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Dissociation between exercise-induced reduction in liver fat and changes in hepatic and peripheral glucose homeostasis in obese patients with Non-Alcoholic Fatty Liver Disease

***Running title*:** Exercise, liver fat and insulin sensitivity in obese patients with NAFLD

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Abstract

Non-Alcoholic Fatty Liver Disease (NAFLD) is associated with multi-organ (hepatic, skeletal muscle, adipose tissue) insulin resistance (IR). Exercise is an effective treatment for lowering liver fat but its effect on insulin resistance in NAFLD is unknown.

We aimed to determine whether supervised exercise in NAFLD would reduce liver fat and improve hepatic and peripheral (skeletal muscle and adipose tissue) insulin sensitivity. Sixty nine NAFLD patients were randomised to 16 weeks exercise supervision (*n*=38) or counselling (*n*=31) without dietary modification. All participants underwent magnetic resonance imaging/spectroscopy to assess changes in body fat, and in liver and skeletal muscle triglyceride, before and following exercise/counselling. To quantify changes in hepatic and peripheral insulin sensitivity, a pre-determined subset (*n*=12 per group) underwent a two-stage hyperinsulinaemic euglycaemic clamp pre- and post-intervention. Results are shown as mean (95% CI).

Fifty participants (30 exercise, 20 counselling), 51 y (40, 56), BMI 31 kg/m^2 (29, 35) with baseline liver fat/water % of 18.8 % (10.7, 34.6) completed the study (12/12 exercise and 7/12 counselling completed the clamp studies). Supervised exercise mediated a greater reduction in liver fat/water % than counselling [Δ mean change 4.7% (0.01, 9.4); $P<0.05$], which correlated with the change in cardiorespiratory fitness (r = -0.34, P = 0.0173).

With exercise, peripheral insulin sensitivity significant increased (following high-dose insulin) despite no significant change in hepatic glucose production (following low-dose insulin); no changes were observed in the control group.

Although supervised exercise effectively reduced liver fat, improving peripheral IR in NAFLD, the reduction in liver fat was insufficient to improve hepatic IR.

Keywords: NAFLD, insulin resistance, exercise, liver fat and magnetic resonance spectroscopy.

Summary statement

In NAFLD, 16 weeks of supervised exercise effectively reduces liver fat and improve peripheral insulin resistance and cardiorespiratory fitness. Greater reductions in liver fat are needed to improve hepatic insulin resistance, requiring higher intensity or longer duration of exercise.

Introduction

Non-alcoholic fatty liver disease (NAFLD) is a spectrum of histopathological abnormalities which increase the risk of chronic liver disease, hepatocellular carcinoma and cardiovascular disease (1). NAFLD arises from accumulation of liver fat, frequently complicating obesity and other insulin-resistant states, co-existing with the metabolic syndrome (2, 3). NAFLD is associated with multi-organ (hepatic, skeletal muscle and adipose tissue) insulin resistance (IR) (4, 5).

Although certain anti-diabetes agents reduce liver fat (6, 7), the cornerstone of therapy is lifestyle modification through dietary intervention and/or physical activity (8, 9). Weight loss through dietary intervention has been shown to normalise moderate hepatic steatosis (12-13%) and hepatic IR (10, 11). Considering that NAFLD patients tend to engage in less habitual leisure-time physical activity and be more sedentary, physical activity is also recommended (12, 13). Various modalities of exercise have been shown to be beneficial in reducing liver fat in NAFLD including aerobic (5, 14, 15) and resistance exercise (13), even without weight loss. A recent study addressing the dose-response relationship between aerobic exercise and reduction in liver fat suggests that even low volume, low intensity aerobic exercise can reduce liver fat without clinically significant weight loss (16). It is unclear to what extent reduction in liver fat following exercise is associated with improvements in hepatic and peripheral IR. This is of particular importance considering the high rates of incident type 2 diabetes mellitus (T2DM) in NAFLD patients.

We set out to determine the efficacy of supervised exercise training in reducing liver fat, and the relationship between reduction in liver fat and improvements in hepatic and peripheral IR using the gold standard method for measuring insulin resistance, a 2-step euglycaemic hyperinsulinaemic clamp.

Experimental materials and Methods

Design

A 16-week randomised controlled trial of NAFLD patients, randomised to supervised moderate-intensity aerobic exercise or conventional counselling (control group) (Clinical Trials.gov NCT01834300).

Participants

Patients were recruited through hepatology clinics where they were undergoing routine clinical care from 4 teaching hospitals, and studied in 2 centres, in Guildford and Liverpool. NAFLD was diagnosed clinically by a hepatologist after exclusion of (steatogenic) drug causes, viral or auto-immune hepatitis (negative hepatitis B and C serology and auto-antibody screen), primary biliary cirrhosis and metabolic disorders (α_1-antitrypin deficiency, Wilson's disease).

Inclusion criteria were a diagnosis of NAFLD, being sedentary (<2 h/week low-intensity physical activity, no moderate- or high-intensity activity), non-smokers, with alcohol consumption <14 (females) and <21 (males) units/week. Exclusion criteria were T2DM, ischaemic heart disease or contraindications to exercise. Participants were excluded from follow-up assessment if they deviated from their habitual diet and lost excessive weight.

The study conformed to the *Declaration of Helsinki* and was approved by the local research ethics committees. All participants provided fully informed written consent.

Protocol

69 patients were randomly assigned on a 1:1 basis using a computer-generated sequence to 16 weeks *supervised exercise* or *conventional counselling* (control group) using SAS v 9.1, PROC PLAN software (Statistical Analysis System Institute, NC, USA). Figure 1 shows the CONSORT diagram.

Supervised Exercise. After a familiarisation session, participants attended the university gymnasium weekly, wearing a heart rate monitor (Polar Electro Oy, Finland) and supervised by a trained exercise physiologist. Training intensity was based on individual heart rate reserve (HRR) ([Maximal HR during cardiorespiratory fitness testing] – [Resting HR]). Participants performed 3/week 30 min moderate (30% HRR) aerobic exercise (treadmill, cross-trainer, bike ergometer, rower) progressing weekly based on HR responses (5/week 45 min at 60% HRR by week 12). Throughout, participants were monitored via the Wellness System^{TM} (Technogym U.K. Ltd., Bracknell, UK), which tracks exercise activity within designated fitness facilities or by repeated telephone or e-mail contact.

No dietary modifications were made, confirmed by standard 3-day food diaries collected immediately before and after the intervention and analysed for macronutrient intake.

Control Group. Participants were provided with advice about the health benefits of exercise in NAFLD but had no further contact with the research team. To minimise disturbance to behaviour, diet and physical activity were not monitored.

Measurements

Measurements were performed before and immediately after the intervention period. After overnight fast, venous blood was taken for measurement of glucose, liver function, lipid profile, adiponectin and leptin.

After full medical history and physical examination, a single person at each centre measured body weight, blood pressure, height, waist (umbilical) and hip (greater trochanter) circumference and performed bioimpedance analysis (Tanita BC-420MA, Tokyo, Japan).

Magnetic resonance methods were as previously described (17). Volumetric analysis of abdominal subcutaneous adipose tissue (SAT) and abdominal visceral adipose tissue (VAT) used whole-body axial T1-weighted fast spin echo scans (10 mm slice, 10 mm gap), the abdominal region being defined

from the slices between the femoral heads, top of liver and lung bases. Proton magnetic resonance spectroscopy (^{1}H MRS) quantified intrahepatocellular lipid (IHCL) and intramyocellular lipid (IMCL) (17). In liver 3 voxels of interest were identified at standardised sites avoiding ducts and vasculature. In skeletal muscle a single voxel was identified in each of the tibialis anterior and soleus muscles, avoiding bone, fascia and neurovascular bundle. Single voxel spectroscopy was conducted at each of these five sites: voxel size was 20×20×20 mm, TE (echo time) 135 msec, TR (repetition time) 1500 msec, with 64 acquisitions. ^{1}H-MR spectra were quantified using the AMARES algorithm in the software package jMRUI-3.0 (18). Data were processed blind. Liver fat is expressed as the percentage of CH_2 lipid signal amplitude relative to water signal amplitude after correcting for T1 and T2 (19), and intramyocellular lipid (IMCL) is expressed as CH_2 lipid amplitude relative to total creatine amplitude after correcting for T1 and T2 (20). NAFLD was defined as mean IHCL > 5·3%, which corresponds in the present units (CH_2/H_20) to the cut off of 5.5% by weight advocated on the basis of a large healthy-population ^{1}H MRS study (21) which took account of tissue density, water content and the relative proton densities of triglyceride and water to express IHCL as % by weight in terms more directly comparable with biochemical measurements. This cutoff is also in accordance with traditional definitions of fatty liver based on biochemical analysis (21). (Any IHCL value expressed here as x% CH_2/H_2O can be converted to y% by weight (i.e. 10 × y mg/g) by using y% = 97.1/[1 + (89.1/x%)], based on assumptions and data detailed in (21, 22))

Clamp. Participants were instructed to avoid strenuous physical activity for 48 h. Upon arrival intravenous cannulae were inserted into both antecubital fossae for blood sampling and infusion of stable isotopes, insulin and glucose. After unenriched blood samples, a primed infusion of [6,6-$^2H_2$] glucose (170 mg; 1.7 mg.min^{-1}) was started. 5 baseline samples were taken from 100-120 min, when a 2-step hyperinsulinaemic–euglycaemic clamp commenced: insulin infusion at 0.3 mU.kg^{-1}.min^{-1} (low-dose) for 120 min to measure insulin sensitivity of hepatic glucose production (HGP), then at 1.5 mU.kg^{-1}.min^{-1} (high-dose) for 180 min to measure insulin sensitivity of peripheral glucose uptake. Euglycaemia was maintained by adjusting a 20% glucose infusion, spiked with [6,6-$^2H_2$] glucose (7 mg.g^{-1} glucose for low-dose, 10 mg.g^{-1} high dose) according to 5 min plasma glucose measurements using a glucose oxidase method (Yellow Springs Analyser). Blood samples were taken every 30 min, except for every 5 min from 210-240 min (low-dose steady-state) and 390-420 min (high-dose steady-state).

Plasma glucose concentration and enrichment time-courses were smoothed using optimal segments analysis (23). HGP and glucose uptake (rate of disappearance, Rd) (μmol.kg^{-1}.min^{-1}) were calculated using non-steady-state equations (24), assuming a volume of distribution of 22% body weight. HGP was calculated at steady-state basally (90-120 min) and following low-dose insulin (210-240 min), corrected for fat-free mass and (since HGP is inversely related to [insulin]) multiplied by mean steady-state [insulin] (pmol.ml^{-1}) at low-dose. Glucose Rd was calculated at steady-state following

high-dose insulin (390-420 min) and metabolic clearance rate (MCR) ($ml.kg^{-1}.min^{-1}$) was calculated at basal and high-dose insulin steady-state (390-420 min) as (glucose Rd)/[glucose]. Glucose MCR and Rd were corrected for fat-free mass and (since they are directly related to [insulin]) divided by mean steady-state [insulin] ($pmol.l^{-1}$) at basal and high-dose.

Cardiorespiratory fitness assessment In Liverpool, cardiorespiratory fitness was assessed on a treadmill ergometer following the Bruce protocol (25). Following 2 min warm up at 2.2 km/h on the flat, initial workload was set at 2.7 km/h at 5° grade, then speed and grade increased step-wise every minute. Heart rate and rate of perceived exertion were monitored throughout. VO_{2peak} was calculated from expired gas fractions (Oxycon Pro, Jaegar, Hochberg, Germany) as the highest consecutive 15 s rate in the last minute before volitional exhaustion, or when heart rate and/or VO_2 reached a plateau (21). In Guildford, VO_{2peak} was performed on an electronically-braked bicycle ergometer (Lode; Excaliber Sport, Groningen, the Netherlands) with breath analyser (Medical Graphics, St Paul, MN, USA). Heart rate was measured throughout. After 2 min warm up at 50 W, resistance increased step-wise at 20 W/min until volitional exhaustion (26). Cardiorespiratory fitness was defined as VO_{2peak} identically at each facility (despite the different exercise modalities), expressed per kg body weight.

Biochemistry. Baseline plasma samples were analysed using an Olympus AU2700 (Beckman Coulter, High Wycombe, UK) in Liverpool and an Advia 1800 Chemistry System (Siemens Healthcare Diagnostics, Frimley UK) in Guildford, with standard proprietary reagents and methods: glucose with hexokinase, total cholesterol and high-density lipoprotein (HDL) with cholesterol esterase/oxidase, triglyceride with glycerol kinase and liver enzymes including alanine aminotransferase (ALT), aspartate aminotransferase (AST) and gamma-glutamyltransferase (GGT) with International Federation of Clinical Chemistry (IFCC) kinetic UV (without pyridoxal phosphate activation). Intra- and inter- assay coefficients of variation were ≤10%. Low-density lipoprotein (LDL) was calculated using the Friedwald formula. At a single centre, serum insulin, plasma adiponectin and leptin were measured by RIA using commercial kits (Millipore Corporation, Billerica, MA; intra-assay CV 6%, 5%, 5% respectively), irisin by ELISA (Phoenix Pharmaceuticals, Inc. Burlingame, CA; intra-assay CV 4.1%), fetuin-A by ELISA (Epitope Diagnostics, Inc. San Diego; intra-assay CV 4.8%) and serum NEFA (Wako Chemicals, Neuss, Germany; inter- assay CV 3.0%). Glucose isotopic enrichment was measured by GC-MS on a HP 5971A MSD (Agilent Technologies, Wokingham, Berks, UK)(27). IR was quantified using HOMA2-IR (28). Indices of *hepatic insulin resistance (Hepatic-IR)* and *adipose tissue insulin resistance (Adipose-IR)* were calculated (29, 30).

Diagnosis of *metabolic syndrome* was based on the National Cholesterol Education Program Adult Treatment Panel III criteria (31). Ten-year cardiovascular risk was calculated using the 10 year Framingham Risk Score (32).

Statistical Analysis

Power calculation. The primary outcome variable was IHCL (% fat/water). Based on mean IHCL of 20%, we considered 30% relative difference between groups to be clinically significant, implying mean IHCL of 20% and 14% in the control and exercise groups respectively. Based on a 2-sample *t*-test, 5% 2-sided significance and standard deviation (SD) of 7.75% from previous studies, 56 patients (28 in each arm) were required to detect this 6% absolute IHCL difference with 80% power (27).

Statistical methods. For the primary comparison of supervised exercise *vs*. control, delta (Δ) change from pre-intervention was calculated and analysed using linear regression (ANCOVA), with pre data as a covariate (33). Linear regression assumptions were assessed using Q-Q plots and scatter plots of studentised residuals versus fitted values. Where linear regression assumptions were not met these were resolved using the natural logarithm transformation. For exploratory and comparison purposes any continuous demographic variable within each group was also estimated using a paired *t*-test. Correlations were quantified using Spearman's Rank correlation coefficient (r_s). Data for continuous demographic variables are presented as median and inter-quartile range (IQR) and changes between supervised exercise compared to control are presented as mean (95% CI). Statistical analyses used Stata 13 (StataCorp. 2013. *Stata Statistical Software: Release 13*. College Station, TX: StataCorp LP). Unless otherwise stated, exact P-values are cited (values of "0.000" are reported as "<0.001"). Results are shown as mean (95% CI).

Results

Baseline characteristics Fifty patients completed the study [*n*=30 exercise (23 males, 7 female) and *n*=20 control (16 males, 4 female)] (Figure 1). The age of the participants was similar in the exercise [50y (46, 58), BMI 30.7 kg/m^2 (29.2,32.9)] vs. control groups [52y (46, 59), BMI 29.7kg/m^2 (28.0,33.8)]. An equal number (*n*=15) completed the exercise in each centre (total exercise=30); 8 controls completed in Liverpool and 12 controls completed in Guildford, Surrey (total controls *n*=20). Pre-intervention characteristics of the groups were similar with respect to age, VO_{2peak}, biochemical and metabolic characteristics, and body composition (Tables 1 and 2).

Changes in dietary intake In the exercise group after 16 weeks, total energy intake and macronutrient composition remained unchanged compared with baseline: energy [0.4 MJ (-0.4, 1.2), *P*=0.40)], protein [0.4 g (-11.6, 12.0), *P*=0.97], carbohydrate [6.4 g (-24.2, 37.0), *P*=0.34], sugars [-9.2 g (-27.2, 30.0), *P*=0.41] and fat [9.8 g (8.5, 22.0), *P*=0.44].

Changes in body composition and biochemistry The primary outcome measure of IHCL in the exercise group was significantly reduced after 16 weeks: 19.4% (14.6, 36.1) vs. 10.1% (6.5, 27.1), but not in the control group: 16.0% (9.6, 32.5) % vs. 14.6 (8.8, 27.3). Supervised exercise mediated a greater IHCL reduction than in the controls [-4.7 % (-9.4, -0.01); *P*<0.05] (Table 2). Changes in ALT, AST and in GGT were not significant.

SAT reduction with exercise was significantly greater than with control [-1.8L (= -3.0, -0.7); *P*=0.003], but changes in VAT were not [-0.7L (-1.6, 0.2); *P*<0.109], and nor were changes in IMCL in soleus and tibialis anterior (Table 1).

The changes in fasting plasma insulin and HOMA2-IR [-0.5 (-1.0, 0.02; *P*=0.06] with exercise were not significantly different compared with control, nor were those in adiponectin, leptin, irisin or fetuin (Table 2).

Changes in cardiorespiratory fitness Cardiorespiratory fitness (expressed as ml/kg/min) significantly improved in the exercise group after 16 weeks: 23.7 ml/kg/min (21.7, 27.8) vs. 32.3 ml/kg/min (27.6, 38.0); there was no significant increase in the control group: 23.2 ml/kg/min (20.9, 25.6) vs. 23.1 ml/kg/min (20.9, 26.9). Exercise mediated a greater improvement compared to control [7.3 ml/kg/min (5.0, 9.7); *P*<0.001].

Cardiorespiratory fitness (expressed as absolute values in l/min) significantly improved in the exercise group after 16 weeks: 2.45 l/min (2.22, 2.69) vs. 3.05 l/min (2.77, 3.34); there was no significant increase in the control group: 2.31 l/min (2.05, 2.63) vs. 2.30 l/min (2.04, 2.57). Exercise mediated a greater improvement compared to control [0.72 l/min (0.42, 1.02); *P*<0.001].

The greater fitness improvement was accompanied by greater reductions in total body weight [-2.5 kg (-3.9, -1.1); *P*<0.001)], waist circumference [-3.0 cm (-5, -1); *P*<0.05] and percentage fat mass [-1.9% (-3.0, -0.7]; *P*<0.01) compared to control (Table 1). Changes in IHCL were significantly correlated with improvements in cardiorespiratory fitness (absolute and relative), total body weight and with reductions in visceral and subcutaneous fat (Figure 2).

Changes in peripheral and hepatic insulin sensitivity In the subset of 24 patients that underwent the 2-stage hyperinsulinaemic euglycaemic clamp, 12 patients in the exercise group and 7 patients in the controls completed the full clamp measurements. The changes in this exercise and control subset were similar to those seen in the whole group: [Liver fat, -9.3% (-18.1, 0.5) *vs.* 3.5% (-11.1, 3.9)] and VO_{2peak} [7.7ml/kg/min (4.0, 11.1) *vs.* -1.4ml/kg/min (-4.4, 1.6)].

Plasma glucose concentration at basal and during the clamp did not differ between interventions (data not shown). In the exercise group glucose infusion rate, corrected for [insulin], during the high-dose insulin infusion was higher post-exercise (*P*=0.009) (Figure 3a) but did not change in the control group. Following high-dose insulin infusion there was a significant increase in glucose Rd and MCR, corrected for [insulin] in the exercise group (*P*=0.02, *P*=0.004 respectively) with no significant change in the control group (Figure 3b and c). The change in glucose MCR was significantly different between groups (*P*=0.03).

There was no significant difference with either intervention in HGP corrected for [insulin] at baseline or after low-dose insulin, (Figure 3d) or in the percentage decrease in HGP following low-dose insulin

in either the exercise group (pre-exercise 50.9±5.3 %; post-exercise 55.3±6.4 %) or the control group (pre 46.5±10.3 %; post 56.0±8.5 %).

Changes in glucose MCR, corrected for insulin, under basal conditions were significantly correlated with changes in fitness (r_s=0.48; P=0.04) but not in IHCL (r_s=0.26; P=0.28). After high-dose insulin, the correlation with IHCL did not reach statistical significance (r_s=0.43; P=0.18).

Discussion

We have demonstrated in a randomised controlled study that 16 weeks of supervised moderate-intensity aerobic exercise in NAFLD reduces liver fat and that this was correlated with an improvement in cardiorespiratory fitness. Using a 2-step euglycaemic hyperinsulinaemic clamp in conjunction with quantification of liver fat, we showed, for the first time in patients with NAFLD, that the exercise-induced reduction in liver fat was accompanied by enhanced skeletal muscle and adipose tissue insulin sensitivity, with no improvement in hepatic glucose production.

Various factors modulate liver fat, particularly regular physical activity (34, 35). Numerous studies have highlighted the therapeutic effects of endurance or resistance exercise in lowering liver fat in NAFLD, even without weight loss (15). However modest weight loss also has clinically significant effects on IHCL. In a study by Coker *et al.*, measuring multi-organ insulin sensitivity in caloric restriction and exercise training (with and without weight loss), exercise with weight loss had the greatest effect both on visceral fat and hepatic glucose output suppression (36). However, liver fat was not measured, precluding direct comparison with the current study.

In the current study, exercising participants lost ~3% of body weight and this will have contributed to the reduction in IHCL. In a 2-week dietary intervention in NAFLD, ~4% weight reduction was associated with 42% reduction in liver fat (37) while in the LOOK-AHEAD study, lifestyle intervention in T2DM resulting in 1-5% weight change produced 33% reduction in hepatic steatosis (14). While there are clearly weight-dependent effects, the correlation between a reduction in liver fat and improvement in cardiorespiratory fitness in the supervised exercise group suggests that the latter also is a major driver of IHCL levels.

A significant improvement in *peripheral* (skeletal muscle and adipose) insulin sensitivity accompanied the reduction in liver fat following exercise. It is well documented that chronic exercise improves peripheral insulin sensitivity (38, 39). The improvement in peripheral insulin sensitivity following exercise training occurred without any change in intramyocellular lipid as has been shown in a previous study of overweight men (23). Petersen *et al*. (40), proposed that skeletal muscle IR promotes hepatic steatosis and metabolic syndrome, by altering post-prandial energy distribution, diverting glucose to the liver for *de novo* lipogenesis (DNL) and triglyceride synthesis. Furthermore, acute exercise through reversal of muscle IR, has been shown to reduce hepatic DNL by 30% and

hepatic triglyceride synthesis by 40% (41). In myostatin-null mice, increased muscle insulin sensitivity also protects against hepatic steatosis during high-fat feeding (42). Thus, skeletal muscle metabolism may influence hepatic triglyceride content and metabolism, with inter-organ 'cross-talk' between skeletal muscle, adipose tissue and liver (43). Although not measured here, myokines secreted by skeletal muscle after contraction appear to mediate this cross talk. Thus a plausible mechanism in our study for the reduction in liver fat is enhanced peripheral insulin sensitivity and increased skeletal muscle glucose uptake reducing the flux of plasma glucose to the liver for triglyceride synthesis. The critical role of adipose IR in the metabolic and histological changes in NAFLD, as well as its reversal using thiazolidinediones, has also been demonstrated (29, 44). In this study, we showed that adipose-IR could also be improved with exercise training.

The lack of effect of the exercise programme on hepatic insulin resistance was surprising given the assumed links between liver fat accumulation and defective insulin suppression of glucose production (4, 45). Other studies have reported reduced hepatic steatosis and improved hepatic insulin resistance with weight loss following low calorie diets in NAFLD (10,11). However, in these studies liver fat was lower than in the current study and was reduced to normal by weight loss, from 12 to 2.5% (10) and from 12.8 to 2.9% (11). Although in our study there was a comparable loss of liver fat in the exercise group (9.3%) because the group had much higher liver fat levels at baseline (median 19.4%) many patients remained above the normal range after 16 weeks exercise. This suggests that greater reductions in liver fat are needed to improve hepatic insulin resistance, possibly to within the normal range. It is likely that this could be achieved by increasing the period of exercise supervision or the intensity of the exercise, or by caloric restriction (46). Sullivan *et al.* noted a similar dissociation between (reduced) liver fat and (unchanged) VLDL triglyceride synthesis rate, a metabolic pathway that also exhibits resistance to insulin, after exercise training in patients with NAFLD. Interestingly in the latter study, % liver fat was similar at baseline to the current study (5). Recent animal data may help provide a mechanistic explanation for the phenomenon of improved peripheral insulin sensitivity, reduced liver fat but impaired hepatic insulin sensitivity of glucose metabolism. This data suggests that within the liver glucose production and *de novo* lipogenesis have different insulin sensitivities: the gluconeogenic pathway is insulin-resistant (thus insulin cannot inhibit hepatic glucose production through gluconeogenesis) while the lipogenic pathway remains insulin-sensitive (thus insulin retains its ability to stimulate fatty acid synthesis) (47). This selective insulin resistance is explained by a bifurcation of the hepatic insulin signalling pathway: control of the repression of gluconeogenesis occurs through FoxO1, while a separate pathway controlling lipogenesis involves SREBP-1c(48). Although this cannot be tested in the current study, this mechanism would provide a plausible explanation for the dissociation of the effects of exercise on hepatic liver fat and hepatic glucose production.

We acknowledge limitations to the study. We used a *per protocol* analysis. The drop-out rate (19/69, 28%) was higher than the anticipated 15-20%, 15 controls and 4 in the exercise group, apparently mainly for practical reasons (e.g. time constraints, excessive research burden) but we believe the disproportionately higher dropout rate in the control group reflects many participants' underlying desire to be randomised to the exercise program. The higher dropout rate in the control group is, we cautiously argue, unlikely to bias our conclusion, and will of course not affect assessment of the effect of the exercise intervention *per se*. A further imitation is that cardiorespiratory fitness was assessed at study sites using two different modalities, treadmill vs. cycle ergometer. Whilst cardiorespiratory fitness may be lower using cycle ergometry, the primary comparison was the change in fitness with intervention, thus this is unlikely to bias our findings. This is likely due to the greater spread of VO_{2peak} results given the improvements post exercise training. While we believe our cohort is representative of the general NAFLD population, there may be a selection bias with only the most motivated patients consenting to participate in an exercise intervention study: this may underlie the high dropout rate of controls. Accepting these limitations, the noteworthy strengths are the application of whole body MRI and ^{1}H-MRS, the most sensitive, non-invasive method to quantitate liver fat, and measurement of corresponding changes in organ-specific insulin sensitivity. Using these gold standard techniques we provide important insight into the mechanism by which exercise mediates reduction in liver fat by enhanced peripheral (skeletal muscle) insulin sensitivity, without this reduction in liver fat being paralleled by improved hepatic insulin sensitivity.

In summary, in patients with NAFLD exercise-induced reduction in liver fat is related to the improvement in cardiorespiratory fitness and accompanied by an improvement of *peripheral* (muscle and adipose) but not *hepatic* IR. The greatest benefit in normalising liver fat, improving both peripheral and hepatic IR and potentially providing the greatest protection against incident T2DM, may require increasing the duration and/or intensity of the exercise supervision, in conjunction with caloric restriction.

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Declaration of interest

The authors have nothing to declare.

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Author contribution statement

DC, FSM, AMU and GJK conceived and designed the study protocol, obtained funding, were involved in collection and analysis of data and wrote the manuscript. VSS, CJP, HJ, MB, PR, MB, NCJ, ELT and JDB were involved in collection and analysis of data and contributed to the editing of the manuscript.

Clinical Perspectives

- NAFLD represents a common obesity-related complication, increasing the risk of type 2 diabetes mellitus, cardiovascular disease and chronic liver disease. Exercise interventions are effective in reducing liver fat, even without significant weight loss.
- We demonstrate exercise supervision is effective at reducing liver fat and this was related to an improvement in cardiorespiratory fitness. As expected exercise was associated with significant improvements in peripheral (skeletal muscle and adipose tissue) insulin resistance.
- Surprisingly, despite significant reductions in liver fat with exercise, we did not observe an improvement in hepatic insulin resistance. We speculate that persisting elevated liver fat even after exercise training, means undiminished hepatic insulin resistance. Exercise training needs to be more prolonged or more intense to achieve a greater reduction in liver fat. These results have potential public health implications considering the associated long-term metabolic, hepatic and cardiovascular complications.

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Figure legends

Figure 1. CONSORT diagram showing flow of participants through the study.

Figure 2. Black circles indicate individuals in the exercise group; open circles indicate individuals in the control group.

A) Relationship between reduction in liver fat (IHCL) and improvement in cardiorespiratory fitness (VO_{2peak} $ml.kg^{-1}.min^{-1}$) (r= -0.34; P=0.02)

B) Relationship between reduction in IHCL and reduction in body weight (r=0.48; P<0.001)

C) Relationship between reduction in IHCL and reduction in visceral adipose tissue volume (VAT) (r=0.37; P=0.008).

D) Relationship between reduction in IHCL and reduction in subcutaneous adipose tissue volume (SAT) (r=0.61; P<0.001).

Figure 3. Rates of a) glucose infusion (GINF) during high dose insulin, b) glucose uptake (Rd) during high dose insulin, c) glucose metabolic clearance (MCR) during high dose insulin and d) hepatic glucose production (HGP) during low dose insulin expressed relative to insulin, before (grey bars) and after (black bars) exercise or controls.

Table 1. Clinical, biochemical and MRI-measured body composition in 50 patients before and after supervised exercise intervention (Ex; *n*=30) and control (Con; *n*=20) (reported as *median* and *interquartile range* as within group comparison). *Mean delta changes* with 95% confidence intervals (with significance values) are shown for each intervention and the delta changes are compared (between group comparison). **P*<0.05; ***P*<0.001

	Within-group comparison				**Between-group comparison**			
	Pre Ex Median (IQR)	**Post Ex Median (IQR)**	**Pre Con Median (IQR)**	**Post Con Median (IQR)**	**Ex Δ Change Mean (95 % CI)**	**Con Δ Change Mean (95% CI)**	**Δ Mean (95% CI)**	***P***
Weight (kg)	95.6 (83.8-104)	90.7 (80.1-101.5)	90.4 (86.5-107.5)	90.7 (86.4-108.5)	-2.5 (-3.5, -1.4)**	0.2 (-0.8, 1.1)	-2.5 (-3.9, -1.1)	**0.001**
BMI (kg/m^2)	30.6 (29.0-32.9)	30.0 (27.9-32.0)	29.7 (28.0-33.8)	29.9 (28.0-33.0)	-0.9 (-1.4, -0.5)**	0.02 (-0.5, 0.6)	-1 (-1.3, -0.3)	**0.007**
Waist (cm)	106 (101-112)	103 (95-109)	102 (99-114)	101 (98-114)	-4.1 (-5.8, -2.4)**	-1.01 (-2.45, 0.34)	-3 (-5, -1)	**0.013**
% fat mass	30.4 (25.9-32.1)	28.0 (24.3-29.8)	31.0 (26.5-37.7)	30.7 (25.8-37.0)	-1.6 (-2.4, -0.7)**	0.2 (-0.6, 1.1)	-1.9 (-3.0, -0.7)	**0.002**
Systolic BP (mmHg)	135 (125-142)	129 (121-137)	125 (118-142)	132 (123-143)	-5 (-9, -1)*	1 (-5, 7)	-4. (-10, 1.0)	0.111
Diastolic BP	83 (75-87)	78 (74-82)	82 (72-92)	83 (72-90)	-4 (-7, -0.3)*	-3 (-9, 3)	-2 (-5, 3)	0.456
VO_2peak(ml/kg/min)^	23.7 (21.7-27.8)	32.3 (27.6-38.0)	23.2 (20.9-25.6)	23.1 (20.9-26.9)	7.2 (5.3, 9.1)**	-0.2 (-1.7, 1.3)	7.3 (5.0,9.7)	**<0.001**
ALT^ (U/l)	45 (36-66)	32 (25-44)	47 (29-63)	34 (24-51)	-14 (-23, 5)**	-12(-19, -4)**	0.99 (0.78, 1.20)	0.760
AST^ (U/l)	33 (25-47)	29 (22-35)	31 (23-41)	27 (23-36)	-8 (-12, -3)**	-4 (-8,1)	0.92 (0.79, 1.07)	0.268
GGT^ (U/l)	47 (35-62)	34 (22-48)	42 (28-66)	41 (26-68)	-18 (-29, -7)**	-8(-18, 2)	0.87 (0.74, 1.02)	0.089
Cholesterol (mmol/l)	5.1 (4.7-5.7)	4.8 (4.4-5.3)	5.2 (4.60-5.49)	5.1 (4.53)	-0.19 (-0.38, 0.01)	0.02 (-0.18, 0.22)	-0.20 (-0.49, 0.09)	0.169
Triglycerides (mmol/l)	1.9 (1.4-2.63)	1.7 (1.3-2.2)	1.5 (1.2-2.7)	1.6 (1.4-2.7)	-0.16 (-0.37, 0.04)	0.05 (-0.40, 0.50)	-0.24 (-0.54, 0.07)	0.123
HDL (mmol/l)	1.2 (0.9-1.4)	1.2 (0.9-1.4)	1.2 (0.9-1.3)	1.1 (0.9-1.3)	0.02 (-0.02, 0.06)	0.00 (-0.06, 0.06)	0.03 (-0.04, 0.09)	0.443
LDL (mmol/l)	3.5 (3.0-3.9)	3.2 (2.8-3.5)	3.4 (2.6-3.7)	3.1 (2.5-3.5)	-0.29 (-0.5, -0.1)*	-0.26 (-0.56, 0.03)	0.06 (-0.29, 0.40)	0.745
Chol:HDL ratio	4.6 (4.0-5.1)	4.0 (3.3-5.0)	4.7 (4.0-5.6)	4.6 (4.0-5.2)	0.3 (-0.0-0.5)*	-0.09 (-0.44, 0.27)	-0.21 (-0.61, 0.18)	0.279
Liver fat (% CH_2/water)	19.4 (14.6-36.1)	10.1 (6.5-27.1)	16.0 (9.6-32.5)	14.6 (8.8-27.3)	-9.3 (-13.1, -5.3)*	-2.5 (-6.2, 1.2)	-4.7 (-9.4, 0.01)	**0.05**
VAT (l)	9.8 (8.0-11.7)	8.6 (7.8-9.6)	7.8 (6.9-9.2)	8.0 (6.9-9.1)	-1.0 (-1.6, -0.4)*	-0.2 (-0.8, 0.5)	-0.7 (-1.6, 0.2)	0.109
SAT (l)	23.1 (19.4-32.0)	20.7 (17.5-28.3)	21.7 (19.6-29.1)	23.1 (19.1-29.3)	-1.4 (-2.6, -1.0)*	0.01 (-0.8, 0.9)	-1.8 (-3.0, -0.7)	**0.003**
Abdominal fat (l)	33.2 (29.1-41.0)	29.9 (26.7-37.2)	30.0 (27.5-38.2)	31.9 (27.1-37.5)	-2.8 (-4.0, -1.6)*	-0.15 (-1.6, 1.3)	-2.7 (-4.6, -0.8)	**0.006**
VAT:SAT ratio	0.4 (0.3-0.6)	0.4 (0.3-0.5)	0.4 (0.3-0.4)	0.3 (0.3-0.4)	-0.01 (-0.03, 0.00)	-0.01 (-0.02, 0.01)	0.00 (-0.03, 0.02)	0.853
IMCL Soleus (CH_2/creatine)	12.3 (9.0-16.8)	12.8 (9.2-15.6)	15.5 (11.7-21.8)	15.0 (12.9-21.4)	-0.8 (-2.7, 1.2)	-1.1 (-1.8, 4.1)	-1.9 (-5.0, 1.3)	0.237
IMCL Tibialis Ant.	9.0 (5.6-11.2)	8.6 (6.8-11.6)	7.3 (5.3-9.5)	8.7 (7.1-11.7)	0.2 (-2.3, 2.8)	-0.9 (-9.3, 7.6)	1.0 (0.7, 1.3)	0.848

Within-group comparisons use paired t-tests, $p < 0.05$ being taken as evidence of a significant change pre- to post-intervention: a negative change indicates reduction pre- to post. Between-group comparisons (final two columns) use linear regression (ANCOVA) comparing post-scores between groups correcting for pre-scores, Δ therefore indicates

the difference between post-intervention means after correcting for pre-intervention scores: a negative difference indicates a lower mean for the exercise group compared with control. ^ indicates that a log transformation was necessary to meet the assumptions of linear regression; here, Δ is the ratio of geometric means post-intervention after correcting for pre-intervention scores, a ratio <1 indicating a lower mean in exercise group relative to control.

Table 2. Metabolic measurements in 50 patients before and after supervised exercise intervention (Ex; *n*=30) and control (Con; *n*=20) (reported as *median* and *interquartile range* as within group comparison). *Mean delta changes* with 95% confidence intervals (with significance values) are shown for each intervention and the delta changes are compared (between group comparison). **P*<0.05.

	Within-group comparison				**Between-group comparison**		
	Pre Ex Median (IQR)	**Post Ex Median (IQR)**	**Pre Con Median (IQR)**	**Post Con Median (IQR)**	**Ex Δ Change Mean (95 % CI)**	**Con Δ Change Mean (95% CI)**	**Δ Mean (95% CI)**
Fasting glucose (mmol/l)	5.4 (4.8-6.1)	5.3 (4.9-5.7)*	5.6 (4.8-6.1)	5.5 (5.0-5.8)*	-0.15 (-0.30, 0.00)	-0.2 (-0.3, 0.0)	0.0 (-0.2, 0.2)
Fasting insulin (pmol/l)	131 (96-162)	115 (72-158)*	119(96-193)	130 (95-195)	-22 (-43, -1)	2 (-19, 23)	-26 (-55, 2)
HOMA2-IR	2.5 (1.8-3.0)	2.1 (1.3-2.9)*	2.2 (1.8-3.6)	2.5 (1.8-3.7)	-0.43 (-0.81, -0.05)	0.03 (-0.3, 0.4)	-0.5 (-0.1.0, 0.02)
Fasting FFA (mmol/l)	0.52 (0.45-0.60)	0.42 (0.35-0.59)	0.56 (0.39-0.71)	0.54 (0.42-0.65)	-0.04 (-0.11, 0.03)	-0.03 (-0.08, 0.03)	-0.03 (-0.1, 0.1)
Adipose–IR (mmol/l.pmol/l)	61 (48-88)	50 (30-86)*	55. (47-87)	60 (44-84)	-15 (-27, -2)	-0.5 (-17, 16)	-18 (-36, 0.5)*
Adiponectin (ng/ml)	5950 (3700-8100)	5450 (3550-7650)	6300 (5200-7950)	6650 (4950-9750)	-260 (-790, 269)	259(-543, 1060)	-630(-1497, 238)
Leptin (ng/ml)	9.2 (6.5-12.6)	7.1 (4.3-11.9)*	11.8 (7.0-18.5)	11.8 (6.9-19.0)	-1.7 (-3.0, -0.4)*	-0.3 (-1.5, 1.0)	-1.7 (-3.5, 0.1)
Irisin (ng/ml)	140 (128-171)	129 (121-173)*	140 (128-179)	145 (123-156)	-10.5 (-18.9, -2.1)	-5.4 (-16, 5.1)	-4.7 (-17, 8)
Fetuin-A *(µg/ml)	483 (412-518)	470(397-506)	424 (393.8 - 4780.0)	428 (394-477)	-1.9 (-15.5, 11.6)	-4.0 (27, 19)	-2. (-28, 24)

Within-group comparisons use paired t-tests, *P*<0.05 being taken as evidence of a change pre- to post-intervention: a negative change indicates reduction pre- to post. Between-group comparisons use linear regression (ANCOVA) comparing post scores between groups whilst correcting for pre-scores, therefore indicates the difference between post intervention means after correcting for pre-intervention scores: a negative difference indicates a lower mean for the exercise group compared with control group.