



RESEARCH ARTICLE

MITOCHONDRIAL DYSFUNCTION DURING DIFFERENT SEPSIS FORMS

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ABSTRACT

**Background:** Sepsis-induced multiple organ failure is the major cause of mortality and morbidity in critically ill patients. The precise mechanisms by which this dysfunction is caused remain to be elucidated. Because ATP production by mitochondrial oxidative phosphorylation accounts for more than 90% of total oxygen consumption, we postulated that mitochondrial dysfunction results in organ failure, possibly due to nitric oxide, which is known to inhibit mitochondrial respiration in vitro and is produced in excess in sepsis.

**Methods:** We did skeletal muscle biopsies on 25 septic patients within 24 h of admission to intensive care. The biopsy samples were analyzed for myosin heavy chains (MHC), isoform gene transcript levels using a real-time PCR technique, respiratory-chain activity and ATP production (complex V) using a real-time QPCR technique for mitochondrial enzyme gene transcript levels citrate synthase (CS), cytochrome C oxidase I-III, NADH and UCPs 2-3.

**Results:** The gene transcription of myosin fiber types I, IIa and citrate synthase (complex V) was significant higher in skeletal muscle biopsy of septic survivors, and demonstrated lower insulin sensitivity using HOMA test. Severity of septic shock (SAPS II) found to be higher in critically ill patients.

**Conclusion:** In critically ill patients, we found an association between the type of myosin fiber and ATPase gene transcription and insulin resistance that relate to organ failure and eventual outcome. These data implicate bioenergetic failure as an important pathophysiological mechanism underlying multiple organ dysfunction.

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INTRODUCTION

Sepsis is the systemic inflammatory response associated with an infectious insult. It is the leading cause of death in critically ill patients, and the predominant cause of multiple organ dysfunction (Deitch, 1992). However, precise mechanisms through which organ dysfunction develops remain unknown, as do reasons for its persistence long after cessation of the acute inflammatory phase. Although microvascular flow redistribution undoubtedly occurs (Ince and Sinaasappel, 1999), we and others have shown increased tissue oxygen tension in the organs of animals and patients with sepsis (Rosser et al., 1995; Boekstegers et al., 1991). This finding suggests that the predominant defect might lie in cellular oxygen use (tissue dysoxia) rather than in oxygen delivery per se. Mitochondrial oxidative

phosphorylation is responsible for over 90% of total body oxygen consumption and ATP generation. This takes place in the mitochondrial inner membrane at the respiratory chain. The respiratory chain (electron-transport chain) includes four individual enzyme complexes (I-IV). These enzyme complexes, notably NADH-ubiquinone oxidoreductase (complex I) and cytochrome C oxidase (complex IV), can be inhibited by reactive oxygen and nitrogen species such as nitric oxide (Clementi et al., 1998; Bolanos et al., 1996; Brown and Cooper, 1994). These reactive species are produced in substantial excess during sepsis and are also generated by the mitochondria (Taylor et al., 1995). Complex I inhibition by nitric oxide is facilitated, in vitro, by depletion of the intracellular reduced glutathione. Concentrations of this antioxidant are also known to decrease in septic states (Minamiyama et al., 1996; Corbucci et al., 1985). We also know that there are three major muscle types, skeletal, cardiac and smooth muscle. Skeletal muscle is a form of muscle tissue which is under the control of the nervous system. It is made up of individual components known as

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fibers. Each fiber has an outer membrane called sarcolemma which surrounds myofibrils. Between them are mitochondria and sarcoplasm. The principle cytoplasmic proteins are actin and myosin. There are two main types of fibers, type I and type II. Type I fibers appear red due to the high levels of myoglobin. They have more mitochondria and greater local capillary density. They are more suited for endurance and are slow to fatigue because they use oxidative metabolism to generate ATP (adenosine triphosphate). They generate energy for ATP re-synthesis by means of a long term system of aerobic energy transfer. On the other hand type II fibers tend to be less oxidative, they are white and a reliance on glycolytic enzymes. Compared to glycolysis oxidative phosphorylation produces 17 to 18 times as much ATP from the same amount of glucose. It is believed there are no sex or age differences in fiber distribution. Sedentary men and women have 45% type II and 55% type I fibers. Endurance athletes show a higher level of type I fibers. On the other hand sprint athletes require large numbers of type II. It has been suggested that various types of exercise can induce changes in the fibers of skeletal muscles. Type II fibers show enhancements of the oxidative capacity after high intensity endurance training which brings them to a level at which they are able to perform oxidative metabolism as effectively as type I fibers of untrained subjects. This would be brought about by an increase in mitochondrial size and number and the associated related changes not a change in fiber type (MacIntosh *et al.*, 2006).

There are two commonly used methods for fiber typing. Histochemical staining for myosin ATPase activity and immunohistochemical staining for myosin heavy chain (MHC) type which results from determination of different MHC isoforms. These methods are closely related as the MHC type is the primary determinant of ATPase activity (Smerdu *et al.*, 1994; Pete *et al.*, 2000). In the inner mitochondrial membrane we can also find uncoupling protein (UCP). They are transporters that work in parallel with ATP synthase, generating heat from the energy that is not used to generate ATP (Nedergaard *et al.*, 2005; Rousset, Sophie *et al.*, 2004). Distal muscle weakness and loss of deep tendon reflexes are usually found. It is an axonal (Latronico *et al.*, 1996) motor-sensory polyneuropathy called Critical Illness Polyneuropathy (CIP). Polyneuropathy may develop as early as one week after the onset of SIRS, but the incidence tends to correlate with the duration of the severe illness. The pathogenesis is not known. Electrophysiological findings are those of pure axonal degeneration affecting more motor than sensory fibers. The axonal damage is due to the transport system which is energy dependent. Nerve biopsy reveals axonal degeneration without demyelination or inflammation. This results in denervation and grouped atrophy of muscle (Kress *et al.*, 2014). To address the questions whether bioenergetic status in septic patients is associated with mitochondrial dysfunction, and whether these abnormalities are related to organ failure, we undertook a systematic study of mitochondrial dysfunction in septic patients admitted to intensive care.

## MATERIALS AND METHODS

### Patients

After obtaining approval of PhD dissertation's protocol from the Medical School committee of UoA and consent to participate the study, patients were recruited from the ICU of Evangelismos G.H. since Apr 2003 to Jul 2006. Patients

with recent-onset severe sepsis or septic shock (as defined by standard criteria) were enrolled. Those with severe coagulopathies (PLTs<30 or INR>2), immunosuppression (endogenous or long-term chemotherapeutic), or both, were excluded. Routine physiological and biochemical variables were recorded, and scores on the simplified acute physiology score (SAPS II) and sequential organ failure assessment (SOFA) calculated.

### Procedures

Within 24 h of patients admission to the intensive care unit, a quadriceps femoris muscle biopsy was done microsurgical via a small incision in the middle of the muscle. Biopsy samples (averaging 750 mg total) were frozen immediately in liquid nitrogen. MHC isoform composition was determined by SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis) silver staining.

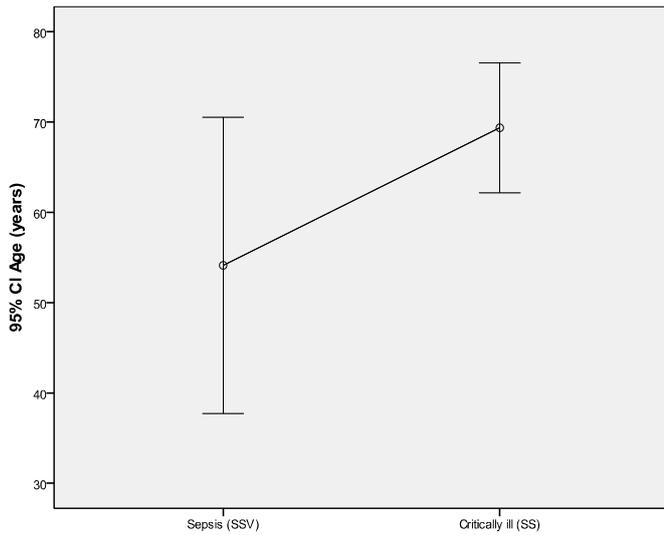
### Statistical analysis

Quantitative variables were expressed as mean values (SD), while qualitative variables were expressed as absolute and relative frequencies. Independent samples Student's t-tests were used for the comparison of mean values between the two groups. For the comparison of proportions, Fisher's exact tests were used. Pearson's correlations coefficients were used to explore the association of two continuous variables. Correlation coefficient between 0.1 and 0.3 were considered low, between 0.31 and 0.5 moderate and those over 0.5 were considered high. All reported p values are two-tailed. Statistical significance was set at  $p < 0.05$  and analyses were conducted using SPSS statistical software (version 19.0).

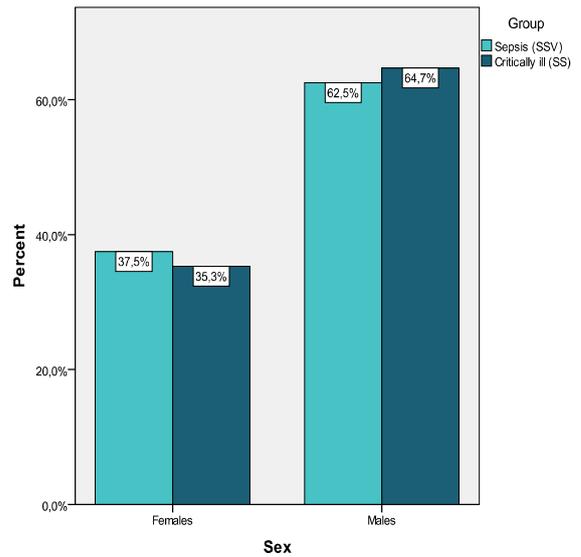
## RESULTS

Data consists of two groups. A study group of all sepsis forms patients (33) and a control group of 10 patients undergone elective orthopedic surgery. We report on the results of dividing study group into two subgroups as important. One of them with septic patients and another one with critically ill ones (severe septic and septic shock). Sample consisted of 25 patients with sepsis, 17 (68.0%) of whom were critically ill. Patients' characteristics are presented in Table 1. Mean age for critically ill patients was 69.4 years (SD=14.0 years) and significantly higher compared to the mean age of the patients with sepsis. In both groups the majority of the patients were men with the percentages being 64.7% for the critically ill group and 62.5% for the septic group. All critically ill patients died while none of the group with sepsis died. Patients' measurements regarding the severity of their sepsis are presented in table 2. Critically ill patients scored significantly in SAPS II, indicating greater severity of their condition, compared to the rest septic patients. SOFA index and all biochemical indexes were similar between the two groups. Owing to variation in the amount of tissue obtained, we were unable to do a full set of analyses on all patients. Information from biopsy regarding patients' insulin tolerance is presented in Table 3. Critically ill patients had significantly lower mRNA-CS, mRNA-MHC I and mRNA-MHC IIA values compared to the rest septic patients (Figures 5-7). There was no difference in biopsies between the activities of mitochondrial complexes but we found significant higher the gene transcription of septic survivors. In all septic

**Demographics**

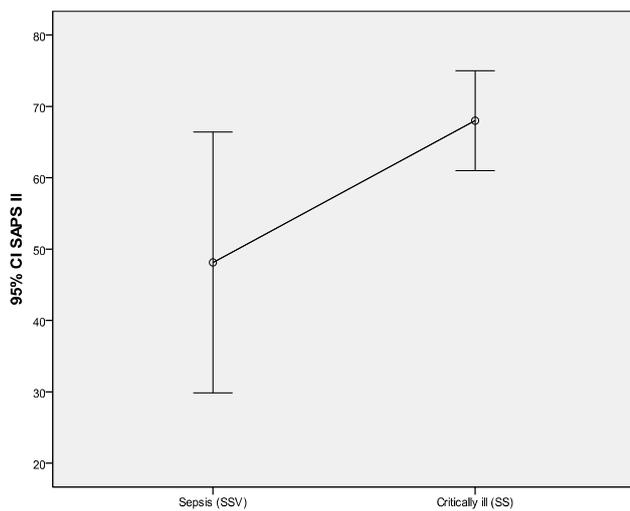


**F1: age**

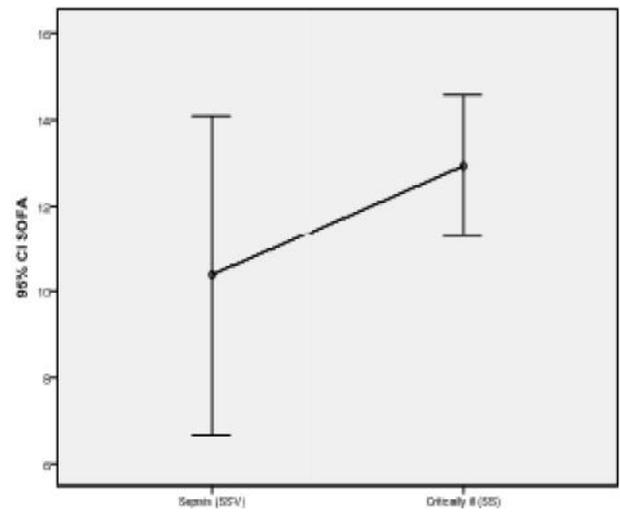


**F2: sex**

**Scores**

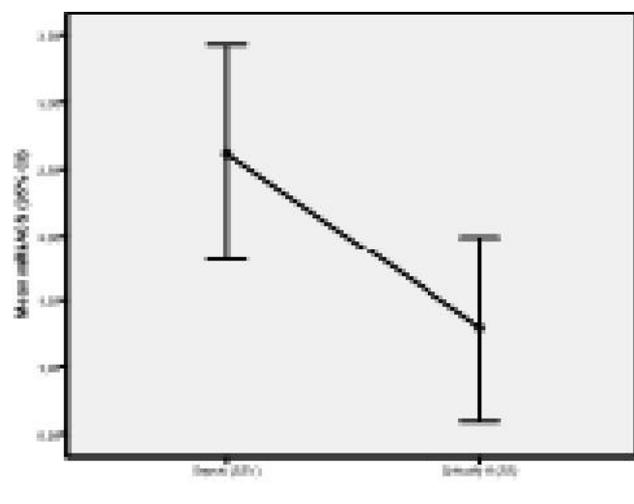


**F3: SAPS II**

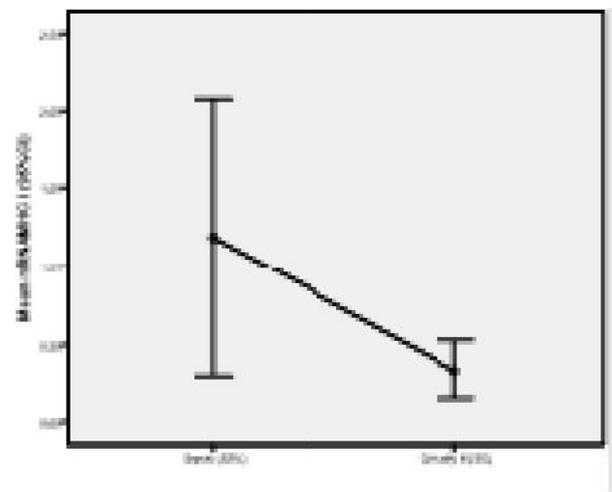


**F4: SOFA**

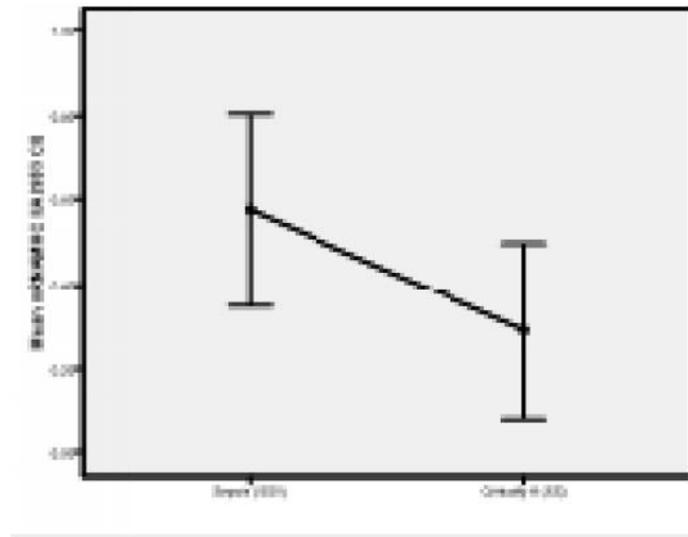
**Biopsy results**



**F5: mRNA CS**

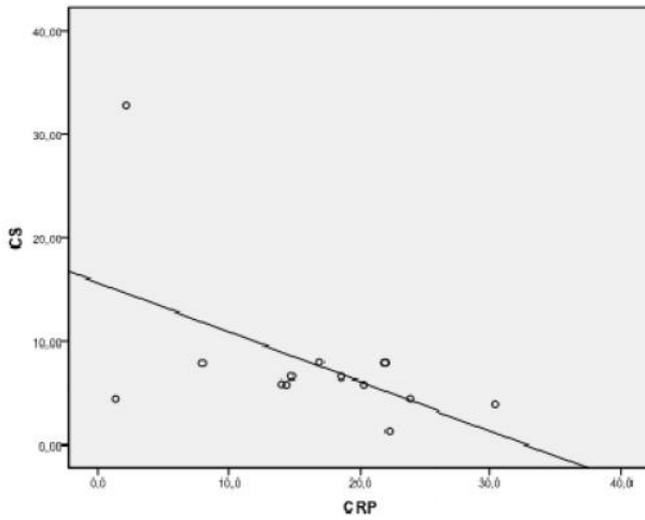


**F6: mRNA MHC I**

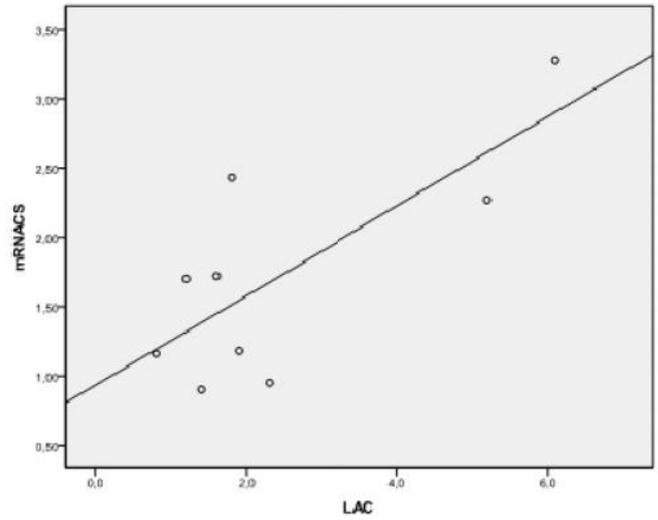


**F7: mRNA MHC IIA**

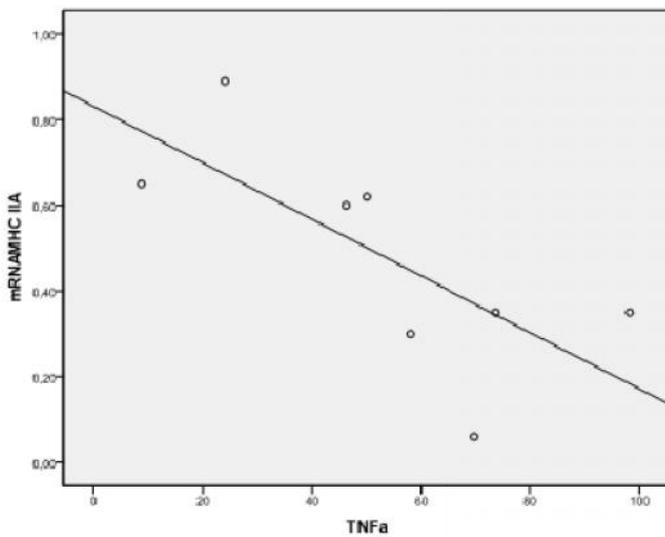
**Pearson correlations**



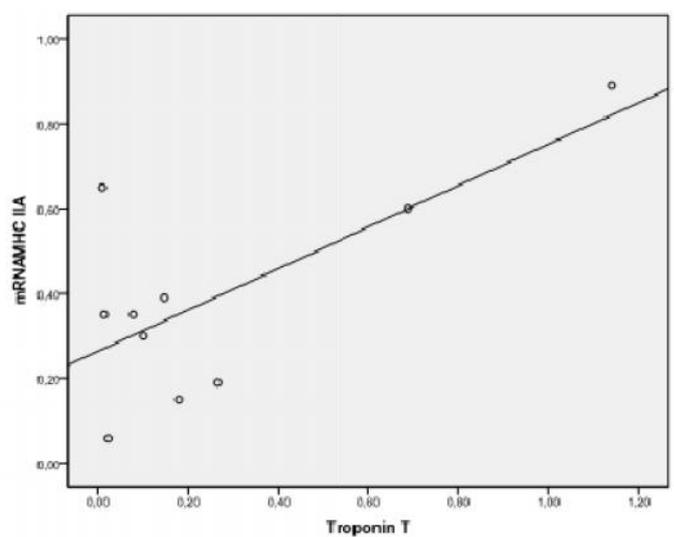
**F8: CS f CRP**



**F9: mRNA CS f lac**



**F10: mRNA MHC IIA f TNFa**



**F11: MRNA MHC IIA f Troponin T**

**Table 1. Sample characteristics for the two study groups**

	Group		P
	Sepsis (SSV)	Critically ill (SS)	
	(N=8)	(N=17)	
Age (years), mean (SD)	54.1 (19.6)	69.4 (14.0)	0.036*
Sex			
Females	3 (37.5)	6 (35.3)	1.000**
Males	5 (62.5)	11 (64.7)	
Death			
No	8 (100.0)	0 (0.0)	<0.001**
Yes	0 (0.0)	17 (100.0)	

\*Student's t-test; \*\*Fisher's exact test

**Table 2. Biochemical indexes in critically ill patients and patients with sepsis**

	Group		P*
	Sepsis (SSV)	Critically ill (SS)	
	Mean (SD)	Mean (SD)	
SOFA	10.4 (4.4)	12.9 (3)	0.112
SAPS II	48.1 (21.9)	68 (12.7)	0.011
CRP	16.1 (7.5)	16.4 (8)	0.925
PCT	7.6 (11.3)	8.1 (14.5)	0.949
TNFa	42.9 (26.3)	63.2 (30.5)	0.227
IL-6	898 (1576.4)	1089.3 (2062.1)	0.859
proBNP	6145.7 (4621.4)	9581 (7556.3)	0.406
Troponin T	0.32 (0.55)	0.3 (0.43)	0.962
LAC	2.24 (2.21)	3.74 (4.69)	0.505

\*Student's t-test

**Table 3. Biopsy results in critically ill patients and patients with sepsis**

	Group				P*
	Sepsis (SSV)		Critically ill (SS)		
	N	Mean (SD)	N	Mean (SD)	
CS	8	6.1 (1.4)	7	9.6 (10.5)	0.373
COX	8	5.5 (2.5)	7	8.7 (7.4)	0.281
COX	6	39.2 (7.5)	4	47.3 (23.9)	0.451
MHC IIA	6	30.7 (6.6)	4	29.5 (14.2)	0.863
MHC IIX	6	27.7 (2.5)	4	24.8 (11.2)	0.545
mRNACS	6	2.6 (0.8)	5	1.3 (0.6)	0.011
mRNACOXI	6	0.70 (0.24)	5	2.01 (2.26)	0.188
mRNACOXIII	6	0.25 (0.18)	5	0.2 (0.18)	0.667
mRNANADH	6	2.53 (2.13)	5	1.67 (1.13)	0.442
mRNAMHC I	6	1.19 (0.84)	6	0.34 (0.18)	0.037
mRNAMHC IIA	6	0.58 (0.22)	6	0.29 (0.2)	0.038
mRNA UCP2	6	0.93 (0.77)	5	0.91 (0.7)	0.963
mRNA UCP3	6	1.35 (0.97)	3	3.49 (3.27)	0.161
mRNAMHC IIX	6	10.6 (10.9)	5	7.8 (7.2)	0.639

\*Student's t-test

**Table 4. Pearson's correlation between severity indexes, biochemical measurements and biopsy results**

	SOFA	SAPS II	CRP	PCT	TNFa	IL-6	proBNP	Troponin T	LAC
CS	-0.01	0.03	-0.53*	-0.23	0.61	-0.15	0.55	-0.22	0.46
COX	-0.03	0.03	-0.53*	-0.31	0.41	-0.01	0.44	-0.16	0.26
MHCI	0.39	0.18	0.49	-0.14	0.11	0.41	0.11	-0.28	-0.25
MHC IIA	-0.49	-0.24	-0.54	-0.07	0.10	-0.35	0.00	0.38	0.24
MHC IIX	-0.32	-0.16	-0.56	0.09	0.30	-0.42	-0.04	0.13	0.28
mRNACS	-0.48	-0.47	-0.32	0.32	0.02	0.04	-0.11	0.04	0.75*
mRNACOXI	0.37	0.29	0.58	-0.18	-0.09	-0.20	-0.21	0.27	-0.21
mRNACOXIII	-0.61*	-0.45	-0.23	0.40	0.12	-0.26	0.26	0.05	0.37
mRNANADH	-0.64*	-0.50	-0.28	0.38	0.22	-0.26	0.21	0.07	0.72*
mRNAMHC I	0.21	0.04	-0.11	-0.20	-0.59	0.04	-0.39	-0.07	-0.38
mRNAMHC IIA	-0.33	-0.31	-0.09	-0.22	-0.72*	-0.42	-0.36	0.71*	-0.27
mRNA UCP2	0.53	0.31	0.38	-0.26	-0.52	0.36	-0.55	0.19	-0.54
mRNA UCP3	0.46	0.43	0.23	-0.21	-0.18	-0.05	-0.53	0.36	-0.29
mRNAMHC IIX	0.32	0.07	0.27	-0.33	-0.48	0.60	0.00	-0.29	-0.60

\*p&lt;.05; \*\*p&lt;.01; \*\*\*p&lt;.001

## DISCUSSION

We have shown, in patients with sepsis and multiple organ failure (critically ill patients), a relation between shock severity and mitochondrial dysfunction. Despite being unable to distinguish clinically between eventual survivors and non-survivors, significant differences were seen in muscle bioenergetic status taken within 24 h of intensive-care admission. Although the number of patients is relatively low and multiple comparisons were made on the data, the findings are nevertheless in keeping with cellular, animal, and the sparse human data previously reported (Brealey David *et al.*, 2002). The respiratory chain is located in the mitochondrial inner membrane and consists of the four complexes plus specialized electron carriers. Passage of electrons down the chain creates a proton gradient across the inner membrane sufficient to drive ATP synthase (complex V) to phosphorylate ADP to ATP. Fink *et al.* (Fink, 2001; Fink Mitchell, 2002) has postulated that impaired cellular O<sub>2</sub> utilisation, rather than inadequate oxygen delivery, may play an important role in sepsis development. We presume that the lengthy course of sepsis and extended exposure to reactive nitrogen species results in irreversible inhibition or permanent damage to the enzyme complex. Brealey *et al.* (2002) showed an association between nitric oxide overproduction, decreased ATP concentrations in skeletal muscles and mitochondrial dysfunction in critically ill patients. That's why bioenergetic failure seems to be an important pathophysiological mechanism due to multiorgan dysfunction (Levy Richard, 2007; Vincent, 2007; Protti Alessandro and Singer Mervyn, 2006; Michael ÉvertonAndrades *et al.*, 2011). The structure of MHC isoforms remained the same but we noticed decreased rate of transcription of genetic information from DNA to mRNA (downregulation of gene transcription) in critically ill patients. Same structure MHC isoforms is also related with decreased ATPase transcription in critically ill patients. Another important result we observed was the association of sepsis severity with the development of insulin resistance and fiber-type transitions (Halvatsiotis *et al.*, 2015). Many studies and animal models suggest a decrease in anabolic insulin signaling within skeletal muscles of critically ill patients (Bierbrauer and Weber-Carstens, 2011; Dhar and Castillo, 2011). Insulin resistance is an adaptive mechanism that prioritizes utilization of energy for immune response in the presence of infection and a better understanding of the complex interactions between metabolism, inflammation and immunity in critically ill will lead to appropriate metabolic and immune support of these patients. In conclusion TNF in the presence of H<sub>2</sub>O<sub>2</sub> as NF- leads to NO overproduction and decrease of ATP production. Fredriksson *et al.* (2006) showed mitochondrial dysfunction in leg muscles of critically ill patients because of low complex I activity. In our study, based on the former results, we proved that mitochondrial dysfunction as decreased ATP production because of low gene transcription of ATPase is correlated to the type of myosin fiber, insulin resistance and pure outcome of critically ill patients.

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