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Exercise training reduces intrahepatic lipid content in people with and people without nonalcoholic fatty liver

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Brouwers B, Schrauwen-Hinderling VB, Jelenik T, Gemmink A, Sparks LM, Havekes B, Bruls Y, Dahlmans D, Roden M, Hesselink MK, Schrauwen P. Exercise training reduces intrahepatic lipid content in people with and people without nonalcoholic fatty liver. *Am J Physiol Endocrinol Metab* 314: E165–E173, 2018. First published November 7, 2017; doi:10.1152/ajpendo.00266.2017.— Exercise training reduces intrahepatic lipid (IHL) content in people with elevated liver fat content. It is unclear, however, whether exercise training reduces IHL content in people with normal liver fat content. Here, we measured the effect of exercise training on IHL content in people with and people without nonalcohol fatty liver. We further measured changes in insulin sensitivity and hepatic energy metabolism. Eleven males with nonalcoholic fatty liver (NAFL) and 11 body mass index-matched individuals without nonalcoholic fatty liver (CON) completed a 12-wk supervised exercise training program. IHL content (proton magnetic resonance spectroscopy), maximal oxidative capacity ($\dot{V}o_{2max}$, spiroergometry), total muscle strength, body composition, insulin sensitivity (hyperinsulinemic-euglycemic clamp), hepatic ATP-to-total phosphorus ratio, and the hepatic phosphomonoester-to-phosphodiester (PME/PDE) ratio (phosphorus magnetic resonance spectroscopy) were determined. IHL content reduced with exercise training ($P = 0.014$) in the whole study population. The relative reduction in IHL content was comparable in NAFL ($-34.5 \pm 54.0\%$) and CON ($-28.3 \pm 60.1\%$) individuals ($P = 0.800$). $\dot{V}o_{2max}$ ($P < 0.001$), total muscle strength ($P < 0.001$), and skeletal muscle insulin sensitivity ($P = 0.004$) increased, whereas adipose tissue ($P = 0.246$) and hepatic ($P = 0.086$) insulin sensitivity did not increase significantly. Hepatic ATP-to-total phosphorus ratio ($P = 0.987$) and PME/PDE ratio ($P = 0.792$) did not change. Changes in IHL content correlated with changes in body weight ($r = 0.451$, $P = 0.035$) and changes in hepatic PME/PDE ratio ($r = 0.569$, $P = 0.019$). In conclusion, exercise training reduced intrahepatic lipid content in people with nonalcoholic fatty liver and in people with normal intrahepatic lipid content, and the percent reduction in intrahepatic lipid content was similar in both groups.

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hyperinsulinemic-euglycemic clamp; insulin resistance; magnetic resonance spectroscopy

INTRODUCTION

The prevalence of obesity and obesity-related diseases has increased dramatically over the past decades (33). Nonalcoholic fatty liver (NAFL) is closely related to obesity and is considered the most common liver disorder in Western society these days (8, 48). NAFL affects 30% of the total adult population (2), and its prevalence further increases to 70% in individuals who are obese (49).

Lifestyle intervention is the current standard treatment for NAFL (15, 27, 28, 42). Dietary intervention studies have shown that a decrease in body weight coincides with a significant reduction in intrahepatic lipid (IHL) content (15, 27, 28, 42). Adding exercise training to dietary restriction has additive effects in reducing IHL content (15). Moreover, exercise training per se can also be an effective way to reduce IHL content, as systematically reviewed previously (23). Previous studies have been focusing on the effect of exercise training on IHL content in people with NAFL (3, 10, 22, 45, 50). These studies showed that aerobic and/or resistance exercise training (reviewed in Ref. 5) and high-intensity interval training (19) all are effective in reducing IHL content in people with NAFL. The single study that measured the effect of exercise training on IHL content in people with normal liver fat content did not find a difference in IHL content (43), but the exercise intervention was short and did not include a group of people with high IHL content, in which a decrease in IHL content with exercise training is well established, to compare with.

It is not clear whether an improvement in insulin sensitivity contributes to the effect of exercise training on IHL content. Exercise training increases insulin sensitivity in insulin-resistant individuals and in patients with type 2 diabetes (9, 31, 32, 53), and people with NAFL are characterized by adipose tissue,

hepatic, and skeletal muscle insulin resistance (6, 13, 21, 25). Only a limited number of studies, however, have measured the effect of exercise training on glucose homeostasis or insulin sensitivity in combination with the effect of exercise training on IHL content (10, 18, 22). In the single exercise training study that performed a hyperinsulinemic-euglycemic clamp and measured IHL content, peripheral insulin sensitivity improved in combination with a 48% reduction in IHL content in individuals with NAFL (10).

Another factor that could contribute to the effect of exercise training on IHL content is improved hepatic energy metabolism. In skeletal muscle, exercise training improves mitochondrial function (32), a feature known to be impaired in insulin-resistant people (40). In liver, γATP levels and γATP synthesis rate have been shown to be lower in patients with type 2 diabetes and were associated with insulin sensitivity and IHL content (39, 47). Moreover, hepatic energy metabolism is influenced by an acute bout of exercise, with a decrease in hepatic ATP/inorganic phosphate ratio concomitant with an increase in IHL content 4 h after exercise (4).

To this end, we here measured the effect of 12 wk of supervised exercise training on IHL content in overweight and obese people with NAFL and in BMI-matched people without nonalcoholic fatty liver (CON). We further measured to effect of exercise training on insulin sensitivity and hepatic energy metabolism. We hypothesized that exercise training would be able to reduce IHL content in both groups, improve insulin sensitivity and alter hepatic energy metabolism.

MATERIALS AND METHODS

Participants. Eleven sedentary, middle-aged (40–70 yr), overweight/obese people with NAFL and 11 body mass index (BMI)-matched (27–35 kg/m^2) people without nonalcoholic fatty liver (CON), all males and with stable dietary habits, completed the study. These individuals participated in a previous study that investigated the metabolic perturbations in people with NAFL and compared those with people with normal IHL and patients with type 2 diabetes (6). As previously described (6), general exclusion criteria were unstable body weight (weight gain or loss >3 kg within 3 mo before start of the study), cardiac disease, impaired renal function, anemia (hemoglobin <7.5 mmol/l), use of β-blockers, use of antithrombotic medication, elevated blood pressure (>160/100 mmHg), claustrophobia, contraindications for MRI, recent participation in a weight loss or vigorous exercise program, history of substantial alcohol use [>3 units (1 unit = 10 ml of alcohol)/day], history of drug abuse, and use of insulin therapy. CON individuals were selected on having low IHL content [all ≤40 mg/g (4.0%)] as measured with proton magnetic resonance spectroscopy (^{1}H-MRS), in the absence of clinical signs of liver disease or liver dysfunction [defined as alanine aminotransferase (ALT) >2.5× normal values (normal values: ALT <45 U/l)] and had to be normoglycemic (fasting plasma glucose <6.1 mmol/l; see Ref. 52). NAFL were selected on having high IHL content [all ≥50 mg/g (5.0%)] as measured with ^{1}H-MRS, with a fasting plasma glucose concentration <7.0 mmol/l.

People were recruited via advertisements in local newspapers and gave written informed consent before participation in the study. The local ethics committee approved the study, which was performed following Declaration of Helsinki principles.

Before onset of the study, routine medical laboratory testing, a medical history, and physical examination were performed, and a resting electrocardiogram was taken. A 120-min 75-g oral glucose tolerance test (OGTT) was performed. Maximal work load (W_{max}) and maximal aerobic capacity ($\dot{V}O_{2max}$) were assessed during a graded cycling test with concurrent ECG until exhaustion (26). Body composition was determined by dual X-ray absorptiometry (Hologic Discovery A, Waltham, MA). Participants were asked to maintain their dietary behavior during the study, and a 3-day food record was collected during the 3 days before the hyperinsulinemic-euglycemic clamp, pre- and postexercise training.

Exercise training protocol. Participants were enrolled in a supervised 12-wk combined aerobic and resistance exercise training program with three exercise sessions per week, as previously described (32). Aerobic cycling exercise (stationary bike) was performed two times a week for 30 min at 70% of W_{max}. Resistance exercise was performed one time a week and comprised three series of 10 repetitions at 60% of the maximal voluntary contraction (MVC), and focused on large muscle groups (chest press, leg extension, lat pull down, leg press, triceps curls, biceps curls, abdominal crunches, and horizontal row). MVC was predicted from five multiple repeated maximum testing, as previously described (38). Total muscle strength was calculated as the sum of the predicted MVC for all eight muscle groups. Warming-up and cooling-down cycling sessions (5 min) were performed at 45% of the W_{max} before and after every exercise session. Every 4 wk, total muscle strength and W_{max} were reassessed to adjust training loads. Training sessions were performed with three to four individuals at a time.

^{1}H-MRS. ^{1}H-MRS was used to quantify IHL content, as described earlier (4), but on a 3T whole body scanner (Achieva 3Tx; Philips Healthcare, Best, The Netherlands) using a five-element coil and a repetition time = 4,000 ms, echo time = 32.5 ms, and number of averages = 64. To minimize motion artifacts, participants were asked to breathe in the rhythm of the measurement and to be at end-expiration during acquisition of spectra. To determine the intensity of the lipid peak, the water signal was suppressed using frequency-selective prepulses. The unsuppressed water resonance was used as internal reference (no. of averages = 32). Spectra were fitted with a home-written script (30) in MATLAB R2014b (Mathworks, Natick, MA). Values are corrected for T2 relaxation per Ref. 17 and given as absolute lipid concentrations by weight (mg/g), per Ref. 46, and as percentage (%).

Laboratory analysis. Arterialized blood samples were collected and immediately centrifuged at high speed. Plasma was frozen in liquid nitrogen and stored at −80°C until assayed. Plasma free fatty acids and plasma glucose were measured with enzymatic assays automated on a Cobas Fara/Mira (FFA: Wako Nefa C test kit; Wako Chemicals, Neuss, Germany) (plasma glucose: hexokinase method; La Roche, Basel, Switzerland). Plasma triglycerides were measured colorimetrically (Roche, Vienna, Austria). Blood parameters during the screening visit were analyzed routinely.

Hyperinsulinemic-euglycemic clamp. All participants underwent a two-step (10 and 40 mU·m^{-2}·min^{-1}) hyperinsulinemic-euglycemic clamp for 6 h (11), as previously described (51), 4 days before the start of the exercise training protocol and 48–72 h after the last exercise bout. In short, after an overnight fast, participants received a primed continuous infusion of [6,6-$^2H_2$]glucose (0.04 mg·kg^{-2}·min^{-1}). After 180 min, 10 mU·m^{-2}·min^{-1} insulin infusion was started for 4 h. Thereafter, 40 mU·m^{-2}·min^{-1} insulin infusions were started for 2 h. During insulin infusion, plasma glucose concentrations were maintained by concomitant infusion of glucose that was enriched with [6,6-$^2H_2$]glucose (hot-GINF) to minimize changes in plasma tracer enrichment (14). Glucose steady state was reached during the last 30 min of both insulin infusion rates.

Blood sampling and indirect calorimetry using a ventilated hood system (Omnical; IDEE, Maastricht, The Netherlands) were performed in the last 30 min of every steady state. Isotopic enrichment of plasma glucose was determined by electric impact ionization gas chromatography-mass spectroscopy as previously described (1).

Calculations. Steele's single-pool non-steady-state equations were used to calculate glucose rate of appearance (R_a) and glucose rate of disappearance (R_d) (n = 9 NAFL, n = 9 CON) (44). Volume of

distribution was assumed to be 0.160 l/kg for glucose. Adipose tissue insulin sensitivity was computed as percent suppression of plasma free fatty acids during low-dose (10 $mU \cdot m^{-2} \cdot min^{-1}$) insulin infusion. Suppression of endogenous glucose production (EGP) was calculated as R_a − exogenous glucose infusion rate (n = 9 NAFL, n = 9 CON). Hepatic insulin sensitivity was computed as percent suppression of EGP during low-dose (10 $mU \cdot m^{-2} \cdot min^{-1}$) insulin infusion. Peripheral insulin sensitivity was measured by insulin-stimulated glucose disposal (ΔR_d) and was computed as the difference between R_d under insulin-stimulated conditions (40 $mU \cdot m^{-2} \cdot min^{-1}$) and R_d under basal conditions. Glucose oxidation was calculated with the assumption that protein oxidation was negligible (n = 7 NAFL, n = 8 CON) (36). Nonoxidative glucose disposal (NOGD) was calculated as R_d (40 $mU \cdot m^{-2} \cdot min^{-1}$) − glucose oxidation ($n$ = 7 NAFL, n = 8 CON) (40 $mU \cdot m^{-2} \cdot min^{-1}$).

Phosphorus magnetic resonance spectroscopy. Phosphorus magnetic resonance spectroscopy (^{31}P-MRS) was performed on a 3T whole body scanner (Philips Achieva 3Tx; Philips Healthcare) (n = 10 NAFL; n = 7 CON). Participants were positioned in a supine position. A 14-cm-diameter circular ^{31}P receive and transmit surface coil was positioned at the level of the liver and secured in place using Velcro bands. Before spectroscopic acquisition, 15 axial, coronal, and sagittal ^{1}H-MR images (slice thickness = 10 mm, field of view = 450 × 450 mm, matrix size = 192 × 96, TE = 1.82 ms, TR = 3.8 ms) from the liver region were acquired using a gradient-echo sequence during one breath hold. The volume of interest was positioned parallel to the coil at 5 cm from the center of the coil. Manual tuning and matching of the ^{31}P-receive-and-transmit surface coil were performed, and pencil bean shimming was performed. Localized phosphorus spectra were obtained using image-selected in vivo spectroscopy. Sequence parameters were: TR = 5,500 ms, number of single acquisitions = 128, number of sample points = 1,024, spectral bandwidth = 3,000 Hz. The carrier frequency was chosen to match the resonance frequency of γATP.

Statistics. All values are reported as means ± SD, unless stated differently. Statistical significance of differences was set at $P < 0.05$. Normality was assessed by the Shapiro-Wilk test. Homogeneity of variances was assessed by Levene's test. A two-way ANOVA model for repeated measures was used with NAFL and CON as between-subject variables and pre- and postexercise training as repeated-measures within-subject variables. An unpaired Student's t-test was used to analyze preexercise training differences in age, aspartate aminotransferase, alanine aminotransferase, γ-glutamyltransferase, 2-h 75-g OGTT glucose values, and compliance. For Pearson' correlations, logarithmic transformation of delta (Δ) IHL content was performed, since the data were skewed. All statistical calculations were performed using JMP 13 (SAS) and Prism 6 (Graphpad).

RESULTS

Participant characteristics. Participant characteristics were previously reported (6) and are summarized in Table 1. NAFL and CON did not differ in age, body weight and body composition, BMI, waist circumference, $\dot{V}O_{2max}$, W_{max}, total muscle strength, aspartate aminotransferase, alanine aminotransferase, γ-glutamyl transferase, hepatic ATP-to-total phosphorus rate, hepatic phosphomonoester-to-phosphodiester (PME/PDE) ratio, fasting plasma glucose, fasting plasma free fatty acids, and fasting plasma triglycerides (all $P > 0.05$).

By design, NAFL had higher IHL content ($P < 0.001$) than CON. Fasting plasma insulin ($P = 0.007$) and plasma glucose concentration after a 120-min 75-g OGTT ($P = 0.021$) was higher in NAFL than in CON. Insulin-stimulated suppression of plasma free fatty acids ($P = 0.018$), insulin-stimulated endogenous glucose production (EGP) ($P = 0.002$), insulin-stimulated glucose disposal (ΔR_d) ($P < 0.001$), insulin-stimulated nonoxidative glucose disposal (NOGD) ($P < 0.001$), and insulin-stimulated carbohydrate (CHO) oxidation ($P = 0.024$) were lower in NAFL than in CON.

Table 1. *Participant characteristics*

	NAFL	CON
Age, yr	54.5 ± 6.8	57.6 ± 8.1
IHL, mg/g	96.6 ± 67.2#	19.4 ± 13.7
IHL, %	9.7 ± 6.7#	1.9 ± 1.4
W_{max}, W/kg	2.0 ± 0.3	2.1 ± 0.5
$\dot{V}O_{2max}$, l/min	2.7 ± 0.5	2.5 ± 0.4
$\dot{V}O_{2max}$, $ml \cdot kg^{-1} \cdot min^{-1}$	26.1 ± 4.7	27.6 ± 4.6
Total muscle strength, kg	89.5 ± 18.0	78.6 ± 14.0
BMI, kg/m^2	30.9 ± 2.6	29.5 ± 2.6
Body wt, kg	102.2 ± 9.6	93.8 ± 11.8
Fat mass, kg	30.7 ± 3.8	27.5 ± 6.4
Fat-free mass, kg	69.0 ± 6.6	64.2 ± 6.4
Fat, %	30.0 ± 2.3	28.8 ± 3.4
Waist circumference	109.1 ± 6.7	106.2 ± 6.2
Fasting glucose, mmol/l	5.6 ± 0.6	5.4 ± 0.6
Fasting insulin, mU/l	17.0 ± 7.4#	8.8 ± 3.4
Fasting free fatty acids, μmol/l	578.6 ± 160.9	680.1 ± 170.3
Fasting triglycerides, mmol/l	1.7 ± 0.6	1.1 ± 0.4
Glucose 120 min, mmol/l	6.7 ± 2.3#	4.8 ± 0.9
AST, U/l	25.5 ± 8.1	22.1 ± 4.1
ALT, U/l	34.5 ± 7.3	30.0 ± 11.9
GGT, U/l	35.4 ± 12.2	34.3 ± 15.5
Hepatic ATP/total phosphorus, %	15.8 ± 1.9	15.5 ± 1.1
Hepatic PME/PDE, %	37.0 ± 14.6	43.2 ± 12.7
Suppression of plasma FFA, %	54.5 ± 16.6#	69.6 ± 10.9
Suppression of EGP, %	30.6 ± 23.2#	62.7 ± 13.2
ΔR_d, $\mu mol \cdot min^{-1} \cdot kg\ FFM^{-1}$	15.2 ± 7.9#	35.1 ± 6.9
ΔNOGD, $\mu mol \cdot min^{-1} \cdot kg\ FFM^{-1}$	7.6 ± 6.6#	23.6 ± 3.7
ΔCHO oxidation, $\mu mol \cdot min^{-1} \cdot kg\ FFM^{-1}$	7.9 ± 3.6#	14.0 ± 4.0
Compliance, %	94.7 ± 5.0	96.0 ± 5.0
Food intake, kcal/day	2,139 ± 349	2,064 ± 453

Data are means ± SD. NAFL, nonalcoholic fatty liver; CON, control; IHL, intrahepatic lipid content; W_{max}, maximal output; $\dot{V}O_{2max}$, maximal aerobic capacity; BMI, body mass index; AST, alanine transaminase; ALT, aspartate aminotransferase; GGT, γ-glutamyl transpeptidase; PME, phosphomonoester; PDE; phosphodiester; FFA, free fatty acids; EGP, endogenous glucose production; ΔR_d, change in glucose disposal; NOGD, nonoxidative glucose disposal; CHO, carbohydrate; FFM, fat-free mass. #$P < 0.05$ NAFL vs. CON.

Compliance was comparable between groups, with an average attendance of 94.7 ± 5.0 and 96.0 ± 5.0% in NAFL and CON ($P = 0.549$), respectively. Food intake was comparable between groups (Table 1).

Intrahepatic lipid content. IHL content reduced with exercise training in both groups (time $P = 0.014$, Fig. 1*A*). The absolute decrease in IHL content with exercise training was not significantly different between groups (time × group $P = 0.077$, Fig. 1*A*), with a reduction in IHL content in NAFL individuals of 21.7 ± 30.2 mg/g (2.2 ± 3.0%) and in CON individuals of 4.0 ± 9.2 mg/g (0.4 ± 0.9%) (Fig. 1*A*). The relative reduction in IHL content with exercise training (%reduction from baseline values) was similar in NAFL (−34.5 ± 54.0%) and in CON (−28.3 ± 60.1%) individuals (time $P = 0.015$, time × group $P = 0.800$, Fig. 1*B*).

Exercise capacity and muscle strength. $\dot{V}O_{2max}$ increased with exercise training in both groups (time $P < 0.001$, time × group $P = 0.277$, Fig. 2*A* and Table 2). W_{max} increased with exercise training (time $P < 0.001$), and the increase in W_{max} was more profound in NAFL than in CON (time × group $P = 0.022$, Table 2). Total muscle strength increased with exercise training in both groups (time $P < 0.001$, time × group = 0.280, Fig. 2*B*).

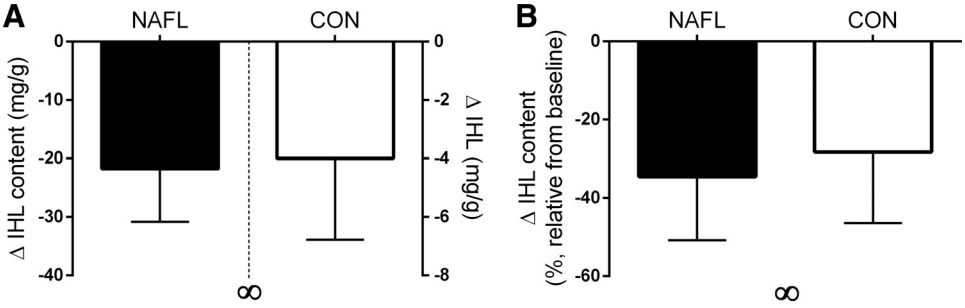

Fig. 1. Changes in intrahepatic lipid (IHL) content with exercise training. Changes in IHL content (*A*) and percent changes in IHL content with exercise training (*B*) in people with nonalcoholic fatty liver (NAFL) and people without nonalcoholic fatty liver (CON). $\infty P < 0.05$ for time effect. Results are means ± SE.

Body composition and food intake. BMI (time $P = 0.432$, time × group = 0.157), body weight (time $P = 0.414$, time × group = 0.163), fat-free mass (time $P = 0.075$, time × group $P = 0.325$) and waist circumference (time $P = 0.424$, time × group $P = 0.157$), and food intake (time = 0.576, time × group = 0.583) did not significantly change with exercise training (Table 2). Fat mass (time $P = 0.009$, time × group $P = 0.550$) and fat percentage (time $P = 0.002$, time × group $P = 0.931$) decreased with exercise training in both groups, although the change in fat mass was small (Table 2).

Circulating metabolites. Fasting plasma glucose (time $P = 0.240$, time × group $P = 0.270$), fasting plasma insulin (time $P = 0.681$, time × group $P = 0.871$), and fasting plasma triglycerides (time $P = 0.729$, time × group $P = 0.277$) did not significantly change with exercise training (Table 2). Fasting plasma free fatty acids decreased with exercise training in both groups (time $P = 0.003$, time × group = 0.121, Table 2).

Adipose tissue, hepatic, and skeletal muscle insulin sensitivity. Suppression of plasma free fatty acids (time $P = 0.246$, time × group $P = 0.525$, Fig. 3*A*) and suppression of EGP (time $P = 0.086$, time × group $P = 0.782$, Fig. 3*B*) did not significantly change with exercise training. Skeletal muscle insulin sensitivity improved with exercise training in both groups (time $P = 0.004$, time × group $P = 0.374$, Fig. 3*C*), and this was because of an increase in NOGD in both groups (time $P = 0.013$, time × group $P = 0.361$, Fig. 3*D*), whereas glucose oxidation was unaffected (time $P = 0.770$, time × group $P = 0.967$, Fig. 3*D*).

Hepatic ATP/total phosphorus ratio and PME/PDE ratio. Hepatic ATP-to-total phosphorus (ATP/total phosphorus) ratio (time $P = 0.987$, time × group = 0.107) and hepatic PME/PDE ratio (time $P = 0.792$, time × group $P = 0.448$), a surrogate marker for liver function, did not change with exercise training (Table 2).

Correlations. Although body weight and hepatic PME/PDE ratio did not change with exercise training, ΔIHL content correlated with Δbody weight (Fig. 4*A* and Table 3) and ΔPME/PDE ratio (Fig. 4*B* and Table 3). When separating participants in a group of individuals that lost weight and a group of individuals that gained weight, ΔIHL content correlated with Δbody weight in the group of individuals that lost weight (Fig. 4*C*), but not in the group of individuals that gained weight (Fig. 4*D*). ΔIHL content did not significantly correlate with $\Delta\dot{V}O_{2max}$, Δtotal muscle strength, Δfat mass, Δfat mass percentage, Δfat-free mass, Δsuppression of plasma free fatty acids, Δsuppression of EGP, and Δ delta R_d (Table 3).

DISCUSSION

We here found that 12 wk of supervised exercise training reduced IHL content in overweight/obese people with NAFL and in BMI-matched people without NAFL. The relative reduction in IHL content with exercise training was similar in both groups, even though people without nonalcoholic fatty liver already had low concentrations of IHL content at the start of the study. Exercise training further improved skeletal muscle, but not adipose tissue or hepatic, insulin sensitivity. Body weight and hepatic energy metabolism were unaffected, but changes in body weight and changes in hepatic PME/PDE ratio correlated with changes in IHL content.

Previous studies have mainly focused on the effect of exercise training in people with NAFL (3, 5, 10, 22, 45, 50). The relative decrease in IHL content with exercise training in people with NAFL (34.5%) in our study was comparable to observations made in previous exercise training studies in which body weight was kept constant (5, 10). In addition, undergoing the same exercise training protocol, we found that people without NAFL showed a similar relative decrease in IHL content (28.3%) as people with NAFL, even though these people already had low concentrations of IHL content at the start of the study. The single study that measured the effect of exercise training on IHL content in people with normal liver fat content did not find a difference in IHL content after 6 wk of exercise training (43). That study, however, did not include a

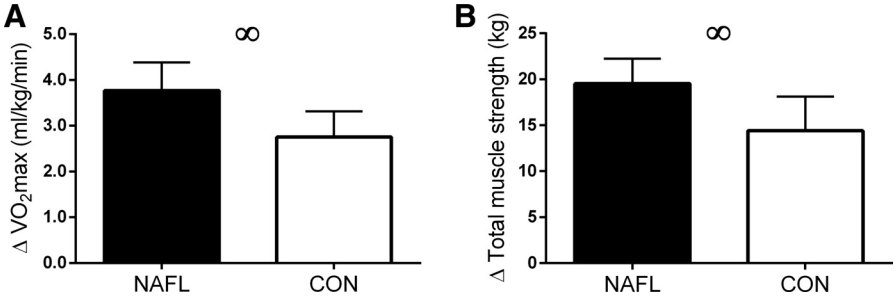

Fig. 2. Changes in maximal oxidative capacity ($\dot{V}O_{2max}$) and total muscle strength with exercise training. Changes in $\dot{V}O_{2max}$ (*A*) and total muscle strength (*B*) with exercise training in people with NAFL and CON. $\infty P < 0.05$ for time effect. Results are means ± SE.

Table 2. *Changes with exercise training*

	ΔNAFL	ΔCON	*P* Value (time)
W_{max}, W/kg	0.51 ± 0.23#	0.31 ± 0.15	<0.001*
$\dot{V}O_{2max}$, l/min	0.35 ± 0.21	0.26 ± 0.16	<0.001*
BMI, kg/m^2	−0.26 ± 0.68	0.07 ± 0.30	0.423
Body wt, kg	−0.85 ± 2.26	0.23 ± 0.96	0.414
Fat mass, kg	−0.99 ± 1.38	−0.65 ± 1.27	0.009*
Fat-free mass, kg	0.21 ± 1.30	0.71 ± 0.98	0.075
Fat, %	−0.75 ± 0.82	−0.71 ± 1.09	0.002*
Waist circumference	−0.65 ± 1.72	0.19 ± 0.77	0.424
Fasting glucose, mmol/l	−0.01 ± 0.29	−0.25 ± 0.64	0.240
Fasting insulin, mU/l	−0.12 ± 2.48	−0.28 ± 1.92	0.681
Fasting free fatty acids, μmol/l	−67.69 ± 129.09	−187.72 ± 208.75	0.003*
Fasting triglycerides, mmol/l	0.19 ± 0.75	−0.10 ± 0.38	0.729
Hepatic ATP/total phosphorus, %	0.71 ± 2.02	−0.70 ± 1.29	0.987
Hepatic PME/PDE, %	−1.15 ± 14.91	−0.67 ± 11.66	0.792
Food intake, kcal/day	−61 ± 242	−1 ± 149	0.576

Data are means ± SD. NAFL, nonalcoholic fatty liver; CON, control; W_{max}, maximal output; $\dot{V}O_{2max}$, maximal aerobic capacity; BMI, body mass index; W_{max}, maximal output; PME, phosphomonoester; PDE; phosphodiester. *$P <$ 0.05. #$P < 0.05$, NAFL vs. CON.

group of people with high IHL content, in which a decrease in IHL content with exercise training is well established, and therefore it cannot be ruled out that the lack of effect on IHL content might have been the result of the shorter duration of the exercise training intervention.

Different exercise intensities can also influence the effect of exercise training on IHL content. Vigorous exercise, but not moderate exercise, has been shown to decrease the chance of having nonalcoholic steatohepatitis (24), and a recent study found reductions in IHL content after 12 wk of high-intensity aerobic training, but not moderate-intensity aerobic training, in obese people with NAFL (34). Along the same lines, higher aerobic capacity has been associated with lower IHL content (20). In the present study, a significant correlation was observed between baseline IHL content and baseline $\dot{V}O_{2max}$ ($r = -0.491$, $P = 0.020$). IHL content reduced with exercise training, whereas $\dot{V}O_{2max}$ improved with exercise training. Changes in IHL content that occurred with exercise training, however, did not correlate with the exercise training-induced changes in $\dot{V}O_{2max}$.

Changes in IHL content did associate with changes in body weight, even though body weight did not significantly change. Thus, one can speculate that with exercise training a small decrease in body weight is mandatory to lower IHL content. This is supported by our observation that, when participants were separated in a group of individuals that lost weight and a group of individuals that gained weight, changes in IHL content only correlated with changes in body weight in the group of individuals that lost weight. Caloric restriction is a very effective way to reduce IHL content (15, 27, 28, 42), and a decrease in body weight of ~4% results in a relative reduction in IHL content of ~40% (7, 28). However, we here obtained a relative reduction in IHL content of ~30%, without statistically significant changes in body weight. Moreover, there is considerable evidence that exercise training in the absence of weight loss reduces IHL content (5, 10, 18). Thus, while even small changes in body weight might have clinical relevance and seem to have contributed to reductions in IHL content, other mechanisms related to exercise training likely have contributed to the reduction in IHL content as well.

Skeletal muscle insulin sensitivity improved with exercise training, in accordance with previous findings in individuals with insulin resistance (31, 32), and in people with NAFL (10). A close relationship between skeletal muscle insulin sensitivity and IHL content has been described before (6, 25), suggesting that NAFL might be part of multiorgan derangements in insulin sensitivity. We could not find a direct association between changes in skeletal muscle insulin sensitivity and changes in IHL content, consistent with previous observations (10). Nevertheless, there is evidence that cross talk between organs has an important role in metabolic diseases. Myokines, for example, are secreted by skeletal muscle and mediate interaction with other organs such as adipose tissue and liver (12, 35). We

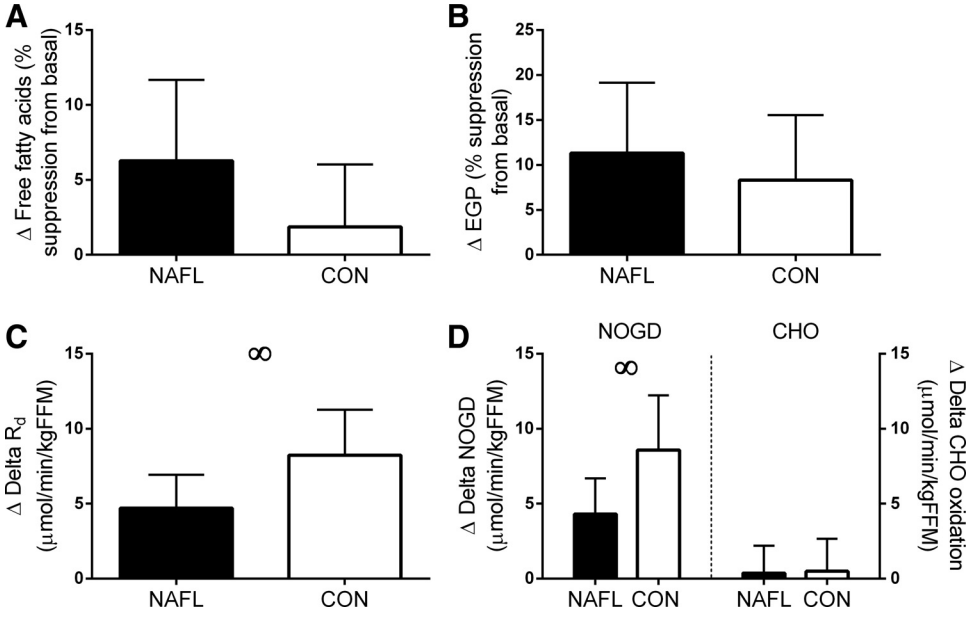

Fig. 3. Changes in adipose tissue, hepatic, and skeletal muscle insulin sensitivity. Changes in percent suppression of plasma free fatty acids as a marker for adipose tissue insulin sensitivity with 10 mU·m^{-2}·min^{-1} insulin infusion (*A*), percent suppression of the endogenous glucose production (EGP) as a marker for hepatic insulin sensitivity with 10 mU·m^{-2}·min^{-1} insulin infusion (*B*), insulin-stimulated glucose disposal (ΔR_d) in skeletal muscle during 40 mU·m^{-2}·min^{-1} insulin infusion (*C*), and insulin-stimulated nonoxidative glucose disposal (NOGD) and insulin-stimulated glucose oxidation in skeletal muscle during 40 mU·m^{-2}·min^{-1} insulin (*D*) in people with NAFL and CON. ∞$P < 0.05$ for time effect. Results are means ± SE.

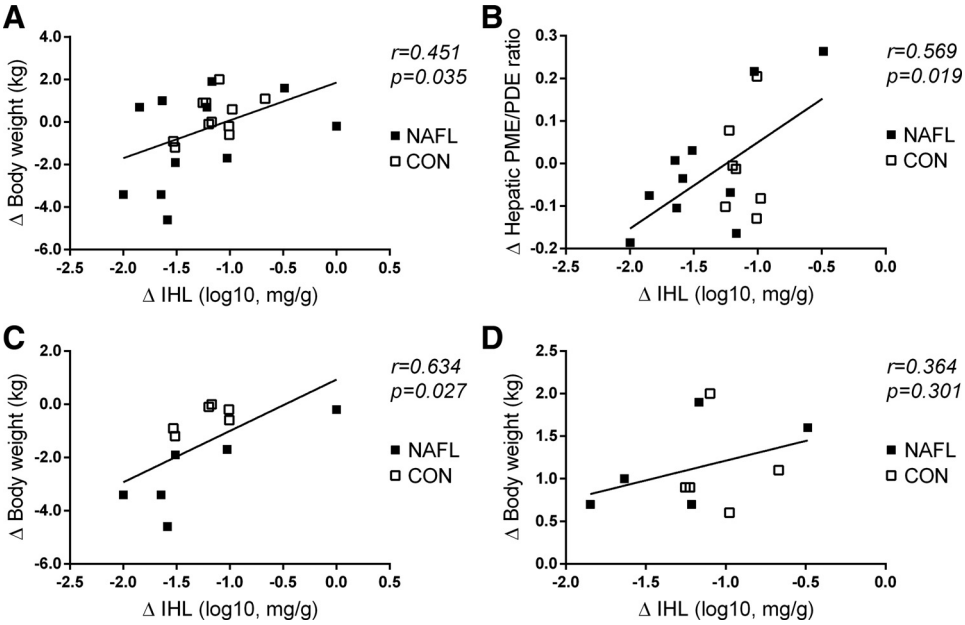

Fig. 4. Correlations. Correlation between changes (Δ) in IHL content and Δbody weight in the whole study population ($r = 0.451$, $P = 0.035$, $n = 22$, *A*), Δphosphomonoester-to-phosphodiester (PME/PDE) ratio in the whole study population ($r = 0.569$, $P = 0.019$, $n = 17$, *B*), Δbody weight in individuals who lost weight with exercise training ($r = 0.634$, $P = 0.027$, $n = 12$, *C*), and Δbody weight in individuals who gained weight with exercise training ($r = 0.364$, $P = 0.301$, $n = 10$, *D*).

did not measure myokines in this study, but it is known that several myokines are released after contraction of the skeletal muscle (12, 35). Future research should elaborate whether interorgan cross talk has an important role in modulating the effects of exercise training on IHL content.

Hepatic insulin sensitivity did not improve with exercise training, and changes in IHL content did not correlate with changes in the suppression of the endogenous glucose production. This is in agreement with a previous exercise training study that found no improvement in hepatic insulin sensitivity despite a reduction in IHL content in people with NAFL (10). There is evidence that exercise training without weight loss can improve hepatic insulin sensitivity in obese healthy individuals, individuals with impaired glucose handling, and patients with type 2 diabetes (31, 32, 43). However, in people with NAFL, reductions in IHL content that are achieved with exercise training in the absence of weight loss have not been linked to improvements in hepatic insulin sensitivity (10). Contrary, reductions in IHL content following low-calorie diets in people with NAFL were paralleled by improvements in hepatic insulin sensitivity (29, 37). Reductions in IHL content following calorie restriction are often greater than what can be achieved with exercise training in the absence of weight loss, with reductions of IHL content to within the normal liver fat range [<50 mg/g (5.0%)] (29, 37). In our study, liver fat content in NAFL individuals stayed elevated compared with the normal range [74.9 mg/g (7.5%)] after exercise training; therefore, larger reductions in IHL content might have been necessary to observe improvements in hepatic insulin resistance.

Table 3. *Correlations for changes in IHL content in the whole study population*

	R	*P*
$\Delta\dot{V}_{O_2max}$	−0.105	0.644
ΔTotal muscle strength	0.218	0.331
ΔBody wt	0.451	0.035*
ΔFat mass	0.267	0.229
ΔFat mass percentage	0.112	0.619
ΔFat-free mass	0.369	0.091
ΔHepatic PME/PDE	0.569	0.019*
ΔSuppression of plasma FFA	−0.101	0.656
ΔSuppression of EGP	−0.039	0.882
ΔR_d	−0.274	0.287

PME, phosphomonoester; PDE; phosphodiester; EGP, endogenous glucose production; ΔR_d, change in glucose disposal. *$P < 0.05$.

We found a significant reduction in fasting plasma free fatty acids with exercise training, but changes in fasting plasma free fatty acids were not associated with changes in IHL content. Increased delivery of free fatty acids released from adipose tissue toward liver is considered to be one of the most important mechanisms contributing to the excessive accumulation of lipids in the liver (49), although associations between fasting plasma free fatty acids and IHL content are not always reported (5). Our data suggest that a reduction in IHL content with exercise training is not necessarily related to a reduction in fasting plasma free fatty acids and agrees with previous exercise training studies in which reductions in IHL content were not paralleled by changes in fasting plasma free fatty acids (18, 45).

Likewise, exercise training did not improve insulin-stimulated suppression of plasma free fatty acid release from the adipose tissue. A previous study reported improved suppression of plasma free fatty acids following 12 wk of exercise training in healthy obese people and patients with type 2 diabetes (32). Suppression of plasma free fatty acids as a measurement of adipose tissue insulin sensitivity is indirect; however, and may depend on the concentration of insulin used (16), which in our study was lower compared with the study by Meex et al. (32).

In the present study, we used ^{31}P-MRS to measure hepatic energy metabolism. Improved hepatic energy metabolism is another factor that could contribute to the effect of exercise training on IHL content. Hepatic ATP levels are associated with insulin resistance and IHL content (39, 47), and we previously found that an increase in IHL content was associ-

ated with a decrease in hepatic ATP/inorganic phosphate ratio in overweight men studied before and after an acute bout of exercise (4). In the present study, hepatic ATP/total phosphorus ratio was not affected by exercise training, suggesting that exercise training did not influence hepatic energy metabolism. It must be noted, however, that hepatic ATP/total phosphorus ratio is a crude index for hepatic energy metabolism, and we did not measure absolute hepatic ATP concentration, or actual hepatic ATP turnover. Measuring absolute hepatic ATP concentration and turnover in future studies will give more insight on whether exercise training influences hepatic energy metabolism.

With the use of ^{31}P-MRS, the hepatic PME/PDE ratio in the phosphorus spectrum can be quantified. It has been proposed that a high hepatic PME/PDE ratio is an indicator for a reduction in hepatic cell membrane synthesis and thereby a surrogate marker of liver function (41). Hepatic PME/PDE ratio did not change with exercise training. However, changes in hepatic PME/PDE ratio did associate with changes in IHL content. Thus, people with larger reduction in IHL content lowered their hepatic PME/PDE ratio, whereas people with smaller reductions in IHL content did not lower their hepatic PME/PDE ratio. These results therefore suggest that a relatively large reduction in IHL content with exercise training seems to be necessary to influence liver function.

In conclusion, we showed that 12 wk of supervised exercise training reduced intrahepatic lipid content in people with nonalcoholic fatty liver and in BMI-matched people with normal intrahepatic lipid content. The relative reduction in intrahepatic lipid content with exercise training was similar in both groups. Our findings highlight the impact of exercise training on hepatic lipid metabolism, in both people with high and low liver fat content.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

B.B., V.B.S.-H., M.K.C.H., and P.S. conceived and designed research; B.B., V.B.S.-H., T.J., A.G., L.M.S., Y.B., and D.D. performed experiments; B.B. and V.B.S.-H. analyzed data; B.B., V.B.S.-H., B.H., M.R., M.K.C.H., and P.S. interpreted results of experiments; B.B. prepared figures; B.B., M.K.C.H., and P.S. drafted manuscript; B.B., V.B.S.-H., T.J., A.G., L.M.S., B.H., Y.B., D.D., M.R., M.K.C.H., and P.S. edited and revised manuscript; B.B., V.B.S.-H., T.J., A.G., L.M.S., B.H., Y.B., D.D., M.R., M.K.C.H., and P.S. approved final version of manuscript.

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