Published in final edited form as:
Epilepsia. 2008 November ; 49(Suppl 8): 117–119. doi:10.1111/j.1528-1167.2008.01854.x.

UCP-mediated free fatty acid uncoupling of isolated cortical mitochondria from fasted animals: correlations to dietary modulations

LM Davis[1], **JM Rho**[2], and **PG Sullivan**[1]

[1] Spinal Cord & Brain Injury Research Center and Department of Anatomy & Neurobiology, University of Kentucky, Lexington, Kentucky 40536, United States

[2] Barrow Neurological Institute and St. Joseph's Hospital & Medical Center, Phoenix, Arizona, 85013, United States

Summary

Uncoupling proteins (UCP) translocate protons from the mitochondrial intermembrane space to the matrix, thereby "uncoupling" electron transport from the production of ATP. It has been shown that these proteins are highly expressed in animals maintained on the ketogenic diet (KD). Although the exact mechanism remains unclear, it is known that these proteins are activated within a protective anti-ROS mechanism by free fatty acids (FFA). In our current studies, mitochondrial samples were probed for the presence of UCP2, which is the most ubiquitously expressed UCP isoform. We found that both traumatic brain injury and fasting up-regulated the expression of UCP2, with a synergistic up-regulation in fasted injured animals. We then used mitochondria from fasted naïve animals to screen a number of FFA for their activation of uncoupling as well as their ability to reduce ROS. We found that arachidonic acid (AA), docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), palmitoleic acid, myristic acid, and butyric acid increased mitochondrial uncoupling when added after oligomycin. These FFA, along with oleic acid, also reduced ROS in mitochondria incubated with oligomycin. In order to correlate our data to KD and fasting, both of which have been shown to be neuroprotective after neurologic insult, we determined the serum levels of FFA in KD and fasted animals using gas chromatography/mass spectroscopy. We also determined brain and CSF FFA levels from fasted animals.

Keywords

calorie reduction; fasting; free fatty acids; mitochondria; reactive oxygen species; trauma; ketogenic diet

Perhaps one of the most important evolutionary occurrences was the development of mitochondrial function. It was at this point that the cell was able to produce enough energy, in the form of ATP, to form highly complex, interconnected networks that developed into the organ systems present in all organisms (Lane 2006). The phosphorylation of ATP is catalyzed by complex V (ATP synthase), which is the final multimeric complex of a series

*Corresponding Author Information: 741 S. Limestone, 375 BBSRB, Lexington KY, 40536, Telephone: 859-323-4684, Facsimile: 859-257-5737, PatSullivan@uky.edu.

DISCLOSURES: The authors have read the journal's policy regarding ethical publishing and agree that this manuscript conforms to those guidelines.

The authors declare that they have no conflict of interests or competing financial interests.

of protein complexes comprising the electron transport chain (ETC). The vast majority of the components of the ETC are located within the inner membrane where electrons are accepted from mitochondrial energy substrates. In response to electron flow, these proteins pump protons from the mitochondrial matrix to the intermembrane space (IMS). This creates a difference in proton concentration between the IMS and the matrix, thereby resulting in an electrochemical gradient, also termed the mitochondrial membrane potential ($\Delta\Psi$). Complex V utilizes $\Delta\Psi$ by allowing the protons to catalyze phosphorylation as they flow back across the inner membrane to the matrix and down their concentration gradient. It is vital to the function of mitochondria that the $\Delta\Psi$ remains within a specific range, and deviations in $\Delta\Psi$ can induce mitochondrial dysfunction and cell death pathways (Nicholls and Ferguson 2002).

As the formation of harmful reactive oxygen species (ROS) is a normal byproduct of mitochondrial function, mitochondria are equipped with endogenous anti-oxidative mechanisms (glutathione/catalase/manganese superoxide dismutase, MnSOD). However, under pathological conditions, the $\Delta\Psi$ can increase above the optimal range, which results in excessive ROS formation due to the escape of electrons from ETC intermediates (Nicholls and Budd 2000; Nicholls and Ferguson 2002). These dangerous molecules have been implicated in the pathological development of epilepsy, indicated by studies showing the susceptibility of MnSOD knockout animals to status epilepticus (SE). These animals show increased hippocampal damage and oxidative damage markers in response to kainate-induced seizures, whereas animals overexpressing MnSOD showed up to 2-fold seizure attenuation (Liang and Patel 2004; Patel and Li 2003; Sleven et al. 2006). It has become increasingly clear that oxidative damage and, in turn, mitochondrial damage is linked to the development and propagation of SE (Liang and Patel 2004). Therefore, to decrease seizure susceptibility, it is necessary to develop therapies to attenuate the cellular pathology that leads to the excessive ROS production.

An intriguing therapeutic target may be uncoupling proteins (UCPs), endogenously regulated mitochondrial proteins which can attenuate increases in $\Delta\Psi$ by independently translocating protons from the IMS to the matrix, effectively bypassing complex V (Echtay et al. 2005). The expression of UCPs is regulated by the peroxisome proliferator activating receptor (PPAR), which is activated by specific free fatty acid (FFA) ligands (Debril et al. 2001; Kiec-Wilk et al. 2005). Free fatty acids are produced within the liver from triglyceride breakdown during fat mobilization (during fasting), or from high dietary fat, such as the ketogenic diet (KD) (Sullivan et al. 2004). Indeed studies have shown an increase in UCP expression in response to the KD, and increased neuroprotection from kainate-induced seizures (Sullivan et al. 2003; Sullivan et al. 2004). The KD, which was originally designed to mimic fasting physiology, can be used as an effective therapy for epilepsy; however the underlying mechanism of this dietary modulation remains unclear.

Recent studies in our laboratory have shown that fasting animals for 24 hrs after traumatic brain injury (TBI) is neuroprotective and decreases mitochondrial oxidative damage (Davis et al. 2008). This neuroprotection is partially due to mitochondrial ketone utilization, which suggests a potential role for UCPs and FFAs in the neuroprotective mechanism of fasting after trauma and perhaps of the KD in epilepsy. Unfortunately, there is limited knowledge of which FFAs activate UCPs. Therefore, our current work has attempted to determine which FFAs are up-regulated in response to fasting, as well as the correlation between their up-regulation and their ability to activate UCPs within mitochondria isolated from fasted animals.

We screened FFA of a wide range of chain lengths and saturation states. We found that palmitoleic acid (C16), myristic acid (C14) and butyric (C4) acid were able to increase

respiration in mitochondria via UCP activation. Arachidonic acid (AA), docosahexaenoic acid (DHA), and eicosapentaenoic acid (EPA), which are all polyunsaturated fatty acids (PUFA), also increased the activation of UCP-mediated respiration in isolated mitochondria. As the overall goal of mitochondrial uncoupling is the reduction of ROS, we investigated the ability of our screened FFAs to decrease ROS production in isolated mitochondria. Results indicated that DHA, EPA, AA, myristic acid, butyric acid, and palmitoleic acid all showed significant decreases in ROS production, which indicates that they are functioning as activators of the endogenous UCP system. Interestingly, while oleic acid only increased respiration to a small degree, it was able to significantly reduce ROS production, which suggests that its ability to attenuate ROS production could either be through a limited activation of UCPs or an alternative mechanism. Valproic acid did not activate UCP-mediated respiration or decrease ROS production.

To correlate the activity of these FFAs with the mechanism of fasting-induced neuroprotection, we measured serum levels of FFAs after 24 hrs of fasting. Compared to 0 hr baseline serum levels of oleic acid, EPA and AA were significantly increased after a 24 hr fast. Linoleic acid was also increased at this time point; however, it did not increase uncoupling or reduce ROS, which may indicate its role in UCP upregulation by the PPAR system. Although not significant, serum DHA trended toward increased levels after 24hrs. Similar to our serum samples, brain tissue linoleic acid and oleic acid were increased in cortical tissue; linoleic acid and AA were also increased in hippocampal tissue. Interestingly, EPA and DHA levels were unchanged in either brain region after 24hrs of fasting. None of the FFAs assayed had increased CSF concentrations.

In light of the importance of FFAs within the KD, and the ability of this diet to increase UCP expression, these findings are an important step to further elucidating the neuroprotective mechanism of both fasting and the KD. The identification of dietary FFAs with the ability to decrease oxidative damage could allow therapeutic supplementation for an increased efficacy of treatment.

Acknowledgments

NS48191 (P.G.S.).

This work has been supported by NIH grant NS48191 (P.G.S.).

Abbreviations

TBI	traumatic brain injury
FFA	free fatty acids
ROS	reactive oxygen species

References

Davis LM, Pauly JR, Readnower RD, Rho JM, Sullivan PG. Fasting is neuroprotective following traumatic brain injury. J Neurosci Res. 2008; 86:1812–1822. [PubMed: 18241053]

Debril MB, Renaud JP, Fajas L, Auwerx J. The pleiotropic functions of peroxisome proliferator-activated receptor gamma. J Mol Med. 2001; 79:30–47. [PubMed: 11327101]

Echtay KS, Pakay JL, Esteves TC, Brand MD. Hydroxynonenal and uncoupling proteins: a model for protection against oxidative damage. Biofactors. 2005; 24:119–130. [PubMed: 16403971]

Kiec-Wilk B, Dembinska-Kiec A, Olszanecka A, Bodzioch M, Kawecka-Jaszcz K. The selected pathophysiological aspects of PPARs activation. J Physiol Pharmacol. 2005; 56:149–162. [PubMed: 15985699]

Lane, N. Oxford University Press pbk. Edition. Oxford. New York: Oxford University Press; 2006. Power, sex, suicide: mitochondria and the meaning of life.

Liang LP, Patel M. Mitochondrial oxidative stress and increased seizure susceptibility in Sod2(−/+) mice. Free Radic Biol Med. 2004; 36:542–554. [PubMed: 14980699]

Nicholls DG, Budd SL. Mitochondria and neuronal survival. Physiol Rev. 2000; 80:315–360. [PubMed: 10617771]

Nicholls, DG.; Fergusonb, SJ. Bioenergetics. Vol. 3. Boston: Academic Press; 2002.

Patel M, Li QY. Age dependence of seizure-induced oxidative stress. Neuroscience. 2003; 118:431–437. [PubMed: 12699779]

Sleven H, Gibbs JE, Heales S, Thom M, Cock HR. Depletion of reduced glutathione precedes inactivation of mitochondrial enzymes following limbic status epilepticus in the rat hippocampus. Neurochem Int. 2006; 48:75–82. [PubMed: 16290321]

Sullivan PG, Dube C, Dorenbos K, Steward O, Baram TZ. Mitochondrial uncoupling protein-2 protects the immature brain from excitotoxic neuronal death. Ann Neurol. 2003; 53:711–717. [PubMed: 12783416]