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 1 day (d) is probably due to these uncoupling proteins among other factors. Studies in UCP2- and UCP1-KOs should clarify this point.

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 pharmacologically 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