Effect of recombinant human tumour necrosis factor α on protein synthesis in liver, skeletal muscle and skin of rats

Yvonne CHARTERS and Robert F. GRIMBLE

Human Nutrition Department, Southampton University, Bassett Crescent East, Southampton SO9 3TU, Hants., U.K.

Bacterial endotoxins cause enhanced protein metabolism in liver, and protein catabolism in muscle and skin. These effects may be mediated by cytokines such as interleukin 1 (IL1) and tumour necrosis factor (TNF). The study investigates the timing and magnitude of effects of recombinant human TNF α on protein synthesis and protein and RNA content of the liver, tibialis muscle and skin of Wistar rats. Intravenous doses of 30 and 300 $\mu\text{g/kg}$ of body weight were used and effects examined 8 h and 24 h after injection. Muscle protein content and synthetic rate were reduced at 8 h post-injection by over 18 % and 20 % respectively. Protein synthesis returned to normal after the lowest dose but remained depressed 24 h after the highest dose due to the accompanying anorexia. Opposite effects were observed in liver. Protein fractional synthetic rate (FSR) was increased by over 26 % at 8 h post-injection and remained elevated 24 h after the higher but not lower dose of TNF. Total protein and RNA contents were significantly higher than controls at this time. Skin protein synthesis was unaffected by TNF; however an increase in protein and RNA content was observed at 8 h post-injection with the lower dose of TNF. Liver and muscle respond in a similar but more rapid way to TNF than to endotoxin. The response of skin is however totally different. While muscle may contribute amino acids for enhanced hepatic protein synthesis following exposure to TNF, skin does not.

INTRODUCTION

Alterations in the pattern and intensity of protein metabolism are major features of the metabolic response to infection, infestation, inflammation and trauma (Beisal, 1977, 1980). The rate of whole body protein turnover and oxidation of amino acids increases together with urinary nitrogenous excretion (Tomkins *et al.*, 1983; Wannemacher, 1977; Garlick *et al.*, 1980a). Protein is lost from muscle. The amino acids thus released are utilized by the liver for gluconeogenesis and acute phase protein synthesis (Beisal, 1977; Kirkpatrick-Smith *et al.*, 1986) and by the immune system to support its enhanced activity (Newsholme *et al.*, 1985). Cytokines such as interleukin 1 (IL1) and tumour necrosis factor (TNF) mediate many of the metabolic effects observed (Dinarello, 1984; Beutler & Cerami, 1987; Cerami & Beutler, 1988).

Changes in protein dynamics within liver, skeletal muscle and skin will influence whole body protein metabolism (Millward *et al.*, 1976; McNurlan *et al.*, 1980) since in rat, these tissues account for more than half of total body protein synthesis. The effects of IL1 on liver and muscle protein metabolism have been characterized (Baracos *et al.*, 1983; Sobrado *et al.*, 1983; Dinarello *et al.*, 1986a, b). IL1 decreases protein synthesis in muscle. In liver it not only stimulates protein synthesis but also changes the profile of proteins produced via alteration in the proportions of mRNA species for serum albumin and acute phase proteins (Perlmutter *et al.*, 1986). TNF α mimics many of the actions of IL1 *in vitro* despite having a different structure and binding to different receptors in target tissues (Dinarello, 1987). In liver, TNF produces similar changes in mRNA profiles

(Perlmutter *et al.*, 1986). Furthermore, increased rates of hepatic protein synthesis of similar magnitude to those produced by IL1 were observed in mice (Moldawer *et al.*, 1988). The action of TNF on muscle protein synthesis is less clear. When TNF was given to cancer patients, an increased outflow of glutamine and alanine from forearm was noted indicating an imbalance between the rates of protein synthesis and breakdown in muscle (Warren *et al.*, 1987). A proportion of the amino acid efflux from the forearm may have emanated from skin as well as skeletal muscle. Indirect evidence suggests that protein metabolism in skin may be affected by TNF. *Escherichia coli* endotoxin, which is a potent stimulator for cytokine production, causes a fall in the fractional synthesis rate (FSR) of protein in rat skin (Wan *et al.*, 1985). Studies *in vitro* of the action of TNF on tissues rich in connective tissue also suggest that TNF may affect protein turnover in skin (Dayer *et al.*, 1985; Tashjian *et al.*, 1987; Tracey *et al.*, 1987). Furthermore, when the fate of ^{125}I -labelled TNF was examined in mice, it was found that skin removed 30 % of the label compared with 31 % by liver (Beutler *et al.*, 1985). We examined the effects of recombinant human TNF upon protein synthesis in liver, skeletal muscle and skin of rats, to provide further information on the effects of this cytokine on tissues which comprise a major portion of body protein. A short report of these measurements has been published previously (Charters & Grimble, 1988).

EXPERIMENTAL

Male Wistar rats from the Southampton University Medical School colony (body weight 151 ± 1 g,

Abbreviations used: TNF, tumour necrosis factor; IL1, interleukin 1; FSR, fractional synthetic rate; K_s , percentage of protein mass synthesized per day; S_A and S_B , specific radioactivities of phenylalanine in free tissue pool and protein respectively.

mean $\pm$ S.E.M.), having a daily photoperiod of 12 h light between 06:00 and 18:00 h, were caged singly, housed at 24 °C, and fed standard laboratory chow (CRM, Labsure, Manea, Cambs.). Rats received intravenous injections of either recombinant TNF α (endotoxin content < 0.137 ng/mg of protein, BASF/Knoll A. G., Ludwigshaven, Germany) or sterile non-pyrogenic saline. TNF was administered at doses of either 30 or 300 μ g/kg of body weight in similar saline (1 ml/kg of body weight) over a 30 s period. Injections commenced at 07:00 h. Food was removed from all rats at this time; however free access to water continued. Protein synthesis was measured *in vivo* 8 and 24 h after injection by administration of [3 H]phenylalanine into the lateral tail vein as a bolus containing sufficient phenylalanine to flood the precursor pool(s) (Garlick *et al.*, 1980b). The injection consisted of 140 μ mol of phenylalanine, including 50 μ Ci of L-[4- 3 H]phenylalanine (specific radioactivity 1.11 TBq/mmol, The Radiochemical Centre, Amersham, Bucks., U.K.) in 1 ml of 0.9% NaCl/100 g of body weight. Exactly 10 min after injection, rats were decapitated and blood collected over the following 15 s. The liver and tibialis muscle and a skin sample were rapidly removed, frozen in liquid N $_2$, weighed and stored at -10 °C. The skin sample was taken from the mid-dorsal region and measured approx. 4 cm $\times$ 4 cm. The remaining skin was removed and weighed. Hair was removed from skin samples by plunging the flattened frozen skin into liquid N $_2$ and scraping with a razor blade. Tissues were pulverized between aluminium blocks cooled by solid CO $_2$ and an accurately weighed portion (approx. 200 mg) was homogenized in 5 vol. of ice-cold 0.2 M-HClO $_4$. The specific radioactivities of phenylalanine in the tissue pool(s) and covalently bound in protein were measured in these homogenates as described by Garlick *et al.* (1980b). The method involved prior hydrolysis of the washed protein precipitates in 6 M-HCl at 110 °C for 24 h, and conversion of phenylalanine to β -phenethylamine. Radioactivity measurements were made in an LKB 1219 scintillation counter after addition of scintillation fluid (Labsint, Lablogic, Sheffield, U.K.), with the use of an external standard. The fractional rate of synthesis (i.e. K_s , the percentage of the protein mass synthesized per day) was calculated from:

$$K_s = \frac{S_B}{S_A t} \times 100$$

where S_A and S_B are the specific radioactivities of phenylalanine in the free tissue pool (i.e. intracellular plus extracellular) and protein respectively, and t is the time of incorporation in days. Tissue protein concentration was measured by the method of Lowry *et al.* (1951), with a bovine serum albumin standard. RNA concentration was measured by the method of Munro & Fleck (1986). Rats treated with TNF and killed after a 24 h interval were allowed free access to food for the last 16 h. The equivalent controls injected with saline were given the same amount of food during this period.

The significance of differences between controls and animals 8 h after receiving TNF was tested by the two-tailed unpaired Student's t -test. Values for the pair-fed controls and animals 24 h after treatment with TNF were examined for significant differences by two-way Analysis of Variance. Values of $P < 0.05$ were taken as being significantly different.

RESULTS

TNF caused anorexia, in that food intakes of 84% and 39% of a typical *ad libitum* intake for rats of similar weight were noted during the period following the 30 μ g and 300 μ g doses of TNF respectively. Mean body weight changes of -6 g and -14 g were noted over the experimental period. Untreated animals of similar body weight with unrestricted access to food normally gain around 4 g/day.

Since considerable anorexia was induced by TNF, pair-fed controls were included in the study. Careful consideration however had to be given to how this was to be done, since healthy rats consume most food during the hours of darkness. Protein synthesis measurements made 24 h after injections would be at a time close to the end of the period of maximal food intake. If controls were pair-fed from the beginning of the whole experimental period, then those controls corresponding to the group receiving 300 μ g TNF/kg would have consumed their limited rations well before the 24 h experimental period was complete. Rats treated with TNF, however, having passed through a period of anorexia, would have consumed their food towards the end of the 24 h period. Pair-fed controls and rats treated with TNF would therefore have been in completely different nutritional states at this time. This problem was minimized by removing food from all rats at the time injections took place. Rats would have full stomachs at this stage. For the following 8 h animals would subsist equally on their stomach contents whatever their degree of appetite following the injections. Food was restored 8 h after injection just prior to commencement of the period of maximum feeding activity. Thus, 24 h after injections, rats from the pair-fed and TNF-treated groups would be in a closer nutritional state than if the pair-fed rats had dissipated their limited amount of food by nibbling during the hours of daylight.

Liver protein FSR was enhanced by at least 26% 8 h after either dose of TNF (Table 1). The rate declined slightly within 24 h in rats receiving the lower dose, but remained elevated at 24 h after the higher dose. Changes in the total amount of protein synthesized and in the efficiency of protein synthesis in terms of RNA content (mg of protein synthesized/mg of RNA/day) mirrored the changes in FRS. Statistically significant differences had occurred in total protein synthesis 8 h after either dose of TNF α , and the effect was still apparent 24 h after the higher dose. This was due to maintenance of the enhanced rate of protein synthesis despite the inhibitory influence of a reduced food intake. Whilst increased efficiency of protein synthesis was apparent 8 h after cytokine administration, values were not significantly different from controls at 24 h. Both doses of cytokine increased total protein and RNA content of liver by 24 h after injection. RNA levels were increased to a greater extent than protein levels by the higher dose of TNF. In muscle, TNF had the opposite effects to those exerted in liver (Table 2). Total tibialis muscle protein synthesis was reduced by over 20% and protein content by up to 18% by 8 h after injection. The decrease was mirrored by apparent reductions in both the fractional rate of protein synthesis and of its efficiency, although differences only reached statistical significance for efficiency in the case of animals given the higher dose of TNF α . Total protein synthesis in tibialis had returned to

Table 1. Effect of recombinant TNF on hepatic protein and RNA content and protein synthesis rates

Total protein was measured by the method of Lowry *et al.* (1951) and total RNA by alkaline hydrolysis as described by Munro & Fleck (1986). The FSR of hepatic protein was determined as described by Garlick *et al.* (1980b). The amount of protein synthesized per day is calculated as the product of the fractional rate and protein mass. The efficiency of protein synthesis represents the total protein synthesized/day per mg of RNA. Values are expressed as means $\pm$ S.E.M. for 6 animals per group. Statistical significance for groups treated with TNF versus the appropriate control is * $P < 0.05$, ** $P < 0.01$.

Group	Protein content (mg)	RNA content (mg)	RNA/protein (μ g/mg)	Protein synthesis		
				Fractional rate (%/day)	Total (g/day)	Efficiency (mg/day per mg of RNA)
Control (8 h)	982 $\pm$ 52	56.2 $\pm$ 1.2	57.8 $\pm$ 2.6	87.8 $\pm$ 4.1	0.86 $\pm$ 0.05	15.2 $\pm$ 1.0
TNF, 30 μ g/kg (8 h)	1017 $\pm$ 56	60.2 $\pm$ 2.5	59.6 $\pm$ 1.9	110.7 $\pm$ 5.1**	1.13 $\pm$ 0.09*	18.7 $\pm$ 1.2*
TNF, 300 μ g/kg (8 h)	1017 $\pm$ 82	54.8 $\pm$ 1.2	55.2 $\pm$ 3.5	112.2 $\pm$ 3.0**	1.15 $\pm$ 0.12*	21.0 $\pm$ 2.2*
TNF, 30 μ g/kg (24 h)	1126 $\pm$ 43	61.5 $\pm$ 2.1*	54.7 $\pm$ 1.0	98.6 $\pm$ 5.7	1.11 $\pm$ 0.69	18.1 $\pm$ 1.3
Pair-fed control (24 h)	1062 $\pm$ 44	57.7 $\pm$ 1.4	57.1 $\pm$ 2.3	93.2 $\pm$ 5.5	1.00 $\pm$ 0.09	17.3 $\pm$ 1.6
TNF, 300 μ g/kg (24 h)	995 $\pm$ 36	59.7 $\pm$ 1.5*	60.3 $\pm$ 1.3**	111.4 $\pm$ 3.2**	1.11 $\pm$ 0.06*	18.6 $\pm$ 0.8
Pair-fed control (24 h)	978 $\pm$ 42	52.2 $\pm$ 2.8	53.4 $\pm$ 1.7	89.4 $\pm$ 4.4	0.87 $\pm$ 0.06	16.8 $\pm$ 1.0

Table 2. Effect of recombinant TNF on protein and RNA content and protein synthesis rates in skeletal muscle of rats

Skeletal muscle was analysed in the same way as described for the liver (Table 1). Results are expressed as means $\pm$ S.E.M. for 6 animals per group. Statistical significance of the groups treated with TNF versus the appropriate control is * $P < 0.05$, ** $P < 0.01$.

Group	Tibialis protein content (mg)	Tibialis RNA content (μ g)	Tibialis RNA/protein (μ g/mg)	Protein synthesis		
				Fractional rate (%/day)	Total (mg/day)	Efficiency (mg/day per mg of RNA)
Controls (8 h)	56.7 $\pm$ 3.3	546 $\pm$ 28	9.8 $\pm$ 0.3	15.9 $\pm$ 1.1	8.0 $\pm$ 0.5	16.1 $\pm$ 0.9
TNF, 30 μ g/kg (8 h)	46.4 $\pm$ 1.9*	462 $\pm$ 27	9.9 $\pm$ 0.3	13.7 $\pm$ 1.0	6.4 $\pm$ 0.6**	13.9 $\pm$ 1.0
TNF, 300 μ g/kg (8 h)	52.2 $\pm$ 3.2	528 $\pm$ 31	10.2 $\pm$ 0.5	13.4 $\pm$ 0.5	6.9 $\pm$ 0.5*	13.2 $\pm$ 0.4*
TNF, 30 μ g/kg (24 h)	60.3 $\pm$ 3.9	618 $\pm$ 44	10.0 $\pm$ 0.6	16.2 $\pm$ 0.5	9.7 $\pm$ 0.6	16.2 $\pm$ 2.0
Pair-fed control (24 h)	58.6 $\pm$ 4.5	558 $\pm$ 41	9.7 $\pm$ 0.8	15.0 $\pm$ 0.5	8.8 $\pm$ 0.7	16.2 $\pm$ 2.0
TNF, 300 μ g/kg (24 h)	50.6 $\pm$ 3.6	521 $\pm$ 32	10.4 $\pm$ 0.4	14.8 $\pm$ 0.9	7.6 $\pm$ 0.8	14.3 $\pm$ 1.3
Pair-fed control (24 h)	50.8 $\pm$ 3.2	483 $\pm$ 36	9.5 $\pm$ 0.2	13.2 $\pm$ 0.9	6.7 $\pm$ 0.5	14.0 $\pm$ 1.0

Table 3. Effect of recombinant TNF on protein and RNA content and protein synthesis rates in skin of rats

Skin was analysed in the same way as described for liver (Table 1). Total protein and RNA content and protein synthesized per day were calculated from values for the composition of biopsy samples and their FSR and from the total skin weight without hair. Results are expressed as means $\pm$ S.E.M. for 6 animals per group. Statistical significance for the groups treated with TNF versus the appropriate control is * $P < 0.05$.

Group	Protein content (mg)	RNA content (mg)	RNA/protein (μ g/mg)	Protein synthesis		
				Fractional rate (%/day)	Total (g/day)	Efficiency (mg/day per mg of RNA)
Control (8 h)	3.91 $\pm$ 0.29	78 $\pm$ 6	20.1 $\pm$ 1.0	42.7 $\pm$ 2.0	1.67 $\pm$ 0.16	21.4 $\pm$ 0.9
TNF, 30 μ g/kg (8 h)	4.71 $\pm$ 0.21*	94 $\pm$ 3*	19.7 $\pm$ 0.5	44.6 $\pm$ 4.1	2.09 $\pm$ 0.18	22.8 $\pm$ 2.2
TNF, 300 μ g/kg (8 h)	3.95 $\pm$ 0.28	82 $\pm$ 4	20.8 $\pm$ 0.5	44.0 $\pm$ 0.9	1.73 $\pm$ 0.12	21.1 $\pm$ 0.6
TNF, 30 μ g/kg (24 h)	4.06 $\pm$ 0.33	85 $\pm$ 7	21.2 $\pm$ 1.5	46.7 $\pm$ 5.8	1.85 $\pm$ 0.22	21.6 $\pm$ 1.4
Pair-fed control (24 h)	4.17 $\pm$ 0.48	81 $\pm$ 10	19.6 $\pm$ 1.1	40.7 $\pm$ 4.4	1.69 $\pm$ 0.27	20.3 $\pm$ 1.2
TNF, 300 μ g/kg (24 h)	4.16 $\pm$ 0.36	79 $\pm$ 6	19.4 $\pm$ 1.7	48.5 $\pm$ 3.6	2.02 $\pm$ 0.21	26.4 $\pm$ 3.5
Pair-fed control (24 h)	4.59 $\pm$ 0.18	96 $\pm$ 5	20.9 $\pm$ 0.6	43.0 $\pm$ 4.7	1.99 $\pm$ 0.26	20.6 $\pm$ 2.4

normal 24 h after injection of the lower dose of TNF; however this effect was not apparent in the rats given the higher dose of TNF. The anorexia caused by the higher dose of TNF prolonged the effect on protein synthesis, since reduced values were also observed in the pair-fed control. TNF had no marked effect on protein synthesis rates in skin nor on the proportion of RNA to protein (Table 3). However, 8 h after injection, the 30 µg/kg dose produced 20% and 21% increases in total protein and RNA content respectively.

DISCUSSION

To elucidate the part played by TNF in the response of protein metabolism to acute inflammation, it is useful to compare the effects of exogenously applied TNF on protein metabolism with those of endotoxins. The latter would lead to the simultaneous production of a range of cytokines. The former exposes animals to high concentrations of one cytokine prior to possible induction of others.

TNF produced similar effects to those of endotoxins. Appetite was reduced, liver protein content was enhanced, and body weight and muscle protein mass fell (McCarthy *et al.*, 1984; Jepson *et al.*, 1986). Jepson *et al.* (1986) and Wan *et al.* (1985) found that protein FSR decreased in muscle and increased in liver 24 or more hours after exposure to endotoxin. The latter authors also observed a decreased rate of synthesis and loss of protein from skin at this time. Although the responses of liver protein metabolism following exposure to endotoxin and TNF are similar in general terms, the timing of the changes and their relationship to tissue RNA content and the efficiency with which it is used differ to some extent. Jepson *et al.* (1986) showed that changes in protein synthesis in muscle during endotoxaemia were accompanied by a change in the ratio of RNA to protein and the efficiency of protein synthesis. A concomitant fall in the efficiency and rate of protein synthesis occurred 24 h after injection; however the ratio of RNA to protein did not decline until 24 h later. In the liver, an increase in the ratio was apparent 18 h before the increase in protein FSR; however, no change occurred in the efficiency.

In our study, changes in protein synthesis in liver and muscle brought about by TNF precede similar changes produced by endotoxin by approx. 16 h. Furthermore, the increase in protein synthesis in the liver precedes any increase in the ratio of RNA to protein. In contrast to the response to endotoxin, a statistically significant change in the efficiency of protein synthesis also occurs. The effects of TNF are more rapid than those of endotoxin since before endotoxin can produce its effect, the molecule must be taken up by macrophages and a range of cytokines released. Beutler *et al.* (1985) demonstrated a rise in plasma TNF levels within 15 min of administration of *E. coli* endotoxin to rabbits. Peak values were observed 2 h after injection. In our laboratory, body temperature changes occur an hour earlier following TNF injection than after endotoxin injection, whereas a difference of 4 h is observed in the time of onset of the fall in serum zinc concentrations which occurs following injection of these inflammatory agents into rats (D. C. Bibby & R. F. Grimble, unpublished work).

Moldawer *et al.* (1988) showed that total liver protein synthesis was stimulated in mice following chronic treatment with human TNF α , IL1 α or IL1 β but not IL2. Liver

RNA content was enhanced in all but IL2-treated mice. This study thus supports the findings of the present investigation that the actions of cytokines on liver protein synthesis and RNA are associated in some way. So, are the differences in the timing of changes in protein and RNA following endotoxin injection, compared with those following TNF injection, due to subsequent circulating concentrations of TNF? Waage (1987) found plasma concentrations of 105 ng of TNF/ml in rats after endotoxin treatment. On the assumption that the rat has a blood volume equal to 8% of body weight and an haematocrit of 45%, then concentrations of approx. 680 ng (30 µg dose) and 6800 ng (300 µg dose) of TNF per ml of plasma would have been achieved momentarily following injection in our study. It is therefore possible that the differences in timing of the change in hepatic RNA and protein can be explained in terms of the quantity of TNF to which liver was exposed. TNF may not be the direct or only mediator of the effects of endotoxin on liver protein metabolism. TNF α has been shown to stimulate IL1 production *in vivo* (Dinarelli *et al.*, 1986b; Moldawer *et al.*, 1988) and *in vitro* (Locksley *et al.*, 1987) from endothelial cells and monocytes (Dinarelli *et al.*, 1986b). Both cytokines have similar actions on liver protein metabolism (Perlmutter *et al.*, 1986; Dinarelli, 1987).

The observation in the present study that injection of TNF causes a substantial, rapid decrease in tibialis muscle protein synthesis does not find unanimous support from previous work. Warren *et al.* (1987) found a doubling of total amino acid flux from the forearm of patients receiving recombinant human TNF, indicating a rapid change in protein dynamics. However Rofe *et al.* (1987) found that incubation of rat hemidiaphragm with recombinant human TNF for up to 2 h had no effect on alanine efflux. Moldawer *et al.* (1987) were unable to demonstrate any effect of either recombinant human IL1 α or β or TNF on extensor digitorum longus muscle protein synthesis or degradation, *in vivo* or *in vitro*, in mice. Differences in the muscle type and species studied may account for these apparent discrepancies. Jepson *et al.* (1986) showed that endotoxin had a smaller effect on protein synthesis in soleus muscle than in gastrocnemius or plantaris muscles.

In contrast to a degree of similarity in the actions of endotoxin and TNF on liver and muscle, protein metabolism in skin was affected in an entirely different way. We have shown previously that endotoxin brings about a 30% fall in protein FSR. TNF has been shown to affect various aspects of connective tissue metabolism *in vitro*, causing proliferation of fibroblasts and an increase in collagenase and PGE₂ concentrations in dermal fibroblasts and human synovial cells (Dayer *et al.*, 1985). Bone resorption is stimulated in mouse calvaria (Tashjian *et al.*, 1987) and proteoglycans are lost from cartilage after exposure to TNF (Tracey *et al.*, 1987). Contrary to these observations, TNF had no catabolic effect on skin. The data in our study suggest a rapid transient anabolic effect of low doses of the cytokine, since both protein and RNA content increased. The increase was not due to animals in the TNF treated-group being larger or having heavier pelts. The body weights of animals, 8 h after treatment with saline or 30 µg or 300 µg/kg of TNF, were 146 g, 138 g, and 137 g respectively; and pelt weights were 24.4 g, 23.6 g and 22.6 g respectively. It is unclear if the increase in

RNA and protein represents an anabolism in skin cells or in sequestered blood cells. Indeed, TNF stimulates endothelial cells in a way that will lead to activation and accumulation of neutrophils (Pober, 1987). Blood flow through the skin may have altered following administration of TNF. We have found that a dose of 30 $\mu\text{g/kg}$ produces a fever, whereas a dose of 300 $\mu\text{g/kg}$ induces a transient hypothermia and no fever. An increased blood flow through the skin may therefore have been expected in animals treated with the lower dose of TNF. Are the changes in muscle protein synthesis alone of sufficient magnitude to provide amino acids for enhanced hepatic protein synthesis? This can be answered semi-quantitatively from the data in the present study by comparing the increase in total protein synthesis following TNF treatment (Table 1) with the product of the decrease in total tibialis protein synthesis (Table 2) and an estimate of whole body muscle mass. If total muscle mass is 35 % of body weight, then the decrease in total muscle protein synthesis of 290–360 mg (300 versus 30 $\mu\text{g/kg}$ TNF) is of the same order of magnitude as the 279–290 mg/day (30 vs 300 $\mu\text{g/kg}$ TNF) increase seen in the liver. This calculation however does not take into account changes in the rate of catabolism of amino acids by liver nor alterations in protein dynamics in other tissues. In addition, it assumes that the tibialis is typical of the total musculature and is therefore open to criticism.

These studies were supported by the Medical Research Council through a grant to R. F. Grimble. The authors acknowledge the generous gift of recombinant human TNF α from BASF/Knoll AG.

REFERENCES

- Baracos, V., Rodemann, H. P., Dinarello, C. A. & Goldberg, A. L. (1983) *N. Engl. J. Med.* **308**, 553–558
- Beisal, W. R. (1977) *Am. J. Clin. Nutr.* **30**, 1236–1247
- Beisal, W. R. (1980) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **39**, 3105–3108
- Beutler, B. & Cerami, A. (1987) *Parasitol. Today*, **11**, 345–346
- Beutler, B. A., Milsark, I. W. & Cerami, A. (1985) *J. Immunol.* **135**, 3972–3977
- Cerami, A. & Beutler, B. (1988) *Immunol. Today*, **9**, 29–31
- Charters, Y. & Grimble, R. F. (1989) *Proc. Nutr. Soc.* **48**, in the press
- Dayer, J. M., Beutler, B. & Cerami, A. (1985) *J. Exp. Med.* **162**, 2163–2168
- Dinarello, C. A. (1984) *New Engl. J. Med.* **311**, 1413–1417
- Dinarello, C. A. (1987) *Immunol. Lett.* **16**, 227–232
- Dinarello, C. A., Cannon, J. G., Mier, J. W., Bernheim, A. A., Lo Preste, G., Lynn, D. L., Love, R. N., Well, A. C., Auron, P. E., Reuben, R. C., Rich, A., Wolff, S. M. & Putney, S. D., (1986a) *J. Clin. Invest.* **77**, 1734–1739
- Dinarello, C. A., Cannon, J. G., Wolff, S. M., Bernheim, H. A., Beutler, B., Cerami, A., Figari, I. S., Pallidino, M. A. & O'Connor, J. V. (1986b) *J. Exp. Med.* **163**, 1433–1450
- Garlick, P. J., McNurlan, M. A., Fern, E. B., Tomkins, A. M. & Waterlow, J. C. (1980a) *Br. Med. J.* **281**, 262–265
- Garlick, P. J., McNurlan, M. A. & Preedy, V. R. (1980b) *Biochem. J.* **192**, 719–723
- Jepson, M. M., Pell, J. M., Bates, P. C. & Millward, D. J. (1986) *Biochem. J.* **235**, 329–336
- Kirkpatrick-Smith, L., Yoder, M. C., Polin, R. A., Douglas, S. D. & Erecinska, M. (1986) *Res. Commun. Chem. Pathol. Pharmacol.* **53**, 381–398
- Locksley, R. M., Heinzl, F. P., Shepard, H. M., Agosti, J., Essolu, T. E., Agarwal, B. B. & Harlan, J. M. (1987) *J. Immunol.* **139**, 1891–1895
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
- McCarthy, D. O., Kluger, M. J. & Vander, A. J. (1984) *Am. J. Clin. Nutr.* **40**, 310–316
- McNurlan, M. A., Pain, V. M. & Garlick, P. J. (1980) *Biochem. Soc. Trans.* **8**, 283–285
- Millward, D. J., Garlick, P. J., Nnanyelugo, D. O. & Waterlow, J. C. (1976) *Biochem. J.* **156**, 185–188
- Moldawer, L. L., Svaninger, G., Gelin, J. & Lundholm, K. G. (1987) *Am. J. Physiol.* **253**, C766–C773
- Moldawer, L. L., Andersson, C., Gelin, J. & Lundholm, K. G. (1988) *Am. J. Physiol.* **254**, G450–G456
- Munro, H. N. & Fleck, A. (1986) *Methods Biochem. Anal.* **14**, 113–176
- Newsholme, E. A., Crabtree, B. & Ardawi, M. S. (1985) *Quart. J. Exp. Physiol.* **70**, 473–489
- Perlmutter, D. H., Dinarello, C. A., Punsal, P. I. & Colten, H. R. (1986) *J. Clin. Invest.* **78**, 1349–1354
- Pober, J. S. (1987) *Ciba Found. Symp.* **131**, 170–184
- Rofe, A. M., Conyers, R. A. H., Bais, R., Gamble, J. R. & Vadas, M. A. (1987) *Biochem. J.* **247**, 789–792
- Sobrado, J., Moldawer, L. L., Bistran, B. R., Dinarello, C. A. & Blackburn, G. L. (1983) *Infect. Immunol.* **42**, 997–1005
- Tashjian, A. H., Voldkel, E. F., Lazzaro, M., Good, D., Bosma, T., Levin, L. (1987) *Endocrinology (Baltimore)* **120**, 2029–2036
- Tomkins, A. M., Garlick, P. J., Schofield, W. N. & Waterlow, J. C. (1983) *Clin. Sci.* **65**, 313–324
- Tracey, K. J., Lowry, S. F. & Cerami, A. (1987) *Ciba Found. Symp.* **131**, 88–109
- Waage, A. (1987) *Clin. Immunol. Immunopathol.* **45**, 348–355
- Wan, J., Grimble, R. F. & Gore, M. (1985) *Proc. Nutr. Soc.* **44**, 71A
- Wannemacher, R. W. (1977) *Am. J. Clin. Nutr.* **30**, 1269–1280
- Warren, R. S., Sarnes, H. F., Gabrilove, J. L., Oettgen, H. F. & Brennan, M. F. (1987) *Arch. Surg. (Chicago)* **122**, 1396–1400

Received 28 June 1988/17 October 1988; accepted 1 November 1988