Effects of acute exercise on lipid content and dietary lipid uptake in liver and skeletal muscle of lean and diabetic rats

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1Biomedical NMR, Department of Biomedical Engineering, Eindhoven University of Technology, Eindhoven, The Netherlands; 2The Netherlands Consortium for Systems Biology, Den Haag, The Netherlands; 3School for Nutrition, Toxicology and Metabolism, Department of Human Movement Sciences, Maastricht University Medical Centre+, Maastricht, The Netherlands; 4Department of Pediatrics, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands; and 5Department of Laboratory Medicine, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands

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Janssens S, Jonkers RA, Groen AK, Nicolay K, van Loon LJ, Prompers JJ. Effects of acute exercise on lipid content and dietary lipid uptake in liver and skeletal muscle of lean and diabetic rats. Am J Physiol Endocrinol Metab 309: E874–E883, 2015. First published October 6, 2015; doi:10.1152/ajpendo.00292.2015.—Insulin resistance is associated with ectopic lipid accumulation. Physical activity improves insulin sensitivity, but the impact of exercise on lipid handling in insulin-resistant tissues remains to be elucidated. The present study characterizes the effects of acute exercise on lipid content and dietary lipid partitioning in liver and skeletal muscle of lean and diabetic rats by use of magnetic resonance spectroscopy (MRS). After baseline measurements, rats were randomized to exercise or no-exercise groups. A subset of animals was subjected to MRS directly after 1 h of treadmill running for measurement of total intrahepatocellular lipid (IHCL) and intramyocellular lipid (IMCL) content (n = 7 lean and diabetic rats). The other animals were administered 13C-labeled lipids orally after treadmill visit (with or without exercise) followed by MRS measurements after 4 and 24 h to determine the 13C enrichment of IHCL and IMCL (n = 8 per group). Total IHCL and IMCL content were fivefold higher in diabetic vs. lean rats (P < 0.001). Exercise did not significantly affect IHCL content but reduced IMCL by 25 ± 7 and 33 ± 4% in lean and diabetic rats (P < 0.05), respectively. Uptake of dietary lipids in liver and muscle was 2.3-fold greater in diabetic vs. lean rats (P < 0.05). Prior exercise did not significantly modulate dietary lipid uptake into muscle, but in liver of both lean and diabetic rats, lipid uptake was 44% reduced after acute exercise (P < 0.05). In conclusion, IMCL but not IHCL represents a viable substrate source during exercise in both lean and diabetic rats, and exercise differentially affects dietary lipid uptake in muscle and liver.

lipid metabolism; exercise; diabetes; liver; skeletal muscle

ONE OF THE EARLIEST DISTURBANCES in the etiology of type 2 diabetes is insulin resistance in major metabolic tissues, such as skeletal muscle and liver, which is generally accompanied by the accumulation of intramyocellular and intrahepatocellular lipids (IMCL and IHCL) (12, 43, 49, 54, 60, 65, 85, 86). Physical activity has been shown to be beneficial in the treatment of insulin resistance and type 2 diabetes (14, 25, 51, 66); therefore, the effect of exercise on ectopic lipids has gained significant interest.

In healthy individuals, the IMCL pool was shown to act as an important substrate source during exercise (32, 68, 75, 77, 79, 83). However, in sedentary obese subjects and type 2 diabetes patients, the capacity to mobilize and oxidize IMCL during exercise appears to be reduced (4, 6, 31, 38, 55, 71, 75). In contrast to the skeletal muscle tissue lipid depots, it is less clear to what extent IHCL is being used as a substrate source during physical activity or exercise. Only a few studies have evaluated the effect of exercise training on IHCL content without any kind of dietary intervention, but in general they indicate that regular exercise training may be effective in reducing IHCL content in obese subjects and type 2 diabetic patients (1, 7, 33, 69, 72). However, the response of IHCL content to acute exercise remains elusive. In healthy rodents, acute exercise of moderate intensity with a duration of up to 1 h does not seem to affect IHCL content (10, 11, 26, 30). In contrast, exhaustive exercise in rats was shown to lead to a decrease in IHCL content in one study (68), but to an increase in another (26). Similarly, there are contrasting findings on the effects of acute exercise on IHCL content in healthy humans, showing either no effect (34) or an increase (8, 20). Besides differences in exercise intensity and duration, variations in baseline IHCL content might also be responsible for these different findings, as exercise seems more effective in reducing IHCL under conditions when baseline liver fat stores are increased (11, 47). However, data on the response of IHCL content to acute exercise in diabetic conditions are greatly lacking.

Although it is well known that dietary fat intake after exercise strongly promotes IMCL repletion (17, 18, 79), little is known about the effect of prior exercise on the partitioning of dietary fat into different tissues. Moreover, it is not clear whether postprandial lipid trafficking after exercise is altered in insulin-resistant conditions. However, knowledge of the metabolic fate of dietary fat in insulin-resistant skeletal muscle and liver after exercise is important to understand the effects of long-term exercise on ectopic lipid accumulation in type 2 diabetes patients.

In the present study, we applied magnetic resonance spectroscopy (MRS) to characterize the extent to which intracellular lipids in liver and skeletal muscle are being used as a substrate source during exercise in healthy and type 2 diabetic conditions. To this end, we included lean, healthy fa/+ Zucker rats, diabetic Zucker fa/− rats, and healthy and diabetic Zucker fa/− rats, which were treated with streptozocin (STZ) to induce insulin resistance. After baseline measurements, rats were randomized to exercise or no-exercise groups. A subset of animals was subjected to MRS measurements after 4 and 24 h to determine the 13C enrichment of IHCL and IMCL. Total IHCL and IMCL content were fivefold higher in diabetic vs. lean rats (P < 0.001). Exercise did not significantly affect IHCL content but reduced IMCL by 25 ± 7 and 33 ± 4% in lean and diabetic rats (P < 0.05), respectively. Uptake of dietary lipids in liver and muscle was 2.3-fold greater in diabetic vs. lean rats (P < 0.05). Prior exercise did not significantly modulate dietary lipid uptake into muscle, but in liver of both lean and diabetic rats, lipid uptake was 44% reduced after acute exercise (P < 0.05). In conclusion, IMCL but not IHCL represents a viable substrate source during exercise in both lean and diabetic rats, and exercise differentially affects dietary lipid uptake in muscle and liver.
diabetic fatty (ZDF) rats and obese, diabetic fa/fa ZDF rats to quantify total IMCL and IHCL before and directly after a single bout of moderate-intensity exercise. Furthermore, we combined the use of proton-observed, carbon-13-edited (1H-13C) MRS with the oral administration of 13C-labeled lipids (35) to investigate the extent to which postprandial lipid partitioning is modulated by prior exercise in lean and diabetic rats.

MATERIALS AND METHODS

Animals. Forty-six male ZDF rats (ZDF/Gmi, Charles River Laboratories, Sulzfeld, Germany) were used for this study: obese, diabetic fa/fa rats at the age of 12 wk (n = 23) and their lean, healthy fa/+ littersmates (n = 23) (56). Animals were housed in pairs at 20°C and 50% humidity, on a 12:12-h light-dark cycle with ad libitum access to Purina 5008 diet (19 energy percent (En%) from fat, 54 En% from carbohydrates, and 27 En% from protein; SM R/M modified 5008 diet, Ssniff Spezialdiäten, Soest, Germany) and water during the entire period of the experiment, unless stated otherwise. All experimental procedures were reviewed and approved by the local institutional animal care committee (Maastricht University, Maastricht, The Netherlands).

Experimental design. Figure 1 shows a schematic overview of the study protocol, and Table 1 provides an overview of the different animal groups. Prior to treadmill visit, all animals were subjected to 1H-13C MRS measurements to determine total (12C and 13C-labeled intracellular lipid concentrations in liver and tibialis anterior (TA) muscle at baseline. Three days after MRS experiments at baseline, rats were placed on a treadmill (1055MSD-E52 Exer-3M Open Treadmill with shocker, Columbus Instruments, Columbus, OH) for familiarization, initially without turning on the running belt and shocker. After 15 min, they ran for 10 min at 10 m/min with the shocker set to 2.9 mA. Next, rats were randomized to an exercise group (n = 15 fa/+ and n = 15 fa/fa rats) or a no-exercise group [n = 8 fa/+ (LEANEXE_NO_SUB) and n = 8 fa/fa (DIABETICEXE_NO_SUB)] rats to determine the effects of acute exercise on total IHCL and IMCL content and postprandial lipid partitioning in liver and muscle. The day after familiarization, rats in the no-exercise groups were placed on the treadmill for 1 h with the speed set to 0 m/min and the shocker to 2.9 mA, while rats in the exercise 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/+ (LEANEXE_NO_SUB) and n = 7 fa/fa (DIABETICEXE_NO_SUB) rats of the exercise groups were subjected to MRS experiments without the administration of 13C-labeled substrate 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-exercise groups (LEANNO_EXE and DIABETICNO_EXE) and the remaining n = 8 fa/+ (LEANEXE) and n = 8 fa/fa (DIABETICEXE) rats in the exercise groups were administered 1.5 g [U-13C]Algal lipid/kg body wt (13C enrichment >98%; fatty acid composition: 53% palmitic acid, 9% palmitoleic acid, 28% oleic acid, and 6% linoleic acid; Cambridge Isotope Laboratories, Andover, MA) orally directly after the treadmill visit. In these rats, no MRS measurements were performed directly after treadmill visit, as the anesthesia would affect the digestion and uptake of dietary lipids. After the administration of 13C-labeled lipids, rats were refrained from food intake for 4 h and then subjected to MRS experiments. Between 4 and 24 h, fa/fa rats were pairwise fed with fa/+ rats; therefore, all rats had access to an equal amount of chow. Final MRS experiments were performed 24 h after treadmill visit.

Blood samples were taken from the saphenous vein directly after the treadmill visit and during each MRS experiment and were used for determination of plasma glucose, nonesterified fatty acid (NEFA), and triglyceride (TG) concentrations and plasma fatty acid (FA) 13C enrichment. At the end of the MRS measurement at the 24-h time point, the anesthetized animals were killed by incising the inferior vena cava.

MRS experiments. During MRS experiments, animals were anesthetized using 1.5–2.5% isoflurane (IsoFlo; Abbott Laboratories, Maidenhead, Berkshire, UK). Body temperature was maintained at 37 ± 1°C using heating pads. Respiration was monitored using a pressure sensor.
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Table 1. Overview of animal groups

<table>
<thead>
<tr>
<th>Lean/Diabetic Rats</th>
<th>$^{13}$C-Labeled Substrate</th>
<th>Exercise</th>
<th>Time Points MRS Measurements</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lean/Diabetic</td>
<td>$^{13}$C-labeled substrate</td>
<td>Exercise</td>
<td>Time Points MRS Measurements</td>
</tr>
<tr>
<td>Lean/Diabetic</td>
<td>$^{13}$C-labeled substrate</td>
<td>Exercise</td>
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</tr>
<tr>
<td>Lean/Diabetic</td>
<td>$^{13}$C-labeled substrate</td>
<td>Exercise</td>
<td>Time Points MRS Measurements</td>
</tr>
</tbody>
</table>

Lean, lean, healthy fa/+ ZDF rats; Diabetic, obese, diabetic fa/fa ZDF rats; $^{13}$C-labeled substrate, oral administration of $[U-^{13}$C]$\text{algal lipid mixture directly post-treadmill visit}$; SUB, substrate; EXE, 1 h of treadmill running; NO_EXE, 1 h of treadmill visit with running belt off; post, directly post-treadmill visit; 4 h post, 4 h post-treadmill visit; 24 h post, 24 h post-treadmill visit.

registering thorax movement (Rapid Biomedical, Rimpar, Germany) and the respiration period was maintained between 800 and 1,200 ms. All MRS experiments were performed on a 6.3 T horizontal Bruker MR system (Bruker, Ettlingen, Germany). In each rat, also in the animals that did not receive $^{13}$C-labeled substrate, localized $^{1}$H-$^{13}$C MRS was performed first on a $4 \times 2 \times 4$ mm$^{3}$ voxel placed in the median lobe of the liver and, after repositioning, on a $3.5 \times 3.5 \times 3.5$ mm$^{3}$ voxel in the TA muscle using the LASER-POCE method as described previously (35). Briefly, 3D localization of a voxel in liver or muscle was achieved by the localization by adiabatic selective refocusing (LASER) sequence (23), which was combined with a proton-observed, carbon-edited (POCE) element (59) for $^{13}$C editing. The $^{13}$C editing pulse was centered on the lipid methylene resonance (as determined from an unlocalized $^{13}$C spectrum). For each voxel, 64 LASER-POCE experiments, consisting of 16 averages each, were performed serially in an interleaved fashion, with the $^{13}$C editing pulse turned on every other experiment. The experiments without the $^{13}$C editing pulse are regular localized $^{1}$H MRS measurements and can therefore also be used to determine total IHCL/IMCL levels (see below). Water suppression was achieved using SWAMP (sequence for water suppression with adiabatic modulated pulses) (16). An unsuppressed water spectrum, consisting of 16 averages, was recorded from the same voxel and was used as internal reference. Other LASER-POCE parameters were as follows: repetition time, 2,000 ms; echo time, 26.8 ms; POCE echo time, 7.9 ms; number of data points, 640; WALTZ-16 $^{13}$C decoupling, and total scan time, 24 min.

MRS data analysis. Spectra from the 32 LASER-POCE experiments with and without the $^{13}$C editing pulse were added separately, and the difference spectrum was calculated using Matlab (R2009b; Mathworks, Natick, MA). Water and IHCL/IMCL methylene (IHCL-CH$_2$/IMCL-CH$_2$ signal at 1.3 ppm) peak areas were quantified from the unsuppressed and suppressed spectra, respectively, using the jMRUI software package (81) as described previously (15). Total ($^{12}$C + $^{13}$C) and $^{13}$C-labeled IHCL/IMCL levels were determined from the LASER-POCE spectra without $^{13}$C editing and the difference spectra, respectively, and are presented as a percentage of the unsuppressed water signal measured in the same voxel. The average relative $^{13}$C enrichment determined at baseline was used to correct the $^{13}$C-labeled IHCL/IMCL levels at 4 and 24 h after $^{13}$C-labeled lipid administration for natural abundance of $^{13}$C.

Plasma analysis. Plasma glucose concentrations were determined in blood using an automatic glucometer (FreeStyle, Abbott, IL). For plasma NEFA and TG concentration analyses, blood samples were collected in paraoxon-coated capillaries (to prevent TG hydrolysis) (87) and centrifuged for 10 min at 1,000 g. Plasma aliquots were then frozen in liquid nitrogen and stored at $-80^\circ$C for analyses using the NEFA-HR(2) kit (Wako Chemicals, Neuss, Germany), and a serum TG determination kit (Sigma-Aldrich, Zwijndrecht, The Netherlands), respectively. $^{13}$C enrichments of plasma FAs were measured in plasma samples taken at 4 h post-treadmill visit and $^{13}$C-labeled lipid administration as previously described (19).

Statistics. All data are expressed as means ± SE. Data were analyzed using mixed-model repeated-measures ANOVA with time point (baseline, directly postexercise and 24 h postexercise; or baseline, 4 h, and 24 h post-treadmill visit and $^{13}$C-labeled lipid administration) as the within-subjects factor, and genotype (lean and diabetic rats) and treadmill use (no-exercise and exercise) as between-subjects factors. Bonferroni corrections were applied when appropriate. In case of a significant interaction term between factors, the effect of time was analyzed using one-way ANOVA or paired Student’s t-tests (for $^{13}$C-labeled IMCL and IHCL), and differences between lean and diabetic rats, and no-exercise and exercise were detected by unpaired Student’s t-tests. Statistical analyses were performed using the IBM SPSS Statistics 19 software package (SPSS, Chicago, IL). The level of significance was set at $P < 0.05$.

RESULTS

Animal characteristics. Body and liver weight data of rats that did not receive $^{13}$C-labeled substrate (LEANEXE_NO_SUB and DIABETICEXE_NO_SUB) are shown in Table 2, and data of rats that did receive $^{13}$C-labeled substrate (LEANNO_EXE, LEANNO_EXE, DIABETICNO_EXE, and DIABETICEXE) are shown in Table 3. At baseline, body weight of diabetic rats was greater than that of lean rats ($P < 0.05$; Tables 2 and 3). The treadmill visit resulted in a decrease in body weight in all groups, but body weight loss was greater in diabetic than in lean rats ($P < 0.05$; Tables 2 and 3). Moreover, it was greater in the exercise groups than in the no-exercise groups ($P < 0.05$; Table 3). Liver weight of diabetic rats was $\sim 60\%$ higher than that of lean rats ($P < 0.05$; Tables 2 and 3).

Plasma analyses. Plasma glucose concentrations of rats that did not receive $^{13}$C-labeled substrate are shown in Table 4. Results of plasma glucose, NEFA, and TG concentration analyses from rats that did receive $^{13}$C-labeled substrate are presented in Table 5. Diabetic rats had elevated plasma glucose levels compared with lean rats at every time point, independent of treadmill use ($P < 0.001$; Tables 4 and 5). In both lean and diabetic rats, plasma glucose was not significantly altered directly after treadmill visit (Tables 4 and 5), either in no-exercise or in exercise groups. In diabetic rats, plasma glucose

Table 2. Animal characteristics of lean fa/+ and diabetic fa/fa ZDF rats in exercise groups without administration of $^{13}$C-labeled substrate

<table>
<thead>
<tr>
<th>LEANEXE_NO_SUB</th>
<th>DIABETICEXE_NO_SUB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight at baseline, g</td>
<td>332 ± 7</td>
</tr>
<tr>
<td>ΔBody weight, g</td>
<td>-7.4 ± 1.2</td>
</tr>
<tr>
<td>Liver weight, g</td>
<td>10.5 ± 0.3</td>
</tr>
</tbody>
</table>

Data are means ± SE; n = 7. ΔBody weight, difference in body weight before and after treadmill visit. *Significantly different from lean ($P < 0.05$).
was ~20% lower at 4 h and 24 h post-treadmill visit compared with baseline and directly post-treadmill visit \( (P < 0.05; \) Tables 4 and 5), which was likely caused by the 4 h of fasting after treadmill visit and the pairwise feeding with lean rats between 4 and 24 h after treadmill visit.

At baseline, plasma NEFA concentrations tended to be higher in diabetic rats than in lean rats \( (P = 0.06; \) Table 5). While at baseline plasma NEFA levels were lower in exercise groups than in no-exercise groups, directly after the treadmill visit plasma NEFA concentrations were higher in exercise groups than in no-exercise groups, independent of genotype \( (P < 0.05; \) Table 5). At 24 h post-treadmill visit, plasma NEFA concentrations had returned to baseline levels in all groups. Plasma TG concentrations were elevated in diabetic rats compared with lean rats \( (P < 0.001) \) but were not significantly affected by treadmill use (Table 5).

The \(^{13}\text{C}\) enrichments of plasma FAs, determined at 4 h post-treadmill visit and \(^{13}\text{C}\)-labeled lipid administration, did not significantly differ among groups and were 12.5 ± 1.4% for palmitic acid, 46.3 ± 3.4% for palmitoleic acid, 17.4 ± 1.8% for oleic acid, and 10.1 ± 1.1% linoleic acid.

**MRS experiments.** Figure 2 displays typical examples of water-suppressed LASER-POCE spectra acquired from liver and TA muscle of a diabetic rat at 4 h after exercise and the administration of \(^{13}\text{C}\)-labeled lipids. The lipid methylene signal at 1.30 ppm in the normal \(^{1}H\) spectra without the \(^{13}\text{C}\) editing pulse was used for quantification of total \((^{12}\text{C} + ^{13}\text{C})\) IHCL and IMCL. The difference spectra contain signals only from \(^{1}H\)-\(^{13}\text{C}\)-coupled resonances and were used to determine the \(^{13}\text{C}\) enrichment of the IHCL and IMCL pools for the groups of animals that did receive \(^{13}\text{C}\)-labeled substrate.

Figure 3 shows total IHCL (Fig. 3A) and IMCL (Fig. 3B) contents at baseline and directly and 24 h after a single bout of exercise in rats that did not receive \(^{13}\text{C}\)-labeled substrate (LEAN\textsubscript{NO_EXE_NO_SUB} and DIABETIC\textsubscript{NO_EXE_NO_SUB}). IHCL and IMCL content were 4.7- and 4.8-fold higher in diabetic rats compared with lean rats \( (P < 0.001) \), respectively, independent of time point. Interestingly, in both lean and diabetic rats, total IHCL content was not significantly affected by a single bout of treadmill running \( (P = 0.15) \). However, directly after treadmill running, total IMCL content was decreased by 25 ± 7% in lean \( (P < 0.05) \) and by 33 ± 4% in diabetic \( (P < 0.01) \) rats compared with baseline. At 24 h postexercise, total IMCL contents had returned to baseline levels in both lean and diabetic rats \( (P < 0.05) \) compared with postexercise.

In Fig. 4, total and \(^{13}\text{C}\)-enriched IHCL and IMCL contents are presented for rats that did receive \(^{13}\text{C}\)-labeled lipids directly after treadmill visit (LEAN\textsubscript{EXE_EXE}, LEAN\textsubscript{EXE_EXE}, DIABETIC\textsubscript{NO_EXE_EXE}, and DIABETIC\textsubscript{EXE_EXE}). In these groups, MRS measurements were performed at baseline and at 4 and 24 h after treadmill visit. Similar to the data of the rats that did not receive \(^{13}\text{C}\)-labeled substrate (Fig. 3A), total IHCL content in rats that did receive \(^{13}\text{C}\)-labeled lipids was not significantly affected by exercise, also not after 4 h (Fig. 4A). Whereas total IMCL content was decreased directly after exercise in both lean and diabetic rats (Fig. 3B), at 4 h after exercise, IMCL content in lean rats was not significantly different from baseline \( (P = 0.43) \) or between exercise and no-exercise groups \( (P = 0.89; \) Fig. 4B). However, in diabetic rats, total IMCL content at 4 h after exercise was still lower compared with the measurement at 24 h after exercise \( (P < 0.001) \).

The \(^{13}\text{C}\)-enriched IHCL and IMCL concentrations in Fig. 4, C and D, represent the amount of lipids stored in liver and muscle originating from the administered \(^{13}\text{C}\)-labeled lipids. Diabetic rats incorporated 2.3- and 4.5-fold more diet-derived \(^{13}\text{C}\)-labeled lipids into their IHCL depots than lean rats at 4 and 24 h after the administration of \(^{13}\text{C}\)-labeled lipids, respectively \( (P < 0.01; \) Fig. 4C), independent of treadmill use. Prior exercise led to a 44% decrease in the uptake of dietary lipids in the liver at 4 h after treadmill visit and the administration of \(^{13}\text{C}\)-labeled lipids, independent of genotype \( (P < 0.05) \). However, at 24 h, the incorporation of \(^{13}\text{C}\)-labeled lipids in the IHCL pool was again similar between exercise and no-exercise groups \( (P = 0.782) \).

Diabetic rats stored 2.3- and 10.5-fold more dietary lipids into the IMCL pool compared with lean rats at 4 and 24 h after the administration of \(^{13}\text{C}\)-labeled lipids, respectively \( (P < 0.05; \) Fig. 4D), independent of treadmill use. \(^{13}\text{C}\)-labeled IMCL in lean rats decreased 75 ± 8% between 4 and 24 h after administration of \(^{13}\text{C}\)-labeled lipids \( (P < 0.01) \), independent of treadmill use. This decrease, however, was not observed in diabetic rats \( (P = 0.503) \). Interestingly, no significant effects of exercise were observed on lipid handling in skeletal muscle tissue of either lean or diabetic rats \( (P = 0.981) \).

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**Table 3.** Animal characteristics of lean fa/+ and diabetic fa/fa ZDF rats in no-exercise and exercise groups with administration of \(^{13}\text{C}\)-labeled substrate

<table>
<thead>
<tr>
<th></th>
<th>LEAN\textsubscript{NO_EXE}</th>
<th>LEAN\textsubscript{EXE}</th>
<th>DIABETIC\textsubscript{NO_EXE}</th>
<th>DIABETIC\textsubscript{EXE}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight at baseline, g</td>
<td>321 ± 6</td>
<td>341 ± 3</td>
<td>388 ± 11*</td>
<td>362 ± 7*</td>
</tr>
<tr>
<td>−Body weight, g</td>
<td>−3.6 ± 0.6</td>
<td>−6.6 ± 0.9$</td>
<td>−7.3 ± 0.5$</td>
<td>−10.8 ± 1.2$</td>
</tr>
<tr>
<td>Liver weight, g</td>
<td>10.5 ± 0.2</td>
<td>10.8 ± 0.1</td>
<td>17.8 ± 0.6$</td>
<td>16.3 ± 0.7*</td>
</tr>
</tbody>
</table>

Data are means ± SE; \( n = 8 \). *Significantly different from lean \( (P < 0.05) \); †significantly different from no-exercise \( (P < 0.05) \).

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**Table 4.** Plasma glucose concentrations of lean fa/+ and diabetic fa/fa ZDF rats in exercise groups without administration of \(^{13}\text{C}\)-labeled substrate, at baseline, immediately after treadmill visit, and 24 h after treadmill visit

<table>
<thead>
<tr>
<th>Plasma glucose (mmol/l)</th>
<th>LEAN\textsubscript{NO_EXE_NO_SUB}</th>
<th>DIABETIC\textsubscript{NO_EXE_NO_SUB}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>7.0 ± 0.2</td>
<td>19.2 ± 1.4*</td>
</tr>
<tr>
<td>Post</td>
<td>7.2 ± 0.4</td>
<td>17.5 ± 1.5*</td>
</tr>
<tr>
<td>24 h post</td>
<td>7.0 ± 0.3</td>
<td>14.4 ± 1.0*</td>
</tr>
</tbody>
</table>

Data are means ± SE; \( n = 7 \). *Significantly different from lean \( (P < 0.05) \); †significantly different from baseline \( (P < 0.05) \); #significantly different from post-treadmill visit \( (P < 0.05) \).
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**Table 5. Plasma glucose, NEFA, and TG concentrations of lean fa/+, and diabetic fa/fa ZDF rats in no-exercise and exercise groups with administration of [13C]-labeled substrate, at baseline, immediately after treadmill visit, and 4 and 24 h after treadmill visit**

<table>
<thead>
<tr>
<th></th>
<th>LEAN&lt;sub&gt;NS,EXE&lt;/sub&gt;</th>
<th>LEAN&lt;sub&gt;EXE&lt;/sub&gt;</th>
<th>DIABETIC&lt;sub&gt;NS,EXE&lt;/sub&gt;</th>
<th>DIABETIC&lt;sub&gt;EXE&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Plasma glucose (mmol/l)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>6.3 ± 0.3</td>
<td>6.7 ± 0.4</td>
<td>19.7 ± 1.4*</td>
<td>19.4 ± 0.5*</td>
</tr>
<tr>
<td>Post</td>
<td>6.3 ± 0.4</td>
<td>6.0 ± 0.3</td>
<td>20.8 ± 1.7*</td>
<td>18.3 ± 1.0*</td>
</tr>
<tr>
<td>4 h post</td>
<td>7.1 ± 0.4</td>
<td>6.7 ± 0.2</td>
<td>15.7 ± 1.6#</td>
<td>15.9 ± 0.3**#</td>
</tr>
<tr>
<td>24 h post</td>
<td>8.3 ± 0.6</td>
<td>7.6 ± 0.2</td>
<td>16.7 ± 1.7**#</td>
<td>15.0 ± 0.8**#</td>
</tr>
<tr>
<td><strong>Plasma NEFA (mmol/l)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>0.39 ± 0.05</td>
<td>0.28 ± 0.04$</td>
<td>0.60 ± 0.10</td>
<td>0.36 ± 0.09$</td>
</tr>
<tr>
<td>Post</td>
<td>0.16 ± 0.06†</td>
<td>0.27 ± 0.03$</td>
<td>0.28 ± 0.06*†</td>
<td>0.54 ± 0.10*$</td>
</tr>
<tr>
<td>4 h post</td>
<td>0.68 ± 0.08†</td>
<td>0.43 ± 0.04#*$</td>
<td>0.56 ± 0.07#</td>
<td>0.43 ± 0.05$</td>
</tr>
<tr>
<td>24 h post</td>
<td>0.34 ± 0.04#</td>
<td>0.25 ± 0.03$*$</td>
<td>0.49 ± 0.08**#</td>
<td>0.39 ± 0.07*$</td>
</tr>
<tr>
<td><strong>Plasma TG (mmol/l)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>0.72 ± 0.08</td>
<td>0.69 ± 0.06</td>
<td>2.30 ± 0.24*</td>
<td>2.06 ± 0.31*</td>
</tr>
<tr>
<td>Post</td>
<td>0.58 ± 0.08</td>
<td>0.49 ± 0.06</td>
<td>2.27 ± 0.52*</td>
<td>2.33 ± 0.31*</td>
</tr>
<tr>
<td>4 h post</td>
<td>0.60 ± 0.06</td>
<td>0.57 ± 0.06</td>
<td>2.16 ± 0.29*</td>
<td>2.22 ± 0.33*</td>
</tr>
<tr>
<td>24 h post</td>
<td>0.64 ± 0.04</td>
<td>0.70 ± 0.07</td>
<td>2.42 ± 0.30*</td>
<td>2.44 ± 0.36*</td>
</tr>
</tbody>
</table>

Data are means ± SE; n = 8. NEFA, nonesterified fatty acids; TG, triglyceride. *Significantly different from lean (P < 0.05); †significantly different from baseline (P < 0.05); #significantly different from post treadmill visit (P < 0.05); $significantly different from 4 h post treadmill visit (P < 0.05); $significantly different from no-exercise (P < 0.05).

**Discussion**

This study aimed to elucidate the effects of exercise on intracellular lipid handling in liver and skeletal muscle of lean and diabetic rats. The application of [1H-13C] MRS (35) allowed for direct in vivo measurements of total and 13C-labeled intracellular lipid content in liver and muscle, at baseline and at multiple time points after a single bout of exercise followed by oral administration of 13C-labeled lipids. The first objective was to characterize the extent to which intracellular lipids in skeletal muscle and liver are being used as a substrate source during exercise. At baseline, total IMCL and IHCL contents were substantially higher in diabetic than in lean rats. Directly after 1 h of treadmill running, IMCL depots of both lean and diabetic rats were significantly reduced. In contrast, physical activity did not seem to affect the IHCL depots. The second objective was to investigate the extent to which 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 than in lean rats. However, prior exercise did not significantly affect postprandial lipid uptake in skeletal muscle of both lean and diabetic rats. In contrast, dietary lipid uptake in the liver in the early postprandial period was significantly reduced after acute exercise both in lean and diabetic rats.

We used the ZDF rat as an animal model of type 2 diabetes. Between 8 and 10 wk of age, fa/fa rats progress from a normoglycemic-hyperinsulinemic to a hyperglycemic-hyperinsulinemic state (13, 21). In this study, we confirmed that 12-wk-old fa/fa rats developed overt type 2 diabetes, characterized by elevated plasma glucose and TG levels in the fed state (Tables 4 and 5). Heterozygous fa/+ rats remained normoglycemic and served as healthy controls (13, 21). The manifestation of diabetes in fa/fa rats is secondary to a genetic mutation in the leptin receptor. Leptin or leptin receptor deficiency is not, however, an important contributor to type 2 diabetes in humans; therefore, although the ZDF rat is extensively used as an animal model of type 2 diabetes, it does not exactly reflect disease etiology in human diabetes patients (82), which is a limitation of the study.

Type 2 diabetes has been associated with increased IMCL content in both animal (13, 36, 42, 44, 84) and human studies (38, 43, 52, 54, 76). This was confirmed in the present study.
as we observed markedly elevated IMCL stores in diabetic rats compared with lean rats (Figs. 3B and 4B). The IMCL pool has been shown to act as a dynamic fuel with an increase in its mobilization and subsequent oxidation during physical activity in lean individuals and animals (8, 20, 30, 32, 67, 68, 75, 77, 79, 83). In line with these findings, the present study demonstrated that, directly after cessation of exercise, the IMCL depot of lean rats had been reduced by $25 \pm 7\%$ (Fig. 3B). Although the literature suggests that the capacity to use IMCL is impaired in sedentary obese subjects and type 2 diabetes patients (4, 6, 31, 38, 55, 71, 75), the present study showed a similar relative reduction ($33 \pm 4\%$) in the IMCL depot following exercise in the diabetic rats (Fig. 3B). In absolute terms, the use of IMCL as a substrate source was even higher in the diabetic rats compared with the lean controls. Therefore, diabetic rats did not show an impaired capacity to utilize the IMCL depot as a substrate source during exercise. This may be attributed to the relatively normal plasma NEFA levels in the diabetic rats (Table 5) as opposed to the substantially elevated plasma NEFA levels in obese type 2 diabetes patients (58, 64). It has been shown that elevated plasma NEFA levels inhibit the capacity to mobilize and, as such, oxidize the IMCL stores (78, 80).

In agreement with previous observations (36, 41, 68, 70), we observed a substantially higher IHCL content in diabetic rats when compared with lean rats (Figs. 3A and 4A).
reports on the acute effects of exercise on IHCL content are inconsistent (8, 10, 11, 20, 26, 30, 34, 68), and it remains unclear to what extent IHCL is used as a substrate source during exercise. We observed that a single bout of exercise does not reduce IHCL content in either lean or diabetic rats (Figs. 3A and 4A), suggesting that IHCL is not being used as a readily available fuel source to provide energy during physical activity. These findings confirm previous studies in healthy rodents reporting that acute exercise of moderate intensity with a duration of up to 1 h does not seem to affect IHCL content (10, 11, 26, 30). Interestingly, longer bouts of exercise have been shown to increase IHCL content (8, 20, 26). It could be speculated that longer exercise bouts stimulate an increase in IHCL storage through a consistent elevation of plasma NEFA levels secondary to prolonged exercise, similarly to the effects observed during fasting conditions (73). Analogously, in humans, IMCL content was shown to increase in inactive muscle tissue following prolonged exercise, which was attributed to the increased plasma NEFA flux (61).

Data on the response of IHCL content to acute exercise in diabetic conditions is greatly lacking. It has been suggested that exercise is more effective in reducing IHCL under conditions when baseline liver fat stores are increased (47), and previous studies showed that a single bout of exercise decreases IHCL content in high-fat diet-fed rats (11, 68). However, in the present study, acute exercise did not significantly affect IHCL content in either lean or diabetic rats; therefore, baseline IHCL levels do not seem to modulate the effect of acute exercise on IHCL content.

Most exercise studies have focused on the depletion of IMCL during exercise, but fewer data are available on postexercise IMCL replenishment. In the present study, we observed replenishment of the IMCL pool back to baseline levels within 24 h of postexercise recovery for both lean and diabetic animals (Fig. 3B). Although it is well known that dietary fat intake after exercise strongly promotes IMCL repletion (17, 18, 79), little is known about the effect of prior exercise on the uptake of dietary fat into skeletal muscle tissue. Moreover, it is not clear whether postprandial lipid trafficking after exercise is altered in insulin-resistant conditions. To investigate the metabolic fate of dietary lipids after cessation of exercise, 13C-labeled lipids were orally administered following a 1-h treadmill visit. In the early postprandial period, lipid uptake in muscle of diabetic rats was 2.3-fold higher compared with lean rats (Fig. 4D), independent of treadmill use. This is in accord with previous reports of increased muscle lipid uptake in diabetic rats by our laboratory (36) and in patients with type 2 diabetes (2, 38, 57) during postprandial conditions. In contrast to lean rats, 13C-labeled IMCL in the diabetic rats did not significantly decrease between 4 and 24 h after treadmill visit and the administration of 13C-labeled lipids, suggesting that IMCL turnover is reduced in diabetic rats at rest. These data are in accord with previous reports of reduced lipid oxidation in insulin-resistant and type 2 diabetic muscle (3, 5, 6, 9, 24, 36–39, 46, 48).

Despite the observed declines in total IMCL contents during exercise and the subsequent replenishment of the total IMCL pools, we did not observe an elevated incorporation of dietary lipids into the IMCL depots after cessation of exercise in either lean or diabetic rats (Fig. 4D). These results are in contrast to a recent study of Hansen et al. (27), showing that prior acute exercise resulted in a greater trafficking of dietary fat to oxidative tissues, i.e., skeletal muscle, in healthy rats. The increased uptake of dietary fat into muscle after exercise was explained by the transient upregulation of muscle lipoprotein lipase (LPL) expression and activity upon exercise (29, 53, 62, 63). However, it was also shown that uptake of dietary fat is not the same for different muscles and that it is higher in more oxidative muscles. Therefore, the effects of exercise on muscle LPL expression and activity seem to be muscle fiber type dependent, which could explain the absence of an effect of prior exercise on dietary lipid uptake in the more glycolytic TA muscle in the current study (15).

Uptake of dietary lipids into the liver was 2.3-fold greater in diabetic rats than in lean rats in the early postprandial period (Fig. 4C). Additionally, liver weights were ~60% higher in diabetic rats compared with lean rats, resulting in an even larger difference in total dietary lipid uptake by the liver. These results are in accord with previous observations of elevated lipid uptake in insulin-resistant and diabetic liver (3, 28, 36, 46, 49, 57). During periods of high lipid influx, such as during the immediate postprandial period, the liver acts as a systemic lipid buffer by taking up NEFA from the spillover pathway and chylomicron remnants, which are later resecreted back into the circulation as very low-density lipoproteins (22, 45). It has been suggested that in the insulin-resistant state, a greater proportion of meal-derived FAs may be handled by the liver in the postprandial period (45).

In contrast to skeletal muscle, prior exercise did affect the uptake of dietary lipids in the liver. We observed that in both lean and diabetic rats dietary lipid uptake in the liver in the early postprandial period was reduced after acute exercise by 44% (Fig. 4C). This result is in contrast to Hansen et al. (27), who showed no effect of prior exercise on dietary lipid uptake in the liver of healthy rats. During exercise, hepatic FA uptake is known to be decreased despite increased plasma NEFA levels (40), which may be attributed to a redistribution of blood flow away from the liver. However, in the early recovery phase after exercise, both hepatic blood flow (50) and hepatic FA reesterification (74) were shown to be increased. It can be speculated that directly after exercise the increased influx of adipose tissue-derived NEFA into the liver suppresses the uptake of diet-derived FAs, which may explain our observation of reduced dietary lipid uptake in the liver at 4 h after the cessation of exercise. However, 13C enrichment of plasma FAs was not significantly affected by exercise, implying that the reduced dietary lipid uptake is not simply caused by a greater dilution of the dietary 13C-labeled lipids.

In conclusion, IMCL, but not IHCL, represents a viable substrate source during exercise in both lean and diabetic rats. Despite the decline in IMCL during exercise and the subsequent replenishment during recovery, prior exercise did not augment the uptake of dietary lipids into skeletal muscle. In contrast, in the livers of both lean and diabetic rats, dietary lipid uptake was significantly reduced after acute exercise.

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EXERCISE EFFECTS ON LIPID PARTITIONING IN LIVER AND MUSCLE

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R E F E R E N C E S

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