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

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<tbody>
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<td>Published Version</td>
<td>doi:10.3892/ijmm_00000288</td>
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Molecular characterization and quantification using state of the art solid-state adiabatic TOBSY NMR in burn trauma

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Received March 26, 2009; Accepted May 11, 2009

DOI: 10.3892/ijmm_0000288

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 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 (‘H-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 ‘H-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) ‘H-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 BioSpin 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=0.0 ppm, 50 mM in D2O) that functioned as a reference for both resonance chemical shift and quantification was introduced into the rotor tube. The samples were secured and tightened in the rotors with a top insert, screw, and cap (Bruker). The HRMAS 1H MRS was performed at -8°C at a MAS speed of 3 kHz to minimize tissue degradation.

The 1D water-suppression spin-echo Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence [90°-(t-180°)-t]m acquisition] was employed (21). Incorporation of the CPMG sequence is preferred over the use of simple Free Induction Decays (FIDs) because it acts as a T2 filter that reduces the interference of very broad features in the spectrum baseline originating from tissue water and macromolecules. The CPMG sequence protocol included an inter-pulse delay (τ=2τ/τn) of 333 μsec, 256 transients, a spectral width of 7.2 kHz, 8k data points, and a 3 sec repetition time (TR). For quantification purposes, we measured the T2 relaxation time by varying the CPMG evolution time (TCPMG = 2τn) [n=7-800 (~5-530 msec)]. We refined the C915 (TOBSY) symmetry-based 13C MAS solid-state NMR pulse sequence for 2D HRMAS 1H-MRS use, and compared the magnetization transfer efficiency and SNR to MLEV-16 (TOCSY) in burn mice specimens. C915 cancels the 1st order average Hamiltonian and minimizes the higher orders’ contributions from chemical shielding anisotropy (CSA), dipolar coupling (D) and offset terms, retaining only the isotropic J-coupling. In both cases, WURST-8 adiabatic inversion pulses were employed for their efficient use of radio-frequency (r.f.) power in the compensation of pulse offsets, in-homogeneity, and miscalibration with reduced r.f. heating. The C915 is a rotor-synchronized sequence with a fixed WURST-8 pulse length to rotor period ratio of 15/18, according to WURST-inverse-WURST (WiW) scheme (22). The 2D TOBSY pulse sequence using C915 is shown in Fig. 1 (a cw water suppression block was omitted) (18). For the 2D [1H,1H] TOCSY experiment, the C915 mixing block in Fig. 1 was replaced by the MLEV-16 scheme. Each 180° pulse of the MLEV-16 (23) was produced from a rotor synchronized WURST-8 adiabatic pulse, as described previously (24).

TOBSY and TOCSY sequences were acquired for the 2D [1H,1H] experiment. 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-s 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 along the direct dimension (7.5 ppm spectral width), 8 scans, 2 dummy scans, a 3 sec repetition time (TR). For quantification

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.
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 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, but were not well resolved using the CPMG sequence, were then averaged over all samples within each group. The data are reported as means ± standard errors. 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 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%). An example of differential performance of the two 2D methods is presented in Fig. 5; note that the full FA (CH2)n spin system (six bonds), including the vinyl proton at 5.33 ppm (CH=CH), is apparent in the TOBSY spectrum but not the TOCSY spectrum.

Averaging of the calculated 2D SNR ratio gains (SNR_C9/SNR_MLEV) for each metabolite over multiple samples revealed that the use of (C9) 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 relative to MLEV-16 in the 2D cross-peak volumes of selected metabolites are shown in Fig. 6. We detected an altered concentration of several water-soluble metabolites in burned samples (b) relative to controls (a).
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 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 biomarkers with 2D TOBSY.

<table>
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<tr>
<th>Metabolite</th>
<th>δH (ppm)</th>
<th>Group</th>
<th>Control</th>
<th>Burn</th>
<th>% Δ from control a</th>
<th>P b</th>
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<td>Lipid components</td>
<td>0.90</td>
<td>CH₃</td>
<td>13.9±8.2</td>
<td>36.9±4.7</td>
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<td>0.06</td>
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<td></td>
<td>1.29</td>
<td>(CH₃)₃</td>
<td>42.6±18.3</td>
<td>155.7±43.9</td>
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<td></td>
<td>1.58</td>
<td>CH₃CO</td>
<td>3.4±2.1</td>
<td>7.5±1.4</td>
<td>-75.2%</td>
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<td></td>
<td>2.03</td>
<td>CH₂C=CH</td>
<td>17.2±11.5</td>
<td>155.4±28.6</td>
<td>-160.3%</td>
<td>0.03</td>
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<tr>
<td></td>
<td>2.24</td>
<td>CH₂=CO</td>
<td>38.2±2.8</td>
<td>77.2±18.2</td>
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<tr>
<td></td>
<td>2.78</td>
<td>CH₃CH=</td>
<td>3.0±1.9</td>
<td>27.1±14.1</td>
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<tr>
<td></td>
<td>5.33</td>
<td>CH=CH</td>
<td>4.1±3.8</td>
<td>11.7±1.9</td>
<td>-96.2%</td>
<td>0.01</td>
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<tr>
<td>OH-Butyrate</td>
<td>1.18</td>
<td>CH₂</td>
<td>6.7±1.3</td>
<td>4.7±0.7</td>
<td>+35.9%</td>
<td>0.13</td>
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<td>Lactate</td>
<td>1.33</td>
<td>CH₃</td>
<td>32.2±13.4</td>
<td>59.5±4.5</td>
<td>-60.0%</td>
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<tr>
<td>Alanine</td>
<td>1.48</td>
<td>CH₂</td>
<td>1.5±0.5</td>
<td>0.6±0.3</td>
<td>+85.7%</td>
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<tr>
<td>Lysine</td>
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<td>δCH₂</td>
<td>2.8±0.4</td>
<td>1.02±0.4</td>
<td>+93.19</td>
<td>0.05</td>
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<td></td>
<td>3.01</td>
<td>CH₃CH</td>
<td>0.6±0.1</td>
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<tr>
<td>Glutamate</td>
<td>2.09</td>
<td>δCH₂</td>
<td>1.1±0.3</td>
<td>&lt;0.14</td>
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<tr>
<td>Glutathione</td>
<td>2.55</td>
<td>γCH₂-Glu</td>
<td>0.8±0.1</td>
<td>nd</td>
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<td>Hypotaurine</td>
<td>2.67</td>
<td>NCH₂</td>
<td>3.8±0.6</td>
<td>1.7±0.5</td>
<td>+76.4%</td>
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<td>P-Creatine</td>
<td>3.02</td>
<td>SCH₂</td>
<td>5.3±2.2</td>
<td>6.8±2.5</td>
<td>-24.8%</td>
<td>0.09</td>
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<td>Creatine</td>
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<td>CH₂</td>
<td>1.5±0.7</td>
<td>10.4±7.5</td>
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<td>Carnosine</td>
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<td>CH₂-ring</td>
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<td>+50.0%</td>
<td>0.02</td>
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<td>Taurine</td>
<td>3.26</td>
<td>S-CH₂</td>
<td>14.1±2.2</td>
<td>36.9±9.4</td>
<td>-89.41%</td>
<td>0.15</td>
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<tr>
<td>Glycine</td>
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<td>CH₂</td>
<td>nd</td>
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<tr>
<td>Myo-inositol</td>
<td>4.08</td>
<td>2-CH</td>
<td>1.5±0.7</td>
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<td>-</td>
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<tr>
<td>Proline</td>
<td>4.17</td>
<td>CH</td>
<td>1.7±0.8</td>
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<td>-</td>
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<tr>
<td>β-Glucose</td>
<td>4.67</td>
<td>1-CH</td>
<td>nd</td>
<td>nd</td>
<td>-</td>
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<tr>
<td>α-Glucose</td>
<td>5.22</td>
<td>1-CH</td>
<td>nd</td>
<td>nd</td>
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<tr>
<td>Glycerol</td>
<td>4.10</td>
<td>1-CH₂</td>
<td>0.4±0.07</td>
<td>1.4±0.6</td>
<td>-111.1%</td>
<td>0.11</td>
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<td></td>
<td>4.30</td>
<td>3-CH₂</td>
<td>nd</td>
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<tr>
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<td>5.24</td>
<td>2-CH</td>
<td>nd</td>
<td>nd</td>
<td>-</td>
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a% Difference, tumor vs. control; bStudent’s t-test; cconcentration in μmol/g from 1D CPMG; dnormalized ratio calculated from 2D TOBSY peak volumes (arbitrary unit); nd, non-detectable or traces.
Performance of the TOBSY method. Use of the rotor-
synchronized WURST-8 adiabatic pulse (C9115) yielded
operationally important improvements in SNR and resolution
of tissue spectra relative to the isotropic mixing pulse
(MLEV-16). Indeed, direct comparison between TOBSY(C9115)
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
centrations 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 C9115 (red) and MLEV-16 (black). (a) HTau, hypotaurine; Tau, taurine; Lac, lactate. (b) Unsaturated acids (CH=CH) and acyl chain methylene (CH2)n.

Figure 5. 1D slices of acyl chain methylene (CH2)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 myo-
cardium 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
degree of 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
production of Tau. Cnr is an endogenously synthesized
dipeptide present in large amounts in skeletal muscle (37). It
scavenges ROS as well as α-β unsaturated aldehydes formed
from peroxidation of cell membrane fatty acids during oxidative
stress (15). Gln is an important mediator in numerous metabolic
pathways and it acts as a regulator of some physiological
processes including glycogen synthesis, gluconeogenesis and
lyposis. Gln plays a critical role as a signaling molecule in
amino acid and glucose-stimulated insulin secretion. Dipeptide
Ala-Gln promotes the turnover of glucose metabolism, signaling
peripheral tissue to increase glucose utilization (38,39).

Our 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 TOBSoB 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


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