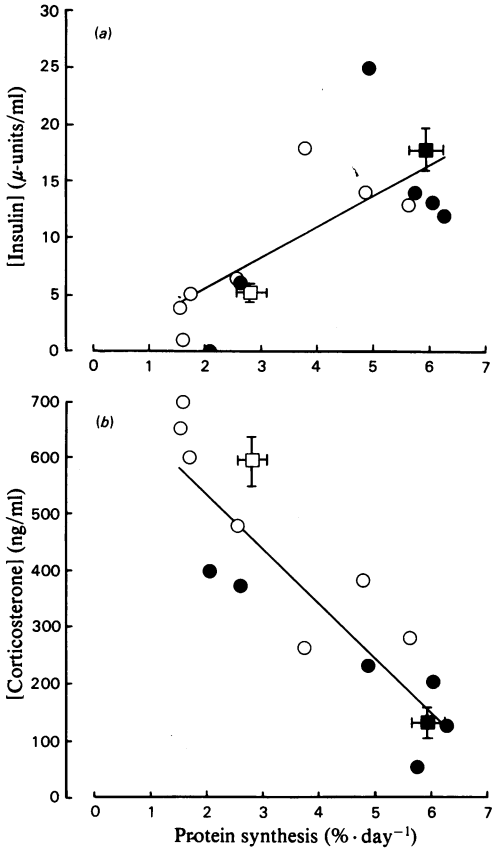

Fig. 1. *Relationship between the increase in muscle protein synthesis and the increase in insulin (a) and the decrease in corticosterone (b) during the first 60 min of refeeding 4-day-food-deprived rats*

Values are for either zero time (□) or after 20 (○), 40 (●), or 60 min of refeeding (■). Values are shown as means ± 1 S.E.M. in the case of 0 ($n=24$) and 60 ($n=19$) min, or as individual values. Protein synthesis, insulin and corticosterone were measured *in vivo* as described in the text. The line is the regression of protein synthesis on insulin (*a*, $r=0.733$) or corticosterone (*b*, $r=0.885$) with the food-deprived-rat and 60 min-refed-rat values excluded from the analysis so that the much larger numbers of values at these two times does not swamp the analysis of the relationship at the intermediate times. However it is apparent that the relationship between the variables is not substantially different at any of the four time points.

deprived' level throughout the refeeding period and so determine the importance of the fall in this hormone on refeeding for the restoration of protein synthesis. The treatment did result in the maintenence of elevated plasma corticosterone levels for at least 3 h after refeeding (Table 1). This resulted in a halving of the response to refeeding at 60 min, which is consistent with corticosterone being partially responsible for the food-deprivation-induced block of protein synthesis. However, the increase in insulin was less at 60 min (*b*4, Table 1) than in the untreated group (*b*3), so that this could have been the reason for the partial reduction in the restoration of protein synthesis rather than the corticosterone treatment. We think that this is unlikely. At 3 h, insulin levels in the corticosterone-treated group were if anything higher than in the untreated group, and the rate of protein synthesis was unchanged from the 1 h value. Furthermore, we have previously shown that even with hyperinsulinaemia, corticosterone depresses RNA activity in muscle (Odedra & Millward, 1982). Because the insulin level achieved in the corticosterone-treated rats was similar to values associated with maximal stimulation of protein synthesis when coupled with a fall in corticosterone (Figs. 1*a* and 1*b*), it would appear that the reduced response to refeeding was indeed due to the prevention of the fall in corticosterone by the treatment. The fact that a significant increase in protein synthesis still occurred ($0.05>P>0.01$) indicates that the hormone can only exert a partial block on muscle protein synthesis. This is consistent with results from our previous studies, which have shown that the depression of protein synthesis by corticosterone treatment results in only a moderate suppression of the RNA activity, the main component being a loss of RNA (Odedra & Millward, 1982; Odedra *et al.*, 1983).

Effect of anti-insulin pretreatment

The anti-insulin serum injected was sufficient to bind all the insulin which could have been released in the 60 min after refeeding and was certainly still in circulation and active after 60 min as judged by the results of the insulin radioimmunoassay (i.e. negetive results were obtained, indicating an excess of antiserum). Protein synthesis increased on refeeding (*c*3, Table 1) ($P<0.002$), although the increase was less than in the untreated group ($P<0.02$). The implication of this is that insulin is required for some, but not all, of the recovery of protein synthesis on refeeding. If the fall in corticosterone activates protein synthesis independently of any increase in insulin, then this could account for the increase in protein synthesis in these treated rats. However, as Table 1 shows, the fall in corticosterone in the anti-insulin-serum-treated rats (*c*3, Table 1) was not marked as in the untreated animals (*c*2, Table 1). In fact, of the seven animals, four had corticosterone levels that were similar to those of the food-deprived animals, whereas three had low levels similar to those of the refed group. Why this difference in the change in corticosterone occurred is not clear. We can only surmise that the anti-insulin treatment

caused a variable amount of stress on the treated animals. Nevertheless, since there was no difference between these two groups in terms of their recovery of protein synthesis, it can be concluded that the increase in protein synthesis in the anti-insulin-serum-treated rats was independent of any change in corticosterone and was mediated by another factor.

Physiological implications

These experiments were designed to examine the reversibility of the vary severe depression of protein synthesis that occurs after 4 days of food deprivation (Henshaw *et al.*, 1971). The 'RNA activity' (4.42g of protein/day per g of RNA) was much lower than the values recently reported by Garlick *et al.* (1983) in their overnight-food-deprived rats. However, Giugliano (1983) has reported values as low as 2.27g of protein/day per g of RNA in zinc-deficient rats, so that there is no reason to believe that pathological changes had occurred. The RNA activity doubled during the first 60min of refeeding, with no further increase up to 6h. Thus the continued increase in insulin between 1 and 6h after refeeding could not promote any further increase in protein synthesis. In the diabetic rat the reduced RNA activity (7.2g) of protein/day per g of RNA (Odedra *et al.*, 1982) can be fully restored to normal values early on during a 6h infusion of insulin (at least when the rats were adrenalectomized; Odedra *et al.*, 1982). Clearly, prolonged food deprivation results in a greater impairment in muscle protein synthesis than occurs in diabetes. This could be due to the decreased tri-iodothyronine levels in food deprivation (Millward *et al.*, 1981), since thyroid status is unlikely to return to normal as fast as do insulin and corticosterone. Although in the absence of insulin tri-iodothyronine has no effect on the RNA activity (Brown *et al.*, 1983), tri-iodothyronine is required for optimal RNA activity (Brown & Millward, 1983), probably for the maintenance of optimal elongation rates (Mathews *et al.*, 1973).

There seem to be at least three factors involved in the acute stimulation of protein synthesis during refeeding (and others needed for the subsequent changes). Insulin does not appear to be an absolute requirement, since it seems that some stimulation of protein synthesis can occur in its complete absence. The only alternative explanation is that the anti-insulin serum is not removing all of the insulin or is reacting in some way with the insulin receptor in muscle. Although we feel this to be unlikely, we cannot rule it out at the moment. However, the conclusion of Garlick *et al.* (1983) that concentrations of insulin below 40μ-units/ml have no effect on muscle protein synthesis is not supported by the results of the present studies, since increases in insulin to a value as low as 12μ-units/ml were associated with an increase in protein synthesis when coupled to a reduction in corticosterone and the changes in the other undefined factors in response to refeeding.

The involvement of other factors as well as corticosterone and insulin in the regulation of muscle protein synthesis complicates the interpretation of these results and the determination of the mechanism of action of corticosterone. The increase in protein synthesis on refeeding in the absence of insulin occured equally in the presence of high or low levels of corticosterone (*c*3, Table 1), indicating, as discussed above, the involvement of a third factor in the restoration of protein synthesis. If there were a direct catabolic effect of corticosterone which was distinct from the effect of the third factor, the increase in protein synthesis on refeeding the anti-insulin-serum-treated group would be expected to be greater in the animals with the low compared with the high corticosterone. This was not observed. Although there were only three rats in one group and four in the other, the measurements are reliable and the variability in the rates of protein synthesis was very small. Further experiments were limited by the supply of anti-insulin serum. Thus it would appear that the catabolic effect of corticosterone does involve modification of insulin stimulation. An interaction with insulin is suggested by the rapidity of reversal of this part of corticosterone's action. Our results show that it is reversible in less than 60min of refeeding, possibly in as little as 30min (Fig. 1*b*), which probably precludes a transcriptional event.

The present results shed no light on the identity of the third factor(s) involved in the acute response to refeeding. As discussed by Garlick *et al.* (1983), we have argued against a regulatory role for amino acids (Millward *et al.*, 1976), and McNurlan *et al.* (1982) have failed to detect an effect of leucine on muscle protein synthesis administered *in vivo*. It seems to us more likely that some other hormonal response to refeeding is responsible, but what it is remains to be determined.

These studies were supported by the British Diabetic Association, the Muscular Dystrophy Group and the Medical Research Council. We are grateful to Ms. Shree Dalal for the steroid-hormone assays and to the Wellcome Foundation for generously providing the anti-insulin serum.

References

Brown, J. G. & Millward, D. J. (1983) *Biochim. Biophys. Acta* **757**, 182–190.

Brown, J. G., van Beuren, J. & Millward, D. J. (1983) *Biochem. J.* **214**, 637–640

Garlick, P. J., Millward, D. J. & James, W. P. T. (1973) *Biochem. J.* **136**, 935–945

Garlick, P. J., McNurlan, M. A. & Preedy, V. R. (1980) *Biochem. J.* **192**, 719–723
Garlick, P. J., Fern, M. & Preedy, V. R. (1983) *Biochem. J.* **210**, 669–676
Giugliano, R. (1983) Ph.D. Thesis, University of London
Goldberg, A. L. (1979) *Diabetes* **28**, 18–24
Henshaw, E. C., Hirsch, C. A., Morton, B. E. & Hiatt, H. H. (1971) *J. Biol. Chem.* **246**, 436–440
Jefferson, L. S. (1980) *Diabetes* **29**, 487–495
Mathews, R. W., Oronsky, A. & Haschemeyer, A. E. V. (1973) *J. Biol. Chem.* **248**, 1329–1333
McNurlan, M. A., Fern, E. B. & Garlick, P. J. (1982) *Biochem. J.* **204**, 831–838
Millward, D. J. & Waterlow, J. C. (1978) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **37**, 2283–2290
Millward, D. J., Garlick, P. J., James, W. P. T., Nnanyelugo, D. O. & Ryatt, J. S. (1973) *Nature (London)* **241**, 204–205
Millward, D. J., Nnanyelugo, D. O. James, W. P. T. & Garlick, P. J. (1974) *Br. J. Nutr.* **33**, 127–142
Millward, D. J., Garlick, P. J., Nnanyelugo, D. O. & Waterlow, J. C. (1976) *Biochem. J.* **156**, 185–188
Millward, D. J., Brown, J. G. & Odedra, B. (1981) in *Nitrogen Metabolism In Man* (Waterlow, J. C. & Stephen, J. M. L., eds.), pp. 475–494, Applied Science Publishers, London
Odedra, B. R. & Millward, D. J. (1982) *Biochem. J.* **204**, 663–672
Odedra, B. R., Dalal, S. & Millward, D. J. (1982) *Biochem. J.* **202**, 363–368
Odedra, B. R., Bates, P. C. & Millward, D. J. (1983) *Biochem. J.* **214**, 617–627
Pain, V. M. & Garlick, P. J. (1974) *J. Biol. Chem.* **249**, 4510–4514
Rennie, M. J., Edwards, R. H. T., Halliday, D., Matthews, D. E., Wolman, S. L. & Millward, D. J. (1982) *Clin. Sci.* **63**, 519–523
Waterlow, J. C., Garlick, P. J. & Millward, D. J. (1978) *Protein Turnover in Mammalian Tissues and in the Whole Body*, North-Holland, Amsterdam and Oxford