

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

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

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35 **Abstract**

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

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

47 Fifty participants (30 exercise, 20 counselling), 51 y (40, 56), BMI 31 kg/m² (29, 35) with baseline
48 liver fat/water % of 18.8 % (10.7, 34.6) completed the study (12/12 exercise and 7/12 counselling
49 completed the clamp studies). Supervised exercise mediated a greater reduction in liver fat/water %
50 than counselling [Δ mean change 4.7% (0.01, 9.4); $P<0.05$], which correlated with the change in
51 cardiorespiratory fitness ($r = -0.34$, $P = 0.0173$).

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

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

57

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

59

60 **Summary statement**

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

64

65 **Introduction**

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

71 Although certain anti-diabetes agents reduce liver fat (6, 7), the cornerstone of therapy is lifestyle
72 modification through dietary intervention and/or physical activity (8, 9). Weight loss through dietary
73 intervention has been shown to normalise moderate hepatic steatosis (12-13%) and hepatic IR (10,
74 11). Considering that NAFLD patients tend to engage in less habitual leisure-time physical activity
75 and be more sedentary, physical activity is also recommended (12, 13). Various modalities of exercise
76 have been shown to be beneficial in reducing liver fat in NAFLD including aerobic (5, 14, 15) and
77 resistance exercise (13), even without weight loss. A recent study addressing the dose-response
78 relationship between aerobic exercise and reduction in liver fat suggests that even low volume, low
79 intensity aerobic exercise can reduce liver fat without clinically significant weight loss (16). It is
80 unclear to what extent reduction in liver fat following exercise is associated with improvements in
81 hepatic and peripheral IR. This is of particular importance considering the high rates of incident type
82 2 diabetes mellitus (T2DM) in NAFLD patients.

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

87 **Experimental materials and Methods**

88 *Design*

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

92 *Participants*

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

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

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

105 *Protocol*

106 69 patients were randomly assigned on a 1:1 basis using a computer-generated sequence to 16 weeks
107 *supervised exercise* or *conventional counselling* (control group) using SAS v 9.1, PROC PLAN
108 software (Statistical Analysis System Institute, NC, USA). Figure 1 shows the CONSORT diagram.

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

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

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

122 *Measurements*

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

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

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

132 from the slices between the femoral heads, top of liver and lung bases. Proton magnetic resonance
133 spectroscopy (^1H MRS) quantified intrahepatocellular lipid (IHCL) and intramyocellular lipid (IMCL)
134 (17). In liver 3 voxels of interest were identified at standardised sites avoiding ducts and vasculature.
135 In skeletal muscle a single voxel was identified in each of the tibialis anterior and soleus muscles,
136 avoiding bone, fascia and neurovascular bundle. Single voxel spectroscopy was conducted at each of
137 these five sites: voxel size was $20 \times 20 \times 20$ mm, TE (echo time) 135 msec, TR (repetition time) 1500
138 msec, with 64 acquisitions. ^1H -MR spectra were quantified using the AMARES algorithm in the
139 software package jMRUI-3.0 (18). Data were processed blind. Liver fat is expressed as the percentage
140 of CH_2 lipid signal amplitude relative to water signal amplitude after correcting for T1 and T2 (19),
141 and intramyocellular lipid (IMCL) is expressed as CH_2 lipid amplitude relative to total creatine
142 amplitude after correcting for T1 and T2 (20). NAFLD was defined as mean IHCL $> 5.3\%$, which
143 corresponds in the present units ($\text{CH}_2/\text{H}_2\text{O}$) to the cut_off of 5.5% by weight advocated on the basis of
144 a large healthy-population ^1H MRS study (21) which took account of tissue density, water content and
145 the relative proton densities of triglyceride and water to express IHCL as % by weight in terms more
146 directly comparable with biochemical measurements. This cutoff is also in accordance with traditional
147 definitions of fatty liver based on biochemical analysis (21). (Any IHCL value expressed here as x%
148 $\text{CH}_2/\text{H}_2\text{O}$ can be converted to y% by weight (i.e. $10 \times y$ mg/g) by using $y\% = 97.1/[1 + (89.1/x\%)]$,
149 based on assumptions and data detailed in (21, 22))

150 *Clamp*. Participants were instructed to avoid strenuous physical activity for 48 h. Upon arrival
151 intravenous cannulae were inserted into both antecubital fossae for blood sampling and infusion of
152 stable isotopes, insulin and glucose. After unenriched blood samples, a primed infusion of $[6,6\text{-}^2\text{H}_2]$
153 glucose (170 mg; $1.7 \text{ mg}\cdot\text{min}^{-1}$) was started. 5 baseline samples were taken from 100-120 min, when a
154 2-step hyperinsulinaemic–euglycaemic clamp commenced: insulin infusion at $0.3 \text{ mU}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ (low-
155 dose) for 120 min to measure insulin sensitivity of hepatic glucose production (HGP), then at 1.5
156 $\text{mU}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ (high-dose) for 180 min to measure insulin sensitivity of peripheral glucose uptake.
157 Euglycaemia was maintained by adjusting a 20% glucose infusion, spiked with $[6,6\text{-}^2\text{H}_2]$ glucose (7
158 $\text{mg}\cdot\text{g}^{-1}$ glucose for low-dose, 10 $\text{mg}\cdot\text{g}^{-1}$ high dose) according to 5 min plasma glucose measurements
159 using a glucose oxidase method (Yellow Springs Analyser). Blood samples were taken every 30 min,
160 except for every 5 min from 210-240 min (low-dose steady-state) and 390-420 min (high-dose steady-
161 state).

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

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

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

183 *Biochemistry.* Baseline plasma samples were analysed using an Olympus AU2700 (Beckman Coulter,
184 High Wycombe, UK) in Liverpool and an Advia 1800 Chemistry System (Siemens Healthcare
185 Diagnostics, Frimley UK) in Guildford, with standard proprietary reagents and methods: glucose with
186 hexokinase, total cholesterol and high-density lipoprotein (HDL) with cholesterol esterase/oxidase,
187 triglyceride with glycerol kinase and liver enzymes including alanine aminotransferase (ALT),
188 aspartate aminotransferase (AST) and gamma-glutamyltransferase (GGT) with International
189 Federation of Clinical Chemistry (IFCC) kinetic UV (without pyridoxal phosphate activation). Intra-
190 and inter- assay coefficients of variation were $\leq 10\%$. Low-density lipoprotein (LDL) was calculated
191 using the Friedwald formula. At a single centre, serum insulin, plasma adiponectin and leptin were
192 measured by RIA using commercial kits (Millipore Corporation, Billerica, MA; intra-assay CV 6%,
193 5%, 5% respectively), irisin by ELISA (Phoenix Pharmaceuticals, Inc. Burlingame, CA; intra-assay
194 CV 4.1%), fetuin-A by ELISA (Epitope Diagnostics, Inc. San Diego; intra-assay CV 4.8%) and serum
195 NEFA (Wako Chemicals, Neuss, Germany; inter- assay CV 3.0%). Glucose isotopic enrichment was
196 measured by GC-MS on a HP 5971A MSD (Agilent Technologies, Wokingham, Berks, UK)(27). IR
197 was quantified using HOMA2-IR (28). Indices of *hepatic insulin resistance (Hepatic-IR)* and *adipose*
198 *tissue insulin resistance (Adipose-IR)* were calculated (29, 30).

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

202 *Statistical Analysis*

203 *Power calculation.* The primary outcome variable was IHCL (% fat/water). Based on mean IHCL of
204 20%, we considered 30% relative difference between groups to be clinically significant, implying
205 mean IHCL of 20% and 14% in the control and exercise groups respectively. Based on a 2-sample *t*-
206 test, 5% 2-sided significance and standard deviation (SD) of 7.75% from previous studies, 56 patients
207 (28 in each arm) were required to detect this 6% absolute IHCL difference with 80% power (27).

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

220 **Results**

221 *Baseline characteristics* Fifty patients completed the study [$n=30$ exercise (23 males, 7 female) and
222 $n=20$ control (16 males, 4 female)] (Figure 1). The age of the participants was similar in the exercise
223 [50y (46, 58), BMI 30.7 kg/m² (29.2,32.9)] vs. control groups [52y (46, 59), BMI 29.7kg/m²
224 (28.0,33.8)]. An equal number ($n=15$) completed the exercise in each centre (total exercise=30); 8
225 controls completed in Liverpool and 12 controls completed in Guildford, Surrey (total controls $n=20$).
226 Pre-intervention characteristics of the groups were similar with respect to age, VO_{2peak}, biochemical
227 and metabolic characteristics, and body composition (Tables 1 and 2).

228 *Changes in dietary intake* In the exercise group after 16 weeks, total energy intake and macronutrient
229 composition remained unchanged compared with baseline: energy [0.4 MJ (-0.4, 1.2), $P=0.40$],
230 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,
231 30.0), $P=0.41$] and fat [9.8 g (8.5, 22.0), $P=0.44$].

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

237 SAT reduction with exercise was significantly greater than with control [-1.8L (= -3.0, -0.7);
238 $P=0.003$], but changes in VAT were not [-0.7L (-1.6, 0.2); $P<0.109$], and nor were changes in IMCL
239 in soleus and tibialis anterior (Table 1).

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

243 *Changes in cardiorespiratory fitness* Cardiorespiratory fitness (expressed as ml/kg/min) significantly
244 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,
245 38.0); there was no significant increase in the control group: 23.2 ml/kg/min (20.9, 25.6) vs. 23.1
246 ml/kg/min (20.9, 26.9). Exercise mediated a greater improvement compared to control [7.3 ml/kg/min
247 (5.0, 9.7); $P<0.001$].

248 Cardiorespiratory fitness (expressed as absolute values in l/min) significantly improved in the exercise
249 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
250 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
251 greater improvement compared to control [0.72 l/min (0.42, 1.02); $P<0.001$].

252 The greater fitness improvement was accompanied by greater reductions in total body weight [-2.5 kg
253 (-3.9, -1.1); $P<0.001$], waist circumference [-3.0 cm (-5, -1); $P<0.05$] and percentage fat mass [-1.9%
254 (-3.0, -0.7); $P<0.01$] compared to control (Table 1). Changes in IHCL were significantly correlated
255 with improvements in cardiorespiratory fitness (absolute and relative), total body weight and with
256 reductions in visceral and subcutaneous fat (Figure 2).

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

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

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

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

273 Changes in glucose MCR, corrected for insulin, under basal conditions were significantly correlated
274 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,
275 the correlation with IHCL did not reach statistical significance ($r_s=0.43$; $P=0.18$).

276 **Discussion**

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

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

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

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

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

315 The lack of effect of the exercise programme on hepatic insulin resistance was surprising given the
316 assumed links between liver fat accumulation and defective insulin suppression of glucose production
317 (4, 45). Other studies have reported reduced hepatic steatosis and improved hepatic insulin resistance
318 with weight loss following low calorie diets in NAFLD (10,11). However, in these studies liver fat
319 was lower than in the current study and was reduced to normal by weight loss, from 12 to 2.5% (10)
320 and from 12.8 to 2.9% (11). Although in our study there was a comparable loss of liver fat in the
321 exercise group (9.3%) because the group had much higher liver fat levels at baseline (median 19.4%)
322 many patients remained above the normal range after 16 weeks exercise. This suggests that greater
323 reductions in liver fat are needed to improve hepatic insulin resistance, possibly to within the normal
324 range. It is likely that this could be achieved by increasing the period of exercise supervision or the
325 intensity of the exercise, or by caloric restriction (46). Sullivan *et al.* noted a similar dissociation
326 between (reduced) liver fat and (unchanged) VLDL triglyceride synthesis rate, a metabolic pathway
327 that also exhibits resistance to insulin, after exercise training in patients with NAFLD. Interestingly in
328 the latter study, % liver fat was similar at baseline to the current study (5). Recent animal data may
329 help provide a mechanistic explanation for the phenomenon of improved peripheral insulin
330 sensitivity, reduced liver fat but impaired hepatic insulin sensitivity of glucose metabolism. This data
331 suggests that within the liver glucose production and *de novo* lipogenesis have different insulin
332 sensitivities: the gluconeogenic pathway is insulin-resistant (thus insulin cannot inhibit hepatic
333 glucose production through gluconeogenesis) while the lipogenic pathway remains insulin-sensitive
334 (thus insulin retains its ability to stimulate fatty acid synthesis) (47). This selective insulin resistance
335 is explained by a bifurcation of the hepatic insulin signalling pathway: control of the repression of
336 gluconeogenesis occurs through FoxO1, while a separate pathway controlling lipogenesis involves
337 SREBP-1c(48). Although this cannot be tested in the current study, this mechanism would provide a
338 plausible explanation for the dissociation of the effects of exercise on hepatic liver fat and hepatic
339 glucose production.

340 We acknowledge limitations to the study. We used a *per protocol* analysis. The drop-out rate (19/69,
341 28%) was higher than the anticipated 15-20%, 15 controls and 4 in the exercise group, apparently
342 mainly for practical reasons (e.g. time constraints, excessive research burden) but we believe the
343 disproportionately higher dropout rate in the control group reflects many participants' underlying
344 desire to be randomised to the exercise program. The higher dropout rate in the control group is, we
345 cautiously argue, unlikely to bias our conclusion, and will of course not affect assessment of the effect
346 of the exercise intervention *per se*. A further imitation is that cardiorespiratory fitness was assessed at
347 study sites using two different modalities, treadmill vs. cycle ergometer. Whilst cardiorespiratory
348 fitness may be lower using cycle ergometry, the primary comparison was the change in fitness with
349 intervention, thus this is unlikely to bias our findings. This is likely due to the greater spread of
350 VO_{2peak} results given the improvements post exercise training. While we believe our cohort is
351 representative of the general NAFLD population, there may be a selection bias with only the most
352 motivated patients consenting to participate in an exercise intervention study: this may underlie the
353 high dropout rate of controls. Accepting these limitations, the noteworthy strengths are the application
354 of whole body MRI and 1H -MRS, the most sensitive, non-invasive method to quantitate liver fat, and
355 measurement of corresponding changes in organ-specific insulin sensitivity. Using these gold
356 standard techniques we provide important insight into the mechanism by which exercise mediates
357 reduction in liver fat by enhanced peripheral (skeletal muscle) insulin sensitivity, without this
358 reduction in liver fat being paralleled by improved hepatic insulin sensitivity.

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

366

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370 and lipoprotein metabolism in patients with NAFLD.

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372 **Declaration of interest**

373 The authors have nothing to declare.

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375

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378

379 **Author contribution statement**

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

384

385 **Clinical Perspectives**

386 • NAFLD represents a common obesity-related complication, increasing the risk of type 2 diabetes
387 mellitus, cardiovascular disease and chronic liver disease. Exercise interventions are effective in
388 reducing liver fat, even without significant weight loss.

389 • We demonstrate exercise supervision is effective at reducing liver fat and this was related to an
390 improvement in cardiorespiratory fitness. As expected exercise was associated with significant
391 improvements in peripheral (skeletal muscle and adipose tissue) insulin resistance.

392 • Surprisingly, despite significant reductions in liver fat with exercise, we did not observe an
393 improvement in hepatic insulin resistance. We speculate that persisting elevated liver fat even after
394 exercise training, means undiminished hepatic insulin resistance. Exercise training needs to be
395 more prolonged or more intense to achieve a greater reduction in liver fat. These results have
396 potential public health implications considering the associated long-term metabolic, hepatic and
397 cardiovascular complications.

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550 **Figure legends**

551

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

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

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

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

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

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

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

566

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)	<i>P</i>
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 ₂ peak(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 /creatinine)	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. \wedge 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 *(μ g/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.