Sequential changes in in vivo muscle and liver protein synthesis and plasma and tissue glutamine levels in sepsis in the rat

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ABSTRACT

We have investigated sequential changes in skeletal muscle and hepatic protein synthesis following sepsis, and their relationship to changes in circulating and tissue glutamine concentrations. Male Wistar rats underwent caecal ligation and puncture (CLP) or sham operation, with starvation, and were killed 24, 72 or 96 h later. A group of non-operated animals were killed at the time of surgery. Protein synthesis was determined using a flooding dose of L-[4-3H] phenylalanine, and glutamine concentrations were measured by an enzymic fluorimetric assay. Protein synthesis in gastrocnemius muscle fell in all groups. Gastrocnemius total protein content was reduced after CLP and at 72 and 96 h after sham operation. After CLP, protein synthesis was lower at 24 h, and total protein content was lower at 72 and 96 h, than in sham-operated animals. CLP was associated with increased liver protein synthesis at all time points, whereas there was no change after sham operation. Liver protein content did not change after CLP, but was lower at 72 and 96 h after sham operation than in non-operated animals. Plasma glutamine concentrations were reduced at 24 h after sham operation, and at 72 and 96 h after CLP. Muscle glutamine concentrations were reduced in all groups, with the decrease being greater following CLP than after sham operation. In the liver, glutamine concentrations were unchanged after CLP, but increased after sham operation. In rats with sepsis, decreases in muscle protein synthesis and content are associated with markedly reduced muscle glutamine concentrations. Plasma glutamine concentrations are initially maintained, but fall later. In liver, protein synthesis is increased, while glutamine concentrations are preserved. These results support a peripheral-to-splanchnic glutamine flux in sepsis.

INTRODUCTION

Sepsis, trauma and major surgery are associated with increased nitrogen losses and whole-body muscle wasting [1,2]. The ensuing muscle weakness delays weaning from ventilatory support and subsequent mobilization, predisposes to nosocomial infections and the complications of prolonged immobility [3,4], and delays recovery. Protein depletion may also compromise immune function and delay wound healing. Despite increased aware-

Key words: animal disease models, caecal ligation and puncture, critical illness, glutamine/metabolism, liver, muscle, protein synthesis, rats, sepsis, surgery.

Abbreviations: CLP, caecal ligation and puncture; k_s, fractional rate of protein synthesis; LAP animals, rats having undergone sham operation (laparotomy).

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ness of these problems and the widespread use of aggressive nutritional support, increased protein loss and muscle wasting continue to prejudice the recovery of critically ill patients.

Muscle catabolism in critical illness may, however, have some short-term advantages, such as the provision of essential substrates for hepatic acute-phase protein synthesis and increased immune cell replication. Glutamine is the most important carrier of ammonia from the peripheral tissues to the splanchnic area, and serves as an oxidation fuel during cell division [5]. It is a donor of nitrogen for DNA and RNA synthesis, and is essential for the proliferation of cells. Glutamine is the principal metabolic fuel for gut mucosal cells [6], lymphocytes and monocytes [7,8]. Although traditionally considered to be a ‘non-essential’ amino acid, glutamine requirements increase markedly during an acute illness, and utilization may exceed endogenous production. Recently there has been considerable interest in the possibility that addition of glutamine to nutritional regimens for critically ill patients may restore depleted plasma and muscle levels, improve nitrogen balance, and, by stimulating protein synthesis, prevent muscle wasting [9] and ultimately improve outcome [10].

Although the effects of surgical stress and sepsis on protein and amino acid metabolism have been studied extensively [11–14], the sequential changes in muscle and liver protein synthesis rates in vivo have not been well characterized, many previous investigations having been carried out in isolated tissues in vitro [15–17]. Furthermore, the relationship between sepsis-induced changes in protein synthesis and circulating, muscle and liver glutamine levels have not previously been investigated. This information is of fundamental importance when planning or interpreting studies investigating the potential value of glutamine supplementation in sepsis. Moreover, other potential anabolic interventions, such as treatment with growth hormone, may interfere with normal glutamine fluxes, possibly with deleterious effects. This mechanism has been cited as a possible cause of the increased mortality observed with growth hormone treatment in long-stay critically ill adults [18].

The diversity of underlying diseases and infecting organisms associated with sepsis, as well as the effects of treatment, makes in vivo studies in humans very difficult, and investigating changes in individual organs is virtually impossible. In the present study we employed caecal ligation and puncture (CLP) in the rat, which is a widely accepted, useful and clinically relevant model of severe sepsis and multiple-organ dysfunction [19,20]. The advantages of this model include its reproducibility and the fact that it produces bacteraemia with a slowly evolving septic insult, which is particularly suitable for metabolic studies. The clinical signs, physiological changes and metabolic responses are well described and are similar to those in human sepsis [20]. The major disadvantage of the CLP model is precisely its similarity to peritonitis in humans, in that it is difficult to control the magnitude of the septic challenge, the response of individual animals is variable, and a proportion of them will die. Because only survivors are studied, it is possible that the overall magnitude of the measured responses will be underestimated.

Here we describe sequential changes in vivo in skeletal muscle and hepatic protein synthesis and content in response to surgery and sepsis, as compared with surgery alone, in the rat, as well as the relationship between these changes and alterations in circulating and tissue glutamine concentrations.

**METHODS**

**Treatment of animals**

Male Wistar rats (body weight 238–280 g) were obtained from Tuck & Co., and housed in a temperature-controlled environment on a 12 h light/12 h dark cycle. Rats were initially housed three to a cage and given access to food and drink ad libitum for 5 days to allow for acclimatization. On the fifth day in the animal house (the first day of the experiment), 99 animals were ranked and back-ranked to produce seven groups of similar mean weight. Three groups of 17 animals each underwent CLP, and were killed 24 h (CLP24), 72 h (CLP72) or 96 h (CLP96) later. Three groups of 12 animals each were subjected to laparotomy alone (sham operation; LAP) and killed after the same time intervals (LAP24, LAP72 and LAP96). Our objective in this study was to determine the effects attributable to sepsis compared with those of surgery alone. As we had found in previous studies of CLP in rats that similar-sized animals consumed less than 5 g of food/day for up to 5 days after surgery [21], in the present study we denied animals access to food (but allowed water) from the time of surgery to the time they were killed. This precluded the need for animals to be pair-fed to the CLP group, and allowed the number of animals required for the study to be reduced without compromising the validity of our conclusions regarding the effects of sepsis. An additional group of 12 animals, housed in standard cages and allowed free access to food and water, was killed on the first day of the experiment to provide baseline data (group ad libitum 80). The study was approved by the U.K. Home Office under the provisions of the Animals (Scientific Procedures) Act 1986, and the care and handling of the animals were in accordance with National Institutes of Health guidelines.

In all animals, a midline laparotomy was performed under inhalational anaesthesia with halothane (1–4 %) in 30 % oxygen and nitrous oxide. The caecum was identified and lifted out of the peritoneal cavity. In CLP animals the caecum was then ligated tightly at its base in
such a manner that bowel continuity was preserved, and punctured in a single pass through the anterior and posterior walls using a 23G needle. The punctured caecum was squeezed gently to extrude faecal matter, and was then returned to the peritoneal cavity. The laparotomy was repaired with a continuous nylon suture. In LAP animals the caecum was squeezed gently only, and then returned to the abdomen and the laparotomy was repaired. At the end of the operation the animals were given 2.5 ml/100 g body weight of 0.9% NaCl containing 0.3 mg/kg buprenorphine intraperitoneally to provide fluid resuscitation and analgesia. All animals were returned to individual wire-bottomed cages post-operatively to prevent coprophagia.

One animal died from group LAP24, and one from group LAP72 was humanely killed following abdominal dehiscence at 12 h. Cannulation/injection failure occurred in other animals, such that eight LAP24 animals, 11 LAP72 animals and 12 LAP96 animals could be evaluated. There was no mortality from CLP at 24 h, but cannulation/injection failure occurred in two animals from this group. Seven animals died from group CLP72 (41%) and seven from group CLP96; there were no cannulation/injection failures in these groups. Mortality plus the cannulation/injection failures thus left 15 CLP24, 10 CLP72 and 10 CLP96 animals that could be evaluated. Animals were killed by decapitation. At 10 min before the planned time of death, animals were immobilized briefly by wrapping them in a porous towel, a tail vein was cannulated and 150 μmol/100 g body weight t-[4-3H]phenylalanine (Amersham International, Amersham, Bucks., U.K.) was injected in a volume equivalent to 1 ml/100 g body weight for measurement of protein synthesis as described below. The animals were then unwrapped and returned to their cages. On sacrifice, blood was collected for 10 s into lithium/heparin tubes on ice using heparinized funnels. Blood was centrifuged at 3000 g for 4 °C for 10 min, and plasma was decanted and stored at −70 °C until analysis was performed on one piece only, with the second piece being used for glutamine assay. Similarly, two slices, each weighing approx. 500 mg, were cut from the liver for measurement of protein synthesis and glutamine concentration.

All processing steps were carried out at 0–4 °C unless stated otherwise. Muscle and liver specimens were homogenized and then precipitated in 10 ml of 0.2 mol/l HClO₄ (perchloric acid). After centrifugation (2000 g, 4 °C, 15 min) the supernatant was decanted for subsequent neutralization and measurement of the specific radioactivity of free phenylalanine (S₀; see below). The protein pellet was washed twice in 10–12 ml of 0.2 mol/l HClO₄ with additional centrifugation (2000 g, 4 °C, 15 min) and aspiration of the supernatant to waste. The protein pellet was then digested in 10 ml of 0.3 mol/l NaOH (37 °C, 60 min) to further disrupt any cellular compartments that still contained free phenylalanine. The protein was then re-precipitated with 2 ml of 2 mol/l HClO₄ and the supernatants were used to measure RNA content. The protein pellets were then washed an additional six to eight times with 12–14 ml of 0.2 mol/l HClO₄. Protein pellets were hydrolysed in 3 ml of 6 mol/l HCl for 36 h at 105 °C. The hydrolysates were vacuum-dried using a rotary HCl evaporator (Genevac, Ipswich, U.K.). Dried residues were then suspended in 3.0 ml of sodium citrate buffer (pH 6.3; 1.5 mol/l) and samples were stored at 0–4 °C until processed for measurement of the specific radioactivity of protein-bound phenylalanine (Sᵣ; see below).

Solutions containing 5 ml of the neutralized acid-soluble fraction of tissue homogenates or 3 ml of the protein hydrolysates were incubated with phenylalanine decarboxylase in 1 ml of sodium citrate buffer (pH 6.3; 1.5 mol/l) at 52 °C for 24 h for conversion of phenylalanine into 2-phenylethylamine. Solutions were then made alkaline by addition of 0.5 ml of 6 mol/l NaOH and vortexed. After addition of 10 ml of n-heptane and additional vortexing, tubes were centrifuged (500 g, 3 min, 20 °C) and placed in a solid-CO₂/ethanol mixture to freeze the aqueous phase. The solvent phase was poured into separate tubes and then acid-extracted by addition of 4 ml of 0.002 mol/l H₂SO₄. After vortexing and centrifugation (500 g, 3 min, 20 °C), the tubes were placed into the solid-CO₂/ethanol mixture and the aqueous phase was frozen. The solvent phase was then poured to waste and the aqueous phase was allowed to thaw. An aliquot of the aqueous phase was then assayed by fluorimetry or counted for radioactivity by scintillation spectrometry.

The biuret method, as described by Gornall et al. [23], was used for determining protein, and is based on the reaction of protein with CuSO₄ in the presence of alkali. DNA was measured by a fluorimetric method similar to that described by Downs and Wilfinger [24]. The basis of

Measurement of protein synthesis rates, RNA and DNA activities, and tissue RNA, DNA and total protein contents

Rates of protein synthesis were measured using a ‘flooding dose’ technique employing t-[4-3H]phenylalanine, as described previously by Garlick et al. [22]. Before being frozen, the gastrocnemius muscle was divided into two pieces of similar weight; subsequently analysis was performed on one piece only, with the second piece being used for glutamine assay. Similarly, two slices, each weighing approx. 500 mg, were cut from the liver for measurement of protein synthesis and glutamine concentration.
this assay is that bisbenzinimiazole (Hoechst 33258) has a high affinity for the large grooves of the DNA helix. This binding and interaction of Hoechst reagent with the DNA produces a large increase in fluorescence.

RNA determinations were carried out using the twowavelength UV spectrophotometric method of Ashford and Pain [25], as described by Siddiq et al. [26]. This method of RNA estimation relies upon the strong UV absorption of purine and pyrimidine bases at 260 nm ($A_{260}$). To correct for any interfering substances, absorbances are also measured at 232 nm ($A_{232}$). The RNA concentration in muscle was determined using the equation:

$$\text{RNA (\mu g/ml) = } [(32.9 \times A_{260}) - (6.11 \times A_{232})]$$

The RNA concentration in liver was determined using the following equation, devised by Munro and Fleck [27]:

$$\text{RNA (\mu g/ml) = } [(3.4 \times A_{260}) - (1.44 \times A_{232})] \times 10.53$$

The fractional rate of protein synthesis ($k_s$), defined as the percentage of tissue protein renewed by synthesis each day, was calculated from the equation:

$$k_s (\% \text{/ day}) = (S_b \times 100)/(S_i \times t)$$

where $S_b$ and $S_i$ are the specific radioactivities of protein-bound and tissue-free phenylalanine in the acid-soluble fraction of tissue homogenates respectively, and $t$ is the period between injection of isotope and immersion of tissue into iced water.

The amount of protein synthesis per unit of RNA or RNA activity ($k_{RNA}$; mg protein day$^{-1}$ mg$^{-1}$ RNA) was calculated by dividing $k_s$ by the RNA/protein (mg/g) ratio. Similarly, protein synthesis per unit of DNA or cellular efficiency ($k_{DNA}$; mg protein day$^{-1}$ mg$^{-1}$ DNA) was calculated by dividing $k_s$ by the DNA/protein (mg/g) ratio.

### Measurement of plasma and tissue glutamine concentrations

Glutamine concentrations in neutralized HClO$_4$ extracts of plasma and tissue homogenates were measured by an enzymic fluorimetric assay modified from the method of Lund [28].

### Statistical analysis

All data are presented as means ± S.E.M. Values for protein, RNA and DNA are presented as total quantity in the tissue. Values for glutamine are presented as concentration per litre of plasma or per g of tissue. Statistical analysis was performed using Statistics Package for Social Sciences (SPSS) version 10.0 (SPSS Inc., Chicago, IL, U.S.A.). Differences between groups were evaluated by one-way ANOVA with Fisher’s protected least significant difference post hoc [29]. Significance was accepted at $P \leq 0.05$. 

## RESULTS

### Body and tissue weights

The seven groups of animals had a similar mean body weight (256 ± 23 g, mean ± S.E.M.). At 24 h after surgery, all animals had lost weight when compared with the ad libitum ($\theta$) animals (Figure 1a), and this effect was slightly greater in the LAP (sham-operated) group. Weight loss continued at 72 h and 96 h, with no differences between septic and non-septic animals at these time points. Weight loss from carcass (all skeletal muscle plus bone, excluding skin) and from skeletal muscle (left and right gastro-
Protein synthesis and glutamine levels in sepsis

Gastrocnemius protein synthesis

When compared with baseline animals, muscle protein synthesis was acutely reduced 24 h after both CLP and sham operation. The magnitude of this decrease was greater in the septic animals, and was associated with a reduced tissue protein content (Figure 2). By 72 and 96 h, rates of protein synthesis were similar in septic and sham-operated animals, although the fall in tissue protein content remained greater in septic animals at both time points. Total tissue RNA content was also reduced in all experimental animals when compared with the baseline group, with sepsis resulting in lower levels than sham operation at 72 and 96 h (Figure 3a). RNA activity (protein synthesis per unit of RNA) was reduced following sham operation, with the level continuing to fall over the experimental period. Following CLP, however, RNA activity was initially lower than in sham-operated animals, and then increased progressively, such that by 96 h it was higher in CLP than in sham-operated animals (Figure 3b).

Liver protein synthesis

The rate of total protein synthesis in the liver in sham-operated animals did not change from baseline throughout the experiment, whereas in septic animals protein synthesis was increased when compared with baseline and with sham operation at each time point (Figure 2a). Liver protein content fell with time in both groups, being significantly lower at 96 h than at 24 h. However, protein...
content in septic animals was not significantly different from baseline at any time point, whereas after sham operation protein content was lower than baseline at 72 and 96 h (Figure 2b).

Liver RNA content was elevated in septic animals and depressed in non-septic animals (Figure 3a). Liver RNA activity was significantly increased at 24 h after CLP only, and this level was greater than mean levels measured in all sham-operated groups (Figure 3b). Liver total DNA content was elevated at 24 h in septic animals and depressed at 96 h in non-septic animals, but unchanged in the other groups (Figure 4a). Cellular efficiency, however, was increased in all septic groups and unchanged in non-septic groups (Figure 4b).

**DISCUSSION**

In this study, sepsis (CLP) was associated with a substantial fall in muscle protein synthesis, as well as in total protein, RNA and DNA contents; these changes were greater than those seen following sham operation (LAP). Conversely, hepatic protein synthesis and DNA and RNA contents and activities were increased after CLP, whereas after sham operation liver protein synthesis was unchanged, while DNA and RNA contents fell. Liver protein content fell with time in both groups. Both sepsis and sham operation were associated with substantial falls in muscle glutamine concentration, which were more marked with sepsis, and the maintenance of (CLP), or even an increase in (LAP), the liver glutamine

Content in septic animals was not significantly different from baseline at any time point, whereas after sham operation protein content was lower than baseline at 72 and 96 h (Figure 2b).

Liver RNA content was elevated in septic animals and depressed in non-septic animals (Figure 3a). Liver RNA activity was significantly increased at 24 h after CLP only, and this level was greater than mean levels measured in all sham-operated groups (Figure 3b). Liver total DNA content was elevated at 24 h in septic animals and depressed at 96 h in non-septic animals, but unchanged in the other groups (Figure 4a). Cellular efficiency, however, was increased in all septic groups and unchanged in non-septic groups (Figure 4b).

**Plasma and tissue glutamine levels**

When compared with baseline, septic animals had reduced plasma glutamine concentrations at 72 and 96 h, whereas following sham operation there was a transient fall in the plasma glutamine concentration at 24 h only (Figure 5a). Both sepsis and sham operation resulted in a reduced gastrocnemius muscle glutamine concentration over the entire experimental period. At each time point, however, glutamine concentrations were significantly lower in septic than in sham-operated animals (Figure 5b).

The liver glutamine concentration did not change in septic animals, but was increased significantly compared with both baseline and septic animals at 72 and 96 h after sham operation (Figure 5b).
Circulating glutamine concentrations were only marginally reduced.

In normal subjects, muscle protein is in a balanced state of continual synthesis and breakdown. Following surgical stress or during sepsis, protein is lost from muscle because of a reduced rate of protein synthesis, increased protein breakdown, or both. Previous studies have shown that both whole-body protein synthesis and breakdown are increased in multiple-organ failure, with breakdown predominating [30]. Measurement of protein breakdown rates is technically difficult in in vivo models. In this study, therefore, we have measured protein content and organ weights in addition to protein synthesis rates, in order to estimate the extent of protein breakdown in individual tissues.

Body weight fell in all animals, and individual tissue weights were invariably lower than in baseline animals (ad libitum). Although there was no difference in total body weights between starved animals with sepsis or subjected to sham operation, sepsis resulted in increased weight loss from skeletal muscle and carcass, while liver weight fell to a greater extent in non-septic than in septic animals. These findings clearly demonstrate that sepsis is a more potent stimulus to catabolism than is either operative stress or starvation. The early fall in skeletal muscle protein synthesis rates after CLP would be unlikely to result in an acute decrease in protein content, however. Consequently, the reduction in protein content in gastrocnemius muscle seen at 24 h after CLP in the present study suggests that muscle protein breakdown rates were increased. Depressed rates of protein synthesis and cellular efficiency persisted at 96 h after CLP, whereas RNA activity had begun to increase, although remaining at significantly lower levels than baseline. This is not surprising, as most deaths following CLP occur between 48 and 72 h, and animals surviving beyond this time usually recover. It might therefore be expected that protein synthetic activity would be beginning to increase by 96 h.

Although liver weight fell in all experimental animals, liver weight and protein synthesis rates, as well as protein, RNA and DNA contents and activities, were all higher in septic than in sham-operated animals. Moreover, protein synthesis rates, RNA content and cellular efficiency were increased significantly above baseline in the presence of sepsis. These observations are consistent with previous studies in septic rats [31–33], and suggest that hepatic protein synthesis has a high priority during sepsis. Indeed, increased hepatic protein synthesis may be the main contributor to the increased whole-body protein synthesis observed in animal studies of severe catabolism [33]. Vary and Kimball [34] found that increased hepatic protein synthesis rates in sepsis resulted from stimulation of the synthesis of both secreted and non-secreted proteins. In contrast with the finding of these authors that protein synthetic capacity increased only in response to sterile inflammation, our septic rats demonstrated both increased protein synthetic capacity and increased translational efficiency, as indicated by increased RNA activities. Synthesis of both albumin and acute-phase proteins (such as α1-acid glycoprotein, complement component C3 and transferrin) has been shown to be responsible for the increase in total hepatic protein synthesis seen in sepsis [32]. A number of studies in humans have also confirmed increased total hepatic protein synthesis and increased albumin synthesis rates in critical illness [35,36].

We have demonstrated, therefore, that muscle protein catabolism in sepsis is associated with reduced protein synthesis and increased protein degradation. Simultaneously, liver protein synthesis is increased. The mechanism driving the catabolic response to sepsis remains obscure. Elevated levels of ‘stress hormones’, cytokines and other inflammatory mediators have consistently been implicated [37], but more recently the importance of fluxes of essential amino acids from peripheral to splanchnic tissues has been recognized [38]. The differential changes in organ protein synthesis rates, protein contents and weights may be considered as being adaptive, to mobilize essential substrates from peripheral, non-essential tissues to central organs and rapidly dividing cells at times of stress. Glutamine is now recognized to be a ‘conditionally essential’ amino acid in stress states [39], and is an important intracellular determinant of muscle protein synthesis and breakdown rates [40,41].

We have demonstrated that plasma glutamine concentrations are relatively better preserved in septic rats than are muscle glutamine levels, suggesting that the physiological response to stress is designed to preserve plasma glutamine, and thus delivery of this substrate to more vital organs and cells. This is achieved at the expense of muscle glutamine levels, the depletion of which may partly explain the depression of muscle protein synthesis and the fall in muscle protein content in sepsis. We have shown that, in non-septic animals, operative stress (combined with starvation) is associated with significant increases in liver glutamine levels. Of interest, however, is the fact that liver glutamine levels were unchanged following CLP. In the liver, glutamine is utilized for the synthesis of urea and glutathione, and as a substrate for gluconeogenesis [42,43]. Hepatic glutaminase activity is increased during starvation, diabetes and consumption of a high-protein diet [42]. Glutamine metabolism in the liver has been shown to be essential for the maintenance of ammonia and bicarbonate homoeostasis [44]. The hepatic extraction of glutamine is increased in sepsis, possibly due to an effect of the pro-inflammatory cytokines tumour necrosis factor α and interleukin-6 [43,45]. This is associated with a concomitant increase in the net release of glutamate, glutathione, glucose and urea [43]. Glutathione is an important antioxidant produced...
from glutamine that may have a vital role in protective responses to sepsis and shock [46]. Although glutamine is an important regulator of the hepatic synthesis of acute-phase proteins [47], the relationship between liver glutamine concentration and liver protein synthesis is not known, and may not be the same as that in muscle. We have shown that, despite the increase in glutamine levels, liver protein synthesis rates were unchanged in sham-operated animals, whereas the significantly increased liver protein synthesis after CLP occurred in the presence of unchanged glutamine levels. It is interesting to speculate that the "normal" response to stress is to increase liver glutamine levels, and that following CLP there is a significant increase in hepatic glutamine demand that may not be met by a limited exogenous supply. Starvation and sepsis increase hepatic glutamine transport independently and synergistically, a mechanism that may act to support key metabolic pathways in critical illness [48].

Our study supports previous evidence suggesting that glutamine is an important nutrient during sepsis, since, in the absence of dietary intake, skeletal muscle glutamine loss was profound. The mechanism that promotes glutamine flux from peripheral to hepatosplanchnic tissues during stress is not known, although glutamine depletion in skeletal muscle appears to be caused by accelerated glutamine release rather than increased degradation or reduced synthesis [49]. It is not clear, therefore, whether provision of additional exogenous glutamine could prevent or reverse muscle catabolism. In a randomized study of acute supplementation with parenteral glutamine in critically ill humans, muscle glutamine levels were not restored [50]. Dietary glutamine supplementation may, however, increase glutamine availability to hepatosplanchnic tissues, with benefit in terms of hepatic, gastrointestinal and immune cell protein synthesis and function [51–53]. In addition, any intervention that restricts peripheral cellular glutamine release, such as growth hormone therapy, may have significantly deleterious effects if not accompanied by glutamine supplements [18].

In the present study we denied animals access to food from the time of operation to the time they were killed. This simulates the situation in most cases of severe sepsis in humans, where nutritional support is often not tolerated in the early stages. Current recommendations for nutritional support in critical illness emphasize the enteral route of delivery [54], but it is well recognized that even when specifically designed enteral feeding protocols are used many patients receive intakes well below target [55]. We had observed previously that, following CLP in rats, food intake was minimal for up to 5 days [21]. Although control animals pair-fed to those undergoing CLP demonstrated significant depression of circulating levels of insulin-like growth factor-1 when compared with sham-operated animals, sepsis induced by CLP resulted in much greater decreases in this growth factor [21]. These findings indicate that, although anorexia is likely to be a significant contributor to the metabolic derangements associated with CLP, sepsis has a considerable additional effect; this has been confirmed by other workers using the same model [15,56]. In the present study we employed starvation because our objective was to determine the effects attributable to sepsis compared with those of surgery alone, in a model relevant to the situation encountered in most cases of sepsis in humans. We were also cognizant of the importance of limiting the number of animals used.

In summary, we have characterized sequential changes in organ protein synthesis and glutamine flux in an in vivo animal model of sepsis. We have confirmed that sepsis is associated with decreases in skeletal muscle protein synthesis, protein content and weight, and increased hepatic protein synthesis. There is marked skeletal muscle glutamine depletion, with relative preservation of plasma glutamine levels. Liver glutamine levels are increased following operative stress, but not during sepsis. This model could be used to study the effects of nutritional support and novel anabolic interventions on protein catabolism and glutamine levels in sepsis.

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