

Changes in protein distribution in normal and protein-deficient rats during an acute-phase 'injury' response

BY G. JENNINGS AND M. ELIA

Dunn Nutrition Unit, Downhams Lane, Milton Road, Cambridge CB4 4XJ

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The present study investigated the effect of the acute-phase 'injury' response, induced by subcutaneous injection of turpentine, on the hydration and protein content of organs and tissues of normally nourished rats receiving a diet containing 200 g protein/kg, and of protein-malnourished rats receiving a diet containing 30 g protein/kg. The measurements were carried out 48 h after turpentine injection, and were compared with both saline-injected animals, and pair-fed control animals. Circulating α_2 -macroglobulin was also measured as an index of the acute-phase-protein response. In normally nourished rats turpentine injection caused a significant increase in the mean masses of the liver, kidney and lung (7–35% compared with saline-injected animals, and 20–44% compared with pair-fed controls), and a small reduction in the mass of extra-abdominal and extrathoracic tissues ('carcass'). In general the protein content of tissues changed in a similar way (for liver, kidney and lung a 16–33% increase compared with saline-injected animals, and 32–49% compared with pair-fed controls). Protein deficiency produced a significant attenuation in the response to turpentine. The change in the mass and protein content of several tissues was reduced (for lung, liver and kidney, the increase in protein content was only 5–15%), and the effects on anorexia (1 v. 41% reduction in food intake) and the α_2 -macroglobulin response (1.28 v. 4.28 g/l; $P < 0.001$) were also reduced. It is concluded that the injury response spares most central thoracic and abdominal organs, but this effect as well as the anorexia and acute-phase-protein response to injury are attenuated by protein deficiency.

Protein deficiency: Acute-phase response: Injury

Disease and injury cause catabolism or loss of body protein (negative N balance), but tissues do not lose protein equally. For example, in a turpentine model of injury, muscle loses protein because of a decrease in muscle protein synthesis, but liver may gain protein because of an increase in protein synthesis (Wusteman *et al.* 1990). Other studies with endotoxin, scalds, or infusion of live *Escherichia coli* bacteria in rats also suggest that muscle is preferentially lost, and liver preferentially preserved (Thelfall *et al.* 1979; Pedersen *et al.* 1987; Ash & Griffin, 1989; Fong *et al.* 1991; Wusteman & Elia, 1991; Wusteman *et al.* 1994, 1995a). The lung may also be involved in the complex changes in protein distribution after injury, since cytokines such as tumour necrosis factor (TNF), which increase after injury, produce an increase in the size and protein content of the lung (Ferrari-Baliviera *et al.* 1989; Grimble *et al.* 1992). Other workers (Stein *et al.* 1977) have shown that there is an increase in protein synthesis in the lung after fracture of the femur in the rat.

However, information about changes in protein distribution during the acute-phase response is incomplete for at least three reasons. First, some studies have failed to include a pair-fed control group, which means that they are unable to distinguish between the effects due directly to injury and those due to reduced feed intake (Carter *et al.* 1988; Jepson *et al.* 1988; Tran *et al.* 1991). Furthermore some studies have exaggerated the dietary effect

by starving the animals (Ardawi, 1992). Second, the information from some studies is incomplete either because of the limited number of tissues examined (sometimes only one) (Thelfall *et al.* 1979; Pedersen *et al.* 1987; Jones *et al.* 1990; Fong *et al.* 1991; Tran *et al.* 1991; Wusteman *et al.* 1995*b*) or two (Wusteman *et al.* 1990), or because changes in the weight of tissues or organs have been given without information about their protein content. It is possible that changes in the wet weight of tissues after injury, which may be influenced by altered dietary intake (Addis *et al.* 1936; Garlick *et al.* 1975), do not reflect changes in their water content. For example, an increase in the protein fraction of the liver has been reported in the starving rat (Garlick *et al.* 1975), whereas an increase in the hydration fraction of the liver has been reported in the septicæmic rat (Pederson *et al.* 1987). Third, virtually all the information about protein distribution after injury has been obtained in normal animals fed on a normal diet. Some studies have assessed the acute-phase effects of endogenous pyrogen (Bell & Hoffman-Goetz, 1983) or cytokines such as interleukin-1 (Drabik *et al.* 1987) in both normal and protein-deficient animals, but have not assessed the effect on tissue protein distribution. Important information about the effects of TNF in protein-deficient animals have been provided by Hunter & Grimble (1994) and Grimble *et al.* (1992). However, this information is limited either because measurements were made in only two tissues (lung and liver, Hunter & Grimble, 1994) or because no measurements were made of the protein and water content of tissues (Grimble *et al.* 1992). In addition the changes induced by single cytokines may be quite different from 'injury' responses. For example, in the study of Grimble *et al.* (1992) TNF injections produced only a 3–10-fold increase in the concentration of the acute-phase protein α_2 -macroglobulin response, compared with a much greater increment (by one order of magnitude) produced in the turpentine model of injury (Jennings & Elia, 1992).

The confounding effects of malnutrition induced by low-protein diets do not appear to have been examined. Protein deficiency attenuates several of the acute-phase changes after injury (aseptic abscesses induced by turpentine), including the acute-phase-protein response (α_2 -macroglobulin response) (Jennings & Elia, 1991, 1992, 1994; Jennings *et al.* 1992*a, b*). Whether changes in protein distribution are also attenuated in this model of injury is unknown.

The purpose of the present study was to undertake a detailed investigation of the effects of injury on the changes in tissue protein distribution in various tissues, to distinguish between the direct effect due to injury and that of anorexia, and to test the hypothesis that protein deficiency not only attenuates the acute-phase protein response but also anorexia and the changes in protein distribution.

METHODS

Thirty-six male rats from our colony of hooded animals were weaned at 21 d of age and fed *ad libitum* on a purified diet containing 200 g protein/kg (Lunn *et al.* 1976). The animals were bred in one environment and raised in another environment whilst our animal housing facilities were being refurbished. At 28 d of age the rats were divided into two equal groups (eighteen rats per group). Group 1 was given the 200 g protein/kg diet, whereas group 2 was given a low-protein diet (30 g protein/kg, in which most of the protein was replaced with sugar and starch in equal proportions so that it was isoenergetic with the 200 g protein/kg diet (16.7 MJ/kg)). The intake of a 30 g protein/kg diet *ad libitum* results in a near normal intake of energy per 100 g body weight; in young rats this will maintain body weight but will prevent growth (Jennings & Elia, 1991).

At 42 d of age six rats from each dietary group were injected subcutaneously on the dorsum with turpentine at a dose of 5 ml/kg body weight. Since this results in a reduction

in feed intake, six rats from each dietary group were pair-fed to the mean daily intake of the corresponding turpentine-injected rats. The final six rats in each dietary group were injected with saline (150 mM-NaCl; 5 ml/kg body weight) and used as controls.

At 44 d of age (i.e. 2 d after injection or 2 d after pair-feeding) the rats were killed by cervical dislocation and blood (3–5 ml) was collected from the heart and placed into 5 ml tubes containing 160 IU heparin. Plasma was separated by centrifugation at 4°, and stored at –20° until analysis. The heart, lungs, kidney, liver and gastrocnemius muscle were removed, blotted on tissue paper and weighed. The small and large intestines were removed, rinsed in saline, blotted on tissue paper and weighed. All tissue samples were then kept at –20° until analysis.

Plasma was analysed for α_2 -macroglobulin by single radial diffusion (Jennings & Elia, 1992). Tissue water content was measured by freeze-drying to a constant weight, and tissue N was determined in duplicate using samples of approximately 5 mg powdered freeze-dried tissue. The measurements were carried out using a Carlo Erba automatic N analyser (model 1500, Fisons Instruments, Crawley, Sussex). The protein content of a tissue was calculated by multiplying its N content by 6.25.

Body weights and feed intake were measured daily throughout the experiment.

RESULTS

Body weights and feed intake

From 28–42 d of age the rats on the 200 g protein/kg diet consumed approximately 140 g/d per kg body weight. They increased their body weight at a rate of about 7 g/d. The rats on the protein-deficient diet, as expected from previous work (Jennings & Elia, 1991), consumed a similar amount of feed on a weight basis, and did not increase their body weight. The feed intake of the saline-injected rats in both dietary groups was similar in the pre- and post-injection periods (Fig. 1). In contrast the feed intake of the turpentine-injected rats on the 200 g protein/kg diet was reduced by 29% on the first day and 54% on the second day, resulting in an overall reduction of 41% during the 48 h following turpentine injection. The rats on the low-protein diet also consumed less feed (22%) in the first 24 h after turpentine injection (Fig. 1), but more on the subsequent day (23%), so that in the entire 48 h period there was essentially no overall change in feed intake.

Body weight in the rats receiving the 200 g protein/kg diet was reduced by turpentine injection, but not saline injection. The loss of body weight, in the turpentine-injected rats was reproduced by pair-feeding (Fig. 2). The body weight of rats receiving the low-protein diet was not significantly affected by turpentine or saline injection, nor was it affected by pair-feeding (Fig. 2). The saline-injected rats on the low-protein diet maintained their pre-injection weight as did the pair-fed and turpentine-treated rats in this dietary group.

Organ size and composition

To minimize differences due to body size, the weights of tissues are expressed as a proportion of body weight (Table 1). In the rats on the 200 g protein/kg diet the lower feed intake of the pair-fed rats caused a significant reduction in relative size of some tissues (liver, small intestine and large intestine) compared with the rats fed *ad libitum* (Table 1). To account for the effects due to reduced feed intake, the turpentine-treated rats within each group were compared with the pair-fed rats. Turpentine caused an increase in the weight of liver ($P < 0.001$), kidney ($P < 0.01$), lungs ($P < 0.05$) and small intestine ($P < 0.01$) of rats fed on the 200 g protein/kg diet. Similar trends were found in the protein-

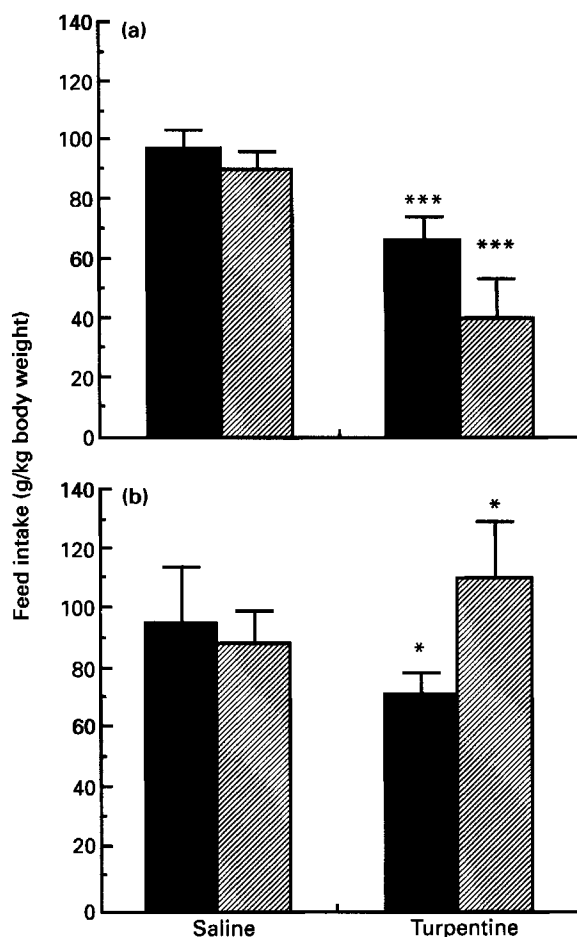


Fig. 1. The effect of turpentine and saline injections on feed intake of rats fed on diets containing (a) 200 g protein/kg or (b) 30 g protein/kg on day 1 (■) and day 2 (▨). Mean feed intake values were significantly different from corresponding saline controls: * $P < 0.05$; *** $P < 0.001$.

deficient rats, and although the changes were attenuated, turpentine still caused an increase in the weight of liver ($P < 0.001$), kidney ($P < 0.01$) and heart ($P < 0.05$). The increase in liver size due to turpentine injection of the rats on the 200 g protein/kg diet (44%) was significantly greater ($P < 0.001$) than that of the rats on the protein-deficient diet (24%).

Turpentine injection in normally nourished animals did not significantly change the hydration fraction of tissues, but pair-feeding caused an independent increase (Table 2). As a result the heart, kidneys and large gut of the animals injected with turpentine had a lower hydration fraction than the same tissues from pair-fed animals (Table 2). The opposite trends were observed in the protein-deficient animals because turpentine administration increased the hydration of several tissues, but pair-feeding did not. The only tissue to show a significant reduction in hydration after turpentine was muscle (Table 2).

Turpentine injection in normally nourished animals significantly increased the protein content of several abdominal and thoracic organs (30–50%), whilst pair-feeding decreased it (Table 3). In protein-malnourished animals turpentine produced smaller increments in protein content of organs (significant only for liver ($P < 0.01$) and kidney ($P < 0.05$)). The

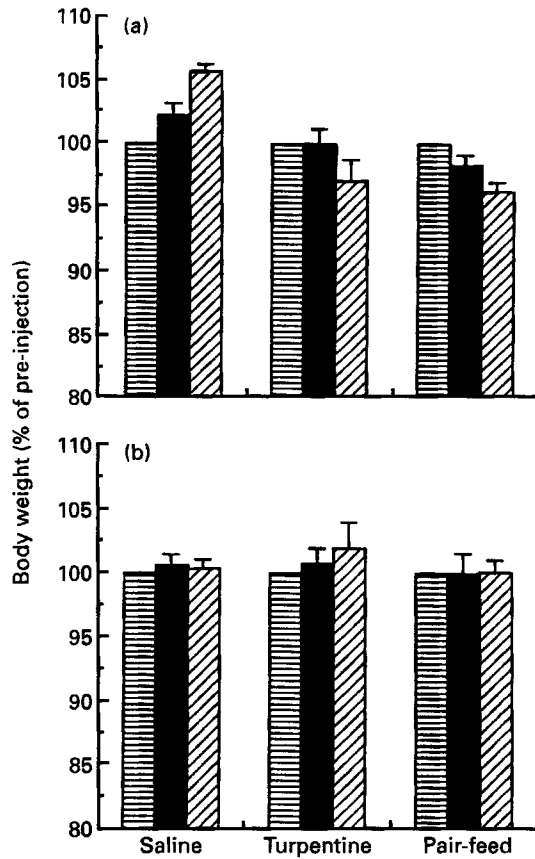


Fig. 2. The effect of turpentine injection, saline injection and pair-feeding on body weight of rats fed on diets containing (a) 200 g protein/kg or (b) 30 g protein/kg on day 1 (■) and day 2 (▨). Weights are expressed as a percentage of the pre-injection weight (▤).

increments in the rats on the adequate-protein diet were significantly greater than those found in the protein-deficient rats e.g. for liver (31.9 v. 12.8%; $P < 0.001$), lung (48.9 v. 17.9%; $P < 0.05$) and kidney (32.8 v. 7.1%; $P < 0.01$). Turpentine injection decreased the protein content of the muscle and 'carcass' of both the normally nourished and protein-deficient animals, but the change in the protein content of the 'carcass' of the normally nourished animals did not reach statistical significance.

α_2 -Macroglobulin response

As in our previous studies (Jennings & Elia, 1991, 1992, 1994; Jennings *et al.* 1992*a, b*), turpentine injection increased the circulating α_2 -macroglobulin concentration to a lesser extent in the protein-deficient animals than in the normally nourished animals (1.28 (SD 0.28) v. 4.85 (SD 0.55) g/l) (Table 4). However, the α_2 -macroglobulin response in both normal and protein-deficient animals was less than reported previously by our group (Jennings & Elia, 1991, 1992; Jennings *et al.* 1991, 1992*a, b*), possibly because the animals were bred in one environment and raised in another environment whilst there was refurbishment of our own facilities, and possibly because the concentration of α_2 -macroglobulin is variable at 2 d (Jennings *et al.* 1992*b*). The protein-deficient diet produced

Table 1. Relative tissue weights (g/kg body weight) in rats injected with turpentine or saline and pair-fed controls, fed on diets containing 200 or 30 g protein/kg‡
(Mean values and standard deviations for six rats per group)

	Saline		Pair-fed		Turpentine	
	Mean	SD	Mean	SD	Mean	SD
200 g protein/kg						
Liver	46.7	1.1	34.7†††	1.1	50.0***†††	1.1
Heart	4.0	1.3	4.2	0.3	4.5	0.6
Kidney	8.8	0.5	8.5	0.4	10.2***†††	0.5
Lung	7.4	1.3	7.5	0.1	10.0*†	2.6
Small intestine	18.4	1.6	15.3††	0.7	17.5**	1.1
Large intestine	5.7	0.5	3.9†††	0.4	4.0†††	0.3
Muscle	7.1	0.2	7.2	0.4	6.9	0.5
Carcass	889.5	3.5	892.2	4.3	870.5***††	5.6
30 g protein/kg						
Liver	39.3	1.3	38.2	2.1	47.2***†††	1.7
Heart	4.5	0.3	4.2	0.2	4.7*	0.4
Kidney	9.1	0.3	8.4	0.8	10.0***††	0.6
Lung	8.0	1.6	7.6	1.0	9.7	3.4
Small intestine	17.9	2.7	16.2	1.2	16.8	1.6
Large intestine	5.9	0.2	5.6	1.0	5.1††	3.8
Muscle	7.3	0.7	6.9	0.5	6.7	0.6
Carcass	890.7	6.8	900.3	3.2	886.4***	4.9

Mean values were significantly different from those for the pair-fed group: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Mean values were significantly different from those for the saline group: † $P < 0.05$, †† $P < 0.01$, ††† $P < 0.001$.

‡ For details of diets and procedures, see pp. 124–125.

a small increase in the basal (pre-turpentine) concentration of α_2 -macroglobulin and pair-feeding had no additional effect.

DISCUSSION

This study provides comprehensive information about the changes in protein distribution produced by turpentine administration, and distinguishes between the effects due to injury and anorexia. It also demonstrates how protein deficiency modifies these responses.

Although 'injury' produces muscle wasting, some central tissues are preferentially preserved (the present study and Thelfall *et al.* 1979; Ash & Griffin, 1989; Ferrari-Baliviera *et al.* 1989; Grimble *et al.* 1992; Hunter & Grimble, 1994; Besler & Grimble, 1995). The present study shows that reduced feed intake in normally nourished animals produces a reduction in protein content of most tissues, but turpentine administration results in preferential preservation of the protein content of most central organs (compared with pair-fed animals (Table 3)). Indeed administration of turpentine to normally nourished rats not only resulted in an increase in the protein content of the lung and liver relative to pair-fed animals (33% and 44% respectively) but also relative to control animals injected with saline (35% and 7% respectively). These trends, which are also observed with respect to the protein content of tissues, seem to occur at the expense of the remaining tissues (carcass) especially muscle, but the contribution of skin is probably also important (Wusteman *et al.* 1994). The increase in the size of organs such as the liver is not due to an acute inflammatory reaction within the tissue because histology is normal (Wusteman *et al.* 1990;

Table 2. Percentage hydration of the tissues of rats injected with turpentine or saline and pair-fed controls, fed on diets containing 200 or 30 g protein/kg‡
(Mean values and standard deviations for six rats per group)

	Saline		Pair-fed		Turpentine	
	Mean	SD	Mean	SD	Mean	SD
200 g protein/kg						
Liver	70.32	0.51	70.41	0.55	70.57	0.77
Heart	80.95	2.07	82.48	0.75	79.67***	1.10
Kidney	77.6	1.44	79.42†	1.16	77.05**	1.29
Lung	82.16	1.89	84.45†	1.27	82.85	2.44
Large intestine	76.09	1.99	82.19†††	1.74	77.32*	5.94
Small intestine	72.15	2.92	67.81	4.12	69.36	1.89
Muscle	76.44	1.02	77.35	0.85	76.41	0.93
Carcass	63.86	1.61	61.67	1.67	63.56	1.92
30 g protein/kg						
Liver	69.93	0.66	69.03	0.58	70.73**	1.10
Heart	73.04	1.47	76.18††	1.08	77.55*†††	0.93
Kidney	75.43	0.74	74.00	1.47	76.50**	1.03
Lung	74.93	4.24	78.68	1.79	80.41††	2.30
Large intestine	72.08	1.97	73.00	4.43	70.28	3.93
Small intestine	72.04	0.84	72.09	1.15	73.08†	0.53
Muscle	70.11	1.39	75.26†††	0.96	73.52*††††	0.69
Carcass	65.98	0.77	68.65†††	1.15	68.57*††††	1.20

Mean values were significantly different from those of the pair-fed group: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Mean values were significantly different from those of the saline group: † $P < 0.05$, †† $P < 0.01$, ††† $P < 0.001$. ‡ For details of diets and procedures, see pp. 124–125.

Jennings *et al.* 1992*b*). The reason for the 'preferential' preservation of central organs, particularly the liver and lung, is not clear but these tissues are major components of the reticuloendothelial system which frequently increases its activity after injury. The activity and energy cost of metabolic processes such as gluconeogenesis and the overall hepatic protein synthetic rate (Wusteman & Elia, 1990), including acute-phase-protein synthesis, are also increased after injury. In addition, the energy cost of maintaining a larger hepatic cell mass (e.g. Na pump activity and protein turnover) will contribute to the energy expenditure of splanchnic tissues, but an increase in metabolic rate per gram of tissue is also possible (Elia, 1991). In normally nourished animals injected with turpentine the loss of large intestine appears to be due to the reduced feed intake, rather than to the systemic effect of injury. However the small intestine of normally nourished animals injected with turpentine shows no significant change compared with that of the saline-injected animals. The present study has not distinguished between mucosal and non-mucosal effects, but a previous study investigating the effects of single (Wusteman *et al.* 1995*b*) or repeated injections of turpentine (Wusteman *et al.* 1994) on the small-intestinal mucosa concluded that there was substantial loss of mucosal mass, which was reproduced by pair-feeding.

The present study also demonstrates that changes in organ size do not necessarily reflect the change in tissue composition after injury. Reduced feed intake in normally nourished animals produced an increase in the hydration fraction of several tissues, whilst the same dietary restriction in animals injected with turpentine produced no such effect. In contrast, turpentine injection in protein-malnourished animals increased the hydration fraction of some tissues but pair-feeding did not. Therefore it is possible for some tissues to show

Table 3. *Tissue protein content (g/kg body weight) in rats injected with turpentine or saline and pair-fed controls, fed on diets containing 200 or 30 g protein/kg†*

(Mean values and standard deviations for six rats per group)

	Saline		Pair-fed		Turpentine	
	Mean	SD	Mean	SD	Mean	SD
200 g protein/kg						
Liver	8.82	0.66	7.8††	0.34	10.29***†††	0.37
Heart	0.59	0.10	0.56	0.04	0.73*	0.15
Kidney	1.38	0.16	1.28	0.12	1.70***††	0.09
Lung	1.01	0.29	0.90	0.14	1.34*	0.34
Large intestine	0.89	0.08	0.49†††	0.07	0.56†††	0.16
Small intestine	3.09	0.44	2.71	0.16	2.85	0.33
Muscle	1.24	0.06	1.19	0.07	1.16†	0.10
Carcass	204.1	17.2	189.8	2.2	189.9	7.7
30 g protein/kg						
Liver	7.06	0.41	7.17	0.18	8.09**††	0.50
Heart	0.89	0.09	0.82	0.04	0.86	0.06
Kidney	1.66	0.07	1.68	0.07	1.80*†	0.10
Lung	1.41	0.21	1.23	0.10	1.45	0.44
Large intestine	0.99	0.08	1.01	0.11	0.98	0.10
Small intestine	3.55	0.36	3.21	0.35	3.41	0.51
Muscle	1.43	0.12	1.35	0.13	1.19*††	0.09
Carcass	206.0	3.9	187.7†††	8.5	182.6†††	8.4

Mean values were significantly different from those of the pair-fed group: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Mean values were significantly different from those for the saline group: † $P < 0.05$, †† $P < 0.01$, ††† $P < 0.001$. † For details of diets and procedures, see pp. 124–125.

Table 4. *Plasma α_2 -macroglobulin concentrations (g/l) in rats injected with turpentine or saline and pair-fed controls, fed on diets containing 200 or 30 g protein/kg†*

(Mean values and standard deviations for six rats per group)

Diet...	200 g protein/kg		30 g protein/kg	
	Mean	SD	Mean	SD
Saline	< 0.05		0.11	0.02
Turpentine	4.85	0.55	1.28***	0.28
Pair-fed	< 0.05		0.10	0.01

*** Mean value was significantly different from that for the corresponding 200 g/kg group: $P < 0.001$. † For details of diets and procedures, see pp. 124–125.

significant changes in their protein content but not their mass (or vice versa). For example, turpentine injection in normally nourished animals provided a significant increase in the protein content of the heart (compared with pair-fed controls), but not in its mass.

Finally, the present study has not only confirmed that protein deficiency attenuates the acute-phase-protein response (Jennings & Elia, 1991, 1992; Jennings *et al.* 1992a), but it has also demonstrated that protein deficiency attenuates two other responses to injury. One of these is the anorectic effect of injury, which was essentially non-existent in the protein-deficient animals during the first 48 h. The second is the attenuation in the protein

distribution induced by turpentine. The cause of these attenuations is not clear, but theoretically it may be due to reduced local reaction to turpentine administration, which was not observed in a previous study (Jennings *et al.* 1992*b*), or to either altered hormonal and/or cytokine responses, or altered tissue responsiveness to these signals.

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