The effect of malnutrition on the metabolic response to surgery

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The effect of previous malnutrition on the metabolic response to surgical hysterectomy was investigated in adult female rats. Malnutrition was achieved by feeding a 20 g protein/kg diet and restricting food intake to 50 % of normal. This dietary regimen was maintained for 3 weeks before surgery and for 4 d after surgery. Unoperated control rats were pair-fed with the hysterectomized rats after surgery. Energy balance was measured by the comparative carcass technique and, in a second experiment, urinary N excretion was measured. Surgery caused energy expenditure to increase by 37 % in *ad libitum*-fed rats but in malnourished rats it increased by only 22 %. Urinary N excretion rose immediately after surgery. In the *ad libitum*-fed rats it was on average 85 % greater in hysterectomized rats than controls for the first 3 d after surgery, whereas in the restricted rats it was 74 % greater on the first day and not significantly elevated thereafter. Thus, malnutrition attenuated the metabolic response to surgery but did not abolish it completely.

Surgery: Malnutrition: Nitrogen excretion: Energy balance

Both accidental injury and surgical trauma cause increases in energy expenditure and urinary N excretion (Cuthbertson, 1932; Tilstone & Cuthbertson, 1970). These responses result from underlying changes in the metabolism of fuels and substrates, including increased gluconeogenesis, lipolysis and net breakdown of muscle protein (Wilmore & Kinney, 1981), which appear to be designed to support the activation of inflammatory and reparative mechanisms, and thus to promote recovery. However, nutrient intake is often also sharply reduced at this time, and if the metabolic responses are too severe or unduly prolonged they can lead to nutritional depletion in vulnerable patients, and thus have a negative effect on clinical outcome (Dempsey *et al*. 1988). Hence it is often considered important to try to minimize protein breakdown and energy loss.

The metabolic response to injury is quite different from the normal response to decreased food intake, which leads to decreases in energy expenditure and urinary N excretion (Benedict, 1915). However, it is not known whether decreased food intake has the same effect in traumatized subjects, and thus whether adaptation to chronically low nutrient intake might actually suppress the normal metabolic response to injury. It is known that malnutrition impairs many aspects of immune function (Chandra, 1991) and this is believed to be one of the reasons for the association of poor nutritional status with poor outcome following surgery (Dempsey *et al*. 1988). Since the same cytokines are thought to be involved in mediating both the metabolic and the immunological responses to injury, it is quite possible that malnutrition would suppress both responses. It is clearly important to establish whether this is so, since it would imply that attempts to minimize the metabolic response might actually have adverse clinical consequences.

There appear to be no reports in the literature of systematic studies to investigate the effects of malnutrition on the metabolic response to injury in patients. This may be largely because of the difficulty in matching groups of subjects for severity of injury and degree of malnutrition, and because it is often difficult to assess the degree of malnutrition separately from the effect of the severity of the underlying disease.

We have therefore used an animal model to examine the effects of previous malnutrition on the metabolic response to surgical injury. Hysterectomy was chosen as the surgical stress since this was found to cause decreased food intake, and elevated energy expenditure and N loss which lasted for up to 4 d (AR Bosagh Zadeh and PW Emery, unpublished results). Nutritional depletion was achieved by restricting the intake of both protein and energy in adult rats. In the first experiment we measured energy expenditure by the comparative carcass technique. Preliminary data from this experiment have been reported in abstract form (Bosagh Zadeh & Emery, 1998). We then carried out a second experiment to provide more detailed information on the loss of protein by measuring urinary N excretion.

Abbreviations: GE, gross energy; ME, metabolizable energy.

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Methods

Mature female Sprague-Dawley rats (initial weight 165–175 g, age 6–7 weeks) were housed individually throughout both experiments at 22° with a 12 h light–dark cycle (lights on at 07.00 hours).

Experiment 1

Twenty-two rats were fed *ad libitum* for 3 weeks on a semipurified diet containing 170 g protein/kg while a further twenty rats were fed on a diet containing 20 g protein/kg in amounts restricted to 50 % of the mean intake of the *ad libitum*-fed rats (see Table 1 for diet composition). After 3 weeks four rats from each group were killed for analysis of initial carcass composition. The remaining rats were paired on the basis of similarity of body weight and growth rate. One from each pair then underwent hysterectomy while the other was kept as an unoperated, pair-fed control.

Hysterectomy was performed under halothane anaesthesia (3 % for induction, 2 % for maintenance, in a 2 : 1 (v/v) mixture of N_2O and O_2). A 70 mm mid-line incision was made into the peritoneum and the uterus was removed but the ovaries were left intact. The muscle layer was sutured with continuous 2/0 silk, and the skin was closed with stainless steel clips. The whole procedure lasted approximately 20 min, and the animals were fully conscious within another 5 min.

After surgery the operated rats were returned to the same dietary treatments as before surgery, while the non-operated controls were pair-fed with the operated rats on an individual basis. Food intake and body weight were measured daily. At 4 d after surgery the rats were killed and their carcasses analysed.

Analytical methods

The stomach, intestines and caecum were removed from the carcasses, their contents were flushed out and they were returned to the carcasses. The carcasses were then oven-dried at 105° for 3 d to determine water content. The dried carcasses were then thoroughly ground using a pestle and mortar and a coffee grinder, and the fat content of 10 g portions was determined from the loss in weight following Soxhlet extraction with light petroleum (b.p. 60–80°). N content of dried, defatted carcass samples was determined by the Kjeldahl method.

Table 1. Composition of the diets

Ingredient	Normal diet (g/kg)	Low protein diet (g/kg)
Casein	198	30
Maize starch	300	385
Sucrose	300	385
Maize oil	100	100
Cellulose	40	40
Mineral mix*	40	40
Vitamin mix†	20	20
DL-Methionine	2	0
Protein content	165	19·4
Metabolizable energy content (kJ/g)	14·4	15·5

* Bernhart & Tomarelli (1966).
† Naismith *et al.* (1969).

Energy balance calculations

The gross energy (GE, kJ/g) content of the diet was determined by ballistic bomb calorimetry, and the N content (g/100 g) was determined by the Kjeldahl method. Metabolizable energy (ME, kJ/g) content was then calculated from the following formula (Miller & Payne, 1959):

$$ME = 0{\cdot}95 \times GE - 0{\cdot}314 \times N.$$

The energy content of the carcasses was calculated from their fat and protein contents assuming values of 38·6 kJ/g fat and 22·7 kJ/g protein (Miller & Dulloo, 1984). The carcass energy content of the rats killed on the day of surgery was used to calculate a value for initial energy content per unit live weight, and this value was then applied to the starting weight of each rat in the experimental groups to determine initial body energy content. Thus the amount of energy stored over the 4 d postoperative period was calculated from the difference between initial and final energy content for each rat.

Energy expenditure was calculated from the difference between ME intake and energy stored in each rat.

Experiment 2

Twelve rats were fed *ad libitum* for 3 weeks on a semipurified diet containing 170 g protein/kg while a further twelve rats were fed on a diet containing 20 g protein/kg in amounts restricted to 50 % of the mean intake of the *ad libitum* fed rats (see Table 1 for diet composition). After 2 weeks the rats were transferred to metabolism cages where they remained for the rest of the experiment. At 1 week after transferring the rats to the metabolism cages the rats were paired on the basis of similarity of body weight and growth rate. One from each pair underwent hysterectomy (see Expt 1 for details) while the other was kept as an unoperated, pair-fed control. After surgery the operated rats were returned to the same dietary treatments as before surgery, while the non-operated controls were pair-fed with the operated rats on an individual basis. Urine was collected for the last 2 d before surgery and the first 4 d after surgery, and analysed for N content by the Kjeldahl method.

Statistics

Differences between operated rats and pair-fed controls were assessed using paired *t* tests. A probability $< 0{\cdot}05$ was considered significant.

Results

Experiment 1

The amount of food offered to the restricted groups of rats during the 3 weeks before surgery was maintained constant at 8 g/d. This amounted to 48 % of the average intake of the *ad libitum*-fed rats over the same period (Fig. 1). The combination of restriction in energy and protein intake resulted in a loss of body weight of 19 % over the 3-week

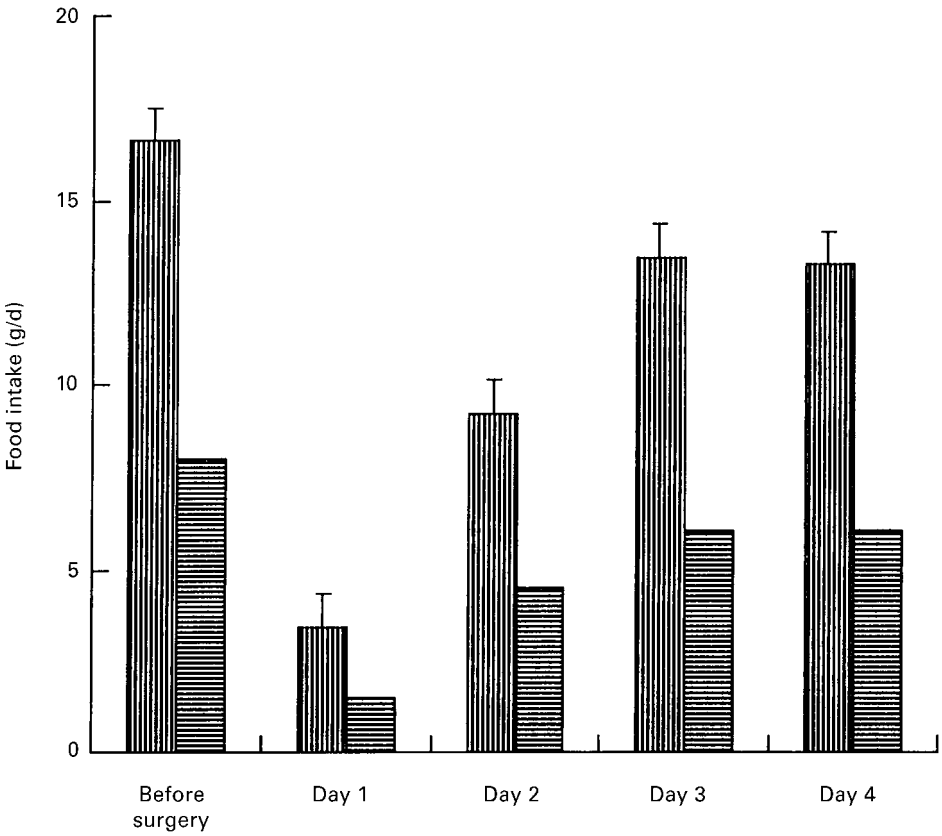

Fig. 1. Food intake of *ad libitum*-fed (▥), and restricted (▤) rats before and after surgery (Expt 1). Restricted rats (*n* 20) were fed on a low protein diet in amounts restricted to 50 % of the mean intake of the *ad libitum*-fed rats who received a normal protein diet (*n* 22). For details of diets, see Table 1.

preoperative period, while the *ad libitum*-fed rats increased their body weight by 31 %.

After surgery food intake fell sharply by 80 % on the first day, then recovered to within 20 % of the preoperative value by day 4 (Fig. 1). Food intake of the restricted groups was maintained at 44–49 % of the values for the *ad libitum*-fed groups postoperatively.

The body weights of all the rats fell immediately after surgery (Table 2). In the *ad libitum*-fed surgical rats it increased slightly over the next 3 d, whereas in the corresponding pair-fed controls it remained constant, so that by day 4 there was a small but significant difference in body weight. In contrast, body weight continued to decrease in both restricted groups throughout the postoperative period.

Table 2. Body weights of rats at the start of the experiment and before and after surgery

	Ad libitum-fed rats†				Restricted-fed rats†			
	Surgical		Control		Surgical		Control	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Expt 1								
No. of rats	9		9		8		8	
Initial body weight (g)	166	5	171	1	167	2	164	1
Body weight before surgery (g)	211	5	210	5	134	1	134	1
Body weight 1 d after surgery (g)	201	6	199	6	127	2	125	1
Body weight 4 d after surgery (g)	207*	6	200	7	120	2	121	2
Expt 2								
No. of rats	6		6		6		6	
Initial body weight (g)	171	3	174	3	173	3	174	3
Body weight before surgery (g)	207	5	209	2	144	1	143	2
Body weight 1 d after surgery (g)	198	6	204	3	135	2	138	2
Body weight 4 d after surgery (g)	205*	4	199	3	124*	1	117	1

Mean values were significantly different from the corresponding control group, *$P < 0{\cdot}05$.
† For details of diets see Table 1. Restricted rats were fed on a low protein diet in amounts restricted to 50 % of the mean intake of the *ad libitum*-fed rats who received a normal protein diet.

Table 3. Body composition of rats 4 d after surgery

	Ad libitum-fed rats†				Restricted-fed rats†			
	Surgical		Control		Surgical		Control	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
No. of rats	9		9		8		8	
Body water (g)	137*	4	130	4	83·6	1·2	83·1	0·9
Body protein (g)	37·3**	1·2	38·6	1·3	24·8*	0·4	25·7	0·3
Body fat (g)	15·6*	0·9	19·8	1·5	2·4	0·2	3·6	0·6

Mean values were significantly different from the corresponding control group: *$P < 0{\cdot}05$, **$P < 0{\cdot}01$.
† For details of diets see Table 1. Restricted rats were fed on a low protein diet in amounts restricted to 50 % of the mean intake of *ad libitum*-fed rats who received a normal protein diet.

The excess body weight in the operated rats appears to have been caused by fluid retention, since body water content was significantly greater in the *ad libitum*-fed surgical rats than the corresponding pair-fed controls when they were killed 4 d after surgery (Table 3). On the other hand there was no significant difference in body water content between restricted surgical rats and their pair-fed controls.

Surgery caused a significant reduction in body fat content by an average of 4·2 g in the *ad libitum*-fed rats (Table 3). In the restricted rats surgery caused a much smaller reduction in body fat content (mean 1·2 g), which was not statistically significant. This may have been because the malnourished rats had already lost virtually all their fat reserves, so that very little more fat could be lost without breakdown of cell membranes with catastrophic consequences. The loss of fat following surgery represented 21 % of the fat reserves of the *ad libitum*-fed animals, and 30 % in the case of the restricted rats.

Surgery also caused a significant reduction in body protein content in both the *ad libitum*-fed rats and the restricted rats (Table 3).

The effects of surgery on energy balance are shown in Table 4. Initial carcass energy content for each rat was calculated from data on matched groups of rats killed on the day of surgery. For rats fed *ad libitum* the mean value for initial carcass energy was 8·21 (SE 0·17) kJ/g live weight, and for restricted rats it was 6·14 (SE 0·13) kJ/g live weight. In the *ad libitum*-fed surgical rats there was a considerable decrease in energy stores during the postoperative period, while the pair-fed controls were in approximate energy balance. Thus, the calculated energy expenditure was significantly greater in the operated rats than in the controls. In the restricted rats surgery also caused an increased loss of body energy stores, again indicating increased energy expenditure in operated rats as compared with pair-fed controls. However, the magnitude of the increase in energy expenditure was much smaller in the restricted rats than those fed *ad libitum* (234 (SE 63) *v.* 64 (SE 17) kJ/4 d, $P < 0{\cdot}05$).

Experiment 2

Dietary restriction achieved a 44 % reduction in food intake before surgery and a 50 % reduction in food intake after surgery (results not shown). The intake of the *ad libitum*-fed rats was on average 33 % lower after surgery than before, similar to the 41 % decrease observed in Expt 1. The effects of malnutrition and surgery on body weight were also very similar to those observed in Expt 1 (Table 2), except that the final body weight of the restricted surgical rats was slightly higher than that of their controls, presumably again because of fluid retention.

Surgery caused a considerable increase in urinary N excretion during the first 2 d after surgery, particularly in the *ad libitum*-fed rats (Fig. 2). In the pair-fed controls urinary N excretion tended to be lower after the surgical group had undergone surgery than before, because of the decrease in intake. Thus, urinary N excretion was significantly higher in the *ad libitum*-fed surgical rats than in the corresponding pair-fed controls on each of the first 3 d after surgery (mean differences between surgical and control rats were 86, 136, 28 and −10 % of the control values on days 1, 2, 3 and 4 respectively). In the restricted rats surgery caused a much smaller increase in urinary N excretion, and the value in the operated rats was only significantly different from that in the pair-fed controls on day 1 (mean differences

Table 4. Energy balance in rats over the 4 d period following surgery

	Ad libitum-fed rats†				Restricted-fed rats†			
	Surgical		Control		Surgical		Control	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
No. of rats	9		9		8		8	
Energy intake (kJ/4 d)	623	29	614	33	278		278	
Energy stored (kJ/4 d)	−238**	46	−26	44	−76**	8	−13	15
Energy expenditure (kJ/4 d)	874**	42	640	33	355**	8	291	15

Mean values were significantly different from the corresponding control group: **$P < 0{\cdot}01$.
† For details of diets see Table 1. Restricted rats were fed on a low protein diet in amounts restricted to 50 % of the mean intake of the *ad libitum*-fed rats who received a normal protein diet.

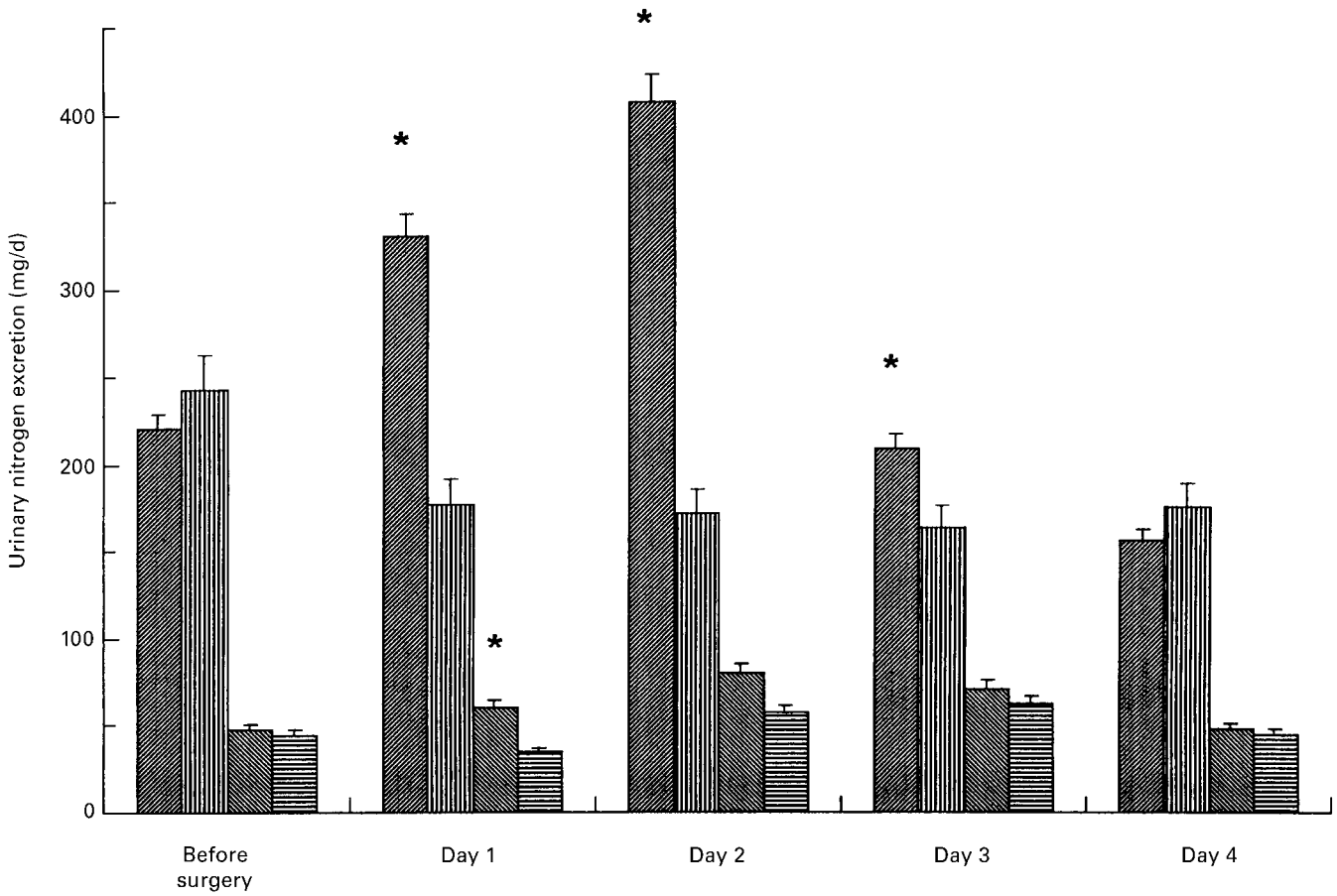

Fig. 2. Urinary nitrogen excretion before and after surgery in *ad libitum*-fed hysterectomized rats (▨), *ad libitum*-fed unoperated controls (▥), dietary restricted hysterectomized rats (▧), and dietary restricted unoperated controls (▤). Restricted rats (*n* 12) were fed on a low protein diet in amounts restricted to 50 % of the mean intake of the *ad libitum*-fed rats who received a normal protein diet (*n* 12). For details of diets, see Table 1. Mean values were significantly different from the corresponding control group: $*P < 0{\cdot}05$.

between surgical and control rats were 74, 40, 14 and 8 % of the control values on days 1, 2, 3 and 4 respectively). Over the first 3 postoperative days the *ad libitum*-fed operated rats excreted 434 mg N more than their pair-fed controls, whereas the restricted operated rats excreted only 57 mg N more than their pair-fed controls.

Discussion

These results clearly show that previous malnutrition reduces the size of both the increase in energy expenditure and the increase in N excretion following surgery, but does not completely abolish either of these features of the metabolic response to trauma. Previous studies (Munro & Cuthbertson, 1943; Cairnie *et al*. 1957) suggested that feeding a protein-free diet for 1 week before fracturing the femur prevented the subsequent increases in metabolic rate and urinary N excretion. However, the first of these reports (Munro & Cuthbertson, 1943) is an abstract with no data while the data in the other (Cairnie *et al*. 1957) actually show that there was a small increase in both metabolic rate and urinary N excretion in the rats fed on the protein-free diet. Unfortunately there were no controls and no statistical information, so it is not possible to evaluate the significance of these data. Data from one controlled study (Munro & Chalmers, 1945) also show a small but significant increase in N excretion following femur fracture in rats fed on a protein-free diet. That study also showed that the increase in N excretion was greater in rats fed on a 250 g protein/kg diet than on a 100 g protein/kg diet.

Previous malnutrition has also been shown to affect the magnitude of the acute-phase protein response to inflammation. Thus Jennings *et al*. (1992*a*) showed that the increase in plasma concentration of α_2-macroglobulin following subcutaneous injection of turpentine was attenuated in rats fed on a low-protein diet, although the fall in serum albumin concentration was at least as great as normal in the protein-depleted animals. On the other hand, the same authors have shown that simultaneous restriction of both protein and energy intakes did not attenuate the acute-phase protein response (Jennings & Elia, 1992). The acute-phase protein response to inflammation is accompanied by anorexia and an increase in the protein content of the liver and some other visceral organs, and these changes are also attenuated by a low-protein diet (Jennings & Elia, 1996).

Both the acute-phase protein response and the metabolic response to trauma appear to be mediated by cytokines. Production of the cytokine interleukin 6 in response to

turpentine injection is delayed but not diminished in magnitude by protein deficiency (Jennings *et al*. 1992*b*). However, dietary protein depletion has been shown to attenuate the acute-phase protein response to injection of endogenous pyrogen (Bell & Hoffman-Goetz, 1982), tumour necrosis factor-α (Grimble *et al*. 1992) and interleukin 1 (Drabik *et al*. 1987). Thus the results of the present study may represent a diminished response of certain metabolic processes to cytokine production in malnourished animals. There was certainly no evidence of any delay in the metabolic response since urinary N excretion peaked on day 1 after surgery in the malnourished rats.

The degree of malnutrition induced in this study was very severe. We have found that rats subjected to this regimen are unable to survive even a brief (24 h) period of postoperative fasting (PW Emery and YH Loh, unpublished results). Although some degree of undernutrition is not uncommon among many groups of patients in hospitals (Lennard-Jones, 1992), patients with a 35 % deficit in body weight would be rare and would have a very high risk of postoperative complications. Such patients clearly require nutritional support, but their small catabolic response to surgery is unlikely to add significantly to their nutritional depletion. It would, thus, not be necessary to attempt to suppress their catabolic response any further, and in fact doing so could potentially be counterproductive.

The increased energy expenditure and N excretion which follow injury are usually taken to indicate mobilization of amino acids and gluconeogenesis to support phagocytosis and wound healing. These processes may be vital for recovery, since peripheral release and central clearance of amino acids has been found to be higher in patients with sepsis who survived than in those who did not (Clowes *et al.* 1985). Our results suggest that these processes have a high biological priority since they still occurred in severely malnourished animals. Thus, the aim of nutritional support should be to support these processes rather than suppress them, since they may be obligatory for optimal recovery from injury.

Acknowledgement

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