Muscle Atrophy Observed in Burns due to Reduced Rate of ATP Synthesis by 31P NMR

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Introduction

When the surface area of the burn is greater than 20 to 30% total body surface area (TBSA), local inflammation generalizes into a severe systemic syndrome accompanied by muscle catabolism/atrophy (1). Although the clinical consequences for the patient with a large burn injury are grave, the mechanisms underpinning post-burn alterations and muscle wasting remain uncertain. *In vivo* NMR spectroscopy allows measurements of physiological biomarkers in intact systems (2, 3) and has recently shown mitochondrial dysfunction in burns (4). In addition, GeneChip microarrays have greatly advanced physiological studies, by providing a snapshot of the transcriptome in a specific organ. Combining NMR and microarray data provides the opportunity to perform "functional genomics". Here we evaluate the rate of ATP synthesis in a clinically relevant mouse burn model by employing *in vivo* ^{31}P NMR, and characterize the concomitant gene expression patterns in burn versus control skeletal muscle tissue.

Materials and Methods

The hind limb muscle of mice was studied with *in vivo* ^{31}P NMR spectroscopy three days after non-lethal burn trauma caused by an extensive burn of approximately 30% TBSA on the dorsum of each mouse. All ^{31}P NMR experiments were performed in a horizontal bore magnet (proton frequency at 400 MHz, 21cm diameter, Magnex Scientific) using a Bruker Advance console. Saturation 90^{o} 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 Pi resonance, symmetrically to the γ-ATP resonance. T1 relaxation times of Pi and PCr were measured using an inversion recovery pulse sequence in the presence of the γ-ATP saturation. An adiabatic pulse (400 scans, sweep with = 10 KHz, 4K data) was used to invert the Pi and the PCr, with an inversion time between 152 ms and 7651 ms. To examine changes in whole muscle gene expression, total RNA was extracted from muscle, harvested at various time points following burns, and hybridized onto MOE430A oligonucleotide arrays, which were subsequently stained, washed, and scanned. All procedures followed standard Affymetrix, Inc. protocols (Santa Clara, CA).

Results

NMR-measured unidirectional ATP synthesis flux primarily reflects flux through the F1F0-ATP synthase enzyme, with the coupled glyceraldehyde-3-phosphate dehydrogenase and phosphoglycerate kinase reactions being negligible. Our in vivo ^{31}P-NMR saturation-transfer spectra data (Figure 1) showed that burn trauma reduces ATP synthesis (Table 2), to suggest a significant reduction in the rate of mitochondrial phosphorylation. Our microarray data validated our NMR findings.

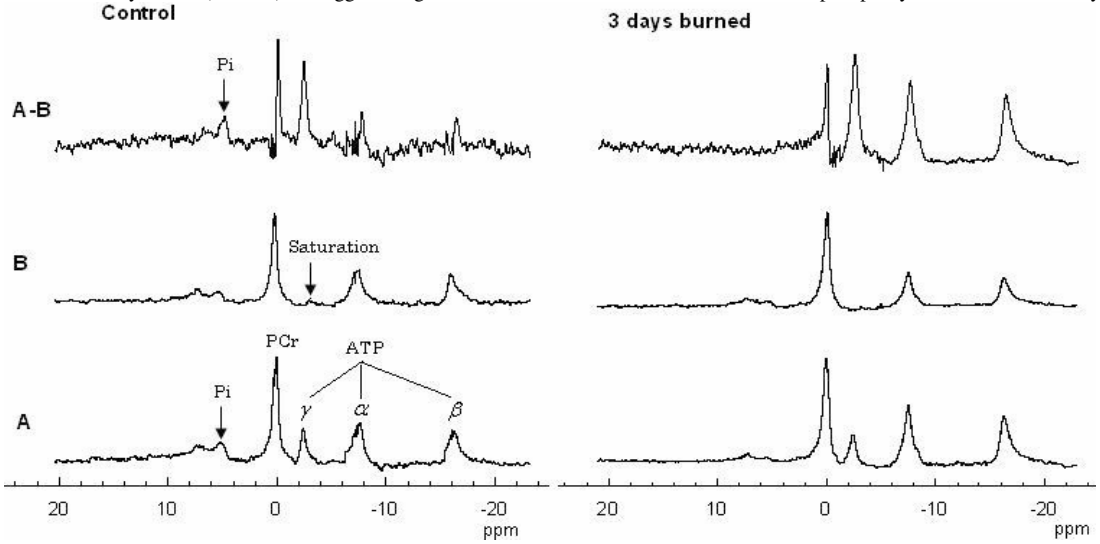

Figure 1. MR spectra of in vivo 31P-NMR saturation-transfer performed on the hind limb skeletal muscle of awake mice. The spectra are acquired from normal and 3 days after burn mice before (A) and after (B) saturation of the γATP resonance, with the difference spectra between the two (A-B). The arrow indicates the position of the saturation by RF irradiation. Pi, inorganic phosphate.

	Normal	Burn 1 day	Burn 3 days	Burn 7 days
$\Delta M/M_0$	0.318±0.025	0.179±0.052[a] (-43.7%)	0.114±0.030[a] (-64.2%)	0.058±0.004[a] (-81.8%)
T_1obs(s)	1.13±0.24	2.42±0.64	3.22±0.50	3.9
K(s^{-1})	0.281±0.022	0.074±0.022[a]	0.035±0.009[a]	0.015±0.001[a]
[Pi] (□mol/g)	0.574±0.250	0.443±0.191[b]	0.425±0.202[b]	0.193±0.014[c]
Flux (□mol/g/s)	0.161±0.069	0.035±0.026[c] (-78.3%)	0.015±0.010[a] (-90.7%)	0.003±0.0004[a] (-98.1%)

Table 1. ATP synthesis flux (reaction Pi → γATP). $\Delta M/M_0$, the fractional change in Pi magnetization as a result of saturation transfer; T1obs, observed spin lattice relaxation time of Pi during γATP saturation in seconds; K, rate constant. ATP synthesis is calculated as [Pi]×K. A Bioluminescence Assay Kit was used to assess ATP concentration.

[a] P<0.001 versus control values (Student's t test)

[b] P>0.05 versus control values (Student's t test)

[c] P<0.05 versus control values (Student's t test)

Discussion

Protons extruded from the mitochondrial matrix during electron transport drive ATP synthesis during re-entry through the F1-ATPase. Our NMR results, in conjunction with our genomic results showing down-regulation of mitochondrial oxidative phosphorylation, and suggest alterations in mitochondrial-directed energy expenditure reactions and indicate a mechanism by which burn results in skeletal muscle inflammation and subsequent atrophy. Future clinical treatments might be targeted to prevent this mechanism.

References

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