Direct multi-tissue assessment of *in vivo* postprandial lipid handling in ZDF rats using localized ^{1}H-[^{13}C] MRS

R. Jonkers[1], L. van Loon[2], K. Nicolay[1], and J. Prompers[1]
[1]Biomedical NMR, Department of Biomedical Engineering, Eindhoven University of Technology, Eindhoven, Netherlands, [2]Department of Human Movement Sciences, Maastricht University Medical Centre+, Maastricht, Netherlands

Objective - Intracellular lipid (ICL) accumulation in liver (IHCL) and muscle (IMCL) is strongly related to the development of insulin resistance and type 2 diabetes (T2D) [1-2]. It is unknown, however, whether excessive lipid accumulation is a consequence of an increased lipid uptake, a decreased lipid mobilization, or a more structural imbalance between uptake and mobilization. Our lab has shown that localized ^{1}H-[^{13}C] MRS in combination with the administration of ^{13}C-labeled lipids allows for direct *in vivo* assessment of changes in ^{13}C-enriched lipid content in rat liver [3]. The aim of the current study was to apply this ^{1}H-[^{13}C] MRS method in a rat model of T2D to investigate *in vivo* lipid handling in insulin-resistant liver and muscle at different stages in the pathogenesis of T2D.

Methods – Four groups of n=6 male Zucker diabetic fatty (ZDF) rats were used for this study: one group of obese, pre-diabetic fa/fa rats at the age of 6 weeks; one group of lean, non-diabetic fa/+ littermates at the age of 6 weeks; one group of obese, diabetic fa/fa rats at the age of 12 weeks; and one group of lean, non-diabetic fa/+ littermates at the age of 12 weeks. MRS measurements were performed at baseline and 4 h, 24 h, and 48 h after oral administration of 1.5 g [U-^{13}C] Algal lipid mixture per kg body weight (Cambridge Isotope Laboratories, Andover, MA, USA). Experiments were performed on a 6.3 T horizontal Bruker MR system using a circular single-turn ^{1}H-surface coil (20 mm) in combination with a single-turn ^{13}C-butterfly coil (40/100 mm). Localized ^{1}H-[^{13}C] MRS was performed using the LASER-POCE sequence (TR = 2 s, TE = 27 ms, TE_{POCE} = 7.9 ms, SWAMP water suppression, ^{13}C WALTZ decoupling, 16 averages, 64 sequential experiments, 34 min) with a 32 µL voxel for the liver and a 27 µL (fa/fa) or 43 µL (fa/+) voxel for the *tibialis anterior* (TA) muscle [3]. Spectra were analyzed using the nonlinear least squares algorithm (AMARES) in the jMRUI software package. Total ICL content and ^{13}C-labeled ICL content were determined from the ICL-CH_2 signal at 1.3 ppm and are expressed as a percentage of the unsuppressed water signal. The absolute ^{13}C enrichment was calculated by subtracting the natural abundance ^{13}C-labeled ICL content determined at baseline from the ^{13}C-labeled ICL content at 4 h, 24 h and 48 h post ^{13}C-labeled lipid administration. Statistical analysis was performed using ANOVA for repeated measures (SPSS).

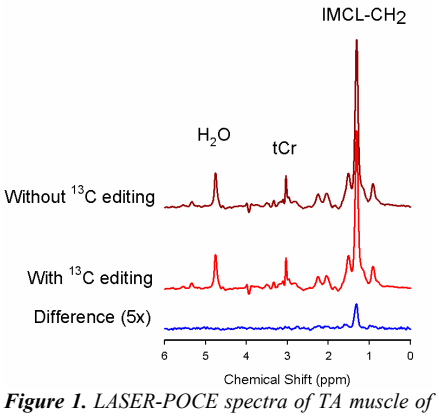

Figure 1. *LASER-POCE spectra of TA muscle of a fa/fa rat at 12 weeks of age, 4 h after the administration of ^{13}C-labeled lipids*

Results – Figure 1 displays LASER-POCE spectra of TA muscle of a fa/fa rat at 12 weeks of age, 4 h after the administration of ^{13}C-labeled lipids. At baseline, total ICL content was higher in fa/fa rats compared with fa/+ rats in both liver and muscle, and at both ages (Fig. 2). Total ICL content did not change after the administration of ^{13}C-labeled lipids (data not shown). Figure 3 shows the absolute ^{13}C enrichments of the IHCL (panels A, B) and IMCL (panels C, D) pools at 4 h, 24 h, and 48 h after the administration of ^{13}C-labeled lipids in 6 (panels A, C) and 12 (panels B, D) weeks old fa/+ and fa/fa rats.

Liver: Four h after the administration of ^{13}C-labeled lipids, ^{13}C enrichment of IHCL was higher in fa/fa rats than in fa/+ rats both at 6 and 12 weeks of age, indicating an increased postprandial lipid uptake in the liver of pre-diabetic and diabetic rats compared with control rats. At 24 and 48 h post, ^{13}C enrichment of IHCL decreased compared with the 4 h time point both in fa/fa and in fa/+ rats and at both ages, suggesting that the ^{13}C-labeled IHCL was exported or oxidized.

Muscle: Four h after the administration of ^{13}C-labeled lipids, ^{13}C enrichment of IMCL was lower in fa/fa rats than in fa/+ rats at 6 weeks of age, indicating a decreased lipid uptake in muscle of pre-diabetic rats compared with control rats. In contrast, at 12 weeks of age, ^{13}C enrichment of IMCL at 4 h post was higher in fa/fa rats than in fa/+ rats, showing that lipid uptake in muscle of diabetic rats was increased compared with control rats. In fa/+ rats, ^{13}C enrichment of IMCL at 24 h post was lower (6 weeks) or tended to be lower (12 weeks) than at 4 h post, indicating that the ^{13}C-labeled IMCL was utilized. In fa/fa rats at 6 and 12 weeks of age, however, ^{13}C enrichment of IMCL did not change significantly between 4 h and 24 h post, suggesting that net IMCL utilization was impaired compared with controls.

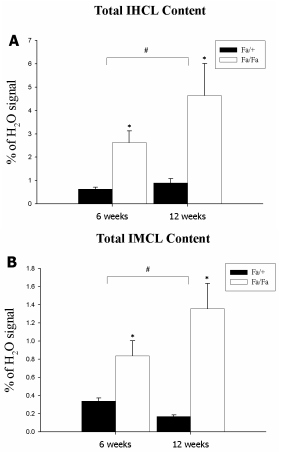

Figure 2. *Total intracellular lipid content in liver (IHCL, A) and muscle (IMCL, B) at baseline. Data are expressed as mean percentages of the unsuppressed H_2O signal ± SD. * Significantly different from fa/+ ($p < 0.05$); # significantly different from 6 weeks ($p < 0.05$).*

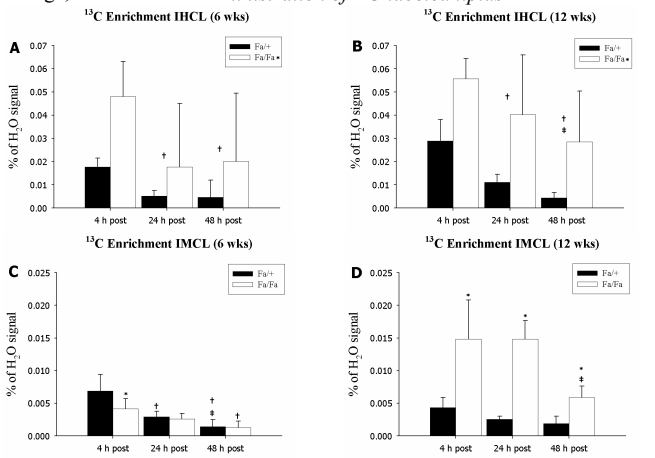

Figure 3. *^{13}C Enrichment of the intracellular lipid pool in liver (IHCL; A and B) and muscle (IMCL; C and D) at the age of 6 weeks (A and C) and 12 weeks (B and D). Data are corrected for baseline natural abundance ^{13}C enrichment and are expressed as mean percentages of the unsuppressed H_2O signal ± SD. * Significantly different from fa/+ ($p < 0.05$); † significantly different from 4 h post ($p < 0.05$); ‡ significantly different from 24 h post ($p < 0.05$).*

Discussion and Conclusion – Ectopic lipid accumulation is a hallmark of insulin resistance and type 2 diabetes, but the causes for this derangement remain unknown. In this study, we determined *in vivo* lipid handling in insulin-resistant rat liver and muscle tissue at different stages in the pathogenesis of type 2 diabetes. Both in pre-diabetic and in diabetic rats, hepatic lipid uptake was increased compared with non-diabetic control rats, whereas the utilization of lipids in the liver did not seem to be largely affected. Likewise, in muscle of diabetic rats, lipid uptake was higher than in muscle of control rats. In contrast, lipid uptake in muscle of younger, pre-diabetic rats was lower than in controls. Whereas in liver of pre-diabetic and diabetic rats, lipid utilization seemed to be normal, in muscle of both pre-diabetic and diabetic rats net lipid utilization appeared to be impaired.

In conclusion, the *novel* application of ^{1}H-[^{13}C] MRS in combination with ^{13}C-labeled lipid administration allows for multi-tissue assessment of *in vivo* lipid handling and will be instrumental to our understanding of disturbed lipid handling in insulin resistant tissues.

References – [1] Kelley DE, et al., AJP Endo Metab 285: E906 – E916, 2003; [2] Krssak M, et al., Diabetolgia, 42:113-116, 1999; [3] Jonkers RAM, et al., Proc ISMRM-ESMRMB, 753, 2010.