High Resolution Magic Angle Spinning MR Spectroscopy Reveals Biomarkers of Insulin Resistance in Burn Trauma

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Introduction

Insulin resistance is a major metabolic abnormality following burn trauma (1) and a common disorder frequently observed in different metabolic diseases, such as obesity, dyslipidemia, arterial hypertension, and type 2 diabetes. Muscle constitutes approximately 40% of the body mass, and insulin resistance results in muscle wasting due to the lack of anabolic effects. Indeed, insulin resistance as documented in skeletal muscle after burn trauma results in enhanced gluconeogenesis and protein catabolism (1, 2). The molecular mechanism of this abnormal insulin function has not been elucidated. Here we report that increased levels of NMR visible intramyocellular lipids (IMCLs) by High-Resolution Magic Angle Spinning (HRMAS) ^{1}H NMR spectroscopy of intact tissue samples of proximal skeletal muscle after burn injury in burns is a biomarker for insulin resistance.

Materials and Methods

Muscle samples collected from burned and normal mice were measured using HRMAS NMR spectroscopy in a Bruker 14.1 Tesla spectrometer at 4 ^{o}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 ^{1}H MAS 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 1.4 PPM to total creatine ratio (IMCLs) and T2 and values between time groups 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. Differences in 1.4 PPM to total creatine ratio (IMCLs) and T2 and values were illustrated by using box plots. 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

Our HRMAS ^{1}H NMR spectra (fig.1) showed differences between burn and controls at 1.4 ppm exhibiting IMCLs, which appear to accumulate in burns as shown by electron microscopy (fig. 2). The Kolmogorov-Smirnov test of normality indicated that only T2 values conformed to a normal distribution. Similarly Levene's test indicated that only T2 had the same variance in every time category. Since the data for ratio 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 T2 and ratio due to differences in their mean values in the four time groups. However only the 1.4 PPM to total creatine ratio (IMCLs) variable could: (a) exhibit a significant linear trend contrast with time (P<0.001) and (b) was significantly different between control and burned animals (P<0.001). Post hoc multiple comparisons between means of variables T2 and IMCLs for different time groups are shown in Table 1. It appears that only the IMCLs variable increase with time until the 1st day. Also our results showed that insulin related genes such as insulin-like growth factor 2, insulin-like growth factor binding protein 3, and insulin-like growth factor binding protein 4 differentially expressed in our burn mouse model. These results were consistent with insulin resistance and are consistent with previous reports in rodents (3).

Fig. 2: EM of burned muscle with IMCL

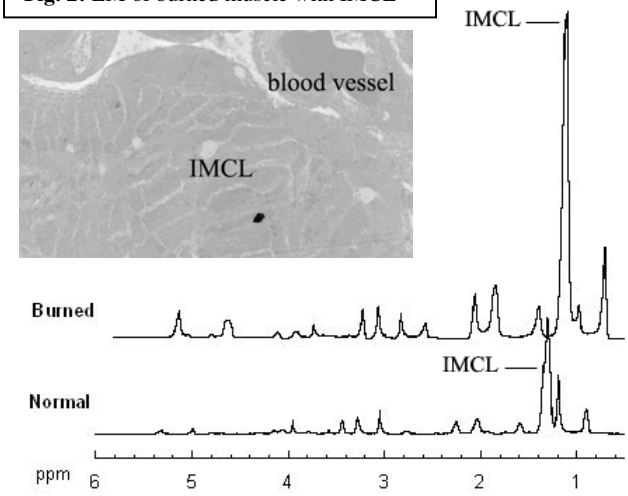

Fig. 1: HRMAS ^{1}H NMR of normal and burned skeletal muscle

Table1: Mean values, standard deviations and Post Hoc multiple comparisons between groups

	Control (n=6)	6hrs (n=6)	1day (n=3)	3days (n=3)	P01	P02	P03	P12	P13	P23
IMCL	1.8±0.1	2.4±0.3	8.3±1.1	9.1±0.8	0.007	0.024	0.007	0.024	0.007	NS
T2	87±11	104±7	86±3	96±13	0.025	NS	NS	NS	NS	NS

NS = Non significant; Control, 6hrs 1day and 3days are assigned the group numbers 0,1,2,3 respectively so that P13 indicates P value for comparison between 1 and 3 group i.e. between 6hrs and 3 days. P values shown have been obtained with Tukeys test for T2 and with the Games Howell test for IMCL variable.

Discussion

Increase in NMR visible IMCLs has been associated with insulin resistance. Here we report for the first time differences in the 1.4 PPM IMCLs of skeletal muscles from control and burned mice by HRMAS ^{1}H NMR spectroscopy. We suggest that increased levels of NMR visible IMCLs in burn trauma is a biomarker for insulin resistance. We also suggest that the IMCLs maintain their intracellular location during burn since their T2 values do not change significantly. This has led us to the concept that suppression of IMCLs may represent a candidate strategy for a partial relief of insulin resistance and muscle wasting (or cachexia) resulting from burn trauma.

References

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