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Derangement in aerobic and anaerobic energy metabolism in skeletal muscle of critically ill and recovering rats

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Abstract

As part of our research into the mechanisms of protein wasting and muscle weakness during critical illness, we here investigate various aspects of energy metabolism. Intraperitoneal injection of zymosan in rats leads to an acute phase of critical illness followed by a prolonged recovery phase. Previously we observed low activities of mitochondrial enzymes, reduced protein synthesis rates and low concentrations of glutamine in skeletal muscle of zymosan-treated rats. In the present study we investigated (1) whether decreases in high energy phosphates are present in skeletal muscle of these rats and (2) whether an impairment in the glycolytic pathway or the tricarboxylic acid cycle leads to these decreases. Concentrations of creatine phosphate and ATP were decreased in zymosan-treated rats to approx. 85% of pair-fed control values respectively on day 2 and on days 4 and 6 after treatment. Concentrations of tricarboxylic acid (TCA) cycle intermediates were decreased to 80% on day 6 after zymosan treatment. Lactate/pyruvate ratio and concentrations of lactate and glycogen were normal at all sampling times. We conclude that no major changes in concentrations of high energy phosphates and in concentrations of intermediates of TCA cycle, glycolysis and glycogenolysis were present. This indicated that, although the maximal oxidative capacity (mitochondrial content) is decreased, no derangement in energy metabolism seems to be present in skeletal muscle of critically ill and recovering rats.

Keywords: Zymosan; Skeletal muscle; Critical illness; Energy metabolism; High energy phosphate; Tricarboxylic acid cycle

1. Introduction

High energy phosphates (e.g., ATP and creatine phosphate) are the direct sources of energy for the cell. Decreased concentrations of ATP and creatine phosphate have been reported in skeletal muscle of critically ill patients and animal models [1–6]. Both muscle contraction and protein synthesis require energy. A decreased availability of high energy phosphates or a reduced capacity to synthesize ATP may, therefore, lead to low protein synthesis rates, loss of muscle mass and muscle weakness. All these features are characteristic for severe illness. Loss of muscle mass as a result of decreased protein synthesis rates occurs during the acute phase of critical illness, whereas muscle weakness may occur during this phase but is mainly manifested during the recovery phase. Patients recovering from severe illness complain of increased fatigability and muscle weakness up to months after leaving the hospital. In the present study derangements in energy metabolism were, therefore, studied during both the acute phase and recovery in a rat model of critical illness.

Recently we described a rat model for critical illness which is characterized by an acute phase of severe illness (2 days) followed by a prolonged recovery phase [7]. The illness was induced by injecting rats with zymosan, an extract of yeast cell wall and a potent activator of macrophages and complement system. A rapid fall in mitochondrial content of skeletal muscle was observed during the acute phase. This low mitochondrial content was maintained during the recovery phase and still present 6 days after treatment [8]. Most ATP is produced by aerobic processes located within the mitochondria. The tricarboxylic acid (TCA) cycle plays a vital role in these processes. Low concentrations of mitochondria may well

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implicate that the concentration of TCA cycle intermediates and mitochondrial ATP production are reduced. A low aerobic capacity to produce ATP may lead to an increased anaerobic production by enhanced glycolysis and glycogenolysis. In the present study we, therefore, investigated (1) whether concentrations of high energy phosphates are decreased in skeletal muscle during the acute phase of critical illness and recovery from it; and (2) whether this is accompanied by reduced concentrations of TCA cycle intermediates and by increased glycolysis and glycogenolysis (decreased glycogen and increased lactate concentrations).

Newsholme and colleagues [9] hypothesized that carbon skeletons obtained via the 'first-half' of the TCA cycle in part are used to synthesize glutamine via 2-oxoglutarate and glutamate. This glutamine may subsequently be released by muscle and used by lymphocytes and macrophages. A decreased availability of TCA cycle intermediates could, therefore, in addition to a deranged energy supply, lead to an impaired glutamine production by skeletal muscle and subsequently an impaired function of the immune system. Decreased concentrations of glutamine in skeletal muscle are a commonly observed feature during severe illness, and were also observed in the zymosan-treated rats [7].

Part of this research has been presented at the 15th Congress of the European Society of Parenteral and Enteral Nutrition (1993).

2. Materials and methods

Male SPF Lewis rats were supplied by the animal house of the University of Limburg. They were individually housed and kept in a controlled environment (12 h light cycle, 21–22°C and 50–60% humidity). Rats were fed a standard lab chow (SRM-A, Hope Farms, The Netherlands) containing (wt/wt) approx. 28% protein, 7% fat, 54% carbohydrates, 4% fibers and 7% minerals with a trace element and vitamin supplement. Rats were allowed to acclimatize to individual housing for one week. All experiments were approved by the Animal Experimental Committee of the University of Limburg.

Recently we have reported concentrations of amino acids and protein synthesis rates in zymosan-treated rats [7]. The same rats were used for the measurements described in the present paper.

Critical illness was induced by intraperitoneal injection of zymosan (50 mg per 100 g body weight) suspended in sterile liquid paraffin (25 mg/ml) as described before [7]. Food intake was substantially reduced after zymosan administration and, therefore, paraffin-injected control rats were pair-fed. Pair feeding was performed in three periods during the day (from 08.00 h till 15.00 h, 15.00 h till 22.00 h, and from 22.00 h till 08.00 h). This was done to ensure that the pair-fed rats had a similar eating pattern and would not eat all food offered at once and would subsequently be starved for the remainder of the day. Measurements were performed 16 h and 2, 4, and 6 days after treatment. Also a control group with free access to the rat chow and no paraffin injections was included in the study. All 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 measurements pair feeding was performed at 07.00 h and food was withheld from 08.00 h till 12.00 h. Sampling of muscle tissue was done between 12.00 h and 14.00 h. Animals were killed by cervical dislocation and the gastrocnemius muscle was rapidly removed, weighed, freeze-clamped in liquid nitrogen and stored at −80°C until analysis.

For metabolite determination muscle was homogenized in ice-cold 1 M perchloric acid (1:5) with an OMNI 1000 mechanical homogenizer. The supernatant was neutralized with 2 M potassium bicarbonate and used for measuring muscle metabolites:

1. ATP, ADP, AMP, creatine phosphate, creatine, pyruvate and lactate were measured as described by Harris and colleagues [10].
2. Citrate, malate and succinate were measured as described in Methods of Enzymatic Analysis [11–13].

All these analyses were modified for measurement on a spectrophotometric centrifugal analyzer (COBAS-BIO, Roche Diagnostica).

A small part (10–60 mg) of the frozen muscle was used to determine glycogen content. Free glucose was separated from glycogen by dissolving muscle in 1 M NaOH (37°C,

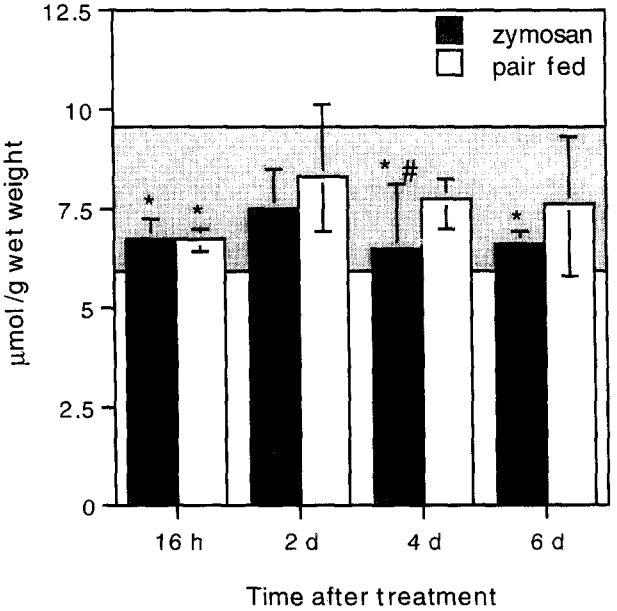

Fig. 1. Concentrations of ATP measured in muscle 16 hours (h) and 2, 4, and 6 days (d) after treatment. Values are given as mean (range) of 6–8 rats. The gray area represents the range of the ad libitum-fed control rats. *Significantly different from ad libitum and #pair-fed control rats.

Table 1
Concentrations of adenine nucleotides (μmol/g wet weight) and the energy charge measured in muscle 16 hours (h) and 2, 4 and 6 days (d) after treatment

		ADP	AMP	AMP + ADP + ATP	Energy charge
	con	0.9 (0.82–0.99)	0.047 (0.041–0.052)	8.8 (7.3–10.4)	0.942 (0.927–0.952)
16 h	zym	0.9 (0.82–0.90) [a]	0.073 (0.066–0.096) [a]	7.6 (7.3–8.1) [a]	0.934 (0.928–0.938) [a]
	pf	0.9 (0.84–0.95)	0.074 (0.064–0.090) [a]	7.7 (7.3–8.0) [a]	0.933 (0.930–0.935) [a]
2 d	zym	1.0 (0.88–1.02)	0.057 (0.052–0.060) [a]	8.7 (7.7–9.5)	0.939 (0.935–0.945) [b]
	pf	0.9 (0.76–1.12)	0.053 (0.041–0.060) [a]	9.2 (7.7–11.1)	0.945 (0.938–0.952)
4 d	zym	0.8 (0.65–0.94)	0.054 (0.045–0.066) [a]	7.4 (5.4–9.1) [a]	0.935 (0.923–0.943)
	pf	0.9 (0.71–1.05)	0.056 (0.041–0.075)	8.6 (7.8–9.1)	0.940 (0.930–0.948)
6 d	zym	0.9 (0.85–0.97)	0.050 (0.044–0.057)	7.6 (7.3–8.0) [a]	0.934 (0.932–0.936) [a]
	pf	0.9 (0.86–0.98)	0.047 (0.043–0.050)	8.5 (6.8–10.3)	0.940 (0.926–0.951)

Energy charge was calculated as [ATP + 0.5ATP]/[ATP + ADP + AMP]. Values are given as mean (range) of 6–8 rats.
Con, ad libitum-fed control; zym, zymosan-treated; pf, pair-fed rats.
[a] Significantly different from control; [b] significantly different from pair-fed.

1 h) and precipitating glycogen with 96% ethanol (80°C for 10 min and then overnight at 4°C). The glycogen pellet was hydrolyzed using 1 M HCl (100°C, 3 h). HCl was neutralized with a KCl saturated KOH/Tris (2.1 M/0.12 M) buffer. Glucosyl units obtained from glycogen were determined using a glucose kit (Hexokinase method, Roche) for a centrifugal analyzer (COBAS-BIO, Roche Diagnostica).

Differences between pair-fed and zymosan-treated rats and between zymosan-treated and ad libitum-fed control rats were analyzed for statistical significance using the Mann-Whitney *U*-test. Significance was set at $P < 0.05$. All measurements were done in duplicate. Values are given as mean (range).

3. Results

Rats injected with zymosan showed both clinical (lethargy, anorexia, diarrhea) and metabolic (muscle wasting, decreased glutamine pools and enlarged liver) signs of critical illness [7]. A mortality rate of 16% was observed in this study. All deaths occurred during the first 36 h. Pair-fed and ad libitum-fed control rats showed none of these signs. Details of the animal model including changes in food intake, body weight and protein synthesis rates have been described elsewhere [7].

3.1. Concentrations of adenine nucleotides (Table 1)

Concentrations of ATP (Fig. 1), the sum of ATP, ADP and AMP and the energy charge were decreased in both

Table 2
Concentrations of creatine and total creatine pool (μmol/g wet weight) measured in muscle 16 hours (h) and 2, 4 and 6 days (d) after treatment

		Creatine	Creatine + creatine phosphate
	con	17.5 (15.7–20.5)	35.4 (33.8–37.9)
16 h	zym	17.4 (15.8–19.1)	31.9 (30.5–33.2) [a]
	pf	16.8 (9.3–21.0)	31.1 (23.4–35.3) [a]
2 d	zym	19.4 (14.7–22.8)	35.0 (26.6–38.8)
	pf	18.0 (16.2–20.1)	33.5 (20.1–39.0)
4 d	zym	18.8 (14.6–21.1) [b]	34.8 (27.7–37.4)
	pf	21.3 (19.9–24.1) [a]	37.6 (34.3–39.9) [a]
6 d	zym	17.7 (15.9–18.9)	36.0 (32.8–38.6)
	pf	18.2 (16.2–19.9)	35.8 (32.4–37.2)

Values are given as mean (range) of 6–8 rats.
Con, ad libitum-fed control; zym, zymosan-treated; pf, pair-fed rats.
[a] Significantly different from control; [b] significantly different from pair-fed.

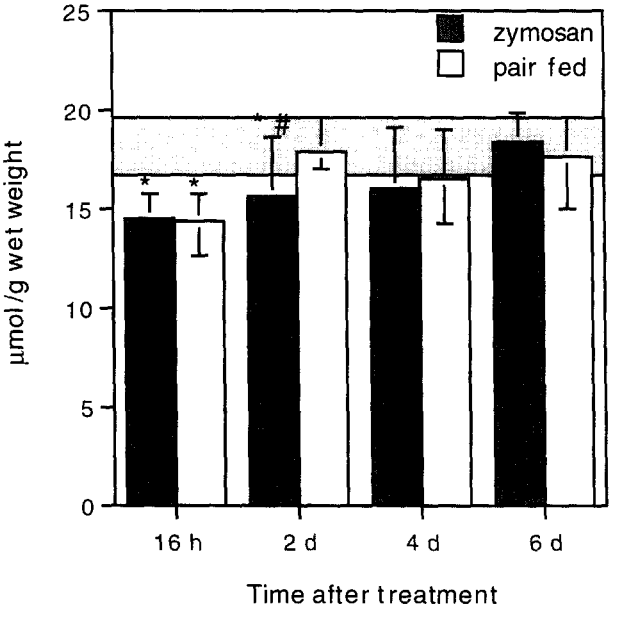

Fig. 2. Concentrations of creatine phosphate measured in muscle 16 hours (h) and 2, 4, and 6 days (d) after treatment. Values are given as mean (range) of 6–8 rats. The gray area represents the range of the ad libitum-fed control rats. *Significantly different from ad libitum and #pair-fed control rats.

pair-fed and zymosan-treated rats in comparison with ad libitum-fed control rats 16 h after treatment. AMP concentrations were increased in both experimental groups after 16 h.

Concentrations of ATP (Fig. 1) were decreased in muscle of zymosan-treated rats on day 4 in comparison with both control groups and on day 6 in comparison with ad libitum-fed control rats. The sum of ATP, ADP and AMP was decreased on days 4 and 6 in comparison with ad libitum-fed control rats. The energy charge was marginally decreased in muscle of zymosan-treated rats on day 2 in comparison with the pair-fed controls and on day 6 in comparison with the ad libitum-fed control rats.

3.2. Concentrations of creatine and creatine phosphate (Table 2)

Concentrations of creatine phosphate (Fig. 2) and the total creatine pool (sum of creatine and creatine phosphate) were decreased 16 h after treatment in pair-fed and zymosan-injected rats in comparison with ad libitum-fed control rats.

Concentrations of creatine were decreased in muscle of zymosan-treated rats on day 4 in comparison with pair-fed control rats. However, creatine concentrations in pair-fed control rats were increased on this day in comparison with ad libitum-fed rats. Concentrations of creatine phosphate (Fig. 2) were decreased 2 days after zymosan treatment in comparison with both ad libitum- and pair-fed control groups.

3.3. Concentrations of tricarboxylic acid cycle intermediates (Table 3)

Of the TCA cycle intermediates citrate, succinate and malate were measured. The sum of these intermediates represents about 70% of the total TCA cycle intermediate pool [14] and, therefore, this value is considered as a good indication of changes in the level of the total TCA-cycle intermediate pool in muscle.

Table 3
Concentrations (nmol/g wet weight) of tricarboxylic acid cycle intermediates (citrate, succinate and malate) measured in muscle 16 hours (h) and 2, 4 and 6 days (d) after treatment

		Citrate	Succinate	Malate
	con	169 (142–198)	270 (213–321)	307 (266–347)
16 h	zym	167 (134–228)	391 (335–495) [a]	338 (285–422) [b]
	pf	173 (149–204)	372 (327–430) [a]	292 (224–345)
2 d	zym	124 (92–155) [a]	285 (201–390)	356 (237–480) [b]
	pf	149 (116–191)	286 (176–406)	292 (225–369)
4 d	zym	100 (50–147) [a]	248 (141–348)	255 (181–343)
	pf	102 (73–121) [a]	258 (195–334)	262 (221–320) [a]
6 d	zym	150 (92–204)	207 (156–274) [a]	222 (204–260) [a,b]
	pf	135 (108–164) [a]	284 (197–420)	314 (253–410)

Values are given as mean (range) of 6–8 rats.
Con, ad libitum-fed control; zym, zymosan-treated; pf, pair-fed rats.
[a] Significantly different from control; [b] significantly different from pair-fed.

Sixteen h after treatment both succinate concentrations and the sum of TCA intermediates (Fig. 3) were increased in zymosan-treated and pair-fed rats in comparison with ad libitum-fed control rats.

Concentrations of citrate were decreased in zymosan-treated rats on day 2 in comparison with ad libitum-fed control rats. Succinate concentrations were decreased 6 days after zymosan treatment in comparison with ad libitum-fed control rats. Concentrations of malate were increased after 16 h and 2 days in comparison with pair-fed controls and decreased on 6 days after zymosan treatment in comparison with both ad libitum- and pair-fed animals. The sum of citrate, succinate and malate (Fig. 3) was decreased on day 6 in comparison with both control groups.

3.4. Concentrations of pyruvate, lactate and glycogen (Table 4)

Concentrations of both lactate and glycogen were decreased in muscle of zymosan-treated and pair-fed rats in

Table 4
Concentrations pyruvate (nmol/g wet weight), lactate (μmol/g wet weight) and glycogen (μmol glucosyl units/g wet weight) measured in muscle 16 hours (h) and 2, 4 and 6 days (d) after treatment

		Pyruvate	Lactate	Lact/pyr	Glycogen
	con	84 (75–100)	4.1 (2.8–5.6)	46 (34–67)	162 (149–181)
16 h	zym	68 (62–84) [a]	2.6 (1.9–3.4) [a]	38 (28–46)	132 (96–154) [a]
	pf	70 (52–81)	2.8 (2.1–4.0) [a]	40 (25–59)	130 (114–154) [a]
2 d	zym	88 (63–111)	2.8 (1.3–4.0)	31 (19–43) [a]	123 (112–138) [a]
	pf	86 (76–99)	3.6 (2.5–5.0)	43 (29–60)	154 (97–202)
4 d	zym	72 (53–97)	3.0 (1.7–4.8) [b]	40 (32–54)	160 (103–214)
	pf	93 (63–114)	4.9 (3.3–6.9)	57 (34–111)	181 (159–214)
6 d	zym	65 (58–69) [a,b]	3.0 (2.1–3.8)	46 (31–65)	214 (193–248) [a]
	pf	84 (78–93)	4.1 (3.0–5.7)	49 (36–73)	206 (185–230) [a]

Values are given as mean (range) of 6–8 rats.
Con, ad libitum-fed control; zym, zymosan-treated; pf, pair-fed rats.
[a] Significantly different from control; [b] significantly different from pair-fed.

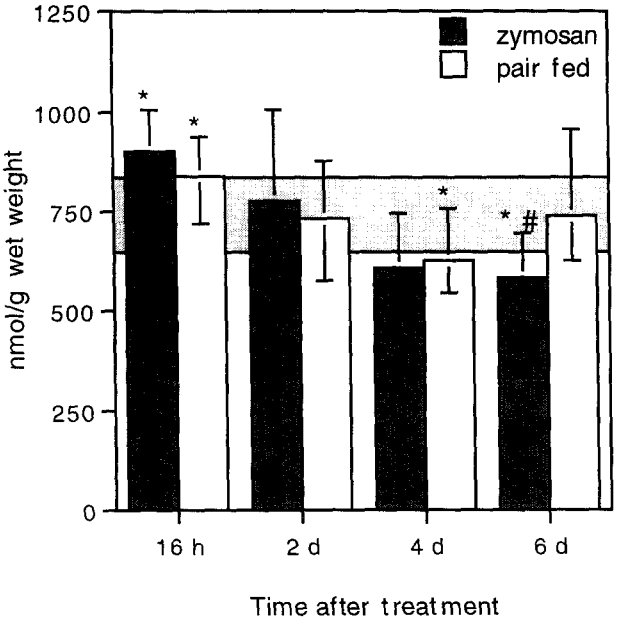

Fig. 3. Concentrations of TCA cycle intermediates (sum of citrate succinate and malate) measured in muscle 16 hours (h) and 2, 4, and 6 days (d) after treatment. Values are given as mean (range) of 6–8 rats. The gray area represents the range of the ad libitum-fed control rats. * Significantly different from ad libitum and #pair-fed control rats.

comparison with ad libitum-fed control rats 16 h after treatment. Glycogen concentrations were increased in both experimental groups on day 6 in comparison with ad libitum-fed control rats.

Concentrations of pyruvate were decreased in muscle 16 h after zymosan treatment in comparison with ad libitum-fed control rats and on day 6 in comparison with both ad libitum- and pair-fed control rats. On day 2 the lactate/pyruvate ratio and the glycogen concentration were decreased in the zymosan-treated rats in comparison with ad libitum-fed control rats.

4. Discussion

In skeletal muscle of both zymosan-treated and pair-fed rats comparable decreases in concentrations of ATP and creatine phosphate, and increases in concentrations of AMP and succinate were observed 16 h after treatment. This indicates that the zymosan-induced changes in muscle energy metabolism during the early phase of acute critical illness are mainly the result of a decreased food intake. The abnormalities present in the pair-fed group tended to normalize after 16 h. During the prolonged recovery period most changes are the result of the zymosan treatment independent of the food intake. The substantial influence of the reduced food intake early after the insult indicates that food intake should be carefully controlled in this kind of metabolic studies.

Concentrations of high energy phosphates were decreased in muscle of zymosan-treated rats to a moderate extent only. Concentrations of creatine phosphate were reduced to 87% of control 2 days after injection. ATP levels were 83 and 85% of the ad libitum-fed control values on day 4 and 6 respectively. In muscle of both patients and animal models dramatic changes in adenine nucleotides have only been observed during critical illness with fairly high mortality rates. Decreased ATP concentrations of approx. 50% have been measured in critically ill patients with mortality rates over 80% [2,3]. In a study of Liaw and colleagues [1] substantial decreases in concentrations of ATP, ADP and creatine phosphate were only observed in the critically ill patients and not in moderately and severely ill patients. Rats with severe burn trauma showed a large (62%) decrease in muscle concentrations of ATP [5]. Other animal models with less severe illness or trauma showed no changes [15–17] or small changes [4,6] in concentrations of adenine nucleotides.

A reduction in TCA cycle intermediates was measured on day 6 after zymosan treatment only, whereas the reduction of mitochondrial content was observed over a longer period [8]. In addition, the decrease in mitochondrial content seems to be larger than that of the TCA cycle intermediates. These result indicate that the concentration of TCA cycle intermediates per mg of mitochondrial protein is increased. The changes in TCA cycle intermediates were in general not paralleled by changes in high energy phosphates and, therefore, do not appear to induce a derangement in the energy metabolism under the circumstances studied. The decreased concentration of TCA cycle intermediates may, however, reduce the availability of carbon skeletons for the production of glutamine via 2-oxoglutarate and glutamate [9]. Glutamine has been suggested to become a conditionally essential amino acid for the critically ill because the body's requirements for glutamine seems to exceed the ability to produce glutamine [18]. Decreased muscle and plasma concentrations of glutamine are commonly observed in critically ill patients and animal models, including the zymosan-treated rats [7].

No substantial differences in glycogen and lactate concentrations were observed between the groups. Decreased glycogen concentrations and increased lactate concentrations are expected when the anaerobic production of ATP via the glycolysis and glycogenolysis is increased. The lactate/pyruvate ratio was not changed in muscle of zymosan-treated rats. This indicates that maintenance of the NADH/NAD ratio by the oxidative phosphorylation was not impaired. In addition, maintenance of the NADH/NAD ratio implies that the oxygen supply is not limited. This rules out the possible occurrence of anoxia in this model. These results indicate that, in spite of the decreased mitochondrial content, the oxidative capacity is adequate to meet the cellular energy needs in skeletal muscle of zymosan-treated rats at rest.

Although the results indicate that the oxidative capacity of the muscle is not limited, decreased concentrations of

ATP were observed on days 4 and 6 after zymosan treatment. These changes are, however, not accompanied or preceded by decreased concentrations of creatine phosphate. Normally an energy deficit will lead to a substantial decrease of the concentration of creatine phosphate before ATP levels start to drop. This supports the conclusion that no derangement in the muscle energy metabolism is present. Most likely, the decrease in ATP is the result of a general loss of adenine nucleotides either by a decreased production or increased breakdown. The same phenomenon has been observed in both severely ill patients [3,19] and animals [6].

Decreased activities of citrate synthase and cytochrome-*c* oxidase were previously observed in muscle of zymosan-treated rats up to 6 days after treatment [8]. This indicates that the mitochondrial content is reduced. In a study of Dudley and colleagues [20] muscle energy metabolism has been studied in rats with different mitochondrial contents in skeletal muscle. Only minor differences were observed in muscle ATP and creatine phosphate/creatine concentrations at rest. However, in situ electrical stimulation resulted in large decreases in high energy phosphates in the low mitochondria group and small decreases in the high mitochondria group. Also in muscle of tumor-bearing rats decreased concentrations of ATP were only observed in the soleus muscle after in situ stimulation and not in the resting state [21]. This indicates that also in muscle of zymosan-treated rats the oxidative capacity may be sufficient to prevent dramatic decreases in concentrations of high energy phosphates at rest but not during an increased contractile activity.

Protein synthesis rates have also been measured in muscle of the same rats as used in the present study [7]. Only at 16 h after treatment protein synthesis rates were decreased. No decreases in concentrations of high energy phosphates were observed at this time point, which seems to suggest that a reduced availability of energy substrates is not the cause of the reduced protein synthesis rates.

In conclusion, modest decreases in concentrations of high energy phosphates have been observed in resting skeletal muscle of zymosan-treated rats both during the acute phase of critical illness and during recovery. These decreases were, however, not accompanied by a derangement in the TCA cycle or by an increased anaerobic production of ATP. We, therefore, conclude that, although the maximal oxidative capacity (mitochondrial content) is decreased in skeletal muscle of zymosan-treated rats, no derangement in energy metabolism seems to be present at rest. The decreased concentration of TCA cycle intermediates observed in skeletal muscle during recovery may have consequences for the glutamine production. Because of the putative clinical importance of glutamine during severe illness this aspect should be investigated in greater detail.

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