P31 NMR demonstrates dysfunction through mitochondrial uncoupling in skeletal muscle after burn injury

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Introduction– Burn trauma of 20%-30% or more of total body surface area (TBSA) results in severe systemic syndrome and muscle catabolism/atrophy [1]. Mitochondria are one of the most complex and important organelles found in eukaryotic cells. In addition to their central role in energy metabolism, mitochondria are involved in many key cellular processes such as the formation of reactive oxygen species and apoptosis. Mutations in mitochondrial DNA lead to a diverse collection of diseases that are challenging to diagnose and treat, and where precise mechanisms of disease pathogenesis remain elusive. Mitochondrial dysfunction has also been implicated in aging and in many chronic disease states including cancer, Parkinson's, diabetes mellitus, Alzheimer's, hepatic and cardiovascular diseases as well as burn injury [1]. Given the central importance of mitochondrial function in human biology, the ability to identify, measure and track the structural and functional basis of mitochondrial heterogeneity in human cells and tissues over the lifespan would transform our understanding of the role of this critical organelle in human health and disease. For this reason, we undertook a systems-biology based approach using ^{31}P NMR, gene and protein expression studies to investigate mitochondrial uncoupling protein 3 mutant mice before and following injury and compare them to controls.

Materials and Methods– NMR spectra of hind limb were acquired 1, 3, and 7 days after 30% TBSA burn trauma. All NMR experiments were performed in a horizontal bore magnet (proton frequency 400 MHz, 21 cm diameter, Magnex Scientific) using a Bruker Avance console. A 90° pulse was optimized for detection of phosphorus spectra (repetition time 2 s, 400 averages, 4K data points). Saturation 90°-selective pulse trains (duration 36.534 ms, bandwidth 75 Hz) followed by crushing gradients were used to saturate the γ-ATP peak. The same saturation pulse train was also applied downfield of the inorganic phosphate (Pi) resonance, symmetrically to the γ-ATP resonance. T_1 relaxation times of Pi and phosphocreatine (PCr) were measured using an inversion recovery pulse sequence in the presence of γ-ATP saturation. An adiabatic pulse (400 scans, sweep with 10 KHz, 4K data) was used to invert Pi and PCr, with an inversion time between 152 ms and 7651 ms. Biopsies were harvested from the left gastrocnemius muscle. RNA was extracted, purified, and quantified and genomic analysis was performed following standard Affymetrix protocols(Affymetrix, CA, USA).

Results– Burn trauma reduces ATP synthesis in control mice (WT, wild type) but less so in mutants (UCP3-KO, uncoupling protein 3 knock-out), (Figure 1). UCP3 mRNA expression was significantly increased at 12 h post-burn. We subsequently posed the question of whether UCP3 protein level also increases following burn in the 30% TBSA mouse model as it does in the local model [2]. Western blot analysis demonstrated that burn injury consistently with the gene expression resulted in increased levels of the 34kDa UCP3 protein by 12 hours. Both mRNA and protein expression data, along with the NMR studies, suggested UCP3 protein contributes to the mitochondrial dysfunction as indicated by the higher ATP synthesis rate baseline (B) levels in UCP-3 KO mice. Also, In UCP3-KOs, the UCP3 gene expression was undetectable as expected, but UCP2 and UCP1 was detected. Therefore, the ATP synthesis rate reduction observed in UCP3-KOs at 1d, 3d and 7d is probably due to these uncoupling proteins among other factors. Studies in UCP2- and UCP1-KOs should clarify this point.

Figure 1. ATP synthesis rate (μmol/g/s) after 30% TBSA burn. Data are from *in vivo* ^{31}P-NMR saturation-transfer experiments performed on the hindlimb skeletal muscle of mice after 30% TBSA burn (solid line, WT; broken line, UCP3-KO). Mean values are shown in squares (WT) and triangles (UCP3-KO); error bars correspond to standard errors. Data were fitted with exponential decays using standard nonlinear fitting procedures in Matlab (MathWorks, Inc, Natick, MA). The characteristic rate of the exponential decay equals 1.15 (WT) and 0.8 (UCP3-KO) [(mmol/g/s)/d]. * $P < 0.001$ for B compared to 1 d; $P < 0.05$ for B compared to 3 d; and $P < 0.05$ for B compared to 7 d (Student's t-Test); B = Baseline.

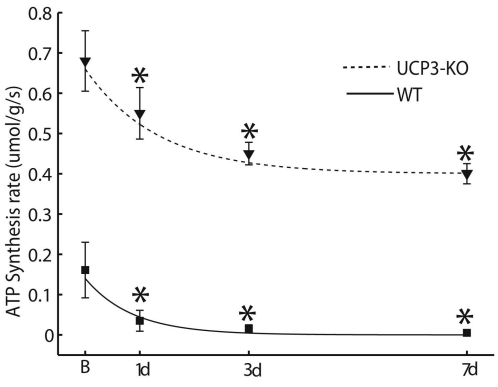
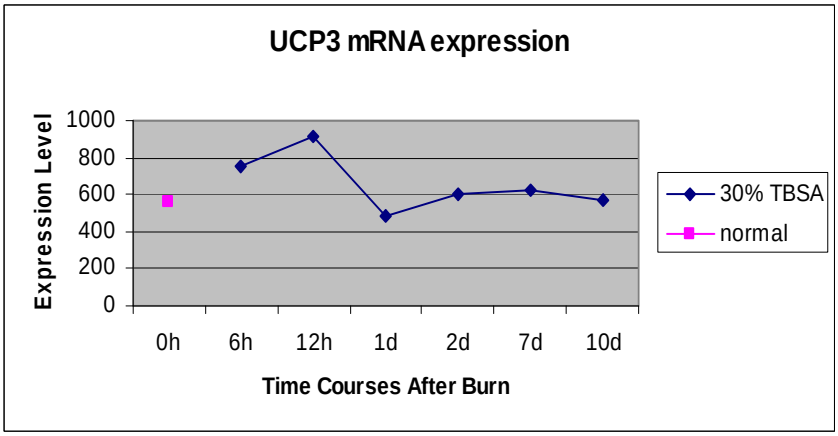


Figure 2. UCP3 mRNA expression levels in skeletal muscle following 30% TBSA burn injury. UCP3 gene expression increases by 12 hours (h) post-burn and returns to normal levels by 1 day (d) (n=6 animals in each group).



Mice were euthanized at 6 and 12 hours and also at 1, 2, 7 and 10 days post-burn; gastrocnemius muscle was isolated from burned and control (non-burned) animals to assess UCP3 protein levels by Western blot, as described previously [2]). Samples at 12, 24, and 48 hours post-burn have increased levels of UCP3 protein versus samples from control animals or from experimental animals at 72 hours and at 7 and 10 days post-burn, and UCP3 levels approach normal at 72 hours or 3 days post-burn. These results demonstrate thermal injury up-regulates the 34kDa UCP3 protein by 12 hours.

Discussion– Our NMR studies along with both mRNA and protein data presented here suggest uncoupling proteins contribute to the mitochondrial dysfunction that underlies the skeletal muscle wasting and general cachexia of burn pathology. Thus, mitochondrial dysfunction is underlined by mitochondrial uncoupling in burns. Uncoupling protein activity could be pharmaceutically targeted to prevent and/or treat skeletal muscle wasting and cachexia in burn patients. Moreover, knowledge obtained in this study should aid in improved treatment, decreased complications, and increased long-term survival of burn victims.

References

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