31P NMR demonstrates reduced ATP synthesis rate and concomitant downregulation of PGC-1β mitochondrial gene expression in skeletal muscle after burn injury

D. Mintzopoulos[1,2], M. N. Mindrinos[3], L. G. Rahme[4], R. G. Tompkins[5], and A. A. Tzika[1,6]

[1]NMR Surgical Laboratory, MGH & Shriners Hospitals, Harvard Medical School, Boston, MA, United States, [2]Radiology, Athinoula Martinos Center for Biomedical Imaging, Boston, MA, United States, [3]Biochemistry, Stanford University School of Medicine, Palo Alto, CA, United States, [4]Molecular Surgical Laboratory, MGH & Shriners Hospitals, Harvard Medical School, Boston, MA, United States, [5]Surgery, MGH & Shriners Hospitals, Harvard Medical School, Boston, MA, United States, [6]Radiology, Athinoula A. Martinos Center of Biomedical Imaging, Boston, MA, United States

Introduction– Burn trauma of 20%-30% or more of total body surface area (TBSA) results in severe systemic syndrome and muscle catabolism/atrophy [1]. Recently a novel mode of regulation for many complex biological programs was revealed, including metabolism by coactivator proteins, best illustrated by the peroxisome proliferator-activated receptor coactivator 1 (PPARγ coactivator-1 or PGC-1) family of coactivators [2,3]. PGC-1 coactivators play a critical role in glucose and lipid, and energy homeostasis and are likely involved in pathogenic conditions such as diabetes [4], and, presumably, burn injury. *In vivo* NMR spectroscopy allows measurements of physiological biomarkers in intact systems [5, 6] and has recently shown mitochondrial dysfunction in burns [7]. Here we present results showing (a) reduced rate of ATP synthesis using *in vivo* saturation-transfer ^{31}P NMR spectroscopy, and (b) downregulation of PGC1-β in a clinically relevant mouse burn model.

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 (Figure 1). PGC-1β expression was significantly downregulated at 6 h post-burn. PGC-1β downregulation was sustained at least until 7 days post-burn and it paralleled the reduction in the ATP synthesis rate (Figure 2). PGC-1β expression was collinear with ATP flux rate with correlation coefficient (Pearson's R) = 0.9523.

Figure 1. ATP synthesis flux (mmol/g/s) after 30% TBSA burn. Mean values ± SE in (black squares) and monoexponential least-squares fit (solid line) with characteristic rate decay rate 0.5541 [(mmol/g/s)/d]. ($\chi^2 = 1.917\times10^{-4}$). *$P$<0.001 for baseline B compared to 1 day; P<0.05 (B compared to 2 days); and P<0.05 for B compared to 3 days (Student's t-test).

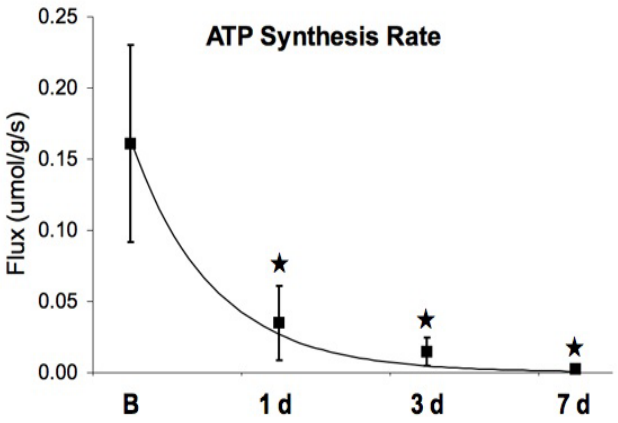
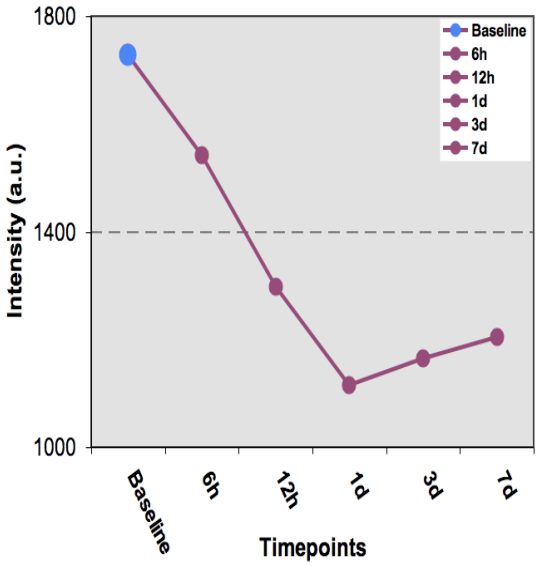


Figure 2. Downregulation of PGC-1β gene expression after 30% TBSA burn



Discussion– NMR-measured unidirectional ATP synthesis flux primarily reflecting rate through the F1F0-ATP synthase enzyme, with the coupled glyceraldehyde-3-phosphate dehydrogenase and phosphoglycerate kinase reactions being negligible. Protons extruded from the mitochondrial matrix during electron transport drive ATP synthesis during re-entry through the F1-ATPase. Our *in vivo* NMR results showed significantly reduced rate of ATP synthesis and were cross-validated by results from analysis of genomics in the same skeletal muscle tissue showing downregulation of the PGC-1β gene expression. As shown in figure 3, there is high correlation between ATP synthesis rate and PGC-1β gene expression. Our findings suggest that the known inflammation and muscle atrophy in burn injury are due to reduced ATP synthesis rate that may be regulated upstream by PGC-1β [8]. These findings implicate mitochondrial dysfunction in distal skeletal muscle following burn injury. PGC-1β is a highly inducible factor in most tissues and responds to common pathways of calcium and cyclic adenosine monophosphate (cAMP) signaling, which strongly suggests that it may be possible to develop drugs to induce PGC-1β.

References

1. Wallace, BH, Caldwell, FT, Cone, JB. J Burn Care Rehab, 15:499, 1994.
2. Spiegelman BM and Heinrich R: Biological control through regulated transcriptional coactivators. Cell 119: 157-167, 2004
3. Handschin C and Spiegelman BM: Peroxisome proliferator-activated receptor gamma coactivator 1 coactivators, energy homeostasis, and metabolism. Endocr Rev 27: 728-735, 2006.
4. Lin J, Handschin C, and Spiegelman BM: Metabolic control through the PGC-1 family of transcription coactivators. Cell Metab 1: 361-370, 2005
5. Ackerman, JJ, Grove, TH, Wong, GG, Gadian, DG, Radda GK. Nature, 283:167, 1980.
6. Hitzig, BM, Prichard, JW, Kantor, HL, Ellington, WR, Ingwall, JS, Burt, CT, Helman, SI, Koutcher, J. Faseb J, 1:22, 1987.
7. Padfield KE, Astrakas LG, Zhang Q, Gopalan S, Dai, G, Mindrinos MN, Tompkins RG, Rahme LR, Tzika AA. *Proc. Natl. Acad Sci. (USA),* 102:5368, 2005.
8. Tzika AA, Mintzopoulos D, Padfield K, Wilhelmy J, Mindrinos M, Yu H, Cao H, Zhang Q, Astrakas LG, Yu YM, Rahme LG, Tompkins RG, Reduced rate of ATP synthesis by in vivo ^{31}P nuclear magnetic resonance spectrosopy and downregulation of PGC-1b in distal skeletal muscle following burn, Int. J. Mol. Med. (in press)