The lipid peroxidation product 4-hydroxynonenal (HNE) is an important mediator of free radical damage [1]. HNE specifically induces uncoupling of mitochondria through the uncoupling proteins (UCPs) and the adenine nucleotide translocase (ANT) although the relative contribution of the two carriers to these effects is unclear [2,3]. To clarify this we studied the sensitivity of HNE-activated proton conductance to GDP (UCPs inhibitor) and carboxyatractylate (ANT inhibitor) in skeletal-muscle and heart mitochondria from mice expressing different amounts of UCP3. Mitochondria were isolated from wild-type and *Ucp3* knockout mice. To increase UCP3 expression, some mice were i.p. injected with LPS (12 mg/kg body weight). HNE activated proton conductance in skeletal-muscle and heart mitochondria. In skeletal muscle, this increase correlated with UCP3 expression levels: it was lower in *Ucp3* knockout mice and higher in LPS-treated wild-type mice. GDP partially abolished HNE effects whereas carboxyatractylate or addition of both inhibitors completely abolished it. In contrast, GDP had no effect on HNE-induced proton conductance in heart mitochondria, but carboxyatractylate or administration of both inhibitors had a partial effect. In skeletal muscle mitochondria, GDP-mediated inhibition of HNE-activated proton conductance was specific for UCP3 since it was not observed in *Ucp3* knockout mice. Carboxyatractylate was able to inhibit UCP3 through an unknown mechanism. We conclude that, in skeletal muscle, HNE-induced increase in proton conductance is mediated by UCP3 (30%) and ANT, whereas in the heart the increase is mediated by ANT and other carriers, possibly including UCP3.

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10P.3 Regulation of H_2O_2 generation by uncoupling protein 1 in thymus mitochondria

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It has been proposed that uncoupling proteins can attenuate mitochondrial production of free radicals and therefore protect against oxidative damage, degenerative diseases and aging. Recently it has been established that uncoupling protein 1 (UCP1) is located in the thymus. We tested a hypothesis that UCP1 can regulate ROS production in rat mitochondria from thymus using an Amplex red/H_2O_2 assay. Our data show that inhibition of UCP1 by GDP caused an increase in ROS production by non-phosphorylating thymus mitochondria respiring on a) succinate and rotenone (1.5 fold increase) and b) glycerol-3-phosphate and rotenone (1.2 fold increase). In parallel with H_2O_2 production measurements, the effect of GDP on membrane potential was monitored by uptake of the fluorescent probe safranine, while the inhibitory effect of GDP on oxygen consumption was measured using an oxygraph respirometer. The observed increase in ROS production upon GDP addition was accompanied by a relative decrease in oxygen consumption. We are currently performing equivalent experiments using thymus mitochondria isolated from *UCP1*$^{-/-}$ mice and their control littermates, to establish that our observations are indeed due to UCP1 activity in the thymus.

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10P.4 Are the novel uncoupling proteins acutely regulated by fatty acids and nucleotides?

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In brown adipose tissue (BAT) mitochondria, uncoupling protein 1 (UCP1) dissipates the protonmotive force to generate heat. UCP1 is activated by fatty acids and inhibited by nucleotides such as GDP, but the precise mechanisms involved remain controversial. Even less is known about the physiological role and regulation of the novel uncoupling proteins, UCP2 and UCP3. Here we present the first demonstration of a conformational change induced by fatty acids for both UCP1 and UCP3 in rat BAT mitochondria. Conformational changes were inferred from the kinetics of proteolysis when isolated mitochondria were treated with exogenous trypsin. Palmitate increased the rate of proteolysis for both proteins, showing that palmitate binds and affects their conformation. Trypsinolysis of UCP1 could be fully rescued by GDP, consistent with its ability to compete functionally with fatty acids. UCP3 degradation, however, was GDP-independent, suggesting that GDP interacts differently (or not at all) with UCP3. Experiments to determine the acute regulation of UCP2 as diagnosed by trypsinolysis are currently being conducted.

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10P.5 *In vivo* gene silencing of uncoupling protein-2 in kidney cortex of diabetic rats results in increased uncoupling, decreased oxidative stress and reduced membrane potential Implications for the adenine nucleotide transporter

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Mitochondria uncoupling *via* uncoupling protein (UCP) 2 may be an important mechanism to reduce oxidative stress and preserve mitochondria function in the diabetic kidney. Short interference (si) RNA to knockdown UCP2 or a non-functional scrambled equivalent (100 μg/rat) was administered to healthy and diabetic (streptozotocin; 60 mg/kg b.w.) Sprague–Dawley rats and the mitochondria isolated 48 h thereafter. Glutamate-stimulated QO_2 in the presence of ATP-synthase inhibitor oligomycin was used to estimate mitochondria uncoupling since proton release across over the inner membrane with subsequent increase in QO_2 will not occur in coupled mitochondria. Diabetes increased UCP2 (192 ± 34% of control corrected for β-actin) and siRNA decreased UCP2 (control + siRNA 70 ± 11 and diabetes + siRNA 88 ± 8% of control). Glutamate-stimulated QO_2 was significantly higher in control + siRNA, untreated diabetics and diabetes + siRNA compared to control (1.6 ± 0.5, 1.5 ± 0.5 and 3.8 ± 0.5 vs. 0.0 ± 0.1 nmol O_2/s/mg protein). The UCP2 inhibitor guanosine diphosphate (GDP) inhibited QO_2 in diabetics (0.9 ± 0.5 vs. 1.6 ± 0.5 nmol O_2/s/mg protein) but not in diabetes + siRNA (4.6 ± 0.6 vs. 3.8 ± 0.5 nmol O_2/s/mg protein). ADP in the presence of oligomycin reduced QO_2 in diabetes + siRNA (2.0 ± 0.5 vs. 3.8 ± 0.5 nmol O_2/s/mg protein) but had no effect in any of the other