Profiling muscle substrate utilization in insulin-resistant subjects using ^{13}C-MRS at 7 Tesla

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Introduction

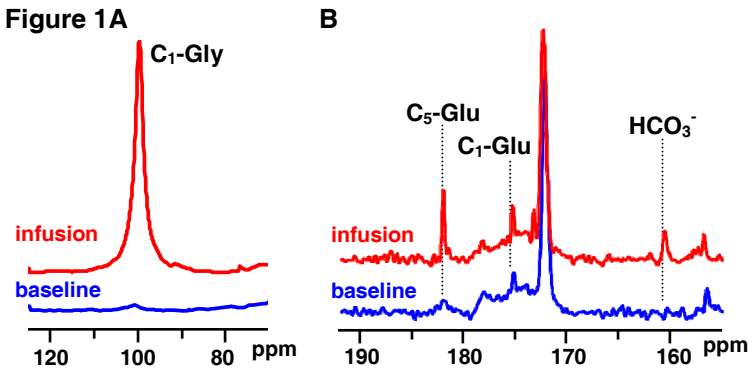
Muscle insulin resistance has been associated with derangements in a variety of parameters of muscle metabolism including decreased inulin-stimulated glucose disposal, impaired glycogen synthesis [1] and reductions in basal rates of mitochondrial metabolism [2]. To date, technological and methodological limitations have meant that each of these parameters have been assessed as distinct studies, which give rise to attendant confounding issues such as disparities in subject population, the muscle group studied, insulin dose or other experimental conditions. By taking advantage of a novel ^{13}C-labelling strategy [3] and the enhanced sensitivity for ^{13}C-MRS available at 7T [4], we have developed an innovative dual ^{13}C-tracer method that has enabled us to comprehensively profile muscle substrate utilization under insulin-stimulated conditions *in vivo* and investigate the effects of insulin resistance.

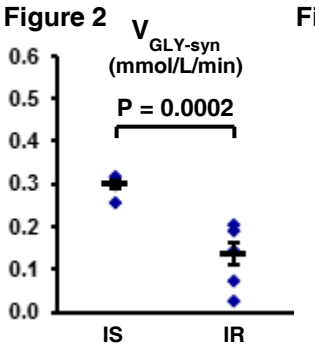
Methods

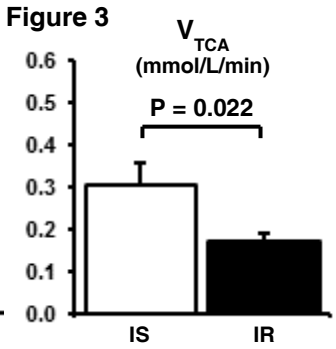
^{13}C-MRS studies were performed at 7T on a Varian DirectDrive system using a custom-built probe consisting of a 5cm diameter ^{13}C surface coil, with a pair of elliptical 9.5 x 7.5cm ^{1}H coils arrayed in quadrature for scout-imaging, shimming and decoupling. Thirteen healthy volunteers underwent a 120-150 min hyperinsulinemic-euglycemic clamp using a variable infusion of [1-^{13}C]-glucose to maintain plasma glucose at ~5mM. Gastrocnemius muscle C_1-glycogen content was assessed at baseline and at regular intervals throughout the clamp using an adiabatic ^{13}C pulse-acquire sequence with WALTZ decoupling and 3-dimensional outer-volume suppression for localization. Sixty minutes into the clamp an infusion of [1-^{13}C]-acetate was initiated and ^{13}C-enrichment of gastrocnemius muscle C_5- and C_1-glutamate was detected by localized ^{13}C-MRS, as above, with nuclear Overhauser enhancement to increase sensitivity. Rates of insulin-stimulated muscle glycogen synthesis were estimated from the increment in the $^{13}C_1$ signal over the final 60-90 min of the clamp. Insulin stimulated muscle TCA cycle flux was determined by metabolic modelling analysis of the kinetics of C_5- and C_1-glutamate turnover [3].

Results

Muscle ^{13}C-spectra demonstrating the incorporation of infused [1-^{13}C]-glucose into C_1-glycogen, and [1-^{13}C]-acetate into C_5- and C_1-glutamate are shown in Figure 1A and 1B. Subjects were categorized as insulin-sensitive (IS, n = 6) or insulin-resistant (IR, n = 7) based on their rate of insulin-stimulated muscle glycogen synthesis ($V_{GLY\text{-}syn}$); individuals with $V_{GLY\text{-}syn}$ < 0.2 mmol/L/min were considered insulin-resistant (Figure 2). Insulin-stimulated rates of muscle TCA cycle flux were significantly lower in insulin-resistant subjects (Figure 3).







Conclusion

We have developed an innovative dual ^{13}C-tracer method to assess muscle glucose utilization and oxidative metabolism during a single ^{13}C-MRS study at 7 Tesla. Using this method we present the first estimates of insulin-stimulated muscle TCA cycle flux in humans, and show that lower rates of insulin-stimulated oxidative metabolism are associated with muscle insulin-resistance. The ability to comprehensively examine muscle substrate utilization *in vivo* will advance our understanding of the pathogenesis of metabolic diseases of skeletal muscle.

References:

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Supported by NIH grants:
R01 DK-085638, R01-NS-087568,
P30 DK-45735, UL1 RR-024139,
a Distinguished Clinical Scientist Award from the ADA (KFP),
and the Howard Hughes Medical Institute.