# Exercise does not modulate postprandial lipid uptake in liver and skeletal muscle of healthy and diabetic rats

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***Target audience:*** This work is relevant to the field of skeletal muscle and liver lipid metabolism in general and in type 2 diabetes in particular.

***Purpose:*** Insulin resistance and type 2 diabetes have been associated with ectopic lipid accumulation [1]. Physical activity improves insulin sensitivity [2], but the impact of exercise on lipid handling in insulin-resistant skeletal muscle and, in particular, liver remains to be elucidated. In the present study, we applied $^{1}$H MRS to characterize to what extent intracellular lipids in liver and skeletal muscle are being used as a substrate source during exercise in a healthy and type 2 diabetic condition. For this purpose, we included lean, healthy *fa/+* Zucker diabetic fatty (ZDF) rats and obese, diabetic *fa/fa* ZDF rats and quantified total intramyocellular lipid (IMCL) and intrahepatocellular lipid (IHCL) content before and directly after a single bout of moderate intensity exercise. Furthermore, we applied $^{13}$C-edited $^{1}$H MRS ($^{1}$H-[$^{13}$C] MRS) in combination with the oral administration of $^{13}$C-labeled lipids to investigate to what extent postprandial lipid handling is modulated by prior exercise in healthy and diabetic rats.

***Methods:*** After baseline MRS measurements, 46 rats were randomized to an exercise group (EXE; n=15 *fa/+* and n=15 *fa/fa* rats) or a no-exercise group (NO_EXE; n=8 *fa/+* ($LEAN_{NO\_EXE}$) and n=8 *fa/fa* ($DIABETIC_{NO\_EXE}$) rats). Rats in the NO_EXE groups were placed in a treadmill for one hour, with the speed set to 0 m/min and the shocker to 2.9 mA, while rats in the EXE groups ran for 10 min at 10 m/min, followed by 40 min at 12.5 m/min and finally 10 min at 10 m/min (total of 1 h), with the shocker set to 2.9 mA. Directly after the treadmill visit, n=7 *fa/+* ($LEAN_{EXE\_NO\_SUB}$) and n=7 *fa/fa* ($DIABETIC_{EXE\_NO\_SUB}$) rats of the EXE groups were subjected to MRS experiments without the administration of $^{13}$C-labeled lipids (NO_SUB) to analyze the direct effect of a single bout of exercise on total IHCL and IMCL content, which was repeated after 24 h. For the assessment of liver and skeletal muscle postprandial lipid handling after exercise, rats in the NO_EXE groups and the remaining n=8 *fa/+* ($LEAN_{EXE}$) and n=8 *fa/fa* ($DIABETIC_{EXE}$) rats in the EXE groups were orally administered 1.5 g [U-$^{13}$C] Algal lipid mixture per kg body weight directly after the treadmill visit. Next, MRS was performed at 4 and 24 h after treadmill visit.

All MRS experiments were executed on a 6.3 T horizontal Bruker MR system with a $^{1}$H surface coil (20 mm) combined with a $^{13}$C butterfly coil (40/100 mm). In each rat, localized $^{1}$H-[$^{13}$C] MRS was performed first on a 4x2x4 mm$^3$ voxel placed in the median lobe of the liver and, after repositioning, on a 3.5x3.5x3.5 mm$^3$ (in LEAN rats) or a 3x3x3 mm$^3$ (in DIABETIC rats) voxel in the *tibialis anterior* (TA) muscle using the LASER-POCE method as described previously [3]. The $^{13}$C-editing pulse (AFP, pulse length=2 ms, bandwidth=5 kHz) was centered on the lipid methylene resonance and the POCE echo time was tuned to $1/^{1}J_{1H\text{-}13C}$ for lipid methylene protons (7.9 ms). Other LASER-POCE parameters were as follows: TR=2 s, TE=26.8 ms, SWAMP water suppression, $^{13}$C WALTZ decoupling, 16 averages, 64 sequential experiments, scan time = 34 min. Spectra obtained with and without the POCE $^{13}$C-editing pulse were subtracted to give a difference $^{1}$H spectrum. Spectra were analyzed using AMARES in jMRUI. The $CH_2$ signal from IMCL/IHCL was used to calculate the total IMCL/IHCL content (from the spectra without $^{13}$C editing) and the $^{13}$C-labeled IMCL/IHCL content (from the difference spectra), which were both expressed as a percentage of the unsuppressed water signal. The average relative $^{13}$C enrichment determined at baseline was used to correct the $^{13}$C-labeled IMCL/IHCL levels at 4 and 24 h for natural abundance of $^{13}$C. All data are expressed as means ± SEM. Statistical analysis was performed using paired ANOVA with repeated measures (SPSS).

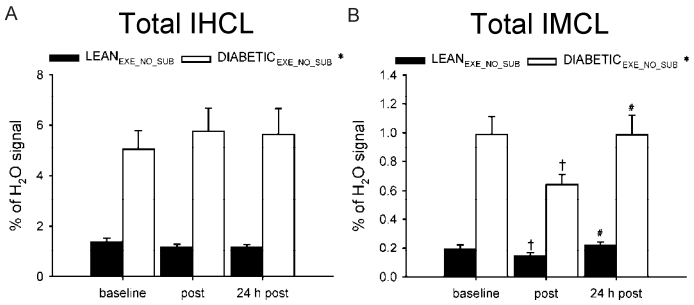


**Figure 1.** Total ($^{12}$C+$^{13}$C) intracellular lipid content in liver (IHCL; panel A) and *tibialis anterior* muscle (IMCL; panel B) of healthy ZDF *fa/+* rats ($LEAN_{EXE\_NO\_SUB}$) and diabetic ZDF *fa/fa* rats ($DIABETIC_{EXE\_NO\_SUB}$) measured at baseline, directly post exercise and at 24 h post exercise (n=7 per group). Data are expressed as a mean percentage of the unsuppressed water signal ± SEM. * Significantly different from LEAN ($P$<0.05); † significantly different from baseline ($P$<0.05); # significantly different from post exercise ($P$<0.05).

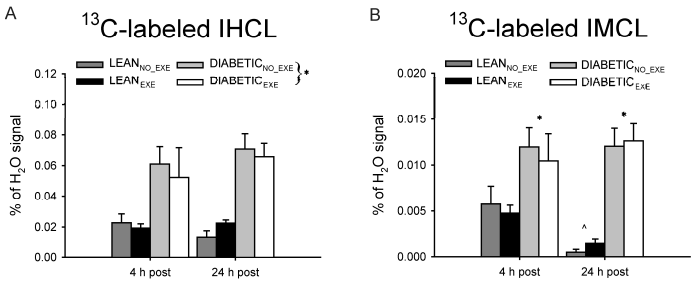


**Figure 2.** $^{13}$C-enriched intracellular lipid content in liver (IHCL; panel A) and *tibialis anterior* muscle (IMCL; panel B) of healthy ZDF *fa/+* rats (LEAN) and diabetic ZDF *fa/fa* rats (DIABETIC) in no-exercise (NO_EXE) and the exercise (EXE) groups determined at 4 and 24 h after treadmill visit and the oral administration of [U-$^{13}$C] algal lipid mixture (n=8 per group). Data are expressed as a mean percentage of the unsuppressed water signal ± SEM. * Significantly different from LEAN ($P$<0.05); ^ significantly different from 4 h post ($P$<0.05).

***Results:*** IHCL content was 4.6-fold higher in $DIABETIC_{EXE\_NO\_SUB}$ rats compared with $LEAN_{EXE\_NO\_SUB}$ rats ($P$<0.001), independent of time point (Fig. 1A). Interestingly, in both $LEAN_{EXE\_NO\_SUB}$ and $DIABETIC_{EXE\_NO\_SUB}$ rats total IHCL content was not affected by a single bout of treadmill running ($P$=0.415). Total IMCL content was 4.7-fold higher in $DIABETIC_{EXE\_NO\_SUB}$ rats than in $LEAN_{EXE\_NO\_SUB}$ rats, independent of time point (Fig. 1B). Directly after treadmill running, total IMCL content was 25±7% decreased in $LEAN_{EXE\_NO\_SUB}$ ($P$<0.05) and 33±4% in $DIABETIC_{EXE\_NO\_SUB}$ rats ($P$<0.01) when compared with baseline. At 24 h post exercise, total IMCL contents had returned to baseline levels in both $LEAN_{EXE\_NO\_SUB}$ and $DIABETIC_{EXE\_NO\_SUB}$ rats ($P$<0.05).

DIABETIC rats incorporated 3.2-fold more dietary derived $^{13}$C-labeled lipids into their IHCL depot compared with LEAN rats, independent of time point and treadmill use ($P$<0.001, Fig. 2A). Prior exercise did not affect lipid handling in liver of both LEAN and DIABETIC rats ($P$=0.604). DIABETIC rats stored 2.1- and 12.7-fold more dietary lipids into the IMCL pool compared with LEAN rats at 4 and 24 h after the administration of $^{13}$C-labeled lipids, respectively ($P$<0.05, Fig. 2B), independent of treadmill use. $^{13}$C-labeled IMCL in LEAN rats decreased 75±8% between the 4 and 24 h time point ($P$<0.01), independent of treadmill use, which was not observed in DIABETIC rats ($P$=0.503). Interestingly, no effects of exercise were observed on lipid handling in skeletal muscle tissue ($P$=0.981) of both LEAN and DIABETIC rats.

***Discussion and Conclusion:*** This study aimed to elucidate the effects of exercise on intracellular lipid handling in liver and skeletal muscle of healthy and diabetic rats. The application of $^{1}$H-[$^{13}$C] MRS allowed for direct *in vivo* measurements of total and $^{13}$C-labeled lipid content in liver and muscle, at baseline and at multiple time points after a single bout of exercise followed by oral administration of $^{13}$C-labeled lipids. The first objective was to characterize to what extent intracellular lipids in skeletal muscle and liver are being used as a substrate source during exercise. At baseline, total IMCL and IHCL content were substantially higher in diabetic than in healthy rats. Directly after one hour of treadmill running, IMCL depots of both healthy and diabetic rats were significantly reduced. In contrast, physical activity did not seem to affect the IHCL depots. The second objective was to investigate to what extent prior exercise affected dietary lipid uptake. In line with total lipid content, dietary lipid uptake in both skeletal muscle and liver was substantially higher in diabetic rats when compared with healthy rats. However, prior exercise did not affect postprandial lipid uptake in liver and skeletal muscle of both healthy and diabetic rats.

***References:*** [1] Samuel VT et al., *Lancet* **375**, 2267-2277, 2010; [2] De Feyter HM et al., *Diabetes Care* **30**, 2511-2513, 2007; [3] Jonkers RAM et al., *Magn Reson Med* **68**: 997-1006, 2012.