The leaky mitochondrion

Mitochondria use proton gradients to make ATP. Sometimes, however, proton 'leak' uses the gradient to produce heat instead. Here, Kent Sahlin and co-workers argue that the balance of these two consequences of fuel oxidation might be under physiological control



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The mitochondrion is the power plant of the cell, where the energy derived from oxidation of fuels is converted to ATP, i.e. oxidative phosphorylation. The process involves pumping of protons across the mitochondrial membrane and the proton gradient this forms drives the synthesis of ATP. Although the major part of oxygen utilization is used for ATP synthesis (coupled respiration), the mitochondrial membrane is leaky and protons may leak back through the membrane. Proton leak will increase oxygen consumption (uncoupled respiration, UCR) and the energy will be dissipated as heat instead of being trapped as 'useful energy', i.e. ATP (Fig. 1). UCR has been estimated to about 40% of basal metabolic rate in the rat (Brand *et al.* 1994) and will reduce the efficiency of oxidative phosphorylation, which is typically measured as the yield of ATP per consumed oxygen (i.e. P/O ratio).

Although the term leak indicates deficiency in system design, UCR may have, or may be associated with, important physiological functions e.g. thermogenesis and body weight regulation, prevention of oxidative stress, prevention of mitochondrial damage induced by fatty acids (FA) and control of oxidative phosphorylation.

The mechanism of UCR in skeletal muscle is not known but is currently an extensive field of research. Proton leak may occur through membrane proteins (e.g. uncoupling proteins, UCP) or by non-specific transmembrane flux, which is dependent on the lipid composition of the mitochondrial membrane (Brand *et al.* 2002). In brown adipose tissue proton leak is mediated by UCP1 and has a well described role in thermogenesis. A homologous protein (UCP3) has been found in skeletal muscle but the significance of UCP3 as a determinant of UCR and basal metabolic rate is under debate. Concentrations of UCP3 increase in parallel with plasma FA during fasting and high fat diets and it has been suggested that the role of UCP3 is to protect mitochondria from an overload of long chain FA (Hoeks *et al.* 2003). The high UCP3 content in fast-twitch glycolytic muscles, where the capacity for FA oxidation is reduced, supports such a role (Hoeks *et al.* 2003).

There is also evidence that UCP3 functions as a mild uncoupler and as such reduces formation of reactive oxygen species. Markers located near the UCP2 and UCP3 gene are strongly associated with basal metabolic rate (Bouchard *et al.* 1997) and suggest a role of UCR in thermogenesis.

An approximate measure of UCR is the oxygen consumption of isolated mitochondria and permeabilized fibres under non-phosphorylating conditions. It has been known for a long time that FA stimulate UCR in isolated mitochondria and that the effect varies between different forms of FA (Matthias *et al.* 1999). An intriguing finding is that endurance training increases the sensitivity of UCR to FA (Tonkonogi *et al.* 2000). FA-evoked UCR increased two-fold after 6 weeks of training (Fig. 2). Diet-induced thermogenesis is increased in endurance trained subjects in proportion to their VO_2max (Lopez *et al.* 2000). It is possible that this relates to the increased mitochondrial uncoupling in the presence of FA and that endurance training may prevent obesity during overfeeding.

A well known adaptation to endurance training is an increased mitochondrial biogenesis. This would in itself increase UCR provided proton leak per mitochondrial volume remains constant. However, UCR, measured in the absence of FA, was reduced after endurance training when related to

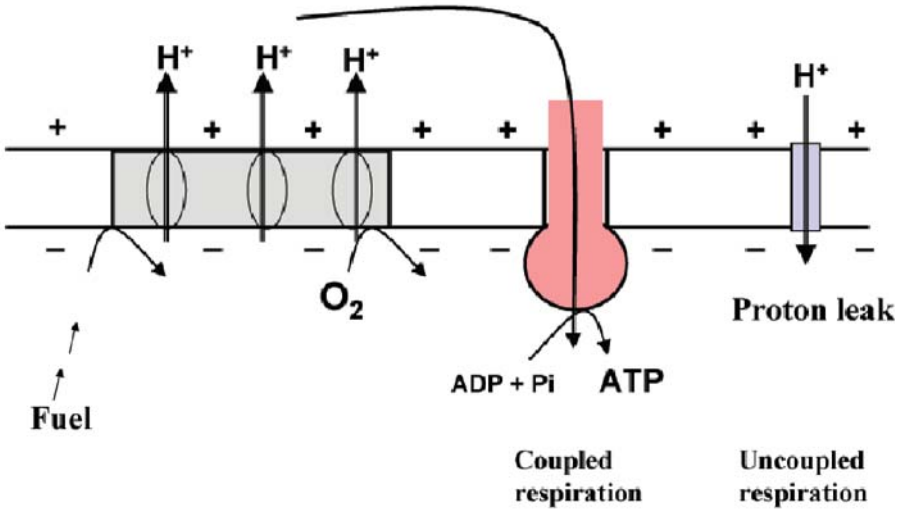

Figure 1. Schematic diagram of mitochondrial inner membrane indicating the mechanisms of coupled and uncoupled respiration. The driving force for proton influx is due to both an electrical and a concentration gradient over the membrane.

mitochondrial volume (Fernstrom *et al.* 2004). The decrease in UCR was paralleled by reduced levels of mitochondrial UCP3 protein. The training-induced reduction in UCR may be a mechanism to avoid unnecessary waste of energy due to increased mitochondrial volume and may also increase efficiency during exercise. This idea is supported by the observed inverse correlation between mechanical efficiency during cycling and UCP3 protein in human skeletal muscle (Schrauwen & Hesselink, 2003).

UCR may contribute to the hitherto unexplained excess oxygen consumption during and after heavy exercise. During prolonged exercise at a constant work rate there is a slow increase of VO_2 (oxygen drift) indicating a decreased mechanical efficiency. UCR, measured in permeabilized muscle fibres (i.e. after removal of the muscle cell membrane), was increased after prolonged exercise (Tonkonogi *et al.* 1998), and the oxygen drift may relate to increased uncoupling. However, UCR measured in isolated mitochondria (with and without FA) as well as UCP3 protein both remained unchanged after prolonged exercise (Fernstrom *et al.* 2004).

Measurements of UCR in muscle fibres and in isolated mitochondria have a number of limitations. During the preparation of mitochondria, potential changes in UCR may be reversed due to reversal of the exercise-induced perturbation of the cellular environment (e.g. acidosis, Ca-overload and hyperthermia). Only the effect of structural changes that remain in the permeabilized fibres and the isolated mitochondria will be observed. In vivo UCR may therefore be entirely different from that measured in vitro under standardized conditions.

UCR may also relate to intermittent opening of large protein pores in the mitochondrial membrane (i.e. permeability transition pores; PTP). PTP opening is stimulated by Ca-overload, oxidative stress and energetic stress, i.e. conditions prevailing during high-intensity exercise. Contrary to our expectations, mitochondria isolated from muscle samples taken immediately after prolonged exercise were more resistant to Ca^{2+}-overload than before exercise. Again this may not give a correct picture of what is occurring in the working muscle since the exercise-induced increase in mitochondrial Ca^{2+} is likely to disappear during the preparation process. However, the results suggest that endurance exercise has a protective role and increases Ca-tolerance of mitochondria.

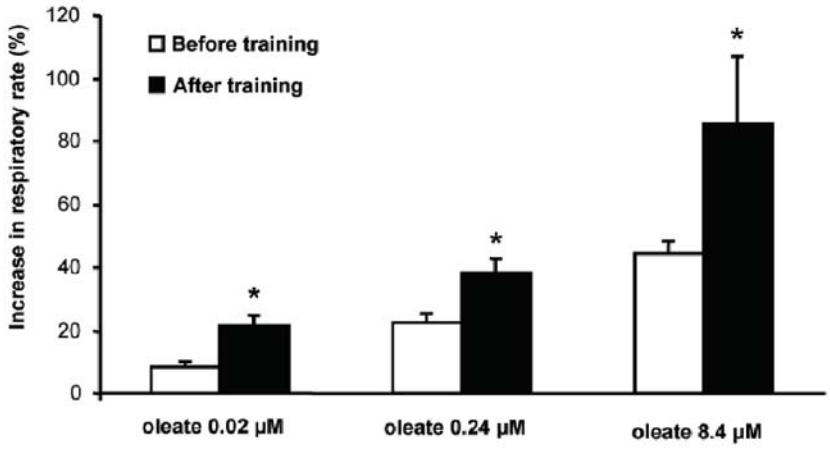

Figure 2. Effect of increased concentrations of long chain fatty acid (oleate) on uncoupled respiration in isolated mitochondria obtained before and after 6 weeks of endurance training. Measurements were performed with pyruvate/malate in the presence of oligomycin (inhibitor of ATP synthase) and atractyloside (inhibitor of adenine nucleotide translocase).

Irrespective of the role of UCP3 in skeletal muscle, UCR is an important factor in muscle energetics and as such important for body weight regulation and work efficiency. The reduced efficiency in oxidative phosphorylation associated with UCR may impair performance during exercise. However, transition from rest to exercise reduces proton leak, and futile proton cycling (pump and leak) may be a mechanism to control oxidative phosphorylation during rapid transitions in energy flux. UCR is thus a parameter under physiological control and can be both upregulated (overfeeding) and downregulated (exercise). The leaky mitochondrion and the physiological role of proton leak deserve further attention.

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