Assessment of Mitochondrial Energy Coupling *In Vivo* Using a Novel $^{13}C/^{31}P$ NMR Approach

B.M. Jucker, S. Dufour, J. Ren, X. Cao, S.F. Previs, B. Underhill, K. Cadman and G.I. Shulman
Yale University School of Medicine, New Haven, CT, USA

Introduction:

Mitochondrial uncoupling proteins (UCP) play an integral role in regulating cellular energy consumption via non-shivering thermogenesis (1). This is accomplished by diminishing the proton motive force across the inner mitochondrial membrane which results in uncoupling of respiration from ATP synthesis. Unlike UCP1 which is expressed exclusively in brown adipose tissue (BAT), the recently discovered homolog UCP3 (2) is expressed primarily in muscle and is encoded in a chromosomal region linked to hyperinsulinemia and obesity (3). Because quiescent skeletal muscle utilizes approximately 33% of whole body oxygen consumption, much attention has been given to the control and function of UCP3 as a means of regulating energy expenditure and body weight. However, investigation into the regulation of uncoupling protein has been hampered by the inability to assess its activity *in vivo*. Here we present a novel method to assess mitochondrial energy coupling in skeletal muscle, noninvasively, by combining ^{13}C NMR spectroscopy to measure rates of mitochondrial substrate oxidation along with ^{31}P NMR spectroscopy to assess rates of ATP synthesis in; (i) awake control rats, (ii) chronic triiodothyronine (T_3) treated rats (a model of increased UCP3 expression) and (iii) acute 2,4 dinitrophenol (mitochondrial uncoupler) treated rats.

Methods:

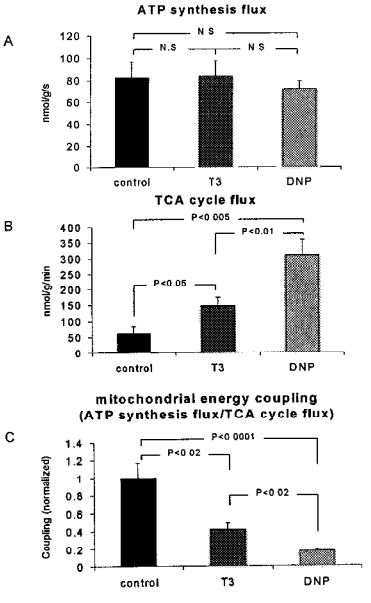
All *in vivo* NMR experiments were performed on a Bruker Biospec 7.0T system (horizontal/22 cm diameter bore magnet). Both ^{13}C observe/^{1}H decouple and ^{31}P observe NMR spectroscopy of the hindlimb skeletal muscles were performed in awake rats.

Non-localized ^{1}H decoupled -^{13}C NMR spectroscopy with lipid suppression was used to detect 2-^{13}C acetate label turnover in the 4-^{13}C (34.4ppm) and 2-^{13}C (55.5ppm) glutamate pools. Broadband ^{1}H Waltz-16 decoupling was applied during acquisition, and additional NOE was achieved using low power decoupling (0.4 W) during the relaxation delay (TR=0.5 s, NS=1800, SW=20 KHz, 4K data). A 15 min baseline spectrum was acquired followed by subsequent 15 min acquisitions throughout the duration of the experiment. Mathematical modeling of the TCA cycle flux was based on a nonlinear least squares fit of the calculated parameters derived from a set of isotopic mass balance equations which describe the label flow in associated pathways to the acquired NMR data (4).

A ^{31}P saturation transfer experiment was used to determine the unidirectional flux of $P_i \rightarrow ATP$. The decrease in magnetization of P_i (ΔM) may be used to determine the kinetics of F1F0 ATP_{ase} activity once the glycolytic contribution of coupled GAPDH and PGK are accounted for. A continuous wave (CW) radio frequency pulse was used to saturate the γ-ATP resonance. Spectra were acquired using a non-selective 90° pulse (TR=4.4 s, NS=128, SW=20 KHz, 4K data). An adiabatic half passage (180°) pulse was used to invert all P_i spins in the inhomogeneous volume of the surface coil (TR=6.0 s, NS=64, SW=20 KHz, 4K data) in the inversion recovery experiment. The 6 variable delay lengths used in the inversion recovery experiment ranged from 10 ms-6 s.

Results and Summary:

The rates of ATP production were not altered by T_3 or DNP treatment as reflected by similar ATP synthesis flux in all three groups (Fig. 1a). However there was a substantial increase in the rate of substrate oxidation required to generate the same amount of ATP (Fig. 1b). The ratio of the measured unidirectional ATP synthesis flux to TCA cycle flux may be used as a qualitative index of the degree of coupling between mitochondrial substrate oxidation and ATP synthesis. Since we do not know the extent of basal mitochondrial uncoupling present as a result of combined proton transport and leaks across the inner mitochondrial membrane this ratio was normalized to the control group (Fig. 1c). When analyzed in this manner it is evident that both T_3 and DNP treatment resulted in substantial decreases in mitochondrial energy coupling activity compared to the control group. The ~60% decrease in mitochondrial energy coupling observed in the T_3 treated group correlated well with the 2-3 fold increase in UCP3 protein content in this group and suggests that increased expression of UCP3 may be a mechanism by which thyroid hormone promotes increased energy utilization and thermogenesis *in vivo*.



This *in vivo* NMR approach should be a useful method for exploring the regulation of uncoupling protein activity in humans and elucidating its role in energy metabolism and obesity.

References:

1) Himms-Hagen, J. *Prog Lipid Res* 28, 67-115, 1989.
2) Boss O. et al. *FEBS Letters*. 408, 39-42, 1997.
3) Gong, D-W. et al. *J. Biol. Chem.* 272, 24129-24132, 1997.
4) Jucker, B.M. et al. Abstract #392, 6th ISMRM meeting 1998.