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Prolonged changes in protein and amino acid metabolism after zymosan treatment in rats

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1. Intraperitoneal injections of zymosan were given to rats, according to a modified procedure, in order to create a pattern of illness with an acute critical phase for 36h followed by a prolonged recovery phase lasting for at least 10 days. Changes in amino acid and protein metabolism were studied in both phases.
2. Differences between this modified and the original zymosan model are a lower mortality (16%), which is limited to the first 36 critical hours, and the absence of signs of severe illness during the prolonged recovery phase.
3. Wasting of muscle protein and decreased protein synthesis rates in muscle were observed during the acute phase of illness. Liver size and liver protein synthesis rates were increased during the same period. The decrease in the total amount of muscle protein and the increase in liver weight were still present 12 days after zymosan treatment, despite a normalization of protein synthesis rates. Large decreases were observed in the concentrations of the conditionally essential amino acids glutamine and arginine in muscle over 6 days. Decreases in plasma glutamine and arginine on day 12 after zymosan indicated that the rats were still not fully recovered on this day.
4. We conclude that injection of a single dose of zymosan in rats leads to metabolic derangements both during the acute phase of critical illness and during the prolonged recovery phase. The model seems suited for investigating the biochemical mechanisms behind these metabolic derangements and for studying therapeutic and nutritional interventions during recovery from critical illness.

INTRODUCTION

During severe illness, such as sepsis and trauma, a complex metabolic derangement often leads to a protracted course in an intensive care unit. Loss of muscle mass and changes in the concentrations of amino acids and other metabolites are characteristic features in this period. Repeated tissue sampling for research purposes is restricted in critically ill patients for ethical reasons. Therefore, several animal models have been developed to investigate these metabolic derangements. However, the few animal models (caecal ligation and puncture, burn injury and repeated endotoxin administration) suitable for the investigation of the long-term metabolic derangements as frequently seen in man all have important limitations. With caecal ligation and puncture [1, 2] and burn injury [3, 4], invasive techniques, notably anaesthesia and surgery, are required to induce the catabolic state. Both models are also characterized by high mortality rates. Endotoxin administration results in a short-lived metabolic response, due to rapid clearance of endotoxin, unless multiple injections or constant infusions are used [5]. Prolonged or repeated administration, however, leads to endotoxin tolerance.

Goris and co-workers [6, 7] described a rat model for long-term illness, with clinical signs of sepsis and multiple organ failure existing for a period of 14 days. Illness in this model was induced by an aseptic intraperitoneal injection of zymosan suspended in liquid paraffin. Zymosan, which is a glucopolysaccharide from the cell wall of yeast, is a potent activator of the alternative pathway of complement [8] and of macrophages [9]. The disadvantages of this model are the high mortality rate and the large variability in severeness of disease and mortality. Here we describe a modification of the zymosan model. This modified model shows a reproducible pattern of illness starting with an acute phase of critical illness with limited mortality rates which is followed by a prolonged recovery phase without mortality and signs of severe illness. In this modified model protein synthesis rates in muscle, liver and small intestinal mucosa and concentrations of amino acids in muscle and plasma have been measured up to 12 days after zymosan treatment.

METHODS

Male Lewis rats, supplied by the central laboratory animal facilities of the University of Limburg, were individually housed and kept in a controlled environment (12h light cycle, 21–22°C and 50–60% humidity). Rats were fed a standard lab chow

Key words: amino acids, critical illness, muscle wasting, protein synthesis rates, zymosan.
Abbreviations: BCAA, branched-chain amino acids; FSR, fractional protein synthesis rate; SA, specific activity.
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(SMR-A; Hope Farms, The Netherlands) containing (w/w) approximately 28% protein, 7% fat, 54% carbohydrate, 4% fibre and 7% minerals with a trace element and vitamin supplement. The rats were allowed to acclimatize for 1 week. The experiments were approved by the animal experimental committee of the University of Limburg.

Rats were given an aseptic intraperitoneal injection of zymosan (50 mg/100 g body weight) suspended in liquid paraffin (25 mg/ml). The zymosan (Sigma Chemical Company) was sterilized by γ-radiation ($\pm$12 000 rad). Suspensions were prepared in small portions using 25 ml aliquots of sterilized liquid paraffin (Merck). An OMNI 1000 homogenizer (OMNI international) was used to provide a homogeneous suspension of zymosan in paraffin after which the suspension was sterilized by incubation at 100°C (water bath) for 90 min.

Food intake is reduced after zymosan administration and therefore paraffin-injected control rats were pair-fed. Pair-feeding was performed in three periods during the day to ensure that the pair-fed rats would not eat all the food at once and would subsequently be starving for the remainder of the day. The three periods were from 08.00 to 15.00 hours, from 15.00 to 22.00 hours and from 22.00 to 08.00 hours.

Protein synthesis rates (muscle, liver and jejunal mucosa), tissue weight and concentrations of protein (muscle and liver) and amino acids (plasma and muscle) were measured 16 h and 2, 4, and 6 days after treatment with zymosan suspended in paraffin or paraffin only (pair-fed).

A control group with free access to rat chow and no paraffin injections was also included in the study. These rats were studied on day 3 to make them closely comparable with all the other experimental groups. In an additional experiment (experiment 2) tissue weights (muscle and liver) and concentrations of the amino acids glutamate, glutamine and arginine (plasma and muscle) were measured 12 days after treatment in zymosan-injected, pair-fed and *ad libitum* fed rats. In both experiments groups were matched for age and initial body weight. During the experiment food intake and body weight were determined daily. On the day of the experiment the rats were starved from 08.00 to 12.00 hours. Pair-feeding was performed at 07.00 hours on this day. Protein synthesis rate measurements and sampling of tissues and blood were performed between 12.00 and 14.00 hours.

To measure protein synthesis rates the phenylalanine flooding dose technique, as described by Jepson et al. [5], was used. Rats were injected intraperitoneally with a large dose of L-[4-3H]phenylalanine (150 μmol of phenylalanine and 20 μCi of labelled phenylalanine/ml; 2 ml/100 g body weight). After 15 min the rats were killed by cervical dislocation, and mixed blood was collected after decapitation. Gastrocnemius muscle, liver and jejunum were collected within 3 min of cervical dislocation. Muscle and liver were weighed and frozen in liquid nitrogen using a pre-cooled pair of tongs. The jejunum was transferred to ice-cold water and rinsed with ice-cold water. The mucosa was separated from the seromuscular layer by scraping with a ice-cold glass slide and was frozen in liquid nitrogen using a pre-cooled pair of tongs.

For measurement of the incorporation of [^{3}H]phenylalanine into tissue protein the method described by Garlick et al. [10] was used. The same samples were used to measure protein concentration using the method of Lowry et al. [11]. Fractional protein synthesis rate (FSR) was calculated from the specific activity (SA; d.p.m./nmol) of [^{3}H]phenylalanine in the precursor pool (intracellular phenylalanine) and the SA of protein-bound [^{3}H]phenylalanine. To calculate the FSR in muscle the SA of intracellular phenylalanine was multiplied by 0.9 as described by Jepson et al. [5] because plateau labelling of the precursor pool in muscle is reached after 2 or 3 min only. For liver this correction factor was 1.0 [5]. It was assumed that the correction factor for small intestine also is 1.0.

$$\mathrm{FSR} = \frac{\mathrm{SA}_{\mathrm{protein\text{-}bound}}}{\mathrm{SA}_{\mathrm{precursor}} \times \mathrm{time\ (days)}} \times 100\%$$

In a pilot experiment it was shown that no difference existed between zymosan-treated and control rats in the time to reach plateau labelling (1–2 min) and in the maintenance of the plateau for at least 20 min in blood after a flooding dose of [^{3}H]phenylalanine.

Free amino acids in muscle and plasma were measured by h.p.l.c. [12]. Plasma was deproteinized with sulphosalicylic acid. Intracellular amino acids in muscle were extracted using a 5% sulphosalicylic acid solution with norvaline (0.5 mmol/l) as an internal standard. Concentrations of glutamate, glutamine and arginine in muscle and plasma from experiment 2 were measured using enzymic assays as described previously [13, 14].

All measurements were performed in duplicate, except for muscle protein synthesis rates, which were performed in triplicate. Values are given as mean (range). The Mann–Whitney *U*-test was used to determine statistically significant differences between the zymosan-treated and pair-fed rats at one time point and between the zymosan-treated and the *ad libitum* fed control rats. Significance was set at $P<0.05$.

RESULTS

The rat model

All rats injected with zymosan showed signs of severe illness, including lethargy, anorexia, diarrhoea and loss of haemorrhagic fluid from nose and eyes for 2 days after zymosan administration. The peritoneal cavity yielded remnants of paraffin, and adhesions between abdominal organs were found 2, 4, 6

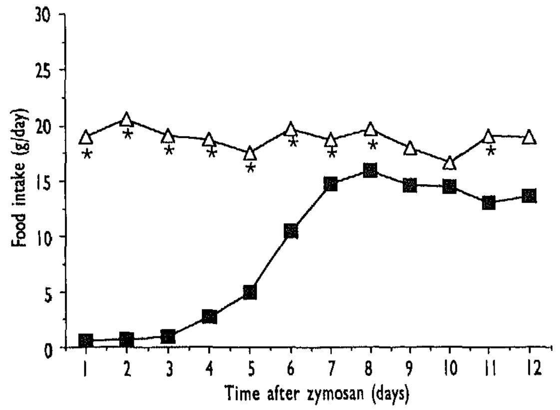

Fig. 1. Food intake of control (△) and zymosan-treated (■) rats. Values from experiment 2 are given, in which rats were studied for 12 days after zymosan treatment. The rats in experiment 1 followed the same pattern of food intake for the 6 days that they were studied. Statistical significance: $^{*}P<0.05$ compared with control rats.

and 12 days after zymosan treatment. The liver was pale and had adhesions with the diaphragm and stomach. The pair-fed rats (injected with paraffin only) and the *ad libitum* fed rats showed none of these abnormalities. Sixteen per cent of the zymosan-treated animals died during the first 36 h after injection. No animals died hereafter. Due to the induction of the illness food intake was dramatically reduced. Intake was reduced to 0.5 g/day on days 1 and 2, after which it slowly increased to 70–80% of the normal intake between days 7 and 12 after zymosan treatment (Fig. 1).

The initial body weights were not significantly different between the experimental groups in both experiments (Tables 1 and 2). The rats from experiment 2, however, had higher initial body weights in comparison with those of experiment 1. Due to the treatment both the pair-fed and the zymosan-treated rats lost weight in comparison with the fed control rats (Fig. 2). A decrease in weight was seen during the first 4 days, followed by a small increase. No catch-up growth was observed. The reduction in body weight in the pair-fed group was larger than in the zymosan-treated rats. Muscle (gastrocnemius) weight (expressed per 100 g of initial body weight) was reduced in the zymosan-treated rats in comparison with the *ad libitum* fed control rats 16 h after treatment and with both the *ad libitum* fed and pair-fed rats on day 2, 4, 6 and 12 (Table 1 and 2). Due to the zymosan treatment liver weight (expressed per 100 g of initial body weight) was increased in comparison with the pair-fed rats during the whole experiment (16 h and 2, 4, 6, and 12 days), decreased in comparison with the fed control rats on 16 h and day 2, but greater than the fed control rats on day 12 (Tables 2 and 3).

Protein metabolism

No differences in muscle protein concentration and wet/dry weight ratio were observed in the zymosan-treated rats in comparison with the *ad libitum* fed and pair-fed control rats (Table 1). Total muscle protein content (expressed per 100 g of initial body weight) was decreased in the zymosan-treated rats 2, 4, 6 and 12 days after treatment in comparison with the *ad libitum* fed and pair-fed control rats (Tables 1 and 2). Total liver protein content (expressed per 100 g of initial body weight) was significantly decreased in the zymosan-treated group in comparison with the *ad libitum* fed control rats 16 h and 2 and 4 days after treatment, but was increased compared with the pair-fed group on days 2, 4 and 6 (Table 3).

FSR in muscle was decreased in the zymosan-treated rats in comparison with the pair-fed and fed control rats 16 h after treatment (Table 4). On days 2 and 4 after treatment FSR was decreased in muscle of the zymosan-treated rats in comparison with the fed control rats. No difference, however, was seen at these time points between the zymosan-treated and pair-fed rats. FSR in liver in the zymosan-treated rats was increased 16 h, 2 days and 4 days after treatment in comparison with the pair-fed rats (Table 4). In comparison with the fed control rats an increase was seen 2 days after treatment. FSR in the mucosal layer of the jejunum was decreased in the zymosan-treated rats on day 6 in comparison with both control groups (Table 4).

Amino acid metabolism

Both in plasma (Table 5) and muscle (Table 6) the sum of the amino acid concentrations was decreased 2, 4 and 6 days after zymosan treatment in comparison with one or both of the control groups. The concentrations of phenylalanine and tyrosine are not included because these are higher than the physiological values due to the flooding dose of phenylalanine given for measuring FSR. Most essential amino acids (threonine, methionine, tryptophan, lysine, valine, leucine, isoleucine) were decreased in concentration in plasma 2, 4 and 6 days after treatment (Table 5). Of the non-essential amino acids, plasma concentrations of glutamate and glutamine were decreased on days 6 and 12 and of arginine on days 4, 6 and 12 after zymosan treatment (Tables 5 and 7). In muscle, of the essential amino acids, threonine was decreased days 2, 4 and 6 after treatment, lysine on days 2 and 4, and the sum of the branched-chain amino acids (BCAA; valine, leucine and isoleucine) on days 4 and 6 (Table 6). Of the non-essential amino acids, both the concentrations of glutamine and arginine were decreased on days 2, 4 and 6. No differences in concentrations of glutamate, glutamine and arginine were observed in muscle 12 days after treatment (Table 7).

DISCUSSION

Many animal models have been developed to study the metabolic response to trauma and sepsis

Table 1. Initial body weight and gastrocnemius weight, protein content, total protein, and dry/wet weight ratio 16 h and 2, 4 and 6 days after treatment. Values are given as mean (range) for six to eight rats. Abbreviations: CON, *ad libitum* fed control rats; ZYM, zymosan-injected rats; PF, pair-fed rats; NA, not analysed. *Significantly different from control rats; †significantly different from pair-fed rats.

	Initial body wt. (g)	Muscle wt. (g/100 g initial body wt.)	Protein (mg/g wet wt.)	Total protein (mg/100 g initial body wt.)	Dry/wet wt. ratio
CON	178 (146–223)	0.74 (0.67–0.98)‡	180.8 (163.5–196.0)	133.3 (119.7–161.0)‡	0.24 (0.22–0.27)
16 h					
ZYM	207 (195–229)	0.55 (0.52–0.59)*	NA	NA	NA
PF	211 (202–230)	0.56 (0.45–0.60)	NA	NA	NA
2 days					
ZYM	184 (157–236)	0.45 (0.42–0.49)*†	162.2 (124.7–186.7)	73.6 (58.2–90.2)*†	0.24 (0.21–0.27)
PF	191 (172–243)	0.54 (0.51–0.55)	175.1 (144.0–187.1)	94.8 (79.0–102.0)	0.24 (0.23–0.25)
4 days					
ZYM	180 (156–228)	0.39 (0.24–0.44)*†	165.8 (157.7–184.2)	69.7 (61.5–75.9)*†	0.25 (0.22–0.29)
PF	197 (169–236)	0.50 (0.44–0.56)	169.3 (152.5–184.2)	84.9 (76.5–93.9)	0.24 (0.23–0.24)
6 days					
ZYM	184 (147–225)	0.44 (0.41–0.50)*†	172.7 (166.2–179.9)	74.8 (68.1–85.5)*†	0.24 (0.23–0.26)
PF	188 (151–227)	0.54 (0.51–0.57)	176.7 (158.7–184.3)	95.9 (86.6–104.3)	0.24 (0.22–0.24)

‡Values of the *ad libitum* fed rats were obtained on day 3 to make them comparable with all other groups. Because growing rats are used these numbers should be slightly smaller for the younger rats and larger for the older rats.

Table 2. Initial body weight, gastrocnemius weight, gastrocnemius protein content and liver weight measured 12 days after treatment. Values are given as mean (range) for six to eight rats. Abbreviations: CON, *ad libitum* fed control rats; ZYM, zymosan-injected rats; PF, pair-fed rats. *Significantly different from control rats; †significantly different from pair-fed rats.

		Muscle			
	Initial body wt. (g)	Wt. (g/100 g initial body wt.)	Protein (mg/g wet wt.)	Total protein (mg/100g initial body wt.)	Liver wt. (g/100 g initial body wt.)
CON	264 (239–295)	0.65 (0.62–0.66)	216.7 (182.7–263.4)	141.2 (113.6–174.0)	4.0 (3.5–4.6)
ZYM	269 (258–277)	0.41 (0.35–0.46)*†	207.9 (182.2–226.1)	86.3 (67.7–100.7)*†	4.8 (4.4–5.1)*†
PF	269 (256–275)	0.54 (0.46–0.61)	225.1 (188.6–296.9)	122.8 (92.5–170.4)	3.1 (2.8–3.5)

(for a review, see [15]). However, most models only show an acute severe and short-lived period of illness. A simple intraperitoneal injection with zymosan suspended in paraffin, as used here, provided a model for studying both an acute phase of critical illness and a prolonged recovery phase.

Zymosan treatment has been used in several studies as a model for critical illness and multiple organ failure. Goris and co-workers [6, 7], who introduced the model in rats, described a triphasic illness. On the first 3 days clinical signs of critical illness were observed, such as lethargy, anorexia, hyperventilation, diarrhoea and loss of haemorrhagic fluid from nose and eyes. A stable intermediate period was then followed by the development of a second phase of severe illness, with signs of multiple organ failure between 7 and 14 days after zymosan treatment. Steinberg et al. [9] described histopathological changes in lung, liver and kidney 7 days after zymosan administration, from which they concluded that the injection of zymosan in rats created a model for multiple organ failure.

Several pilot experiments performed in our laboratory showed that the model as described by Goris and co-workers [6, 7] led to varying degrees of illness and variable mortality rates in repeated experiments. Much effort, therefore, has been put into improvement of the reproducibility of the model. The procedure to prepare the zymosan suspension has been modified (see the Methods section) and the dose of zymosan was reduced to half the dose administered by Goris and co-workers [6, 7]. This resulted in a decrease in acute mortality to 16% (35% in the studies of Goris and co-workers [6, 7]), whereafter no additional rats died (another 15% in the studies of Goris and co-workers [6, 7]). This modified model has led to a reproducible pattern of illness in six different experiments performed in our laboratory over the last 2 years with a total of 132 rats. Rats showed signs of severe illness over 2–3 days, after which they slowly recovered, as indicated by increasing food intake and gain in body weight. During the phase of severe illness characteristic changes in protein metabolism with muscle protein wasting, decreased muscle protein synthesis rates, increased liver weight and enhanced liver protein

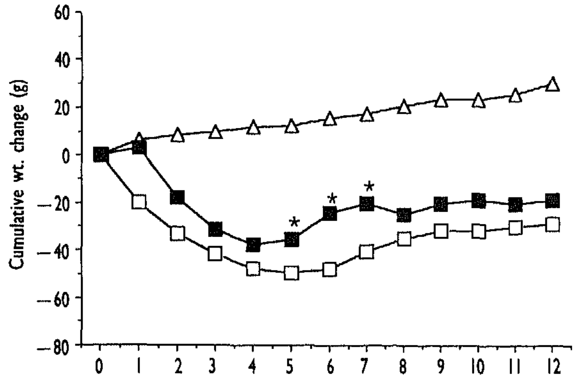

Fig. 2. Cumulative changes in body weight of control (△), pair-fed (□) and zymosan-treated (■) rats. Values from experiment 2 are given, in which rats were studied for 12 days after zymosan treatment. The rats in experiment 1 followed the same pattern of cumulative weight change for the 6 days that they were studied. Rats were injected with either zymosan suspended in paraffin or with paraffin only (pair-fed). Control rats were not injected. Changes were calculated from initial body weight and body weights attained every morning during the experiment. Sixteen hour values were measured just before the rats were killed. All values for the zymosan-treated and the pair-fed rats are significantly different from those of the control rats, except on day 0 and 1 for the pair-fed rats. Statistical significance: $^*P<0.5$ between zymosan-treated and pair-fed rats.

Table 3. Liver weight, protein and total protein 16 h and 2, 4 and 6 days after treatment. Values are given as mean (range) for six to eight rats. Abbreviations: CON, *ad libitum* fed control rats; ZYM, zymosan-injected rats; PF, pair-fed rats. *Significantly different from control rats; †significantly different from pair-fed rats.

	Liver wt. (g/100 g initial body wt.)	Protein (mg/g wet wt.)	Total protein (mg/100 g initial body wt.)
CON	5.2 (4.2–6.0)‡	194.8 (186.3–208.7)	1008 (830–1135)‡
16 h			
ZYM	3.7 (3.4–3.9)*†	202.4 (187.7–218.0)†	744 (642–828)*
PF	3.1 (3.0–3.2)	224.9 (215.3–231.3)	700 (666–729)
2 days			
ZYM	3.9 (3.8–4.3)*†	187.3 (174.7–196.4)†	738 (679–778)*†
PF	2.9 (2.4–3.2)	205.3 (182.0–218.3)	589 (517–636)
4 days			
ZYM	4.8 (4.6–5.2)†	168.5 (125.4–189.5)*	811 (606–949)*†
PF	3.3 (2.8–3.6)	180.6 (166.5–195.9)	592 (504–650)
6 days			
ZYM	5.1 (4.6–5.8)†	176.9 (132.7–199.3)	895 (718–998)†
PF	4.0 (3.2–4.4)	173.5 (165.4–186.8)	687 (552–757)

‡Values of the *ad libitum* fed rats were obtained on day 3 to make them comparable with all other groups. Because growing rats are used these numbers should be slightly smaller for the younger rats and larger for the older rats.

Table 4. FSR in gastrocnemius, liver and mucosal layer of the jejunum 16 h and 2, 4 and 6 days after treatment. Values are given as mean (range) for six to eight rats. Abbreviations: CON, *ad libitum* fed control rats; ZYM, zymosan-injected rats; PF, pair-fed rats. *Significantly different from control rats; †significantly different from pair-fed rats.

	FSR (%/day)		
	Muscle	Liver	Mucosa
CON	8.2 (6.8–9.8)	78.7 (66.7–102.6)	83.1 (67.9–91.6)
16 h			
ZYM	5.3 (4.3–6.5)*†	74.1 (60.1–87.7)†	105.2 (67.2–142.7)
PF	9.5 (7.5–10.5)	53.4 (46.3–58.4)	92.5 (38.7–145.4)
2 days			
ZYM	5.1 (3.4–6.8)*	113.5 (78.8–143.9)*†	74.2 (60.6–87.8)
PF	5.7 (3.7–8.0)	77.4 (59.6–99.5)	78.4 (67.9–87.1)
4 days			
ZYM	5.8 (4.2–9.2)*	88.6 (73.7–100.4)†	72.9 (61.9–88.4)
PF	5.7 (3.8–7.2)	72.9 (58.8–85.2)	78.2 (59.8–99.9)
6 days			
ZYM	6.9 (5.6–8.1)	81.9 (67.2–99.1)	66.1 (52.4–77.0)*†
PF	6.5 (3.9–8.1)	70.0 (55.8–88.9)	85.5 (59.3–98.4)

synthesis rates were observed. Although the rats did not show signs of critical illness between days 6 and 12, changes in food intake, body weight, tissue weight and protein (days 6 and 12), concentrations of glutamine and arginine in plasma (days 6 and 12) and muscle (day 6) and mucosal protein synthesis rates (day 6) indicate that the zymosan-treated rats were not fully recovered. There were, however, no signs of severe illness or multiple organ failure during this period in the modified model.

Muscle mass was found to be decreased on days 2, 4, 6 and 12 after zymosan treatment, and, since both the dry/wet weight ratio and the protein concentrations in the gastrocnemius muscle at these time points were unchanged, this suggests that the net loss was due to a reduction in muscle tissue. Both the pair-fed and the zymosan-treated rats lost muscle tissue; however, in the pair-fed rats this was in proportion to the decreased body weight due to the semi-starvation. The larger loss in the zymosan-injected rats indicates that the wasting of muscle is the result of net protein catabolism induced by severe illness. If this protein wasting applies to the whole skeletal muscle compartment of the rat then the smaller loss of body weight in the zymosan-treated rats in comparison with the pair-fed rats may be the result of increased water retention in the zymosan-treated rats. In critically ill patients water is often sequestered as non-functional extracellular fluid [16].

Muscle wasting is a common feature during severe illness in humans and is often, at least in part, the result of decreased protein synthesis rates [17]. In most animal models of sepsis and critical illness wasting of muscle mass is accompanied by reduced protein synthesis rates [5, 18, 19]. In the zymosan-injected rats muscle protein synthesis rates were reduced 16 h after treatment in comparison with the pair-fed animals. Also, altered protein breakdown rates may contribute to the changes in protein content, but these were not measured because no reliable method for measurements *in vivo* is available in rats. The difference in total muscle protein content between zymosan-injected and pair-fed rats did not increase further after day 2.

Table 5. Plasma concentrations of amino acids 2, 4 and 6 days after treatment. Values are given as mean (range) for six to eight rats. *Significantly different from control; †significantly different from pair-fed rats.

Amino acid	Concn. (μmol/l)						
	2 days		4 days		6 days		Control
	Zymosan-injected	Pair-fed	Zymosan-injected	Pair-fed	Zymosan-injected	Pair-fed	
Glu	103 (58–166)	113 (60–170)	94 (70–125)	82 (73–93)	70*† (60–79)	90 (68–115)	124 (81–198)
Asn	58* (48–75)	64 (50–78)	33† (22–41)	47 (41–59)	31* (24–33)	40 (23–62)	40 (30–50)
Ser	255† (201–395)	316 (246–418)	192*† (152–267)	352 (316–434)	221† (140–376)	381 (327–422)	262 (210–302)
Gln	635 (542–740)	754 (524–917)	664 (625–705)	648 (564–762)	552† (361–670)	740 (600–894)	637 (500–683)
Gly	342 (194–594)	361 (256–439)	308*† (279–373)	492 (439–604)	238*† (214–257)	477 (376–577)	395 (333–483)
Thr	177† (125–308)	266 (184–320)	100*† (85–119)	263 (229–310)	174† (122–253)	230 (194–265)	208 (155–232)
His	73 (45–97)	63 (46–88)	60 (55–66)	67 (59–79)	52† (40–57)	65 (51–76)	60 (47–73)
Cit	72 (49–105)	81 (59–102)	75 (57–84)	78 (65–94)	90 (73–126)	98 (74–134)	82 (60–106)
Ala	344 (227–603)	307 (237–427)	276 (224–314)	359 (283–510)	208 (182–242)	347 (290–397)	242 (197–270)
Tau	126* (65–253)	156 (124–182)	147 (80–285)	223 (136–392)	108*† (79–162)	288 (224–376)	210 (136–274)
Arg	133 (85–210)	160 (116–207)	81*† (73–89)	171 (136–212)	74*† (55–107)	152 (100–200)	146 (112–185)
Met	44 (35–52)	46 (36–59)	27*† (23–30)	45 (38–56)	21*† (18–25)	38 (28–48)	39 (30–47)
Trp	56† (44–77)	69 (51–89)	34*† (26–45)	80 (62–101)	42*† (32–56)	61 (43–73)	59 (42–69)
Lys	329† (274–427)	421 (323–517)	269* (176–352)	326 (203–415)	347 (330–559)	342 (246–476)	372 (324–423)
Val	175 (130–232)	183 (117–222)	104*† (91–110)	163 (126–192)	114*† (98–139)	183 (137–219)	158 (112–188)
Ile	71 (56–91)	81 (51–108)	44* (40–48)	52 (39–63)	41*† (36–46)	63 (49–75)	63 (49–72)
Leu	124 (97–167)	127 (82–172)	70*† (61–77)	98 (67–122)	69*† (59–83)	104 (84–124)	102 (75–121)
BCAA	369 (283–482)	390 (250–495)	219*† (193–234)	313 (232–374)	225*† (193–268)	351 (270–410)	323 (236–381)
Sum	2932† (2674–3387)	3567 (2898–4167)	2580*† (2381–2829)	3525 (2983–4431)	2471*† (2175–2680)	3697 (3001–4189)	3194 (2508–3660)

In contrast to muscle, liver protein synthesis rates were increased not only in the critical phase but also on day 4. Increases in total liver protein content and liver protein synthesis rates have also been observed in rats treated with endotoxin and turpentine [5, 18]. This response may, in part, be a reflection of the increased synthesis rate of (retained) acute phase and endogenous proteins in liver.

Decreased concentrations of BCAA in plasma and muscle were seen 4 and 6 days after zymosan treatment in comparison with pair-fed and fed controls. During severe illness a reduction in plasma BCAA concentration has been observed [20, 21]. In the study by Stinnett et al. [22] decreased plasma and intracellular muscle concentrations of BCAA were seen in both patients and animals with severe burn injury. Enhanced whole body and muscle oxidation rates of BCAA, reported during sepsis, burn injury and trauma [23, 24], may be the cause of these reduced concentrations.

Concentrations of the non-essential amino acid glutamine in muscle were reduced in comparison with the pair-fed and fed control rats until 6 days after zymosan treatment. Plasma concentrations of glutamine were maintained until 6 days after zymosan treatment and were reduced on day 12 after treatment. Decreased concentrations of glutamine in muscle have been repeatedly reported in critically ill patients [25, 26] and in animal models [1, 3, 18]. Glutamine levels in human muscle stay low during a prolonged period (30 days) in contrast to changes in concentrations of other amino acids [27]. Glutamine has more metabolic functions than any other amino acid [28] and is, therefore, suggested to become essential during severe illness [29].

A positive correlation has been reported between

Table 6. Gastrocnemius concentrations of amino acids 2, 4 and 6 days after treatment. Values are given as mean (range) for six to eight rats. *Significantly different from control; †significantly different from pair-fed rats.

Amino acid	Concn. (nmol/g wet wt.)						
	2 days		4 days		6 days		Control
	Zymosan-injected	Pair-fed	Zymosan-injected	Pair-fed	Zymosan-injected	Pair-fed	
Glu	1432 (775–2133)	1470 (1425–1532)	1326 (1061–1520)	1146 (629–2279)	1442 (400–2221)	1332 (1155–1596)	1646 (979–2471)
Asn	118*† (62–163)	160 (138–187)	98 (61–149)	151 (57–340)	74 (48–91)	92 (61–120)	81 (67–97)
Ser	387*† (297–537)	691 (555–880)	467*† (377–598)	1033 (489–2028)	506† (310–834)	866 (747–1015)	711 (372–1001)
Gln	1042*† (627–1374)	2151 (1717–2534)	1957 (1515–3072)	2522 (1123–5291)	1637*† (1144–2022)	2272 (1894–2727)	2459 (1884–2872)
Gly	1284*† (876–1586)	3053 (2421–3627)	2940* (2099–4306)	3098 (1745–4533)	3397 (1798–4416)	5125 (3140–7447)	4475 (2066–6521)
Thr	190*† (162–247)	376 (316–485)	133*† (97–192)	309 (239–368)	213*† (138–307)	343 (280–446)	347 (304–408)
His	83*† (49–108)	125 (107–149)	106 (71–182)	182 (62–414)	92 (73–112)	112 (75–135)	125 (71–200)
Cit	33*† (24–41)	110 (56–160)	89* (71–144)	132 (74–244)	122 (96–173)	154 (150–191)	142 (82–216)
Ala	740† (601–928)	1207 (918–1627)	893 (780–1092)	1029 (555–1464)	662† (241–1021)	1020 (917–1292)	834 (638–978)
Tau	7431† (3998–11003)	10348 (7628–14146)	9155 (8035–11708)	10216 (5169–12772)	8938 (7632–9982)	9866 (6647–13000)	9443 (6634–12024)
Arg	76*† (45–100)	229 (155–281)	73*† (46–110)	249 (109–496)	87*† (64–118)	192 (143–235)	185 (133–235)
Met	58* (37–78)	49 (42–58)	29 (19–42)	37 (26–44)	22* (16–29)	29 (17–38)	32 (22–41)
Trp	48 (18–82)	35 (15–66)	51 (14–122)	43 (15–117)	77 (17–191)	73 (12–141)	46 (7–85)
Lys	155*† (126–194)	542 (432–663)	259*† (173–370)	450 (218–807)	412 (228–631)	416 (281–580)	442 (400–476)
Val	129 (98–176)	145 (108–200)	72* (51–99)	86 (60–104)	78*† (48–110)	125 (116–143)	114 (104–125)
Ile	68* (55–98)	68 (42–94)	33* (20–42)	30 (20–37)	29* (14–46)	43 (35–52)	42 (31–48)
Leu	235* (160–306)	247 (143–346)	135* (87–192)	160 (65–232)	129 (83–197)	186 (143–266)	180 (138–200)
BCAA	432* (308–579)	460 (286–634)	240* (158–333)	276 (145–360)	236*† (145–342)	355 (303–456)	337 (297–362)
Sum	13448*† (8399–17312)	20469 (17733–23954)	17345* (15382–19292)	20865 (12906–28571)	17917*† (16425–21211)	21868 (20979–23330)	22148 (17871–25441)

Table 7. Gastrocnemius and plasma concentrations of glutamate, glutamine and arginine measured 12 days after treatment. Values are given as mean (range) for six to eight rats. Abbreviations: CON, *ad libitum* fed control rats; ZYM, zymosan-injected rats; PF, pair-fed rats. *Significantly different from control rats; †significantly different from pair-fed rats.

	Muscle			Plasma		
	Glu (μmol/g wet wt.)	Gln (μmol/g wet wt.)	Arg (nmol/g wet wt.)	Glu (μmol/l)	Gln (μmol/l)	Arg (μmol/l)
CON	2.5 (2.2–3.0)	3.4 (2.7–4.4)	259 (161–360)	174 (130–219)	727 (588–810)	201 (188–220)
ZYM	2.1 (1.9–2.9)	3.3 (2.9–4.0)	333 (212–402)	89 (69–97)*†	545 (493–594)*†	125 (104–148)*†
PF	2.3 (2.0–2.6)	3.4 (3.3–4.2)	368 (247–568)	127 (108–165)	692 (561–915)	190 (160–231)

concentrations of glutamine and protein synthesis rates in muscle both *in vitro* [30] and *in vivo* [31]. Rennie et al. [32] hypothesized that a fall in muscle glutamine concentration is causally related to muscle wasting in patients. No such correlation ($P = 0.4904$) was found in the zymosan-treated rats (Fig. 3) due to the normalization of protein synthesis rates on day 6 in the zymosan-treated rats in combination with reduced muscle concentrations of glutamine. Also, Wusteman et al. [18] came to the conclusion that this relation is not a universal one.

Concentrations of arginine were decreased in

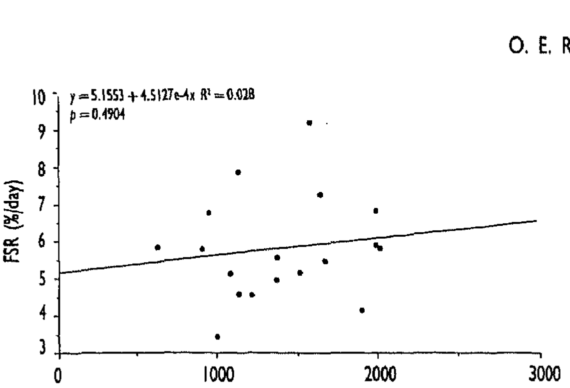

Fig. 3. Relationship between muscle concentrations of glutamine and muscle FSR in the zymosan-treated rats measured on days 2, 4 and 6. The Spearman rank correlation was used for statistical analysis.

muscle 2 and 6 days after zymosan treatment. Plasma concentrations, however, were reduced on days 4, 6 and 12. Arginine has also been suggested as an indispensable dietary amino acid in clinical conditions such as starvation, injury or stress due to its metabolic functions during growth, wound healing and tumour cell killing [33]. The decreased concentrations of arginine in the zymosan-treated rats both in plasma and muscle, therefore, may lead to an impaired recovery.

We conclude that injection of a single dose of zymosan in rats leads to metabolic derangements both during the acute phase of critical illness and during the prolonged recovery phase. The model seems suited for investigating the biochemical mechanisms behind these metabolic derangements and for studying therapeutic and nutritional interventions during recovery from critical illness.

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