Molecular characterization and quantification using state of the art solid-state adiabatic TOBSY NMR in burn trauma

Citation

Published Version
doi:10.3892/ijmm_00000288

Permanent link
http://nrs.harvard.edu/urn-3:HUL.InstRepos:11717627

Terms of Use
This article was downloaded from Harvard University’s DASH repository, and is made available under the terms and conditions applicable to Other Posted Material, as set forth at http://nrs.harvard.edu/urn-3:HUL.InstRepos:dash.current.terms-of-use#LAA

Share Your Story
The Harvard community has made this article openly available. Please share how this access benefits you. Submit a story.

Accessibility
Molecular characterization and quantification using state of the art solid-state adiabatic TOBSY NMR in burn trauma

VALERIA RIGHI1,2, OVIDIU ANDRONESI1,2, DIONYSSIOS MINTZOPoulos1,2 and A. ARIA TZIKA1,2

1NMR Surgical Laboratory, Department of Surgery, Massachusetts General Hospital and Shriners Burn Institute, Harvard Medical School, Boston; 2Athinoula A. Martinos Center of Biomedical Imaging, Department of Radiology, Massachusetts General Hospital, Boston, MA 02114, USA

Received March 26, 2009; Accepted May 11, 2009

DOI: 10.3892/ijmm_00000288

Abstract. We describe a novel solid-state nuclear magnetic resonance (NMR) method that maximizes the advantages of high-resolution magic-angle spinning (HRMAS), relative conventional liquid-state NMR approaches, when applied to intact biopsies of skeletal muscle specimens collected from burn trauma patients. This novel method, termed optimized adiabatic TOtal through Bond correlation SpectroscopY (TOBSY) solid-state NMR pulse sequence for two-dimensional (2D) 1H-1H homonuclear scalar-coupling longitudinal isotropic mixing, was demonstrated to provide a 40-60% improvement in signal-to-noise ratio (SNR) relative to its liquid-state analogue TOCSY (TOtal Correlation SpectroscopY). Using 1- and 2-dimensional HRMAS NMR experiments, we identified several metabolites in burned tissues. Quantification of metabolites in burned tissues showed increased levels of lipid compounds, intracellular metabolites (e.g., taurine and phosphocreatine) and substantially decreased water-soluble metabolites (e.g., glutathione, carnosine, glucose, glutamine/glutamate and alanine). These findings demonstrate that HRMAS NMR Spectroscopy using TOBSY is a feasible technique that reveals new insights into the pathophysiology of burn trauma. Moreover, this method has applications that facilitate the development of novel therapeutic strategies.

Introduction

Burn lesions are produced by a direct transfer of energy from any source of heat to body tissues. Severe thermal injuries are associated with marked metabolic alterations due to the liberation of inflammatory mediators and hormonal disturbances induced by stress. When a burn is sustained, there is a systemic release of adrenal stress hormones (catecholamines and glucocorticoids) to physiologically support the victim's ability to fight and escape the threat (1). There is an increase in energy expenditure in burn injury attributable to metabolic processes, such as gluconeogenesis, ureagenesis, fatty acid (FA) synthesis, FA breakdown, Cory cycles, and processes working to compensate for loss of body heat through injured skin (2). Burn trauma that affects skeletal muscle has both local and systematic effects. Functionally debilitating changes were documented at local and distant sites; these changes are especially pronounced when the burn size exceeds 30% of the total body surface area (TBSA) (3).

NMR spectroscopy-based metabolomics analysis detects a wide range of metabolites in biological samples, enabling precise molecular screening (4-6). It was used extensively in studies of schizophrenia (6,7), Alzheimer's disease (8), human brain tumors (9,10), and other human tumors (11,12). High Resolution Magic Angle Spinning (HRMAS) 1H magnetic resonance spectroscopy (1H-MRS) represents a promising, non-destructive tool for metabolic profiling of unprocessed tissue (13). NMR spectroscopy has been used to explore metabolic change after burn trauma in liver tissue extracts (14-16) and the HRMAS technique was recently used in combination with in vivo NMR to examine lipid accumulation following burn trauma (17).

Currently, HRMAS 1H-MRS of tissue biopsies employs conventional liquid-state pulse sequences. This approach assumes that MAS alone is sufficient to remove residual anisotropic interactions present in partially immobilized samples. This assumption holds true for simple one-dimensional (1D) 1H-MRS. However, in multidimensional experiments that rely on 1H-1H homonuclear scalar-coupling (J-coupling)
mediated magnetization transfer (i.e., TOtal Correlation Spectroscopy or TOCSY), residual anisotropic interactions are reintroduced unintentionally by pulse sequences. This degradation dramatically alters transfer efficiency, diminishing sensitivity, which is crucial in order for HRMAS 1H-MRS to become a routinely used diagnostic technique.

The diminished sensitivity problem is critical because multidimensional spectroscopy is necessary for unambiguous assignment and quantification of metabolites present in ‘crowded’ and overlapping 1D spectra. An optimized adiabatic TOtal through Bond correlation SpectroscopyY (TOBSY) solid-state NMR pulse sequence for two-dimensional (2D) 1H-1H homonuclear scalar-coupling mixing may yield a substantial signal-to-noise (SNR) gain relative to its liquid-state analogue TOCSY sequence (18). To this end, we developed and implemented the adiabatic 2D TOBSY solid-state NMR method in order to investigate burn metabolic injury. We compared 2D TOBSY to more conventional liquid-state NMR approaches and quantified the metabolites detected in burn trauma.

**Materials and methods**

**Burn trauma mouse model.** C57 mice were injured using an established burn trauma model (19,20). The experimental protocols were approved by the Massachusetts General Hospital Institutional Animal Research Review Board Committee. Mice were anesthetized by intraperitoneal injection of 40 mg/kg phenobarbital sodium. An area of the left leg corresponding to 5% of the total burn surface area (TBSA) was shaved and the burn injury was inflicted by immersing the left leg of mice in 90°C water for 4 sec. Three days after infliction of the burn, mice were sacrificed and the skeletal muscle tissue underlying the burn and contralateral muscle from the non-burned leg were harvested, immediately frozen in liquid nitrogen, and stored at -80°C.

**Ex vivo 1H HRMAS MRS.** All HRMAS 1H MRS experiments were performed on a wide-bore Bruker Bio-Spin Avance NMR spectrometer (600.13 MHz) using a 4-mm triple resonance (1H, 13C, 2H) HRMAS probe (Bruker). The tissue samples (15-25 mg) were placed into a zirconium oxide (ZrO2) rotor tube (4 mm diameter, 50 μl). A 10-μl aliquot of external standard trimethylsilyl- propionic-2,2,3,3-d4 acid (TSP), (Mw=172, δ=0.00 ppm, 50 mM in D2O) that functioned as a reference was reintroduced unintentionally by pulse sequences. This interference of very broad features in the spectrum baseline Decays (FIDs) because it acts as a T2 filter that reduces the sensitivity, which is crucial in order for HRMAS 1H-MRS to become a routinely used diagnostic technique.

**Figure 1. Pulse sequence for 2D [1H-1H] TOCSY (a) and TOBSY (b).** In the TOCSY sequence, the core of the experiment is a spin-lock time (MLEV-16); this mixing time must be held long enough to complete the transfer process to all of the spins. The TOBSY experiment employs an adiabatic C915 rotor-synchronized pulse sequence. The mixing time for TOBSY and TOCSY are similar, on the order of 45 min.

In all experiments, identical acquisition and processing parameters were used, 2k points along the direct dimension (13 ppm spectral width), 200 points along the indirect dimension (7.5 ppm spectral width), 8 scans, 2 dummy scans, 1-sec CW low-power on-resonance water pre-saturation, 2-sec total repetition time period, 45-min mixing time (to allow maximum buildup signal for most metabolites), 56-min total acquisition time; QSINE = 2 window function in both dimensions, FT with 2k points in the direct dimension and zero-filling to 1k in the second dimension, phase correction in both dimensions, and baseline correction in the second dimension. Spectra were acquired using XWINNMR 3.5 software (Bruker Biospin Corp, Billerica, MA).
Ex vivo 1H HRMAS MRS data processing. MR spectra of specimens were analyzed using MestReC software (Mestrelab Research). A line-broadening apodization function of 0.5 Hz was applied to CPMG HRMAS 1H FIDs prior to Fourier transformation (FT). MR spectra were referenced with respect to TSP at ±0.0 ppm (external standard), manually phased, and a Whittaker baseline estimator was applied to subtract the broad components of the baseline. The 1D slices of metabolites, extracted along the indirect dimension from 2D TOBSY and TOCSY, were scaled to a common noise level and the peaks were integrated using the XWINNMR software package (XWINNMR 3.5, Bruker Biospin Corp, Billerica, MA).

The 2D process parameters were, QSINE = 2 window function in both dimensions, FT with 2k points in the direct dimension and zero-filling to 1k in the second dimension, phase correction in both dimensions, and baseline correction in the second dimension. Spectra were processed using XWINNMR 3.5 software (Bruker). The 2D spectra were quantified using the Sparky program (T.D. Goddard and D.G. Kneller, SPARKY 3, (USCF, http://www.cgl.ucsf.edu/home/sparky)).

Quantification of metabolites. Metabolites were quantified using the ‘external standard’ technique in order to achieve more accurate values. We initially used 1D spectra for quantification. All peaks that appeared to be separate in the TOBSY spectrum were not well resolved using the CPMG sequence but were well resolved using the CPMG sequence. We used the ratio of the Cross Peak Volume of the Metabolites [CVP(M)] to the TSP Diagonal Peak Volume [DPV(TSP)], as described previously (27). This ratio was then corrected for T2 relaxation, using lic(100) = li(x) x exp[T2(x)/T2(100)]/n, where li(x) is the measured intensity, T2(x) is the CPMG echo time, and n is the number of protons in the functional group and corresponds to the resonance of the metabolite. In accordance with the ‘external standard’ technique employed (26), metabolite concentrations were quantified relative to the absolute concentration (μmol) of the respective metabolite [M] = lic(M)/(iC(TS)(M) x wt), where wt is the weight of the sample in grams.

Absolute quantification of metabolites from 1D CPMG spectra. Resonance intensities were measured for -CH 3 protons of the TSP and compared to the resonance intensities measured for metabolites. The peak intensities for most of the metabolites, as well as TSP, were calculated from the intensity of the respective resonance (X) measured from the T2-filtered HRMAS 1H MR spectrum. The calculated peak intensities were then corrected for T2 relaxation, using lic(X) = lic(x) x exp[T2CPMG/T2(X)]/n, where lic(X) is the measured intensity, T2CPMG is the CPMG echo time, and n is the number of protons in the functional group and corresponds to the resonance of the metabolite. In accordance with the ‘external standard’ technique employed (26), metabolite concentrations were quantified relative to the absolute concentration (μmol) of the respective metabolite [M] = lic(M)/(iC(TS)(M) x wt), where wt is the weight of the sample in grams.

Relative quantification of metabolites from 2D TOBSY spectra. To quantify more metabolites, we used the ratio of the Cross Peak Volume of the Metabolites [CVP(M)] to the TSP Diagonal Peak Volume [DPV(TSP)], as described previously (27). This ratio was divided by the source biopsy specimen’s weight (wt) to yield normalized metabolite intensities, lic(M) = (1/wt) x CVP(M)/DPV(TSP). Performance gains were calculated by dividing (C9 1 2 15) TOBSY SNR values by (MLEV-16) TOCSY SNR values (SNRCPM/SNRMLEV16) for each metabolite within each sample; the calculated values were then averaged over all samples within each group.

Statistics. The data are reported as means ± standard errors. Statistical analysis was done using the Student’s t-test. A p-value <0.05 was considered statistically significant in all cases.
As illustrated in Fig. 4, 1D slices were extracted along the indirect dimension of 2D TOBSY (red) and 2D TOCSY (black) experiments that analyzed control skeletal muscle specimens to confirm that the transfer efficiency predicted for the TOBSY sequence was met. 1D slices corresponding to both small metabolites (i.e., Lac, Tau, HTau) and large molecules [i.e., vinyl protons of FA chains (CH=CH) and intramyocellular lipids (CH2)n at 5.33 and 1.33 ppm, respectively] are shown in Fig. 4 (slices scaled to a common noise level and peaks integrated). C9 15 yielded higher signal intensities for low molecular weight metabolites (Fig. 4a), namely HTau and Tau (>50%), and Lac (>80%), as well as for high molecular weight metabolites (Fig. 4b), such as unsaturated acids (CH=CH) and intramyocellular lipids (CH2)n (>80%).

Averaging of the calculated 2D SNR ratio gains (SNR_C9/SNR_MLEV_16, respectively) for each metabolite over multiple samples revealed that the use of (C915) TOBSY afforded a substantial SNR benefit over the use of (MLEV-16) TOCSY. The SNR gain for large molecules, such as FA components, was ~60%, while that for low-molecular-weight, faster-tumbling metabolites (i.e., Tau, HTau and Lac) was slightly less, though still substantial, in the range of 40-50%. SNR gains for C9 15 relative to MLEV-16 in the 2D cross-peak volumes of selected...
metabolites in control and burned skeletal muscle samples are illustrated in Fig. 6.

**Discussion**

In the present study, we demonstrate the utility of a novel 2D HRMAS NMR TOBSY method for gaining sensitivity in the detection of both small metabolites and lipids in burn trauma tissue. The novel (C9 1\(\text{H}_{15}\)) TOBSY method decreased acquisition time and reduced metabolite concentration variability relative to (MLEV-16) TOCSY. Furthermore, it enabled us to detect new biomarkers of burn trauma with 2D TOBSY.

**Table I.** Chemical shift (\(\delta, \text{ppm}\)) and quantity of selected metabolites in burned versus control skeletal muscle specimens.

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>(\delta) (ppm)</th>
<th>Group</th>
<th>Control</th>
<th>Burn</th>
<th>(% \Delta) from control(^a)</th>
<th>(P^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipid components</td>
<td>(0.90)</td>
<td>(\text{CH}_{3})</td>
<td>(13.9\pm8.2^c)</td>
<td>(36.9\pm4.7^c)</td>
<td>-90.6%</td>
<td>0.06</td>
</tr>
<tr>
<td>Lipid</td>
<td>(1.29)</td>
<td>(\text{(CH}_{3}\text{)})</td>
<td>(42.6\pm18.3^c)</td>
<td>(155.7\pm43.9^c)</td>
<td>-114.1%</td>
<td>0.05</td>
</tr>
<tr>
<td>Lipid</td>
<td>(1.58)</td>
<td>(\text{CH}_{2}\text{CO})</td>
<td>(3.4\pm2.1^c)</td>
<td>(7.5\pm1.4^c)</td>
<td>-75.2%</td>
<td>0.12</td>
</tr>
<tr>
<td>Lipid</td>
<td>(2.03)</td>
<td>(\text{CH}_{2}\text{C}=-\text{C})</td>
<td>(17.2\pm11.5^c)</td>
<td>(155.4\pm28.6^c)</td>
<td>-160.3%</td>
<td>0.03</td>
</tr>
<tr>
<td>Lipid</td>
<td>(2.24)</td>
<td>(\text{CH}_{3}\text{-CO})</td>
<td>(38.2\pm2.8^c)</td>
<td>(77.2\pm18.2^c)</td>
<td>-67.6%</td>
<td>0.01</td>
</tr>
<tr>
<td>Lipid</td>
<td>(2.78)</td>
<td>(=\text{CCH}_{2}\text{C}=)</td>
<td>(3.0\pm1.9^c)</td>
<td>(27.1\pm14.1^c)</td>
<td>-160.1%</td>
<td>0.01</td>
</tr>
<tr>
<td>Lipid</td>
<td>(5.33)</td>
<td>(\text{CH}=\text{CH})</td>
<td>(4.1\pm3.8^c)</td>
<td>(11.7\pm1.9^c)</td>
<td>-96.2%</td>
<td>0.01</td>
</tr>
<tr>
<td>OH-Butyrate</td>
<td>(1.18)</td>
<td>(\text{CH}_{3})</td>
<td>(6.7\pm1.3^c)</td>
<td>(4.7\pm0.7^c)</td>
<td>+35.9%</td>
<td>0.05</td>
</tr>
<tr>
<td>Lactate</td>
<td>(1.33)</td>
<td>(\text{CH}_{3})</td>
<td>(32.2\pm13.4^c)</td>
<td>(59.5\pm4.5^c)</td>
<td>-60.0%</td>
<td>0.13</td>
</tr>
<tr>
<td>Alanine</td>
<td>(1.48)</td>
<td>(\text{CH}_{3})</td>
<td>(1.5\pm0.5^c)</td>
<td>(0.6\pm0.3^c)</td>
<td>+85.7%</td>
<td>0.05</td>
</tr>
<tr>
<td>Lysine</td>
<td>(1.69)</td>
<td>(\text{CH})</td>
<td>(2.8\pm0.4^c)</td>
<td>(1.02\pm0.4^c)</td>
<td>+93.19</td>
<td>0.05</td>
</tr>
<tr>
<td>Glutamate</td>
<td>(3.01)</td>
<td>(\text{CH}<em>{2}\text{CH}</em>{2})</td>
<td>(0.6\pm0.1^c)</td>
<td>(\text{nd})</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Glutamine</td>
<td>(2.09)</td>
<td>(\text{CH}<em>{3}\text{CH}</em>{2})</td>
<td>(1.1\pm0.3^c)</td>
<td>&lt;0.14</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Glutathione</td>
<td>(3.05)</td>
<td>(\text{CH}<em>{2}\text{CH}</em>{2}\text{CH}_{2}\text{Cys})</td>
<td>(0.8\pm0.1^c)</td>
<td>(\text{nd})</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Hypotaurine</td>
<td>(2.67)</td>
<td>(\text{NCH}_{2})</td>
<td>(3.8\pm0.6^c)</td>
<td>(1.7\pm0.5^c)</td>
<td>+76.4%</td>
<td>0.12</td>
</tr>
<tr>
<td>P-Creatine</td>
<td>(3.02)</td>
<td>(\text{SCH}_{2})</td>
<td>(5.3\pm2.2^c)</td>
<td>(6.8\pm2.5^c)</td>
<td>-24.8%</td>
<td>0.09</td>
</tr>
<tr>
<td>Creatine</td>
<td>(3.04)</td>
<td>(\text{CH}_{3})</td>
<td>(5.4\pm1.0^c)</td>
<td>(10.4\pm7.5^c)</td>
<td>-63.3%</td>
<td>0.01</td>
</tr>
<tr>
<td>Carnosine</td>
<td>(3.10)</td>
<td>(\text{CH}_{2}\text{-ring})</td>
<td>(2.0\pm0.4^c)</td>
<td>(1.2\pm0.6^c)</td>
<td>+50.0%</td>
<td>0.02</td>
</tr>
<tr>
<td>Carnosine</td>
<td>(3.23)</td>
<td>(\text{CH}_{2}\text{-CO})</td>
<td>(14.1\pm2.2^c)</td>
<td>(36.9\pm9.4^c)</td>
<td>-89.41%</td>
<td>0.15</td>
</tr>
<tr>
<td>Carnosine</td>
<td>(3.42)</td>
<td>(\text{NCH}_{3})</td>
<td>(3.5\pm1.6^c)</td>
<td>(\text{nd})</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Glycine</td>
<td>(3.55)</td>
<td>(\text{CH}_{3})</td>
<td>(4.0\pm1.0^c)</td>
<td>(\text{nd})</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Myo-inositol</td>
<td>(3.54)</td>
<td>(2\text{-CH})</td>
<td>(1.5\pm0.7^c)</td>
<td>(\text{nd})</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Proline</td>
<td>(4.17)</td>
<td>(\text{CH})</td>
<td>(1.7\pm0.8^c)</td>
<td>(\text{nd})</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Proline</td>
<td>(3.43)</td>
<td>(\text{CH})</td>
<td>(3.3\pm1.3^c)</td>
<td>(\text{nd})</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>β-Glucose</td>
<td>(4.67)</td>
<td>(1\text{-CH})</td>
<td>(3.2\pm0.8^c)</td>
<td>(\text{nd})</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>α-Glucose</td>
<td>(5.22)</td>
<td>(1\text{-CH})</td>
<td>(3.4\pm0.8^c)</td>
<td>(\text{nd})</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Glycerol</td>
<td>(4.10)</td>
<td>(1\text{-CH}_{2})</td>
<td>(0.4\pm0.7^c)</td>
<td>(1.4\pm0.6^c)</td>
<td>-111.1%</td>
<td>0.11</td>
</tr>
</tbody>
</table>

\(^a\%\text{ Difference, tumor vs. control; }^b\text{Student's t-test; }^c\text{concentration in }\mu\text{mol/g from 1D CPMG; }^d\text{normalized ratio calculated from 2D TOBSY peak volumes (arbitrary unit); }^\text{nd, non-detectable or traces.}
Performance of the TOBSY method. Use of the rotor-synchronized WURST-8 adiabatic pulse (C9\textsuperscript{15}) yielded operationally important improvements in SNR and resolution of tissue spectra relative to the isotropic mixing pulse (MLEV-16). Indeed, direct comparison between TOBSY(C9\textsuperscript{15}) and TOCSY(MLEV-16) spectra (Fig. 3) demonstrated enhanced sensitivity of the TOBSY NMR method to detect, identify, and quantify metabolites. The SNRs obtained with this new method were significantly better than those obtained using the conventional TOCSY sequence for both low- and high-molecular weight compounds in both control and burned skeletal muscle samples (Fig. 6). The improved metabolic profile of burned skeletal muscle achieved with 2D TOBSY indicates that this method is well suited to complement 1D CPMG in qualitative and quantitative analysis of metabolite concentrations in burned tissues as it will enhance evaluation of burn-associated metabolic dysfunction.

Molecular changes associated with burn injury. The presently observed increase in mobile lipid molecules detected in the tissues 3 days after burn injury could be associated with cellular process such as inflammation, apoptosis and necrosis in agreement with a previous study (17). The elevated levels of IMCLs, in particular, in the burn tissue serve as a substrate for oxidative metabolism. This increase may be due to down-regulation of lipid oxidizing enzymes (29). Triglycerides (TGA),

Figure 4. 1D slices of selected metabolites were extracted along the indirect dimension from 2D experiment overlays of C9\textsuperscript{15} (red) and MLEV-16 (black). (a) HTau, hypotaurine; Tau, taurine; Lac, lactate. (b) Unsaturated acids (CH=CH) and acyl chain methylene (CH\textsubscript{2}n).

Figure 5. 1D slices of acyl chain methylene (CH\textsubscript{2}n) and the relative spin system row for TOBSY (upper row) and TOCSY (lower panel).
oxidant injury. Recent studies report that Tau has protective effects on mitochondria and their enzyme activities in myocardium in rats that were subjected to a severe burn. These protective effects may be attributable to Tau's ability to improve oxygen free radical eradication and alleviate Ca\(^{2+}\) overload in the mitochondria (35).

GSH, a cysteine-containing tripeptide synthesized from glutamate, cysteine and glycine, is a metabolite involved in oxidative stress. The significantly reduced GSH observed in the burned tissues relative to control tissues is likely attributable to general oxidative stress or oxidative damage. GSH is a major component of the cellular antioxidant system and plays an important role in the antioxidation of ROS and free radicals. Moreover, vulnerability to free radical damage was reported following GSH depletion in a number of cell systems (32).

The levels of HTau and Tau are probably determined by the redox balance; HTau is a precursor of Tau, the main product of Cys metabolism in mammals, and is thought to share the same physiological function (36). We speculate that HTau levels are reduced in burned tissues in favor of the oxidative stress. The significantly reduced GSH observed in a number of cell systems (32). Moreover, vulnerability to free radical damage was reported following GSH depletion in a number of cell systems (32).

The levels of HTau and Tau are probably determined by the redox balance; HTau is a precursor of Tau, the main product of Cys metabolism in mammals, and is thought to share the same physiological function (36). We speculate that HTau levels are reduced in burned tissues in favor of the oxidative stress. The significantly reduced GSH observed in a number of cell systems (32). Moreover, vulnerability to free radical damage was reported following GSH depletion in a number of cell systems (32).

The observation of increased PCr (Table I) in burn tissue provides support for the notion that PCr is increased in response to burn injury (40). Both the PCr and creatine increases that we observed are probably due to an elevated activity of creatine phosphokinase enzyme (CPK) which mediates the conversion of ATP to PCr and PCr's breakdown to creatine and phosphorous. Indeed, CPK is elevated in burn victims, probably due to keratinocyte necrosis and leakage of CPK into circulation (40).

**Apoptosis.** More research is required to resolve the relative importance and timing of apoptosis in muscle atrophy following a burn injury. Thermal injury was found to induce apoptosis in the skeletal muscle of rats as early as one day after burn injury (41). Correspondingly, Argiles and colleagues recently suggested that activation of apoptosis signaling is essential to and precedes protein degradation in skeletal muscle wasting during catabolic conditions (42). Skeletal muscle atrophy following burn injury was mostly due to a protein degradation mechanism principally involving the ubiquitin-proteosome pathway (43). It was also postulated that mitochondrial dysfunction and deregulation of apoptotic signaling plays a critical role in the development of sarcopenia of aging (44,45). The present observation of increased ceramide, a key apoptotic second messenger, in burn tissue presumably reflects burn-induced apoptosis. The presence of increased ceramide levels leads to the activation of stress-activated protein kinase, leading ultimately to activation of the pro-apoptotic factors caspase-1,-3, and -9 (41). Thus, growing evidence suggests that up-regulated expression and proteolysis is the result, rather than the cause, of burn-associated apoptosis.
In conclusion, we demonstrated that the presently introduced solid-state HRMAS TOBSY NMR method is a sensitive tool in the molecular characterization of metabolic perturbations in skeletal muscle after burn trauma. Increased FA levels detected in burn tissue reflects activation of inflammatory and apoptotic mechanisms that are directly relevant to mitochondrial dysfunction. Burn injury produced changes in metabolite levels that are attributed to oxidative stress. These findings provide insight into the pathophysiology of burn trauma studies and such findings can be used to direct research into novel therapeutic strategies.

Acknowledgements

This work was supported in part by the National Institutes of Health (NIH) Center Grant (P50GM021700) to Ronald G. Tompkins (A. Aria Tzika, Director of the NMR core) and a Shriners’ Hospital for Children research grant (#8893) to A. Aria Tzika. We also thank Dr Ann Power Smith of Write Science Right for editorial assistance.

References


