

Local Inflammatory Response due to Burn Coincides with Intramyocellular Lipid Accumulation

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Introduction

Burn injury is a clinical insult that produces both muscle wasting and altered lipid metabolism. Burns have both local and systemic effects. Skin and deeper structures are injured by direct thermal contact followed by an intense local inflammatory response. For the patient with a large burn injury, the clinical consequences of this process are prolonged ventilator dependence, loss of mobility, and need for extensive and expensive rehabilitation. Although the focus of intensive study, the molecular mechanisms underpinning post-burn hypermetabolism and muscle wasting remain uncertain. We reported that local burn trauma causes a drastic alteration in mitochondrial transduction of fuel into useful work (ATP) (1) and that these burn induced changes in the mitochondrion may be accompanied by increased accumulation of intramyocellular lipids (2), perhaps as a consequence of both increased delivery of non-esterified fatty acids (patients with severe burns experience increased peripheral lipolysis and decreased lipid flux through the mitochondria. Because uncoupling protein 3 (UCP3) has been implicated in free fatty acid handling by myoskeletal mitochondria and because burn injury results in decreased mitochondrial ATP synthesis (1), we hypothesized that a burn injury would evoke increased intramyocellular lipids and increased expression of myoskeletal UCP3.

Materials and Methods

Mice were studied at 6 hours, 1 day and 3 days after non-lethal burn injury caused by immersion of the left hind limb of anesthetized mice in 90°C water for 3 seconds. Muscle samples collected from burned and normal mice were measured using HRMAS ¹H NMR spectroscopy in a Bruker 14.1 Tesla spectrometer at 4 °C. The Carr-Purcell-Meiboom-Gill (CPMG) spin-echo pulse sequence with a total spin-spin relaxation delay of 10 ms was used to measure spin-echo HRMAS ¹H NMR spectra on all samples. Typically, 256 transients were collected into 32 K data points. Total RNA was extracted from muscle and hybridized onto MOE430A oligonucleotide arrays, which were subsequently stained, washed, and scanned. All procedures followed standard Affymetrix, Inc. protocols (Santa Clara, CA). Kolmogorov-Smirnov test Levene's test were used to assess normality of the variables and homogeneity of variances respectively. Differences in the 1.4 PPM peak to total creatine ratio (IMCLs) were evaluated by using the nonparametric Kruskal-Wallis test. Analysis of variance (ANOVA) was also performed by using parametric and nonparametric methods to ensure that differences or lack of differences between groups was consistent. Statistical analysis was conducted by using SPSS, version 12.0, (SPSS, Chicago, Ill). A two-tailed value of less than 0.05 was considered to indicate a statistically significant difference.

Results

1) Our HRMAS ¹H NMR spectra showed differences between burn and controls at 1.4 ppm (peak 2, fig.1), exhibiting IMCLs, which appear to increase in burns (fig. 1). Since the data for the IMCL variable were heavily skewed and failed to meet the assumption of homogeneity of variances a natural logarithmic transformation was used to normalize them when needed. Kruskal-Wallis test (df=3, P(T2) = 0.03, P(ratio) = 0.002) and ANOVA analysis (df=3, P(T2) = 0.21, P(ratio) <0.001) demonstrated that time is significant related both to IMCLs due to differences in their mean values in the four time groups (normal, 6 hours, 1 day and 3 days). The IMCLs exhibited a significant linear trend contrast with time (P<0.001) and was significantly different between control and burned animals (P<0.001).

2) Figure 2 shows that UCP3 expression increases by 6hrs post-burn and returns to normal levels by 24hrs. We subsequently asked if UCP3 protein expression also increases following burn in the mouse model. Two mice per group were euthanized at 6 hours, and at 1 and 3 days post-burn; and gastrocnemius muscle was isolated from burned and control (non-burned) animals to assess UCP3 protein levels by Western blot. Our results showed that UCP3 protein levels increase in skeletal muscle following burn. Samples at 6 hours and 1 day post-burn have increased levels of UCP3 protein versus samples from control animals or from experimental animals at 3 days post-burn, and UCP3 levels approach normal at 3 days post-burn.

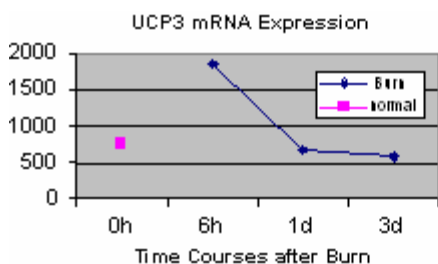


Fig. 2: UCP3 mRNA expression levels in skeletal muscle following hind limb thermal injury. UCP3 gene expression increases by 6hrs post-burn and returns to normal levels by 24hrs.

mitochondrial UCP3 protein by 6 hours. Both mRNA and protein data, along with the NMR studies here and suggest UCP3 protein promotes the mitochondrial dysfunction that underlies the skeletal muscle wasting and general cachexia of burn pathology. Also our results suggest that FFAs might signal altered UCP3 expression. Understanding the link between FFAs and UCP3 expression could provide new information about how processes related to energy metabolism are controlled in burn.

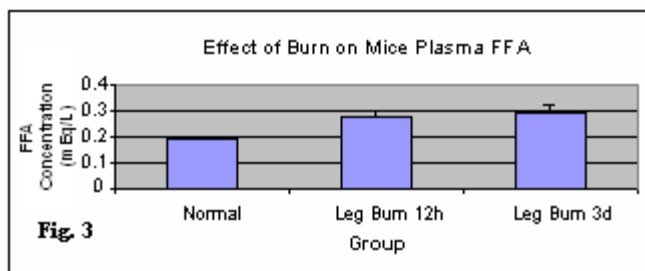
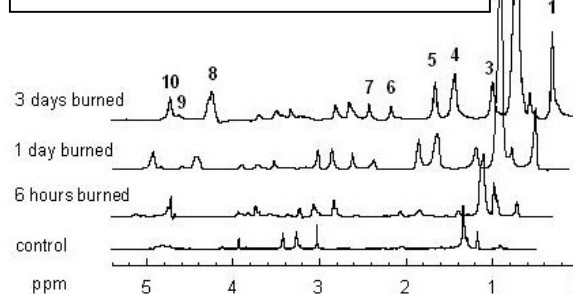


Fig. 3

Figure 1. Water Suppressed high resolution magic angle spinning (HRMAS) ¹H MRS of skeletal muscle tissue after burn trauma. Peaks: 1, CH₃-lipids; 2, (CH₂)_n lipids; 3, CH₂-CH₂-CO lipids; 4, CH=CH-CH₂-CH₂ lipids; 5, CH₂-CH₂-CO lipids; 6, =CH-CH₂-CH= lipids; 7, total creatine; 8, residual water; 9, =CH- lipids; 10, -CH= lipids. Lipids resonances (peaks 1-6, 9, 10) increase over time after burn.



3) We have performed studies to ask if burn injury increases the plasma free fatty acid (FFA) levels in the hind limb burn model. Since various physiological and pathological states associated with raised UCP3 mRNA levels in skeletal muscle are characterized by elevated plasma FFA levels, we investigated whether burn injury increases the FFA levels in our model. Figure 3 shows FFA levels increase by 12 hours and remain elevated through 3 days post-burn (P=0.014; Mann-Whitney U-test).

Discussion

These results demonstrate burn trauma up-regulates IMCLs and the 34kDa and suggest UCP3 protein promotes the mitochondrial dysfunction that underlies the skeletal muscle wasting and general cachexia of burn pathology. Also our results suggest that FFAs might signal altered UCP3 expression. Understanding the link between FFAs and UCP3 expression could provide new information about how processes related to energy metabolism are controlled in burn.

References

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