Wasting power

Professor Olav Rooyackers leads a research programme investigating the causes of multiple organ failure in intensive care patients. Their findings point towards mitochondrial degradation as a key contributor

What prompted the formation of the Intensive Care Unit Metabolism and Nutrition research group?

Professor Jan Wernerman started the group, including the analytical lab, about 25 years ago, when he became head of the clinic and department for intensive care. This was a response to the many clinical questions he had when treating patients in the ICU, including: why are these patients losing so much muscle mass at such an accelerated rate and can we influence this by the way we feed our patients?

Why is the study of Multiple Organ Failure (MOF) important?

MOF, which is characterised by the failing of vital organ systems – respiratory, kidney, liver, cardiovascular, neural – is a relatively new syndrome so little is known about the causes. Most ICU patients develop MOF and many would die without support. Current treatment is basically to support the organ systems and wait for them to recover.

In simple terms, can you explain how mitochondrial function is impaired in ICU patients?

The amount of mitochondria is decreased by about 40 per cent in their skeletal muscle, which leads to problems with cellular energy. The energy substrates cells use for their metabolic processes (adenosine triphosphate and creatine phosphate) are less abundant. This decrease is not due to a slowing down in synthesis of mitochondria, but to their dramatically increased degradation, most likely via autophagia. We are currently studying how well the remaining mitochondria function, because we think that even those are compromised.

Can you outline the methodology that you employ?

To study mitochondria we measure four things:

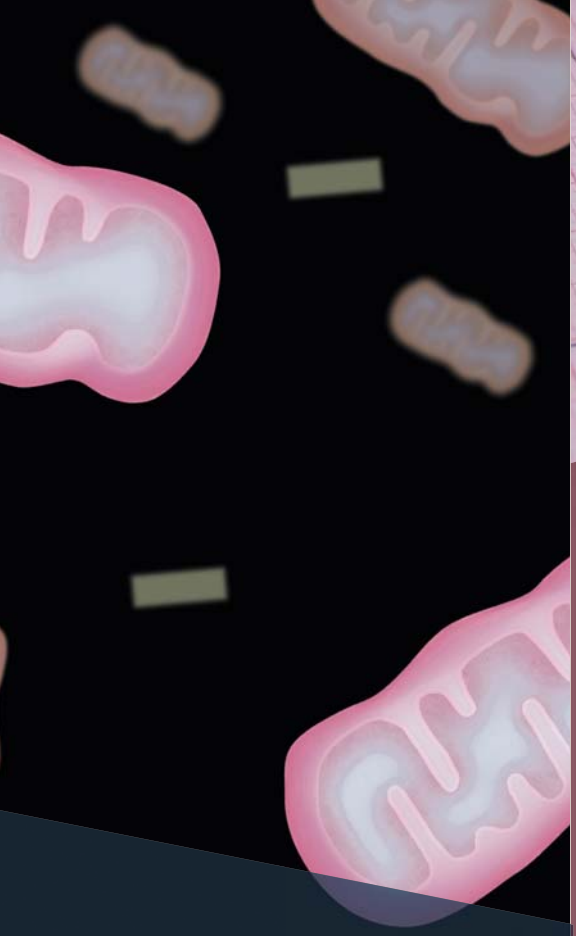
- First, the maximal activity of several enzymes located in them. This also gives us an indication of whether specific mitochondrial functions are compromised or whether there are just fewer mitochondria
- Second, the amount of cellular energy substrates produced by them. This gives us an indication that the changes in mitochondria have a negative effect on cells in an organ
- Third, their synthesis rate. For this, we give a labelled amino acid to patients and see how much of it is built into new mitochondria over several hours. This gives us an indication of whether compromised mitochondrial function is the result of diminished synthesis of new mitochondria. We often combine this with measurements of mitochondria-specific gene expression
- Lastly, we measure mitochondrial function. We do this in the lab, using either cells obtained from patients or mitochondria isolated from patients' organs, to assess the capacity of mitochondria to use oxygen to produce cellular energy.

What preventative methods might safeguard mitochondrial function?

No methods are currently available to specifically safeguard mitochondrial function.

It has been hypothesised that damage to mitochondria with MOF is due to increased oxidative stress, but proof is not very strong in patients. Several very preliminary studies with animals or cells have explored treatment with antioxidants to prevent mitochondrial damage.

A treatment that ICU patients receive more commonly nowadays might also affect



Vital signs

Research at the **Karolinska Institute** into mitochondrial function in intensive care patients with sepsis has found that over-stimulation of mitochondria results in heightened mitochondrial degradation which may then cause multiple organ failure. Further studies are seeking additional suspects with the aim of informing treatment development

PEOPLE ARE, SELF-EVIDENTLY, admitted to an intensive care unit (ICU) when their condition is life-threatening – maybe as a planned admission after surgery or an emergency due to acute illness or accident – and so their prognosis is uncertain. In intensive care, patients are often sedated for comfort and to aid treatment and therefore are usually fed intravenously or via a feeding tube while bedridden. If the patient stays in the ICU long enough,the combination of severe illness, sedation, restricted physical movement and artificial nutrition leads to a unique complex pathophysiology.

The prognosis for a patient admitted to ICU worsens when they have sepsis, an infection to which the body responds with systemic inflammatory response syndrome (SIRS). SIRS often causes one or more major organs to fail, sometimes with fatal results. From studies at the Karolinska Institute and University Hospital in Huddinge, Sweden, it is estimated that 30 to 35 per cent of sepsis patients, and more than 50 per cent of patients with persistent sepsis, die from multiple organ failure (MOF): "Mortality for MOF patients ranges from about 20 per cent with one failing organ to about 80 per cent with four or more," states Olav Rooyackers, a Professor in the Department of Clinical Science, Intervention and Technology and joint Principal Investigator with Professor Jan Wernerman in ICU-based research at the Karolinska Institute.

On average, sepsis patients are treated in the ICU for eight days and, even if treatment is apparently successful, the chances of full recovery are poor, with up to 41 per cent mortality within a year of release from hospital. The life expectancy of those that do survive longer is reduced; they may have physical disabilities and tend to suffer from post-traumatic stress disorder. Rooyackers and the Karolinska ICU research group have therefore been attempting to identify what causes patients with sepsis in intensive care to contract MOF: "There is an urgent need to understand more about the causes for MOF so we can find new treatment strategies," states Rooyackers.

INTEGRATED ICU, RESEARCH GROUP AND LABORATORY

The Rooyackers/Wernerman Metabolism and Nutrition research group is attached to the ICU at the Karolinska University Hospital. The group, which comprises permanent medical and basic scientists and a number of doctorate and postgraduate students, has the resources of an on-site state-of-the-art laboratory. This highly integrated setup makes for clinically relevant and responsive research, according to Rooyackers: "Clinicians and researchers work side by side. Researchers have access to the patients in the ICU and the support of a group of excellent research nurses. We can perform most analyses ourselves and ensure the high quality

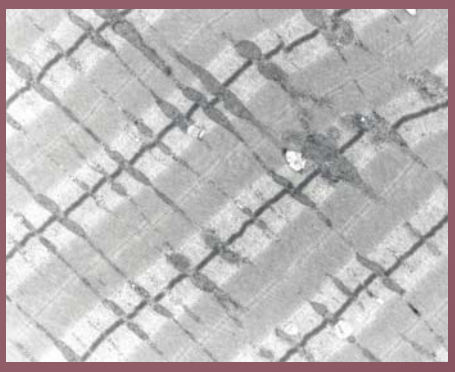
mitochondria: an increasing percentage of patients receive physiotherapy in the unit, to preserve muscle function. This could potentially have an effect on the mitochondria in skeletal muscle, but it has not been studied specifically.

Moving forward, what areas require further investigation and how do you hope to achieve these goals?

For MOF we need to investigate other organ systems in patients with MOF to ascertain whether this mitochondrial problem is a common cause for organ failure. In addition, we need to look at other potential causes to ensure we are not studying something that is caused by a larger problem. Once we have taken these steps, we shall need to investigate what causes mitochondrial damage and whether oxidative stress is really a contributing factor in the organs of the patients with MOF. Only then, we can start to look for and test potential treatments.

Finally, what makes your research group unique?

We are a truly integrated group with clinicians and pure scientists employed by and located in the clinic. This ensures that our research addresses important clinical questions with state-of-the-art techniques. The fact that the research group is led by a clinical professor (Jan Wernerman) and a pure science professor (me) is rather unusual. That we have our own lab that is supported by the clinic is unique.



The electron microscopic (EM) structure of the contractile apparatus and the mitochondria are damaged in skeletal muscle of patients with MOF (left) compared with normal (right).

MITOCHONDRIAL FUNCTION IN SEPSIS WITH MULTIPLE ORGAN FAILURE

OBJECTIVES

To elucidate derangements in the metabolism of critically ill patients and try to find the mechanisms leading to these changes. In addition the group will study the possibilities of the patients to utilise nutrition and if this has any effect on the metabolic derangements.

PARTNERS

Professor James Timmons, Loughburough University, UK

Assistant Professor Thomas Gustafsson, Clinical Physiology, Karolinska University Hospital Huddinge

Professor Folke Hammarqvist, Surgery, Karolinska University Hospital Huddinge

Professor Luc van Loon, University of Maastricht, NL

Dr Nicolaas Deutz, Texas A&M University, USA

FUNDING

Swedish Research Council (VR)

NIH

European Society of Clinical Nutrition and Metabolism (ESPEN)

Stockholm County Council

EU Seventh Framework Programme (FP7)

CONTACT

Professor Olav Rooyackers
Project Coordinator

Anesthesiology and Intensive Care
Clintec
Karolinska Institutet and University Hospital Huddinge, Sweden

T +46 8 58586182
E olav.rooyackers@ki.se

www.icu-metabolism.se

OLAV ROOYACKERS was born in The Netherlands. After studying Biology at the Agricultural University in Wageningen, he received a PhD from the University in Maastricht in 1995. Following a postdoc at the Mayo Clinic in the USA and at the University of Edinburgh in Scotland, he started a researcher position at Karolinska Institutet in January 2000. He is currently Professor in Experimental Anesthesiology and Intensive Care at Karolinska institutet and Karolinska University Hospital in Huddinge.

we need," he explains. Where techniques are only used occasionally, the group has set up collaborative arrangements with experts in those techniques.

The laboratory has two high performance liquid chromatography systems and two gas chromatography-mass spectrometry systems. The main techniques used by the research group are biochemical analyses, gene expression analysis – in collaboration with expert help of Professor James Timmons – Western blot analyses and also analyses of stable isotope tracers, which they consider their speciality. Stable isotope tracers are used to quantify rates of metabolic pathways in real-time in humans: eg. glucose turnover rates, whole body protein turnover, synthesis rates of immune cells, muscle tissue, albumin and mitochondria and turnover rates of specific amino acids. The laboratory is also currently acquiring a liquid chromatography system coupled to tandem mass spectrometry so that they can expand on their current metabolomics analysis capability.

MITOCHONDRIAL FUNCTION AND MOF

MOF is normally assessed according to respiratory, cardiovascular and central nervous system function, blood coagulation and kidney and liver function. However, MOF also causes failure in other areas, such as the immune and skeletal muscle systems, in which it has a negative effect in terms of mortality and morbidity on patients with severe sepsis.

The mitochondria are the organelles that generate the chemical energy that powers cells. They are involved in a number of other important functions, such as signalling and cell differentiation, during the cell life cycle. Dysfunction or degradation of mitochondria therefore fundamentally affects key cell processes but, most damagingly, it decreases cellular energy.

Since a growing body of evidence points to an association between a deficit of cellular energy and MOF, and to mitochondrial dysfunction in the skeletal muscle of patients with sepsis, the ICU research group's focus has been on elucidating the mechanisms of mitochondria in the skeletal muscle of patients with sepsis and MOF. They have specifically been addressing the questions of why ICU patients lose skeletal muscle mass and develop muscle fatigue, which are part of the MOF syndrome, and why this persists after discharge from the ICU. To study this, ICU patients and controls have been recruited in several studies. The group has found that in critically ill patients with sepsis, the function of mitochondria was dramatically decreased by 30-40 per cent. In addition, the rate of production of new mitochondria was not affected, from which Rooyackers concluded that there must be a heightening of the degradation of mitochondria. Other studies however suggest an increased activation of mitochondria early during the critical illness, suggesting an early burn out leading to a later drop in their number. Rooyackers has hypothesised from this that the mitochondria remaining would also suffer oxidative damage. Therefore the group is now investigating the means by which this happens and seeking to confirm whether and how mitochondrial function is also degraded.

> There is an urgent need to understand more about the causes for multiple organ failure so we can find new treatment strategies

Consequently, the key questions that the group are currently addressing are whether remnant mitochondria are properly functional; whether the same 30 to 40 per cent mitochondrial decrease – and functional degradation – as found in skeletal muscle, is found in other tissues; whether there are means of boosting mitochondrial production; and, last but not least, what happens at the molecular level. Early group findings in investigations of molecular processes show that while the body attempts to generate more mitochondria to compensate for losses, it does not happen in the skeletal muscle of patients with sepsis; and that gene expression is increased in inactive skeletal muscle.

WIDENING THE SEARCH

While they have made significant progress, Rooyackers is clear that there is still much to be done: "We have shown that mitochondrial dysfunction and cellular energy problems are present in one organ – skeletal muscle – in patients with MOF," he states. "We have also learned that this compromised function is due to increased degradation of mitochondria in skeletal muscle." These findings suggest that other organs might have the same problem and could explain why organs are failing. They are now seeking to validate this hypothesis by investigating mitochondrial dysfunction in other organs.

The group has also widened the scope of their search for causes beyond mitochondria: "We are starting other studies to try to identify new mechanisms by measuring changes in expression of all genes in several tissues at the same time in patients with severe inflammation and MOF," Rooyackers explains.