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MUSCLE MITOCHONDRIA IN SEPSIS

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Det finns inget som är nytt under solen,
men det finns många gamla saker vi inte vet.
Ambrose Blake

ABSTRACT

Patients treated in the intensive care unit (ICU) for sepsis induced multiple organ failure often suffer from skeletal muscle fatigue after ICU discharge. Since mitochondria are the main determinants of muscle fatigability, their function in muscle of these patients was the theme of this thesis. Decreased muscle mitochondrial activity has been related to mortality in patients suffering from acute sepsis. The present thesis has two major aims. The first is to describe mitochondrial derangements in muscle from septic patients and the second one was to elucidate the underlying mechanisms for these mitochondrial problems.

To describe the mitochondrial derangements in muscle from septic patients, muscle biopsies were obtained from respiratory and leg muscle of mechanically ventilated septic patients and healthy control patients. In both muscle tissues a decreased mitochondrial content was found in comparison to controls. In addition, leg muscle had lower concentrations of energy rich phosphates and an increased lactate concentration. The second study relating to this problem was performed in a human model for studying the very early phase of sepsis. In this study leg muscle biopsies were obtained at baseline and 2 and 4 hours after an intravenous endotoxin injection. Mitochondrial enzyme activities increased 2 hours after endotoxin and went back to baseline at 4 hours. The concentration of ATP did not change between baseline and the two consecutive biopsies, however an increase in activity was found between 2 and 4 hours after endotoxin.

The second aim was to characterize underlying mechanisms that may cause mitochondrial derangements in septic patients. The effect of inactivity on muscle mitochondria was evaluated in diaphragm muscle from mechanically ventilated piglets. The mitochondrial enzyme activity of complex IV was significantly decreased after 5 days of mechanical ventilation while the other mitochondrial enzymes and content did not change. In the last study mitochondrial protein turnover and biogenesis were evaluated in leg muscle from septic ICU patients. Mitochondrial protein synthesis and mRNA levels of mitochondrial proteins were not different between patients and controls. Some of the mitochondrial transcription factors increased in mRNA levels, whereas the others did not change in comparison to controls. The mRNA levels of the active subunits of two mitochondrial matrix proteases increased significantly while the membrane bound proteases did not change.

In summary, the mitochondrial content is decreased in respiratory and leg muscle from septic ICU patients. In leg the lower mitochondrial content was accompanied by low concentrations of energetic phosphates. A human model of sepsis indicated a biphasic development of mitochondrial derangements were an initial increase in mitochondrial enzyme activity is followed by a decrease. The cause of the decrease in mitochondrial content does not seem to be related to inactivation by mechanical ventilation as evaluated in piglets. Decreased mitochondrial content cannot be explained by a decreased mitochondrial protein synthesis or biogenesis. It is more likely that an increased mitochondrial protein breakdown is responsible for the decreased mitochondrial content in patients with sepsis induced multiple organ failure.

LIST OF PUBLICATIONS

I. **Derangements in mitochondrial metabolism in intercostal and leg muscle of critically ill patients with sepsis-induced multiple organ failure**.
Fredriksson K, Hammarqvist F, Strigård K, Hultenby K, Ljungqvist O, Wernerman J, Rooyackers O.
Am J Physiol Endocrinol Metab 291:E1044-E1050, 2006

II. **Muscle mitochondrial activity and energetic status in a human model of sepsis.**
Fredriksson K, Fläring U, Guillet C, Wernerman J, Rooyackers O.
Manuscript

III. **Effect of prolonged mechanical ventilation on diaphragm muscle mitochondria in piglets.**
Fredriksson K, Radell P, Eriksson LI, Hultenby K, Rooyackers O.
Acta Anaesthesiol Scand 49: 1101-1107, 2005

IV. **Mitochondrial protein turnover in muscle from patients with sepsis induced multiple organ failure.**
Fredriksson K, Tjäder I, Ahlman B, Scheele C, Wernerman J, Timmons JA, Rooyackers O.
Manuscript

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LIST OF ABBREVIATIONS

ICU	Intensive care unit
MOF	Multiple organ failure
mtDNA	Mitochondrial DNA
ATP	Adenosine triphosphate
ADP	Adenosine diphosphate
AMP	Adenosine monophosphate
AMPK	AMP kinase
PGC	Peroxisome proliferators activated receptor gamma coactivator
NRF	Nuclear respiratory factor
TFAM	Mitochondrial transcriptionfactor alfa
TFB1&2M	Mitochondrial transcriptionfactor 1 and 2 beta
NO	Nitric oxide
SOFA	Sepsis related organ failure assessment score
COPD	Chronic obstructive pulmonary disorder
SS	Subsarcolemmal mitochondria
IMF	Intermyofibrilar mitochondria
SOD	Superoxide dismutase
CS	Citrate synthase
ROS	Reactive oxygen species

1 INTRODUCTION

1.1 PATIENT BACKGROUND

Critically ill patients with sepsis admitted to the general intensive care unit (ICU) usually have threatening or established failure in one or several vital organs. Organ failure is a common consequence of severe infection, surgical complications or trauma. As the disease progresses more organs may be affected and approximately 10-15% of the patients admitted to the ICU will ultimately suffer from multiple organ failure (MOF) [Awad SS, 2003, Sharma S et al., 2003, Singer M et al., 2004, Vincent JL et al., 1996]. ICU treatment is needed to support the failing organs for the patients to survive, and usually a prolonged stay of more than 5 days is needed when MOF ensues. Patients staying more than 5 days and suffering from MOF have a one-year mortality rate of almost 50% in comparison to 5% in patients staying for a period <5 days (Wernerman J, unpublished data). These longstayer patients consume a large part of the ICU budget despite the relatively low number of patients. In addition, the patients that do recover have a long way back to normal life.

1.1.1 ICU patients and muscle fatigue

When the patients are recovering from their initial illness, decreased muscle strength (muscle weakness) and increased tiredness (muscle fatigue) of the muscle are common problems. An extreme case scenario is that the patient suffers from total paralysis due to critical illness polyneuropathy or myopathy [Bolton CF, 2005, de Letter MA et al., 2001, Friedrich O, 2006, Friedrich O et al., 2005, Latronico N et al., 1996].

Most patients in the ICU are treated with mechanical ventilation because of respiratory failure. As the patients recover from their initial illness the aid from

mechanical ventilation is progressively decreased and the patients are weaned off the ventilator. If the respiratory musculature is weak or easily fatigued a prolonged weaning off period occurs. In these patients an increased risk of developing ventilator-induced pneumonia is apparent [MacIntyre NR, 2005, Meade MO et al., 2001, Sprague SS et al., 2003].

In locomotive skeletal muscle (such as vastus lateralis) the dysfunction is a more obvious problem after ICU discharge. The patients usually need rehabilitation, including physiotherapy, for a long period of time after ICU treatment. In ICU patients treated with mechanical ventilation for acute respiratory distress syndrome, a six minute walking test 3, 6 and 12 months after discharge disclosed severe walking problems [Herridge MS et al., 2003]. After one year, these patients could only walk 66% of the distance a normal healthy person would walk during these six minutes. Whether this is a problem of muscle weakness or fatigue or a combination of the two is not known, however subjectively all patients attributed it to muscle fatigue. The overall aim of this thesis was to investigate derangements in cellular energy and mitochondrial metabolism specifically as a potential reason for the muscle fatigue in these patients.

1.2 CELLULAR ENERGY PRODUCTION AND THE MITOCHONDRION

1.2.1 The mitochondrion

The mitochondrion is thought to originate from bacteria invading the eukaryotic cell millions of years ago. The fact that it is the only organelle that has its own DNA, protein synthesis and protein import system supports this theory. The mitochondrial DNA (mtDNA) is circular, just like bacterial DNA, and encodes 13 proteins important for the mitochondrial oxidative phosphorylation system. All the other proteins necessary for mitochondrial function, as well as the regulation system for

mtDNA transcription, are encoded by the nuclear DNA. Thus the mitochondrion cannot function properly without the nuclear DNA. When mitochondria first were described it was thought that they were separate units within the cell.

The mitochondrion plays part in cell signaling and apoptosis initiation, but its main function is to produce energy. It is therefore commonly termed the power plant of the cell. Cells in the body use the energy dense phosphate bonds of ATP (adenosine triphosphate) to store energy for their different processes. Some cells, such as red blood cells, do not have mitochondria and they produce ATP through a less efficient anaerobic route. However, most cells rely on the mitochondrion to produce the major part of the energy needed for their functions.

1.2.2 Mitochondrial energy production

The energy produced in the mitochondrion comes from the oxidation of several metabolites, such as glucose and fat. These are broken down in the cell to respectively pyruvate and free fatty acids. The mitochondrion takes up pyruvate and free fatty acids from the cytosol of the cell. Within the mitochondrial matrix these metabolites are transformed into acetyl coenzyme A and go through the citric acid cycle (Figure 1). In this cycle two reducing factors, NADH and $FADH_2$, are produced along with some ATP. However the major part of the ATP produced in the mitochondrion is produced in the respiratory chain, or electron transport chain. The respiratory chain consists of 5 enzyme complexes that transport the donated electrons in-between each other. Electrons are donated from the NADH and $FADH_2$ produced in the citric acid cycle to complex I and II, the first and second complex of the chain (Figure 1). While transporting electrons, protons are being pumped from the mitochondrial matrix to the intermembrane space, in between the two membranes of the mitochondrion, thus building up a proton gradient across the inner membrane. These protons need to be

transported back to the matrix to restore equilibrium. The 5th complex (ATP synthase) is responsible for this and while doing so ATP is produced. In addition to this process, the electron transport chain also produces H_2O and CO_2. However, under certain conditions the electron transport chain is uncoupled and the protons leak back through an uncoupling protein in the inner membrane. This process does not produce any ATP and the energy leaks out as heat instead. Compromised cell function occurs when the cell is subjected to low ATP concentrations for a prolonged period of time. This may in turn lead to adverse effects for the affected organ.

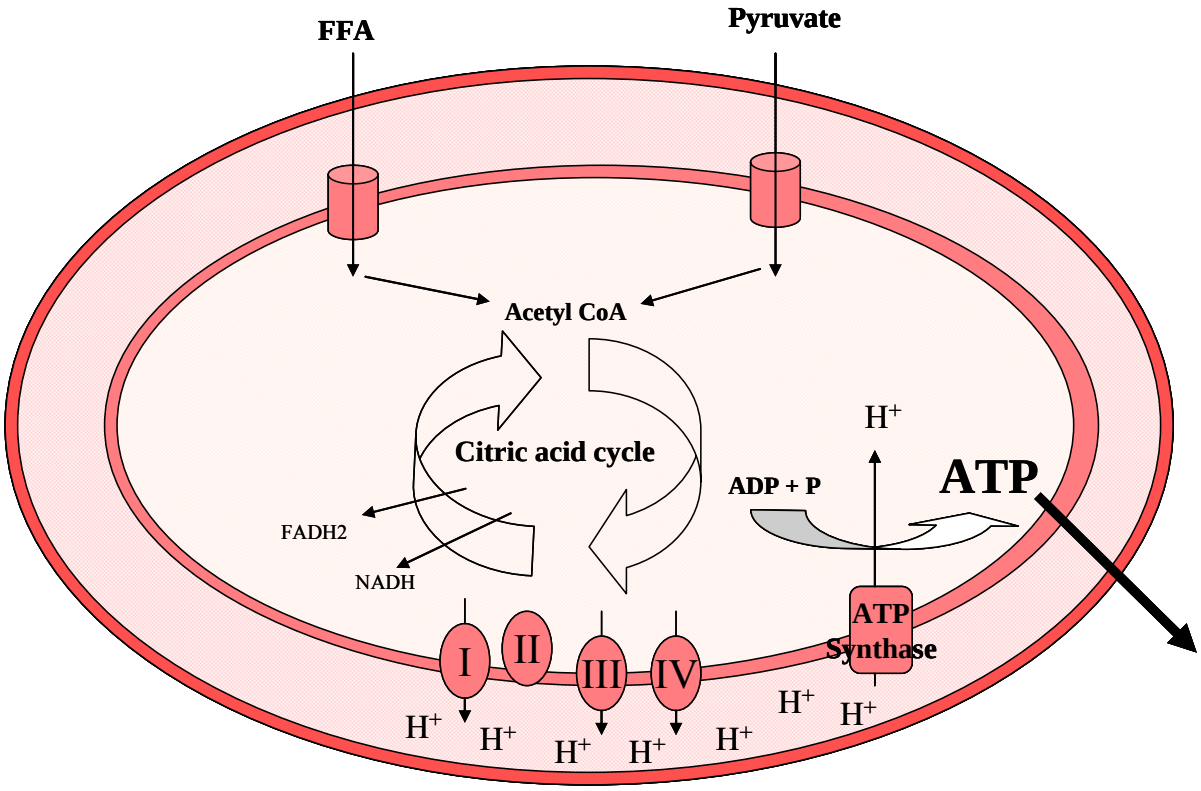

Figure 1: *Energy (ATP) production in the mitochondrion. FFA; Free fatty acid, $FADH_2$; flavin adenine dinucleotid, NADH; nicotineamide adenine dinucleotid, ADP; Adenosine diphosphate, ATP; Adenosine triphosphate, I, II, III and IV; respiratory chain complexes I-IV, H^+; hydrogen*

1.2.3 Mitochondrial biogenesis

During increased demand for ATP, such as during exercise there is a need for a rapid increase in ATP production and this can usually be covered by the mitochondria present in the cell. However if the concentration of ATP gets to low the mitochondrial copy number needs to increase to cover for this lack of energy. The cell is equipped with systems that can cover these events and increase the copy number when needed; this phenomenon is called mitochondrial biogenesis. One of the signals that are important for mitochondrial biogenesis is the increase of a breakdown product of ATP, the AMP molecule. An increased content of AMP activates the enzyme AMP-activated protein kinase (AMPK) that initiates mitochondrial biogenesis, increases glucose uptake and fatty acid oxidation [Hardie DG et al., 2006, Hood DA, 2001, Ojuka EO, 2004]. Ca^{2+} is another factor that controls mitochondrial biogenesis in skeletal muscle. Exactly how these factors control gene expression of mitochondrial related genes is not clear yet. However, it is believed that they interact with nuclear encoded transcription factors that control transcription of nuclear and mitochondrial encoded genes. A coordinated gene expression and protein synthesis of the nuclear and mitochondrial-encoded genes needs to occur in order to increase mitochondrial content.

The synthesis of mitochondrial proteins as well as mtDNA copy number is regulated by transcription factors encoded in the nuclear DNA. These transcription factors are regulating transcription of the mitochondrial related genes encoded in the nuclear DNA, and in addition they control the synthesis of factors responsible for initiating mtDNA transcription (Figure 2). This is a complex system that has not been fully explored yet, but several of these factors are well known and established [Fernandez-Silva P et al., 2003]. There are mainly two transcription factors termed nuclear respiratory factor (NRF) 1 and 2 that in combination with the coactivators PGC-1α and -1β (peroxisome proliferator activated receptor gamma (PPAR-γ)

coactivator) are responsible for transcription of the nuclear encoded mitochondrial proteins [Goffart S et al., 2003, Scarpulla RC, 2002, Scarpulla RC, 2006]. In addition they control the synthesis of factors that initiates mtDNA transcription and replication such as TFAM (Mitochondrial transcription factor A), TFB1M, TFB2M (Mitochondrial transcription factor 1 & 2 B) and RNA polymerase [Falkenberg M et al., 2002, Gleyzer N et al., 2005, Shoubridge EA, 2002]. Mitochondrial copy number and protein synthesis is thus controlled from the cell nucleus and a well coordinated effort is necessary to increase the mitochondrial content in the cell.

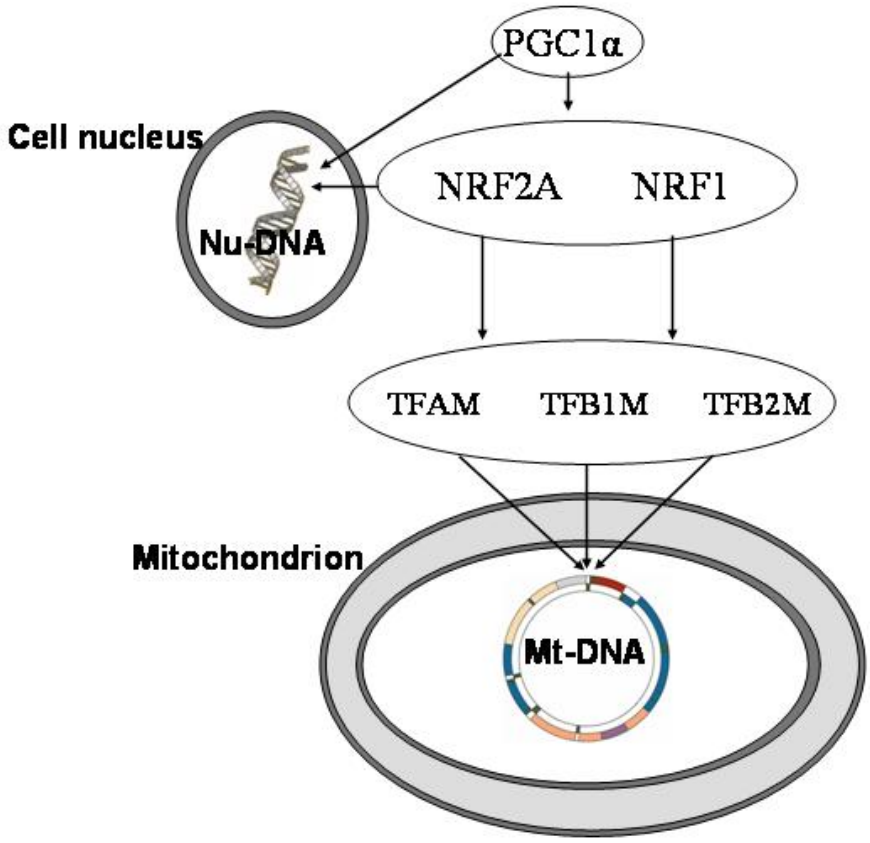

Figure 2: *Transcription factors regulating gene transcription of the mitochondrial and nuclear encoded mitochondrial proteins. Nc DNA, nuclear DNA; mt-DNA, mitochondrial DNA; PGC1α, Peroxisome Proliferator Activated Receptor gamma (PPAR-γ) coactivator; NRF, Nuclear Respiratory Factor; TFAM, Mitochondrial transcription factor A; TFB1&2M, Mitochondrial transcription factor 1 & 2 B*

1.2.4 Mitochondrial and oxidative stress

The system of energy production in mitochondria is complex and needs to be tightly regulated to function properly. Each individual enzyme needs to function well to be able to obtain an optimal energy production.

During the process of building up a proton gradient across the inner mitochondrial membrane, oxygen is utilized. In fact, mitochondria use more than 90% of the oxygen taken up by the body. Under optimal conditions all the oxygen should be utilized to produce energy, water and CO_2. However, some of the oxygen ends up as free oxygen radicals and this is suggested to be prominent in diseased states [Fridlyand LE et al., 2006]. Free oxygen radicals are highly reactive molecules that could oxidize proteins, DNA and lipids in their close surrounding. When proteins are oxidized their confirmation changes and this may affect or even inhibit their function. An inhibition can sometimes be reversed, but this does not always work, leading to a degradation of the protein instead. Since the mitochondrial enzyme complexes are very close to the site of free radical production these radicals are very prone to damage them. Under normal conditions, there is a control system consisting of several proteins and enzymes such as glutathione, superoxide dismutase, catalase as well as vitamins C and E and several other antioxidants. However during increased oxidative stress this system is sometimes not sufficient and irreversible damage may be done to the mitochondrion. The damaged proteins can then be degraded by proteases within the mitochondrion [Bota DA et al., 2001, Käser M et al., 2000].

1.2.5 Mitochondria in skeletal muscle

Muscle is depending on a highly adaptable energy production system to be able to increase energy supply during increased contractile activity. When the supply of substrates is plentiful glycogen, fat and creatine phosphate is stored within

the muscle. These substrates are then easily accessible when extra energy is needed for muscle contraction.

How the energy is produced largely depends on the fiber type present in the muscle. There is slow-twitch (type I) and fast-twitch (type II) fibers and these have different functions within the muscle. The fast twitch muscle fibers are mainly active during short periods of exercise such as sprinting and weight lifting and the energy necessary for contraction is mainly produced through anaerobic pathways. The slow twitch muscle on the other hand is used during endurance exercise such as walking and long distance running. To maintain the muscle activity during a long period of time the energy is mainly derived from substrates supplied by the blood or from substrate stores within the muscle. These substrates are taken care of by the mitochondrion that is equipped to keep on working for a long period of time, as long as oxygen and metabolic substrates are available. When the mitochondrion is no longer able to produce sufficient amounts of energy for the muscle to contract the muscle will get fatigued. If there are less or not well functioning mitochondria in the muscle it will be more easily fatigued and a normal function cannot be maintained. In general, the mitochondrial function improve with endurance exercise [Hood DA, 2001, Irrcher I et al., 2003, Wibom R et al., 1992, Zoll J et al., 2002] and mitochondrial content decrease with a sedentary lifestyle [Berg HE et al., 1993, Ferretti G et al., 1997, Häggmark T et al., 1981, Rifenberick DH et al., 1973]. In addition, the mitochondrial content decrease with ageing [Conley KE et al., 2000, Rooyackers OE et al., 1996a, Tonkonogi M et al., 2003, Trounce I et al., 1989] as well as in patients suffering from type 2 diabetes [Kelley DE et al., 2002].

1.3 MUSCLE MITOCHONDRIA IN SEPTIC PATIENTS

When this thesis work was initiated not much was known about the role of the mitochondrion in skeletal muscle of ICU patients. In fact to this day most of the knowledge about the impact of sepsis and multiple organ failure on mitochondria are based on studies of animal models simulating these conditions. Mainly rats have been used in which injections of endotoxin or zymosan at different doses have been given resulting in sepsis. The effect of sepsis on skeletal muscle mitochondria in these rat models indicates a decreased mitochondrial function, a depletion of ATP and ADP stores, a decreased mitochondrial protein synthesis and damage to the mitochondrial transcription machinery [Boczkowski J et al., 1999, Brealey D et al., 2004, Crouser ED et al., 2002, Rooyackers OE et al., 1996c, Schumer W et al., 1971]. However, these changes seem to be time and dose dependent and in most of these models multiple organ failure has not been achieved. It is also difficult to simulate the effect that different drugs and treatment options could have on mitochondrial function and ATP synthesis.

The few human studies that exist have shown similar results as the rat models, but have not fully elucidated the underlying cause and time perspective of the mitochondrial derangements. In recent years one study has been published were a correlation of mitochondrial dysfunction and mortality was established [Brealey D et al., 2002]. The study shows that mitochondrial complex I activity is decreased while complex IV activity is increased in the non-survivors of sepsis. In addition a decreased ATP content and an increased NO production was found. Apart from that study two older studies on patients suffering from acute cardiogenic and septic shock have been published. In these two studies a decrease in mitochondrial enzyme activity was also found [Corbucci GG et al., 1985, Gasparetto A et al., 1983]. However it is not clear whether the control groups were well matched for the patients in terms of age and

gender in these two articles. Only the early phase of critical illness was described in these three studies and what happens in later stages of sepsis and during MOF has only been published in abstract form [Helliwell TR et al., 1990]. In this abstract muscle biopsies were obtained at different time points during the disease showing a progressive decrease of mitochondrial enzyme activities over time.

The two main goals of this thesis were 1) to better describe the mitochondrial derangements in skeletal muscle of septic patients with MOF with a focus on the time perspective and the difference between muscle types and 2) to elucidate the underlying molecular mechanisms for these changes.

2 AIMS

The thesis has two major aims covered in the four included studies. The general aim of studies I and II was to describe muscle mitochondrial derangements caused by sepsis in man. The underlying mechanisms of these mitochondrial derangements were evaluated in studies III and IV.

The specific aims of the individual studies were:

Study I: To elucidate and describe changes in mitochondrial metabolism in two different muscle types in septic patients.

Study II: To evaluate the very early effects of sepsis on mitochondrial function and metabolism in a human endotoxemia model for sepsis.

Study III: To describe the effects of prolonged mechanical ventilation on mitochondrial function in diaphragm muscle from mechanically ventilated piglets.

Study IV: To evaluate the effect of sepsis and multiple organ failure on muscle mitochondrial turnover in ICU patients.

3 MATERIAL AND METHODS

All included studies were approved by the appropriate ethical committee as stated in the respective papers.

3.1 STUDY SUBJECTS AND STUDY PROTOCOLS

3.1.1 Septic patients (study I and IV)

In total 28 ICU patients were included in studies I and IV (Table 1). The patients suffered from sepsis according to the Bone criteria [Bone RC et al., 1992]. All patients were mechanically ventilated and suffering from single or multiple organ failure (MOF). MOF is defined as a sepsis related organ failure assessment score (SOFA) [Vincent JL et al., 1996] of ≥2 corresponding to 2 or more organ systems. All patients were recruited from the ICU at Karolinska University Hospital in Huddinge and informed consent was obtained from their next of kin. The inclusion criterion was that the patients should be mechanically ventilated. Patients with known preexisting neuromuscular disorders, chronic obstructive pulmonary disorder (COPD) or severe coagulopathy were excluded from the study.

All patients were treated according to the normal routines at the ICU at Karolinska Universtiy Hospital Huddinge, including full nutrition from day 2 and onwards. The patients were included on different days of ICU stay and biopsies were obtained in study I from both respiratory and vastus lateralis muscle, while in study IV only from vastus lateralis muscle. The respiratory muscle obtained in study I was mainly serratus anterior muscle obtained in-between the 5^{th} and 6^{th} rib and is mainly involved in inspiration [Reid DC et al., 1976]. See table 1 and the respective papers for detailed information about the included patients.

Table 1: *Characteristics of subjects/animals included in studies I-IV. Values are given as means ±SD. SOFA, sepsis related organ failure assessment score; ICU, intensive care unit; F, female; M, male; mech vent, mechanical ventilation*

	n	Gender	Age	SOFA	ICU stay	Intervention
Study I						
Septic patients	10	3F/7M	65±13	7±2	7±6	-
Control patients	10	3F/7M	66±14	-	-	-
Study II						
Healthy volunteers	7	7M	26±3	-	-	Endotoxin
Study III						
Mech vent piglets	8	8F	2-4 months	-	-	5 d mech vent
Control piglets	7	7F	2-4 months	-	-	4-6 h mech vent
Study IV						
Septic patients	18	8F/9M	64±14	6±3	7±12	-
Control patients	10	1F/9M	64±10	-	-	-

3.1.2 Control patients (study I and IV)

All control patients were metabolically healthy and underwent elective surgery at Karolinska University Hospital Huddinge (study I) or Ersta Hospital (study IV). In study I the controls were matched for age and gender and in study IV they were selected for being of similar age as the ICU patients (Table 1). Biopsies were obtained just after induction of anesthesia, but before surgery had started, from the serratur anterior muscle in study I and from vastus lateralis muscle in both studies. The control subjects underwent elective surgery for hernia repair, ileostomy closure, recurrent diverticulitis or colorectal resection. All control patients gave informed consent to participate in the respective studies.

3.1.3 Healthy volunteers (study II)

Seven young healthy male volunteers were recruited for the study. After an over night fast the subjects received intravenous endotoxin (4 ng/kg body weight). Blood pressure, heart rate and body temperature were monitored continuously throughout the study.

Vastus lateralis muscle biopsies were obtained before administration of endotoxin as well as 2 and 4 hours after endotoxin. Detailed information is given in paper II as well as in table 1.

3.1.4 Piglets (study III)

Female piglets were randomly divided into a ventilated and a control group. The ventilated group was subjected to volume controlled mechanical ventilation for 5 days and the control group was mechanically ventilated for 4-6 hours as described in paper III. The piglets were continuously anaesthetized throughout the study, but no muscle relaxants were given. Balanced parenteral nutrition was given. Diaphragm muscle biopsies were obtained at the end of the study protocol. Detailed information is given in paper III as well as table I.

3.2 METHODOLOGICAL CONSIDERATIONS

All details about the methods used can be found in the respective papers. In this section the choice of different methods will be discussed.

3.2.1 Isolation of mitochondria

In skeletal muscle there are two different subpopulations of mitochondria located either just under the cell membrane of the muscle, termed subsarcolemmal mitochondria (SS), or in between the myofibrils of the muscle, termed intermyofibrillar mitochondria (IMF) (Figure 3) [Cogswell AM et al., 1993, Elander A et al., 1985, Krieger DA et al., 1980, Palmer JW et al., 1977]. In this thesis mainly the SS mitochondria were isolated for reasons specified below. The SS mitochondria are suggested to be responsible for producing energy for active transport and phosphorylation processes, while the IMF mitochondria are thought to be responsible

for energy production for muscle contraction [Elander A et al., 1985, Krieger DA et al., 1980].

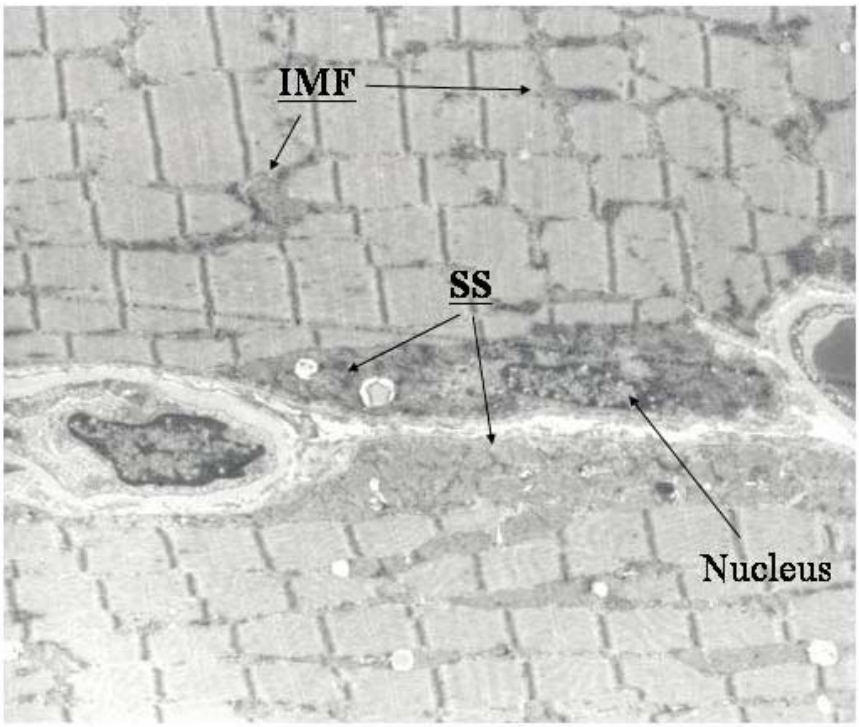

Figure 3: *Electron microscopic image of 2 muscle fibers with the nucleus, subsarcolemmal (SS) and intermyofibrilar (IMF) mitochondria indicated*.

In rats the two mitochondrial subpopulations have slightly different biochemical properties and it has been suggested that the IMF mitochondria have a higher respiratory rate and protein synthesis rate than the SS mitochondria, but that the ability to produce ATP is the same [Cogswell AM et al., 1993, Krieger DA et al., 1980, Palmer JW et al., 1977]. In general in humans these differences have not been observed but the results from different studies are diverse [Elander A et al., 1985, Fischer JC et al., 1985]. The study from Elander et al. shows that mitochondrial respiration is lower in IMF mitochondria, whereas the study from Fischer et al. shows no difference between the two mitochondrial subpopulations in human skeletal muscle. This difference in between studies might be attributed to the isolation techniques used. In the

animal studies as well as in the first human study [Elander A et al., 1985] a proteolytic enzyme called nagarse was used to isolate the IMF mitochondria. With this method the samples are incubated with nagarse for a short period of time and then the activity is stopped through centrifugation to get rid of the enzyme. In the other paper with human subjects [Fischer JC et al., 1985] the IMF fraction was obtained using trypsine. With the trypsination method the incubation time with the enzyme is better controlled through the addition of a specific protease inhibitor. Both these methods are aiming at degrading the myofibrilar proteins to get the IMF mitochondria to detach. However, these proteases are not specific and therefore work on any protein within the tissue and may also degrade mitochondrial proteins. This could potentially influence the biochemical properties of the IMF mitochondria and thus a false difference between the two subpopulations might be detected.

3.2.2 Assessment of mitochondrial content

The mitochondrial content can be assessed in several different ways. The mitochondrial density can be estimated by maximal citrate synthase activity measurements,or by measuring the ratio between mtDNA and nuclear DNA as well as by quantitatively counting mitochondrial content using electron microscopic images. All these measurements have limitations and none can be considered as giving an absolute estimation of mitochondrial content. The quantification of mitochondrial volume using electron microscopy is semi-quantitative. In addition, if the mitochondria swell, as has been shown in liver from ICU patients [Vanhorebeek I et al., 2005], this method would give rise to an increased mitochondrial content even though the function of the mitochondria is decreased. Another aspect to take into consideration is if the mitochondria are damaged, should they be counted at all, or just ignored? The method in which the relationship between mitochondrial and nuclear DNA is evaluated also has

limitations in that the mitochondrial DNA has several copy numbers within the mitochondrion. This does not necessarily mean that all of these represent the well functioning part of the mitochondrion. The mitochondrial genes may not be regulated simultaneously and the results can thus depend on which gene is chosen for analysis. The method of using maximal citrate synthase activity measurements as an estimation of mitochondrial content also has some complications. Immediately after acute exercise, citrate synthase activity increases by 43 % [Fernstrom M et al., 2004] and stays increased 3 hours after exercise. It is not likely that this acute exercise will give rise to increased mitochondrial content immediately after exercise and therefore it is more likely that the enzyme activity has increased on its own. However, we chose this method because it has the advantage of giving an estimation of the amount of functioning mitochondria within the muscle. It would also be possible to assess one of the other mitochondrial enzymes, such as complex I or complex IV of the respiratory chain, to make this estimation. However, these two enzymes have been shown to be acutely down regulated under certain conditions such as during oxidative stress [Brealey D et al., 2002], while there are no such suggestions concerning the citrate synthase activity. Therefore the maximal activity of citrate synthase has been used as an estimation of mitochondrial content in all included studies.

3.2.3 Evaluation of mitochondrial function

To evaluate the mitochondrial function we measured the activity of two key enzymes of the respiratory chain, the complex I and complex IV. These two enzymes are the first and the last enzymes of the chain and their function is crucial for the chain to work properly. These are convenient methods that give a good estimation of the mitochondrial function. However, the golden standard within the field is to measure mitochondrial respiration in isolated mitochondria. In septic patients this

method is not practical to use since the measurements need to be done on fresh muscle tissue samples. The problem with obtaining samples from patients is that the timing of biopsies is difficult to control and immediate preparation and measurements is therefore not always practical in septic ICU patients. Another problem with this method is the isolation procedure of mitochondria as discussed above. To avoid mitochondrial isolation problems an alternative is to measure mitochondrial respiration in skinned muscle fibers, however, the muscle tissue still need to be fresh [Tonkonogi M et al., 2003]. As stated above the isolation of IMF mitochondria poses some problems and it is not clear whether damaged mitochondria can be isolated properly. Therefore the measurements presented here were performed in both isolated mitochondria and in total muscle homogenate.

3.2.4 Measurements of mitochondrial superoxide dismutase (SOD) activity

There are two main forms of SOD, the Cu,Zn SOD and the MnSOD. The MnSOD is located in the inner matrix of the mitochondrion while the Cu, Zn SOD is found both in the cytosol of the cell and in the intermembrane space of the mitochondrion [Weisiger RA et al., 1973]. To evaluate the specific activity of MnSOD the Cu,ZnSOD can be inhibited with potassium cyanide (KCN). The activity can be inhibited up to 90% by the addition of 1 mM KCN, however if the concentration is too high the MnSOD may also be inhibited [Crapo JD et al., 1978]. To test this method, KCN was added at different concentrations ranging from 0.5 mM to 3.0 mM and SOD activity was measured in muscle homogenate. At KCN concentrations of 0.5 and 1 mM no inhibition of SOD could be detected. When the KCN concentration was increased further a decrease in the SOD activity was detected, but it was not linear and therefore

not obvious which concentration would be the optimal. In order to avoid these problems isolated mitochondria were used to measure the mitochondrial SOD activity.

4 DICUSSION OF RESULTS

In the present thesis mitochondrial derangements in muscle due to sepsis were evaluated. The first aim of the thesis was to evaluate and describe mitochondrial derangements in muscle of ICU patients with sepsis induced multiple organ failure (MOF). This was performed in two different muscle groups from septic patients in study I and in study II a human endotoxemia model was used to study the very early phase of sepsis. The second aim was to elucidate the underlying mechanisms that may cause the mitochondrial derangements. The effects of inactivity of respiratory muscle on mitochondrial derangements were evaluated in mechanically ventilated piglets (study III). Study IV concerns the underlying molecular aspects that may cause mitochondrial derangements during sepsis in man.

4.1 DESCRIBING THE PROBLEM OF MUSCLE MITOCHONDRIA IN SEPTIC ICU PATIENTS

Not much work has been done on mitochondrial derangements in skeletal muscle from septic patients. There are a few studies involving patients suffering from acute septic shock and patients with cardiogenic shock [Brealey D et al., 2002, Corbucci GG et al., 1985, Gasparetto A et al., 1983]. These studies all show derangement in muscle mitochondrial enzyme activities. The aspect of prolonged disease and MOF was, however, not evaluated in these studies since the biopsies were taken early on in the diseased state. Study I in the present thesis was designed to evaluate the effect of multiple organ failure and in particular respiratory failure on muscle mitochondria of two different muscle groups, leg muscle and respiratory muscle. Expressed per muscle weight, the respiratory muscle activity of citrate synthase

was 53% lower and that of complex I was 60% lower in the septic patients as compared to the controls. In leg muscle only the activity of complex IV was lower (38%), although citrate synthase had a tendency to be lower in leg as well in septic patients. When these measurements were repeated in leg muscle from septic patients in study IV, significantly lower activities of citrate synthase (25%), complex I (49%) and complex IV (33%) per muscle weight were found. Combining the results from the two studies showed significantly lower activity in all three enzymes in the septic patients as compared to controls (Figure 4).

Expressing the mitochondrial enzyme activities per citrate synthase activity is a way of correcting for mitochondrial content [Wibom R et al., 1992]. When the activities of complex I and IV were expressed per citrate synthase activity no differences between the septic patients and controls were present (see papers I and IV).This indicates that these patients suffered from a decreased mitochondrial content rather than specific changes in mitochondrial enzyme activities. As was also confirmed in isolated mitochondria were the complex I and complex IV activities did not differ between septic patients and controls (see papers I and IV).

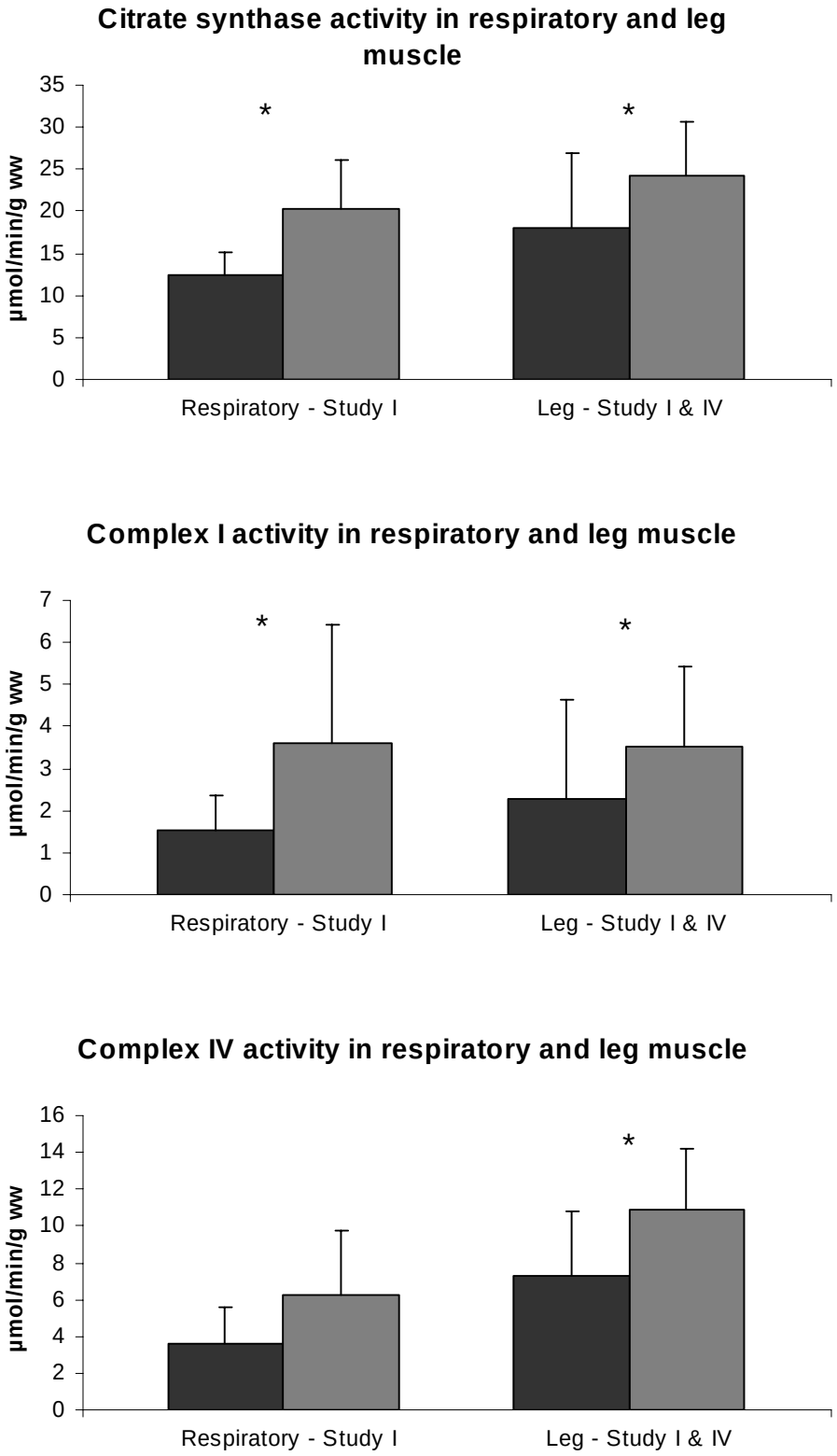

Figure 4*: The dark grey bars to the left represent the septic patients (n=27 for leg muscle and 10 for respiratory muscle) while the light grey bars represent the control patients (n=20 for leg and 10 for respiratory muscle). * Statistically different from controls p<0.05*

The decreased mitochondrial content in leg skeletal muscle is accompanied by a decrease in the concentrations of energy rich phosphates and an increased concentration of lactate (Figure 5). In animal models of sepsis decreased concentrations of energy rich phosphates are only found in severely ill animals [Brealey D et al., 2004]. Also in patients with acute septic shock a lower ATP concentration is found in the patients that died in the ICU, survivors on the other hand have higher ATP concentration in comparison to controls, as measured during the first 24 hours of ICU stay [Brealey D et al., 2002]. This early phase of sepsis was not evaluated in study I, however in study II the initial phase of sepsis was studied in a human endotoxemia model. The ATP concentration increased (18%) between 2 and 4 hours after an endotoxin injection. However, neither the ATP concentration at 2 nor 4 hours were different from the baseline value taken before endotoxin injection (Figure 8).

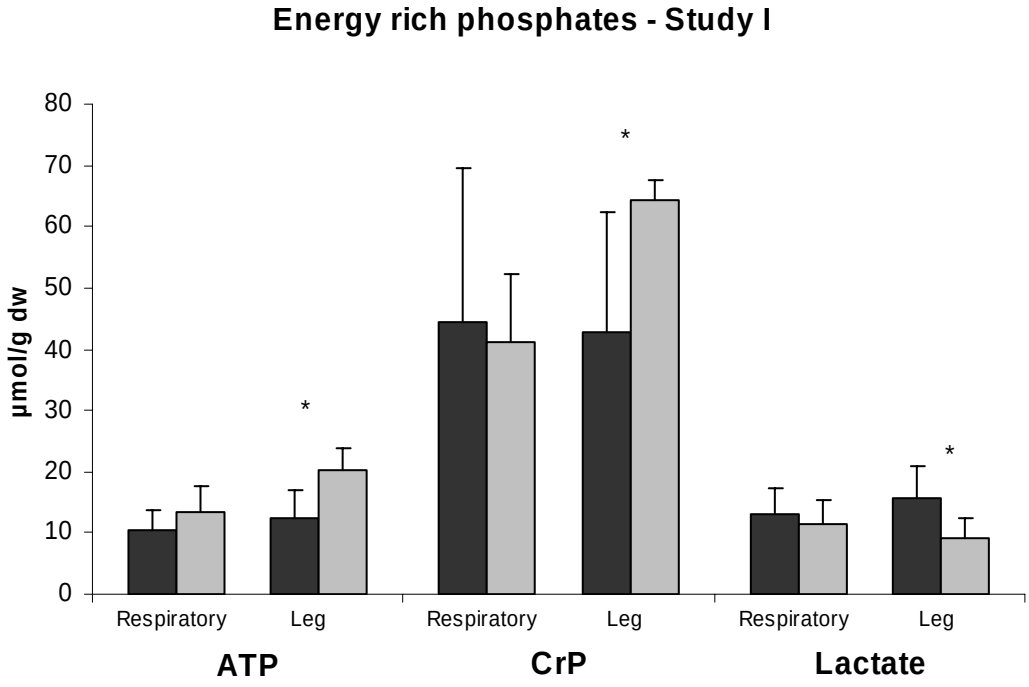

Figure 5: *Concentrations of ATP, creatine phosphate and lactate in respiratory and leg muscle from study I. The dark grey bars represent the septic patients (n=10) and the light grey bars the controls (n=10).* ***** *significantly different from control values (p<0.05).*

The low concentration of ATP taken together with the low mitochondrial content in septic patients, may lead to an acute lack of energy when the muscle is activated again after ICU discharge. Normally muscle can compensate for an increased demand of energy by increasing its mitochondrial activity. However, when mitochondrial content is low from start even an increased mitochondrial activity may not be able to fully compensate for the energy demand. Rats with a low mitochondrial content has a decreased ability to produce ATP during titanic stimulation, causing a decreased ATP concentration and increased fatigue in the muscle [Dudley GA et al., 1987]. In septic patients the ATP concentration was already lower from start, leading to an even greater depletion of ATP content during muscle activation. It is therefore likely that septic patients are more prone to get muscle fatigue after ICU discharge.

4.1.1 Mitochondrial derangements a time perspective

In the studies I and IV the mitochondrial enzyme activities were measured at different time points of ICU stay. Correlation analysis of the effect of time in the ICU on mitochondrial content was performed. Even though there was not a statistically significant correlation, a trend to decrease with time could be observed in both citrate synthase activity and complex IV activity (citrate synthase: R^2=0.0827, p=0.13, Complex IV: R^2=0.0893, p=0.12). Complex I activity did not show the same trend (R2=0.0025, p=0.80). Citrate synthase activities evaluated at two different time points of ICU stay decreased by ~32% (p=0.028) over two weeks [Radell P et al., 2005]. In figure 6 the activity of citrate synthase is plotted against time of ICU stay from studies I and IV together with data from Radell et al (2005). As a reference the mean and standard deviation of the citrate synthase activity in the young healthy controls from study II and in the healthy controls from studies I and IV are given. From this figure it is clear that the citrate synthase activity is low in these patients, that the

activity decreases over time and that age has a clear effect on mitochondrial enzyme activity.

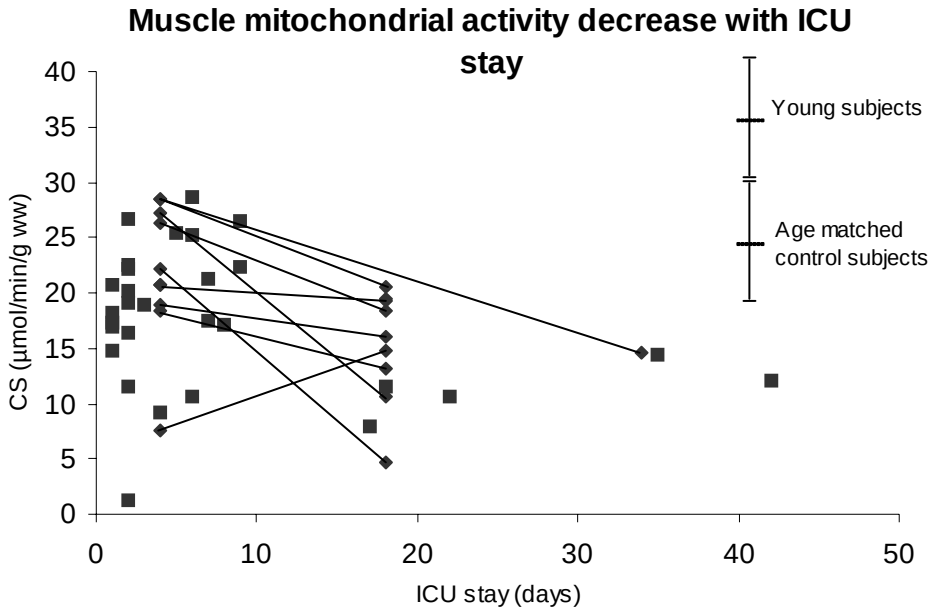

Figure 6: *The activity of citrate synthase (CS) in relation to ICU stay in patients from studies I and IV together with data from Radell et al. (2005). For comparison citrate synthase activity from young healthy subjects (26±3 years; n=7) and control subjects of similar age as the patients (65±12 years; n=20) are included in the figure.*

In study II a human model of the very early stage of systemic inflammation was used to evaluate the effect of time on mitochondrial derangements in septic patients. It is not possible to obtain samples from septic patients at this early stage of the disease as the patients do not reach the hospital until later. In this study we therefore evaluated the effects of endotoxin on mitochondrial derangements in muscle from young healthy volunteers. This is a well validated and extensively used model to study the metabolic effects of early sepsis [Lin E et al., 1998, Martich GD et al., 1993]. The mitochondrial enzymes citrate synthase, complex I and IV all increased two hours after endotoxin and the activities returned to normal 4 hours after endotoxin (Figure 7).

On the other hand the ATP concentration showed a trend to decrease after 2 hours (p=0.08) and then increased by 18 % at 4 hours (Figure 8). Creatine phosphate and lactate concentrations did not change after endotoxin.

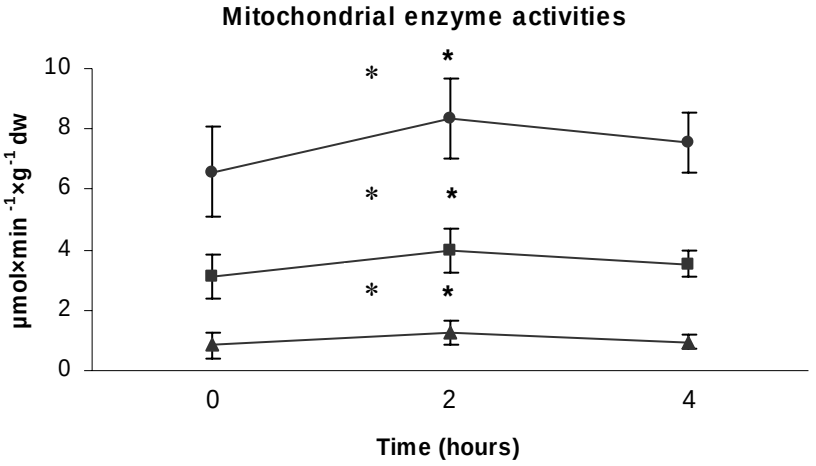

Figure 7: *The activity of citrate synthase (●) and mitochondrial complex I (▲) and IV (■) before endotoxin injection as well as 2 and 4 hours after in healthy human volunteers (n=7). Presented here as mean and standard deviation.* Significantly different from 0 hours (p<0.05)*

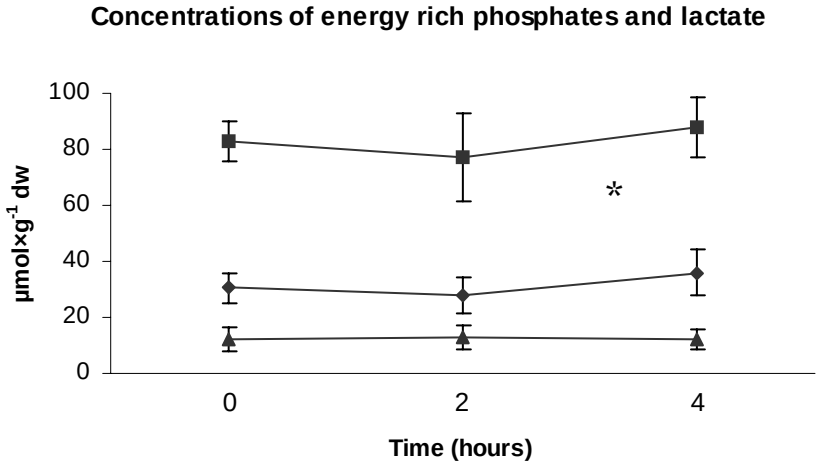

Figure 8: *The concentrations of ATP (●), creatine phosphate (■) and lactate (▲) before as well as 2 and 4 hours after an endotoxin injection, in healthy human volunteers (n=7). Presented here as means and standard deviation. *Significantly different from 2 hours (p<0.05).*

The results from the human model of sepsis (study II) differ from both patient studies and animal models of sepsis. The mitochondrial content in septic patients were low (studies I and IV) and also in many animal models of sepsis there is a decrease in mitochondrial enzyme activity [Brealey D et al., 2004, Crouser ED et al., 2002, Rooyackers OE et al., 1996b]. However, a few animal studies report an increase in mitochondrial enzyme activity early (<16 hours) after sepsis induction [Dahn MS et al., 1995, Dawson KL et al., 1988]. The increased enzyme activities in study II might be explained by fever and shivering. All volunteers had mild fever and the peak came 3 hours after the endotoxin injection (Figure 9). In rat cardiac muscle an increased body temperature to around 42°C during 25 minutes was shown to enhance mitochondrial enzyme activity [Sammut IA et al., 2001]. We speculate that the mitochondrial derangements found in healthy volunteers following an endotoxin challenge was due to a dual phase during sepsis, where an initial increase in enzyme activity is followed by a progressive decline.

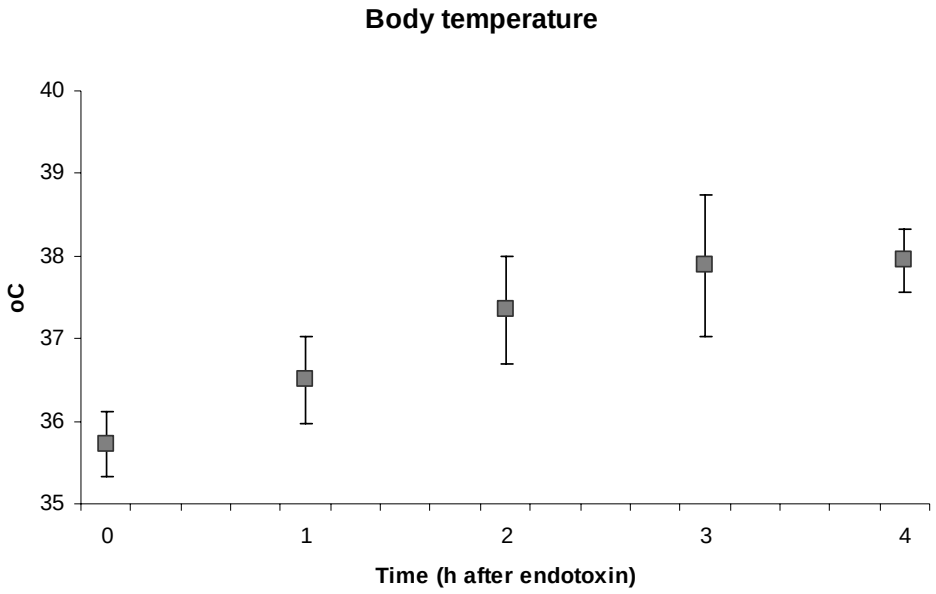

Figure 9: *Change in body temperature in healthy volunteers (n=7) given endotoxin at time 0 hours, presented as means and standard deviation.*

4.1.2 Different response to sepsis in leg and respiratory muscle

The mitochondrial content was significantly lower in two different muscles (respiratory and leg muscle) in septic patients as compared to controls (study I). However when the concentrations of ATP, creatine phosphate and lactate were evaluated differences between the two muscle groups became apparent. In the controls there was a lower concentration of these compounds in the respiratory muscle as compared to leg muscle. Compared to the controls, the septic patients had lower concentrations of ATP and creatine phosphate and higher lactate concentrations in leg muscle. However, in respiratory muscle there were no differences between the septic patients and controls (Figure 5). Similar results are reported for patients with chronic obstructive pulmonary disorder (COPD) suffering from acute respiratory failure where the concentration of energy rich phosphates are lower in leg muscle but not in intercostal muscle in the patients as compared to controls [Gertz I et al., 1977]. Furthermore the healthy controls were reported to have lower ATP levels in intercostal muscle than in leg muscle. The reasons for the differences between the two muscles are not known, but the respiratory muscle is passively stretched during mechanical ventilation while the leg muscle is not stimulated at all. The stretching in itself might stimulate energy production or possibly increase the blood flow and thereby stimulate the transportation of substrates to and from the muscle tissue. These results showed that the metabolic alterations found in one muscle group may not be the same as in another muscle group. Care should therefore be taken when extrapolating results from one muscle type to another.

4.2 POTENTIAL CAUSES AND UNDERLYING MECHANISMS OF MUSCLE MITOCHONDRIAL DERANGEMENTS DUE TO SEPSIS

Mitochondrial content in skeletal muscle of humans is very variable and dependent upon many factors. In the patient, several factors, such as age, underlying disease, medication, treatment, inactivity and a sedentary lifestyle, may have an impact on skeletal muscle mitochondria. Ageing has been shown to decrease mitochondrial content in humans [Rooyackers OE et al., 1996a, Tonkonogi M et al., 2003]. Most septic patients included in studies I and IV were older (mean age 64.4 years) and therefore we carefully selected controls of similar age (mean age 64.9 years). The other factors are unfortunately more difficult to control for in patients and some of these factors will be discussed in further detail below. In addition changes in mitochondrial protein metabolism and gene expression will be discussed as potential mechanisms leading to the decreased mitochondrial content.

4.2.1 Inactivity and mechanical ventilation

ICU patients are always bed-bound and this inactivation can in itself decrease the mitochondrial content in muscle. However, the mitochondrial enzyme activity in septic patients was actually lower than in immobilized healthy volunteers (Figure 10). The healthy volunteers were subjected to 30 days of 6° head down bed-rest which decreased citrate synthase activity by 18% [Berg HE et al., 1993, Hikida RS et al., 1989], while septic patients had a 29% lower citrate synthase activity than the age-matched controls (studies I and IV). Furthermore the septic patients were in the ICU for a median time of seven days and still showed a greater decrease in citrate synthase activity than the 30 days immobilized healthy volunteers. Therefore immobilization of septic patients may contribute to the decreased mitochondrial content in muscle, but it is not the only cause.

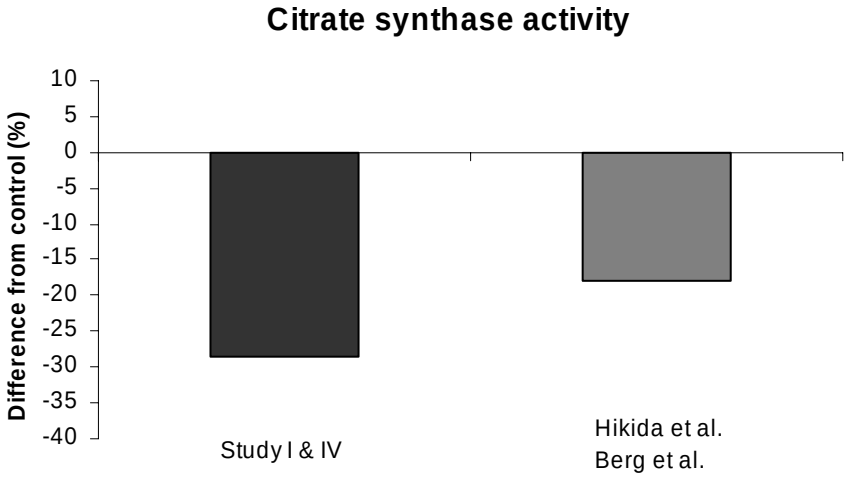

Figure 10: *Difference in citrate synthase activity in septic patients in studies I and IV as compared to age-matched controls in comparison to the decrease in activity after 30 days of immobilisation in young healthy volunteers [Berg HE et al., 1993, Hikida RS et al., 1989].*

Patients with acute respiratory distress may end up in the ICU needing mechanical ventilation to aid respiration. Mechanical ventilation may be regarded as a form of unloading for the respiratory muscles which could also affect muscle mitochondria. In study III, piglets were mechanically ventilated for 5 days and the mitochondrial derangements in the diaphragm muscle were evaluated. To put healthy humans through mechanical ventilation would not be safe or ethically appropriate. After mechanical ventilation a lower activity of mitochondrial respiratory chain complex IV was found in the piglets, but the other mitochondrial enzymes did not change (see paper III). Mitochondrial content was unaltered as evaluated using electron microscopy. These results indicate that there is a specific decrease in complex IV activity in diaphragm muscle of mechanically ventilated piglets. The mechanism behind this specific inhibition is not known. However, a reversible inhibition of complex IV by NO has been suggested [Moncada S et al., 2002]. In the mechanically ventilated piglets no

changes in whole muscle SOD activity or glutathione concentrations in whole muscle were found, but we can not exclude an increased oxidative stress inside the mitochondrion in these piglets.

4.2.2 Mitochondrial protein turnover

The decrease in mitochondrial content demonstrated in septic patients may be the results of an increased mitochondrial protein breakdown, decreased synthesis or a combination of both. Therefore the in vivo mitochondrial protein synthesis rates in septic patients were investigated (study IV). In an animal model for sepsis, mitochondrial as well as mixed muscle protein synthesis decrease [Rooyackers OE et al., 1996c]. However, the hypothesis that the same would be true also in septic ICU patients could not be validated. In study IV mitochondrial protein synthesis did not differ between septic patients and controls (Figure 11). To further back up these findings the gene expression of key mitochondrial proteins that were either nuclear or mitochondrial encoded was examined (see paper IV for details). In none of these key mitochondrial genes a difference in mRNA levels between septic patients and controls was found. Also these results differ from results obtained in animal models of sepsis, were a decrease in gene expression of several mitochondrial enzyme subunits is found in diaphragm muscle of rats treated with endotoxin [Callahan LA et al., 2005]. However, there are several differences between animal models of sepsis and septic ICU patients. Even though the animal models generally show signs of systemic inflammation, they do not present signs of multiple organ failure [Rooyackers OE et al., 1996c]. This may be the reason for the differences between results found in septic patients and in animal models. In general mitochondrial protein synthesis was not decreased in septic patients. This is in line with the finding that total muscle protein synthesis measurements in septic patients is not decreased, even though a significant

muscle protein loss is seen [Tjäder I et al., 2005]. Thus the mitochondrial content as well as the total muscle protein content is decreasing in these patients, despite an unchanged protein synthesis rate. This indicates that the protein breakdown in total muscle as well as in mitochondria could be increased in septic patients.

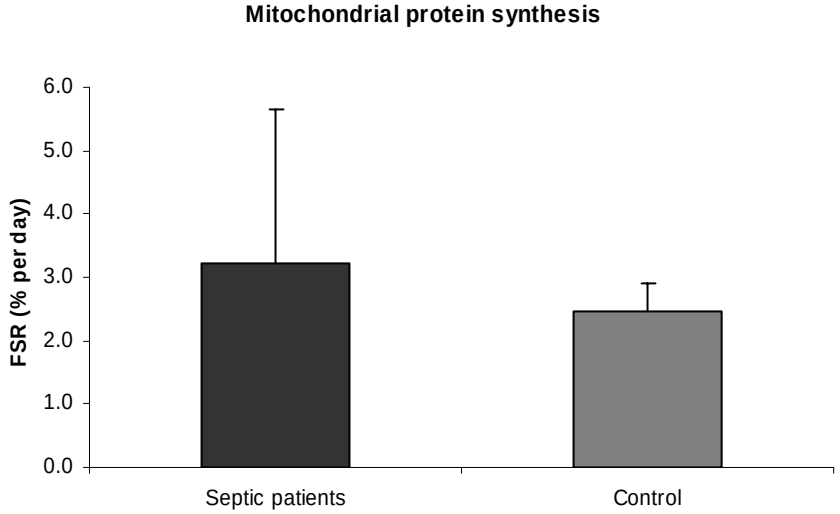

Figure 11: *Mitochondrial protein synthesis in septic patients (n=17) and healthy controls (n=10) presented as means and standard deviation. FSR, fractional synthesis rate*

4.2.3 Molecular control of mitochondrial content

The content of mitochondria is well regulated in muscle by several nuclear encoded transcription factors. PGC1 is a coactivator of mitochondrial genes encoded in the nucleus [Goffart S et al., 2003, Scarpulla RC, 2002, Scarpulla RC, 2006]. PGC1 in turn regulates NRF1 and NRF2α, which are known to be the overall transcription factors of mitochondrial genes (see figure in introduction). The NRFs, in combination with PGC1, control the transcription of nuclear encoded mitochondrial proteins as well as the mitochondrial transcription factors TFAM, TFB1M and TFB2M [Falkenberg M et al., 2002, Gleyzer N et al., 2005, Shoubridge EA, 2002]. These factors are in turn responsible for initiating the transcription of mitochondrial-encoded

genes (Figure 12). Results are obtained from cell cultures of myocytes and other cell types, but so far these regulating factors have not been characterized in muscle from septic patients. In study IV the mRNA levels of these transcription factors and co-activators were examined. The mitochondrial transcription factors (TFAM, TFB1M and TFB2M) were all increased in the septic patients, while only NRF2α was increased among the transcritption factors for nuclear encoded genes (Figure 12). None of the genes that these transcription factors control were increased, suggesting an uncoupling of the transcription machinery in muscle from septic patients.

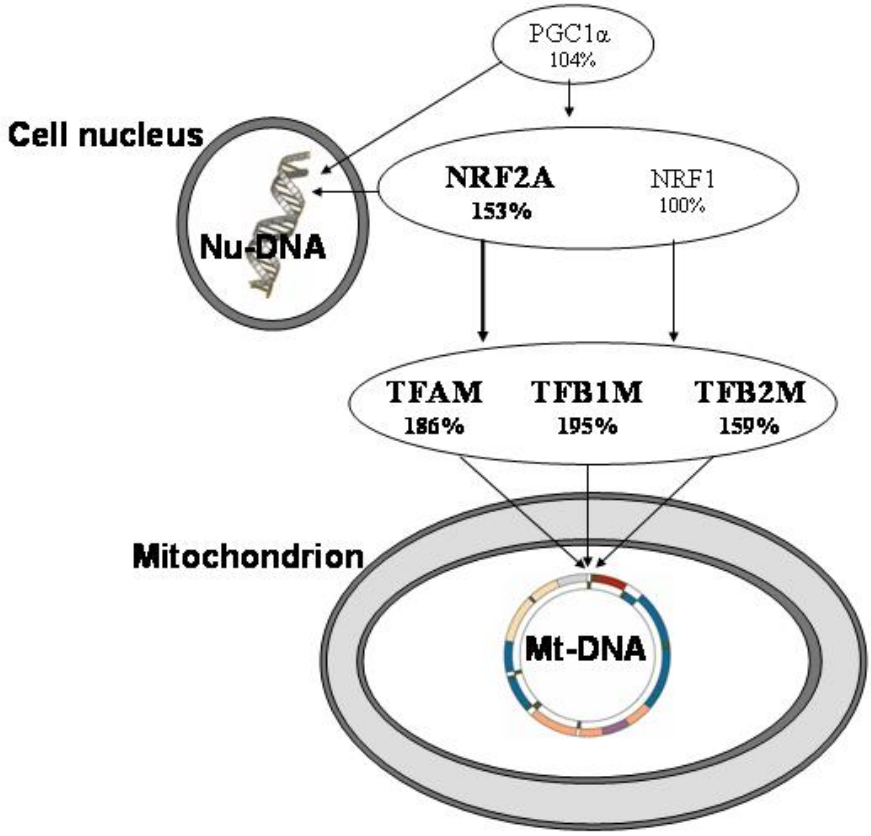

Figure 12: *Regulation of mitochondrial transcription factors in septic ICU patients. The transcription factors in bold were significantly up-regulated in muscle from septic patients in comparison to controls. Numbers are difference in mRNA levels from control in percent. Nu-DNA, nuclear DNA; Mt-DNA, mitochondrial DNA; PGC1α, Peroxisome Proliferator Activated Receptor gamma (PPAR-γ) coactivator; NRF, Nuclear Respiratory Factor; TFAM, Mitochondrial transcription factor A; TFB1&2M, Mitochondrial transcription factor 1 & 2 B*

4.2.4 Mitochondrial protein breakdown

The mitochondrial derangements discussed in this thesis do not seem to be caused by a decreased mitochondrial protein synthesis, nor a decreased mitochondrial biogenesis as discussed above. The most likely explanation is that mitochondrial protein breakdown is increased. However, protein breakdown is difficult to quantify, due to a lack of good methods. Therefore mitochondrial protein breakdown was evaluated in muscle from septic patients through the analysis of gene expression of the four known mitochondrial proteases (study IV). The gene expression of subunits of the two matrix located proteases, Lon and ClPP, as well as the two proteases located in the inner mitochondrial membrane, iAAA and mAAA was evaluated [Bota DA et al., 2001, Käser M et al., 2000]. An increased gene expression of the two matrix located proteases (Lon and CLpp) was found (Figure 13).

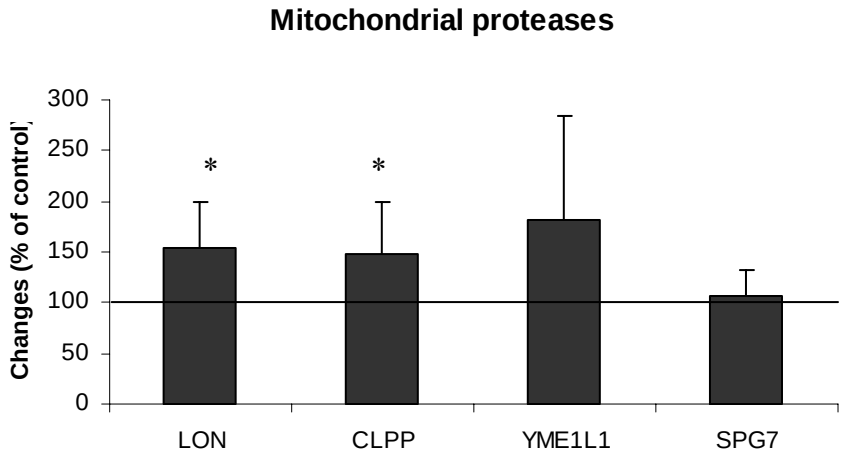

Figure 13: *Gene expression (mRNA levels) of proteases active in the matrix, Lon and CLPP, and inner membrane, YME1L1 and SPG7, of the mitochondrion. The changes are expressed in percent of control values. *p<0.05*

The most well known and examined mitochondrial protease is the Lon and it has been suggested that this protease is particularly important in degradation of oxidatively damaged mitochondrial proteins [Bota DA et al., 2002, Bulteau AL et al., 2006]. Thus the increased gene expression of these two mitochondrial proteases indicates an increased mitochondrial protein breakdown, but further investigation is necessary to fully elucidate this finding.

4.2.5 The impact of oxidative stress on mitochondrial function and content

As suggested, an increase in mitochondrial protein breakdown is the most likely explanation for the decreased mitochondrial content in septic patients. The reason for this increased breakdown could be diverse. However, an increased oxidative stress has been suggested to modulate mitochondrial proteins. Oxidative stress is an event that is very difficult to assess due to the short-lived nature of reactive oxygen species (ROS). It is possible to assess the damaged proteins per se, but these are generally degraded rapidly to protect the cell. Another possibility is to evaluate the concentration or activity of the scavengers (such as vitamin E, glutathione and others). We have focused on measurements of a specific enzyme scavenger, the superoxide dismutase (SOD). This enzyme is present in 2 distinct forms within the cell, whereof one of them (the Mn-SOD) is specifically located within the mitochondrion. The ICU patients in studies I and IV had increased activity of the mitochondrial SOD while the SOD activity in total muscle homogenates did not differ from controls. The mitochondrial SOD increase was highly significant and one of the most obvious changes found in septic patients. This suggests an increased oxidative stress in muscle mitochondria of septic patients, but what the impact is on mitochondrial enzyme activity is not known. To evaluate this, the mitochondrial SOD activity was correlated to the activities of the

mitochondrial enzymes in leg muscle from septic patients (study I, IV and Radell P. et al. 2005). A negative correlation between muscle citrate synthase and mitochondrial SOD (R^2=0.69, p <0.001) as well as complex IV activity (R^2=0.34, p <0.001) in whole muscle homogenates was observed (Figure 14). However, there was no significant correlation between the activity of complex I and mitochondrial SOD in septic patients (R^2=0.08, p=0.062). Taken together there was a correlation between oxidative stress and mitochondrial derangements, however this issue will need more attention in future studies in order to draw any definite conclusions.

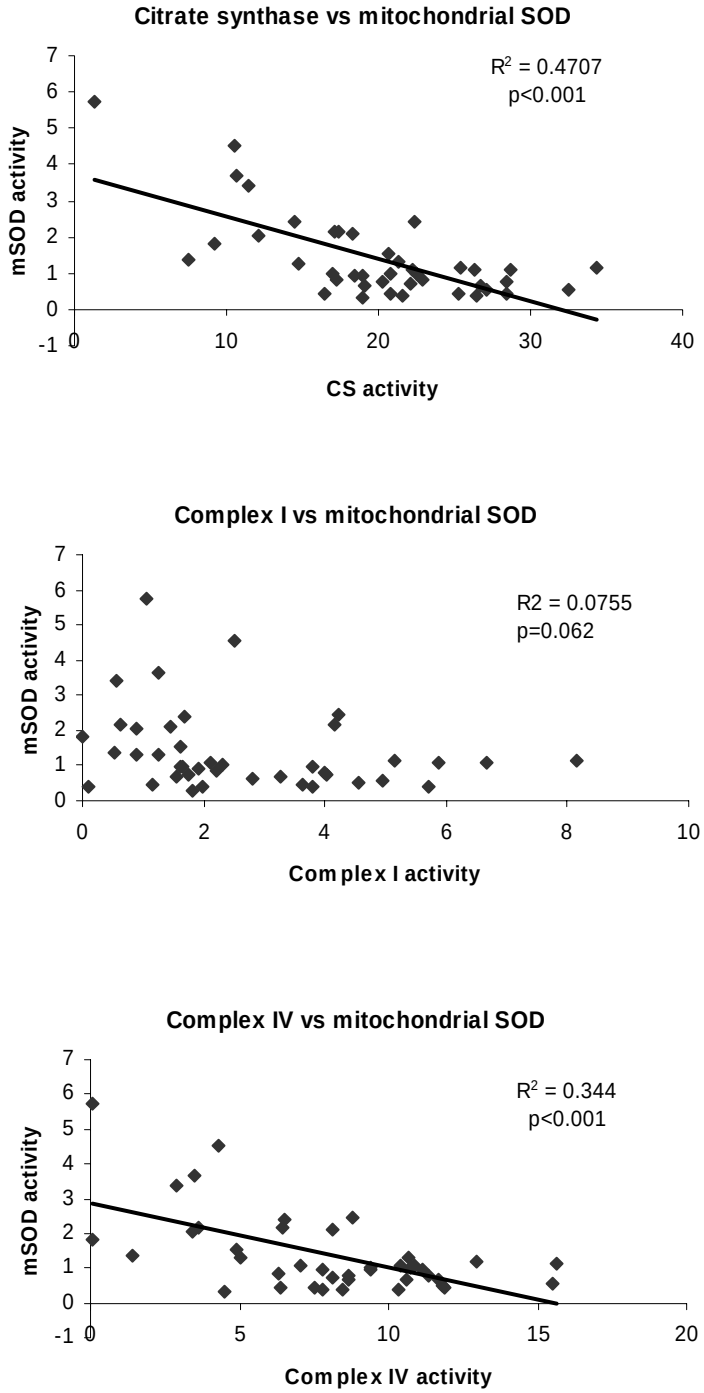

Figure 14: *Correlation between the mitochondrial enzymes, citrate synthase as well as complex I and complex IV of the respiratory chain, measured in whole muscle homogenates and mitochondrial SOD activity in leg muscle from septic patients.*

5 GENERAL DISCUSSION

5.1 INSULIN RESISTANCE AND MUSCLE MITOCHONDRIA

The cause of mitochondrial dysfunction in septic patients is complex and may dependent on many different factors. In the present thesis a few of these contributing factors have been assessed. The mitochondrial protein synthesis, transcription of mitochondrial genes and the increased oxidative stress are all factors that influence mitochondrial content. These molecular aspects can be influenced by hormones and drugs in the septic patient. Several hormones, such as thyroid and sex hormones, insulin, glucocorticoids and leptin, have been shown to have a positive influence on mitochondrial energy production, protein synthesis and biogenesis [Stump CS et al., 2003].

One of the most characteristic hormonal changes in septic and other critically ill patients is insulin resistance. Insulin resistance leads to high blood glucose levels. Administration of insulin to normalize blood glucose levels decreases mortality and morbidity in critically ill patients [Van den Berghe G et al., 2006, van den Berghe G et al., 2001]. The decreased mortality was most evident in patients suffering from sepsis induced MOF and staying in the ICU for more than 5 days. Whether this is an effect of insulin administration itself or an effect of the lowered glucose concentrations is not known.

Insulin administration during glucose clamp in healthy volunteers increase ATP production, gene expression of mitochondrial genes, mitochondrial protein synthesis and the activities of complex IV and citrate synthase [Boirie Y, 2003, Stump CS et al., 2003]. However, when the same experiments were performed in insulin resistant diabetic subjects no effect on muscle mitochondrial function was

observed [Stump CS et al., 2003]. Septic ICU patients are often hyperglycemic and treated with insulin to decrease blood glucose concentrations; however, the mitochondrial derangements are still present in these patients (study I and IV) indicating that insulin might not have an effect on muscle mitochondrial function in septic patients. However, when study I was performed the finding that strict blood glucose control lowers mortality was not yet published and patients were only treated with insulin if the blood glucose levels were higher then 11 mmol/l. The routines have become stricter since then and in study IV blood glucose levels were kept lower, but still very few patients had normoglycemia. However, a strict blood glucose control did not influence muscle mitochondrial function and morphology in ICU patients studied after they died, while liver mitochondria were improved by strict blood glucose control in the same patients [Vanhorebeek I et al., 2005]. Also in other studies, has insulin been shown to have an effect on liver insulin resistance, but not on peripheral insulin resistance. An increased endogenous glucose production is evident causing hyperglycemia in critically ill trauma patients [Thorell A et al., 2004]. Insulin administration to the critically ill trauma patients together with total parenteral nutrition leads to normoglycemia and decreased endogenous glucose production, while glucose uptake by the peripheral tissues, such as muscle, is not affected. Hyperglycemia has a detrimental effect on β-cell function mediated by superoxide production in the mitochondrion [Brownlee M, 2003, Krauss S et al., 2003], leading to a further decrease in insulin production and an even greater increase in hyperglycemia. From these studies it should be noted that the effect of hyperglycemia and insulin administration in septic patients on mitochondria might be different in muscle and other tissues such as liver and pancreatic β-cells. However, more studies are needed to validate this finding.

5.2 MUSCLE MITOCHONDRIAL DYSFUNCTION - WHAT DOES IT MEAN FOR THE PATIENT?

A decreased mitochondrial content in muscle from septic ICU patients was found in studies I and IV. The remaining question is whether and to what extend this influences muscle function in septic patients. It is not only the decreased mitochondrial content that could influence muscle function in critically ill patients. Major loss of muscle protein is also evident, as much as 10 % of muscle protein content is lost per week during ICU stay [Gamrin L et al., 1997]. This state is accompanied by changes in muscle amino acid pattern, of which the loss of muscle glutamine is the most prominent [Gamrin L et al., 1996]. The muscle myofibrilar structure is also deranged in septic patients suffering from acute quadriplegia, were a specific decrease in myosin to actin ratios has been reported [Larsson L et al., 2000]. The findings of decreased muscle protein content, myofibrilar derangements as well as loss of muscle mitochondria could lead to both muscle weakness and fatigue in critically ill patients. However, it is not clear whether the loss of mitochondrial content found in studies I and IV actually leads to a diminished production of energy during rest. Two studies performed in rats have shown that a 25-40 % decrease of muscle mitochondrial enzyme activity does not influence ATP and creatine phosphate levels at rest [Dudley GA et al., 1987, Rooyackers OE et al., 1996b]. However, when the muscles were activated by electrical stimulation, ATP levels decreased much faster when the rats had decreased mitochondrial enzyme activities. In leg muscle of the septic patients in our study, ATP and creatine phosphate concentrations were already low and lactate levels high at rest, indicating that these patients probably will face great problems coping with the increased energy demand during muscle activation. No human data are, however, available to confirm this hypothesis and more studies are needed.

The second question is whether the mitochondrial derangements are specific for muscle tissue in these patients. Loss of mitochondrial function in muscle as well as other tissues have been postulated to contribute to multiple organ failure in septic patients [Crouser ED, 2004, Fink MP, 2002, Protti A et al., 2006, Singer M, 2005]. This so-called cytopathic hypoxia theory suggests that septic ICU patients have problems with oxygen utilization due to mitochondrial dysfunction. As mitochondrial dysfunction progresses the affected organ suffers from lack of ATP and ultimately fails to function [Singer M, 2005]. An association between muscle mitochondrial dysfunction and increased mortality have been found during the first 24 hours of ICU stay in septic patients [Brealey D et al., 2002]. Thus, mitochondrial dysfunction is related to decreased muscle function as well as survival in septic ICU patients.

6 CONCLUSIONS

From this thesis it can be concluded that the mitochondrial content is decreased in muscle of ICU patients suffering from sepsis and organ failure.

Study I: Mitochondrial content was 30-40% lower in leg and respiratory muscle of septic patients as compared to age-matched controls. In leg muscle this decrease was associated with a cellular energy deficit.

Study II: An increased mitochondrial enzyme activity was observed 2 hours after an endotoxin challenge of healthy volunteers.

Study III: Prolonged mechanical ventilation in a piglet model leads to a specific decrease in the activity of mitochondrial complex IV, but did not affect the other mitochondrial enzymes or mitochondrial content.

Study IV: The decreased mitochondrial content found in septic ICU patients was not related to a decreased mitochondrial biogenesis, but was related to an increased gene expression of two mitochondrial proteases suggesting an increase mitochondrial protein breakdown as a mediator for the mitochondrial content decrease.

7 ACKNOWLEDGEMENTS

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