Assessment of Diabetic Skeletal Muscle Metabolism Using Hyperpolarized 13C MR Spectroscopy

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Target Audience Researchers and clinicians interested in type 2 diabetes.

Purpose Studies often ascribe alterations in the non-oxidative metabolic pathways as the primary mechanism behind the disrupted glucose homeostasis in type 2 diabetes mellitus (T2DM), in part because ^{13}C MR spectroscopy (MRS) experiments have observed most of the reduced glucose utilization in T2DM corresponds with a lower glycogen synthesis rate[1]. Any impairment in oxidative metabolism appears to contribute negligibly. Nevertheless, some researchers have hypothesized that a defective or insufficient mitochondrial function can still play a potentially role in T2DM pathogenesis. The relative contribution of the mitochondrial dysfunction in T2DM, however, remains unclear[2]. Recently a new rat model (UCDT2DM)[3], which develops diabetes with age and obesity, has presented a unique opportunity to investigate the altered biochemical mechanism in T2DM. We have performed *in vivo* experiments using hyperpolarized [1-^{13}C]lactate (Lac), [2-^{13}C]pyruvate (Pyr), and dichloroacetate (DCA)[4] to examine in control (CRL) and T2DM skeletal muscle the pyruvate dehydrogenase (PDH) and tricarboxylic acid (TCA) cycle, which reflects oxidative metabolism activity. The results indicate that control vs. T2DM muscle reacts differently with the Lac, Pyr, and DCA, consistent with an altered oxidative metabolism in the pathogenesis of T2DM

Methods Sprague-Dawley (SD) rats with UCDT2DM (459-640g, n=8) and age-/weight-matched CRL SD rats (517-681g, n=9) were anesthetized and scanned using a 3T GE clinical MR scanner and a ^{13}C surface coil (Ø = 28mm, placed on top of right rectus femoris). Immediately after an injection of 40-mM hyperpolarized [1-^{13}C]Lac bolus, ^{13}C MR signal was acquired from CRL (n=6) and UCDT2DM rats (n=5), and 3 of the UCDT2DM rats were additionally scanned following another 40-mM Lac injection 1h after a DCA infusion (200mg/kg). A separate group of animals were scanned after injecting 80-mM hyperpolarized [2-^{13}C]Pyr (n=3 for UCDT2DM and n=3 for CRL) For all scans, dynamic free induction decay (FID) sequence (10° hard pulse, temporal resolution=3s, spectral width/points=10kHz/4096, T_{acq}=4 min) was used to acquire time-resolved ^{13}C MR spectroscopic data. Metabolite ratios relative to total carbon (tC) and apparent conversion rate constants using a modified multi-site exchange model[5] were used as metrics to analyze [1-^{13}C]Lac data. For [2-^{13}C]Pyr analysis, metabolite ratios as compared to the integrated mitochondrial metabolites were compared between CRL and UCDT2DM rats.

Results and Discussion Alanine (Ala, 0.15±0.02 for T2DM and 0.15±0.01 for CRL) and Pyr (0.027±0.004 for T2DM and 0.032±0.002 for CRL) were detected at similar levels from both groups when [1-^{13}C]Lac was injected. Bicarbonate (Bic), which reflects the PDH activity, was significantly lower (P<0.02) in T2DM (0.002±0.002) than in CRL (0.013±0.004). However, DCA increases Bic to 0.092±0.017, indicating that PDH in T2DM muscle can be activated. On the other hand, post-DCA Ala (0.076±0.007) and Lac (0.012±0.005) decreased in T2DM rats. When [2-^{13}C]Pyr was injected, neither CRL nor T2DM muscle showed reliable mitochondrial metabolite peaks. However, with the addition of DCA, [1-^{13}C]acetyl-carnitine (ALC), [1-^{13}C]acetoacetate (ACC), [5-^{13}C]glutamate (Glu) appear in CRL and T2DM muscle. Surprisingly, PDH was more activated by DCA infusion in diabetic models than in CRL (Fig.2). While Glu/tC was comparable between T2DM (0.014±0.004) and CRL (0.013±0.002), ALC/tC (0.096±0.023) and ACC/tC (0.051±0.005) in T2DM muscle were about twice higher than in CRL (ALC/tC: 0.056±0.01, ACC/tC: 0.022±0.005). The results indicate that PDH activity differs in T2DM vs. CRL skeletal muscles, consistent with a difference in oxidative metabolism.

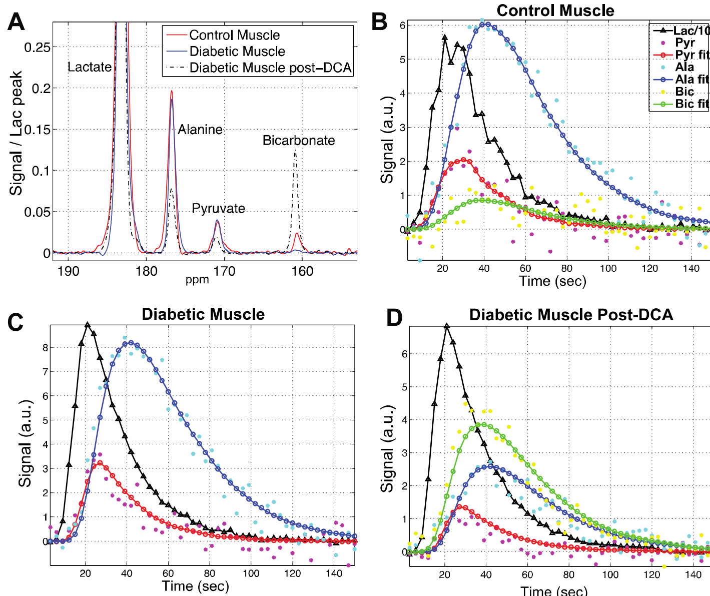

Fig.1 (A) Time-averaged spectra and time-curves of metabolites after an injection of **40-mM [1-^{13}C]Lac** acquired from (B) a healthy control and (C) a diabetic rat muscle. (D) is acquired 1hr after a dichloroacetate injection from the same diabetic rat muscle. Dots and lines indicate measured and fitted time-curves, respectively.

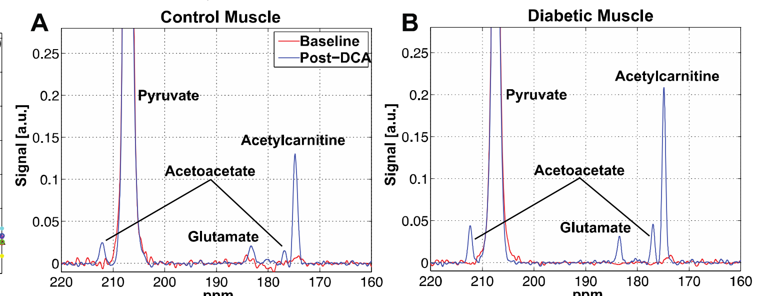

Fig.2 Time-averaged spectra after an injection of **80-mM [2-^{13}C]Pyr** acquired from (A) a healthy control and (B) a diabetic rat muscle.

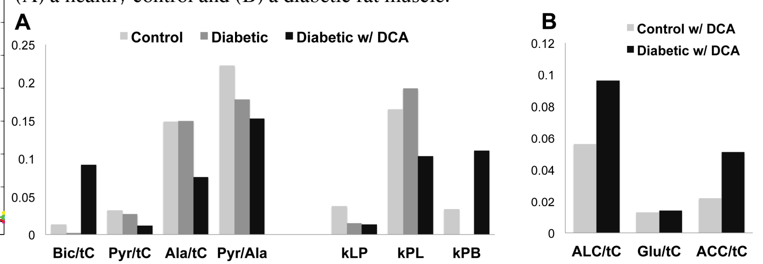

Fig.3 (A) Metabolite ratios and apparent conversion rate constants [Hz] when [1-^{13}C]Lac was injected before and after DCA; kLP: Lac-to-Pyr, kPA: Pyr-to-Lac, kPB: Pyr-to-Bic apparent conversion rate constants. (B) Metabolite ratios when [2-^{13}C]Pyr was injected after DCA.

Conclusion The metabolism of hyperpolarized [1-^{13}C]Lac in the muscle was different in UCDT2DM as compared to CRL rats, especially with respect to PDH activity. The contrasting change in PDH activity with DCA suggests a contribution of oxidative metabolism impairment in diabetes and a potential role for PDH activation to restore glucose homeostasis.

References **1.** Shulman GI et al, *NEJM*. 1990; 322(4):223-8, **2.** Patti ME, *Endocr Rev.* 2010; 31(3):364-95, **3.** Cummings BP et al, *Am J Physiol*. 2008; 295(6):1782-93, **4.** Backshear PJ et al, *Biochem J.* 1975; 146(2):447-56, **5.** Park JM et al, *ISMRM*, 2013; 655

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