Exercise-induced improvements in liver fat and endothelial function are not sustained 12 months following cessation of exercise supervision in non-alcoholic fatty liver disease (NAFLD)

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Running title: Liver fat, endothelial function and exercise in NAFLD

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Word Count (abstract): 218

Word count (excluding abstract, figures, tables and references): 1291

This study was funded by the European Federation for the Study of Diabetes (EFSD).

Clinicaltrials.gov identifier: NCT01834300

The authors have nothing to declare.

Abstract

Aims Supervised exercise reduces liver fat and improves endothelial function, a surrogate of cardiovascular disease risk, in non-alcoholic fatty liver disease (NAFLD). We hypothesised that after a 16-week supervised exercise program, patients would maintain longer-term improvements in cardiorespiratory fitness, liver fat and endothelial function.

Matherials and Methods. Ten NAFLD patients [5/5 males/females, age 51±13years, BMI 31±3kg.m^2 (mean±SD)] underwent a 16-week supervised moderate-intensity exercise intervention. Biochemical markers, cardiorespiratory fitness (VO_{2peak}), subcutaneous, visceral and liver fat (measured by magnetic resonance imaging and spectroscopy respectively) and brachial artery flow-mediated dilation (FMD) were assessed at baseline, after 16 weeks supervised training and 12-months after ending supervision.

Results Despite no significant change in body weight, there were significant improvements in VO_{2peak} [6.5ml.kg^{-1}.min^{-1} (95% CI 2.8, 10.1); *P*=0.003], FMD [2.9% (1.5, 4.2); *P*=0.001], liver transaminases (P<0.05) and liver fat [-10.1% (-20.6, 0.5); *P*=0.048] immediately after the 16-weeks supervised training. Nevertheless, 12-months after ending supervision, VO_{2peak} [0.9ml.kg^{-1}.min^{-1} (-3.3 5.1); *P*=0.65], FMD [-0.07% (-2.3, 2.2); *P*=0.95], liver transaminases (*P*>0.05) and liver fat [1.4% (-13.0, 15.9); *P*=0.83] were not significantly different from baseline.

Conclusions Twelve months following cessation of supervision, exercise-mediated improvements in liver fat and other cardiometabolic variables had reversed with cardiorespiratory fitness at baseline levels. Maintenance of high cardiorespiratory fitness and stability of body weight are critical public health considerations for the treatment of NAFLD.

Introduction

Non-alcoholic fatty liver disease (NAFLD) increases liver-related morbidity and mortality[1], yet cardiovascular disease (CVD) is the leading cause of its mortality[2]. We need effective sustainable interventions to reverse NAFLD and reduce cardiovascular risk. In the absence of approved pharmacological treatment, structured exercise and/or dietary modification are recommended first-line treatment in NAFLD[3]. The cardiometabolic benefits of supervised exercise, which include reduced liver fat, enhanced peripheral insulin sensitivity and microvascular and conduit-artery endothelial function [4, 5], do not require weight loss. Parallel improvements in liver fat and cardiac structure and function [6] emphasise the role of exercise as an intervention to reduce both hepatic and CVD risk.

We hypothesised that after a 16-week supervised exercise program, patients would maintain the longer-term improvements in cardiorespiratory fitness, liver fat and endothelial function. To test this we re-examined a subset of previously-reported patients [4, 5] a year after ending exercise supervision.

Methods

At baseline, NAFLD was diagnosed by a hepatologist based on raised transaminases (after exclusion of secondary causes) with confirmation of elevated liver fat (≥5.5%) by magnetic resonance spectroscopy (^{1}H MRS). All participants were physically inactive (<2 h/week low-intensity physical activity) Caucasians, with no history of excessive alcohol intake (males <21, females <14 units/week); normotensive, normoglycaemic non-smokers with no contraindications to exercise; females were post-menopausal.

Patients who completed a 16-week structured and supervised exercise intervention were offered the opportunity to repeat assessments 12-months later. From the original study cohort, 10 patients who completed the exercise intervention [4, 5] (5 males, 5 females; 51±13y; BMI

86 31±3kg.m^{-2}) underwent repeat assessments 12-months later. All participants remained with
87 similar alcohol intake and as normotensive, normoglycaemic non-smokers. Liverpool Central
88 Research Ethics Committee approved the study, and all participants gave written informed
89 consent.
90 Measurements were performed fasted at baseline, after 16-weeks supervised exercise training
91 and 12-months after its end[5]. Anthropometric measurements were taken and blood samples
92 collected for plasma glucose, lipid profiles and liver enzymes.

93 Magnetic resonance scanning at 1.5T was as previously described[5]. Abdominal visceral
94 (VAT) and subcutaneous adipose tissue (SAT) were calculated from whole-body axial T1-
95 weighted fast spin echo scans. Total abdominal adipose tissue (AT) = VAT + SAT. Liver fat
96 was measured using ^{1}H MRS and expressed as % CH_2 lipid amplitude relative to water signal.

97 High-resolution ultrasound (Terason, t3000, Aloka, UK) was used to image the brachial
98 artery after 30min supine rest. Endothelial-dependent function was assessed as flow-mediated
99 dilation (FMD): brachial artery diameter, flow and shear stress were measured before and
100 after 5min forearm cuff inflation, and FMD is peak artery diameter following hyperaemia,
101 expressed as % increase using an allometric model. Endothelium-independent function was
102 assessed by imaging 1min before and 10min after sublingual (400 μg) glyceryl trinitrate
103 (GTN)[7].

104 Cardiorespiratory fitness[5] was assessed on a treadmill ergometer, initially 2.7 km.h^{-1} at 5°
105 gradient, with step-wise increments every minute. VO_{2peak} was calculated from expired gas
106 (Oxycon Pro, Jaegar, Germany) as the highest consecutive 15s periods of oxygen uptake in
107 the last minute before exhaustion. No self-reported or objective assessment of physical
108 activity and/or exercise was made following the cessation the 16-week structured exercise
109 intervention.

For the exercise training intervention, an exercise physiologist provided supervision and guidance. Based upon individual basal fitness, participants underwent 30min moderate intensity aerobic exercise 3 times/week at 30% heart rate reserve (HRR), progressing weekly based on HR responses in the initial 4-weeks. Intensity increased to 45% HRR for the following 4-weeks, until week 8, where HRR remained at 45% but each session increased to 45min. From week 12, participants were exercising 5 times/week for 45min at 60% HRR. Upon completion of the supervised exercise patients had no contact from the research team for 12-months.

A general linear model with repeated measures was employed to evaluate differences between baseline, immediate and 12-months post-training data. Analyses were performed using SPSS 21.0 (SPSS, Chicago, Illinois). All data in the text, figure and table, including changes, are presented as mean (95% confidence intervals), except age and BMI (presented as mean and standard deviation). Intra-observer coefficients of variation for measurements of liver fat and FMD were 6.0 [8] and 6.7 % [9], respectively.

Results

Body weight did not change significantly from baseline over the training period [change = -1.9kg (-1.5, 5.2); *P*=0.29], or 12-months following its completion [-0.2kg, (-3.6, 3.1); *P*=0.90; Figure 1].

VO_{2peak} increased [6.5ml.kg^{-1}.min^{-1} (95% CI 2.8, 10.1); *P*=0.003] and waist circumference decreased [-6cm (-9, -2); *P*=0.004] following training, but had returned to baseline 12-months later [0.9ml.kg^{-1}.min^{-1} (-3.3, 5.1); *P*=0.67; Figure 1 & -1cm (-7, 5); *P*=0.60; Table 1 respectively].

Liver fat [-10.1% (-20.6, 0.5); *P*=0.048], ALT [-20u/L (-41, 1); *P*=0.05] and AST [-11u/L (-21, -1); *P*=0.04] decreased following training but had returned to baseline 12-months later [1.4% (-13.0, 15.9); *P*=0.83]; Figure 1; 10u/L (-21, 41); *P*=0.48 & 2u/l (-11, 16); *P*=0.70; Table 1 respectively]. There were no significant changes in VAT, SAT or total AT (*P*>0.20; Table 1).

FMD improved [2.9% (1.5, 4.2); *P*=0.001] following training, but had returned to baseline 12 months later [-0.07% (-2.3, 2.2); *P*=0.95; Figure 1]. There were no significant differences in endothelium-independent (GTN-mediated) dilation (*P*=0.74; Table 1).

Patients who lost the most weight during the 16-week intervention period had the smallest gain in liver fat between weeks 16 and 68 (*P*=0.03); 1kg reduction in body weight at 16-weeks reduced the change in liver fat by ~4.5% in the following 52-week period.

Conclusion

Longitudinal data suggest that whilst vigorous physical activity can prevent liver fat accumulation, adherence to current national and international physical activity guidelines alone is not sufficient to prevent NAFLD [10]. A recent study demonstrated that 8-weeks aerobic exercise can reduce liver fat, irrespective of exercise volume and intensity [11]. Following 16-weeks of supervised exercise training in the present cohort, liver fat significantly decreased and FMD increased by 2.8%, extrapolated from meta-analysis data to confer a CVD risk reduction of ~17% [12]. Nevertheless, this improvement had disappeared 12-months after cessation of exercise supervision.

To the authors' knowledge, no study to date has undertaken longer-term follow-up of the exercise-induced improvements in liver and vascular health following cessation of

supervision. This study suggests that short-term exercise interventions have only short-term benefits.

By contrast, improvements in liver transaminases, liver fat and insulin resistance observed after a 6-month hypocaloric diet with dietary counselling, were maintained for 17-36 months after ending counselling, despite modest weight regain [13]; but this study did not examine the effects on CVD risk, the leading cause of mortality in NAFLD [2, 14]. In our study, changes in liver fat and FMD were strongly associated with changes in cardiorespiratory fitness, suggesting that maintenance of exercise-induced improvements in cardiometabolic parameters depends upon sustained cardiorespiratory fitness. It therefore appears that exercise and hypocaloric diet interventions modulate liver fat content across different time courses and perhaps via distinct mechanisms. Indeed, as little as 7 consecutive days of 60min treadmill walking improves liver fat and increases insulin sensitivity in obese individuals with NAFLD [15]. These data suggest that an increase in levels of physical activity with exercise training dynamically modulates liver fat, and that to achieve prolonged cardiometabolic benefits, higher levels of fitness must be maintained. Although the patients were counselled on the benefits of exercise and encouraged to maintain their exercise training without further guidance, physical fitness returned to pre-intervention level, suggesting that long-term supervision or alternative strategies of exercise provision are required.

Limitations of this exploratory pilot study include a relatively small patient cohort, and a lack of intermediate post-intervention assessments and measures of insulin resistance. Follow up assessments were based on patient choice and thus there is the possibility of cohort bias.

In summary, whilst 16-weeks of supervised exercise effectively improves liver fat and endothelial function in NAFLD, the cardiometabolic benefit of training is not sustained 1 year after ending supervision. To overcome the NAFLD epidemic we need an effective mechanism to promote long-term maintenance of fitness.

Acknowledgements Thank you to all the patients for their participation in the study**.**

Funding We would like to thank the European Federation for the Study of Diabetes (EFSD) for funding the study.

Duality of interest **Nil**

Contribution statement G.J.K., P.R., A.M.U., D.J.G., N.T.C., H.J., and D.J.C. conception and design of research; C.J.P., V.S.S., F.S.-M., H.J., and D.J.C. performed experiments; C.J.P., V.S.S., G.J.K., F.S.-M., A.M.U., D.J.G., N.T.C., H.J., and D.J.C. analyzed data; C.J.P., V.S.S., G.J.K., P.R., F.S.-M., A.M.U., D.J.G., N.T.C., H.J., and D.J.C. interpreted results of experiments; C.J.P., V.S.S., P.R., H.J., and D.J.C. prepared figures; C.J.P., H.J., and D.J.C. drafted manuscript; C.J.P., V.S.S., G.J.K., P.R., F.S.-M., A.M.U., D.J.G., N.T.C., H.J., and D.J.C. edited and revised manuscript; C.J.P., V.S.S., G.J.K., P.R., F.S.-M., A.M.U., D.J.G., N.T.C., H.J., and D.J.C. approved final version of manuscript.

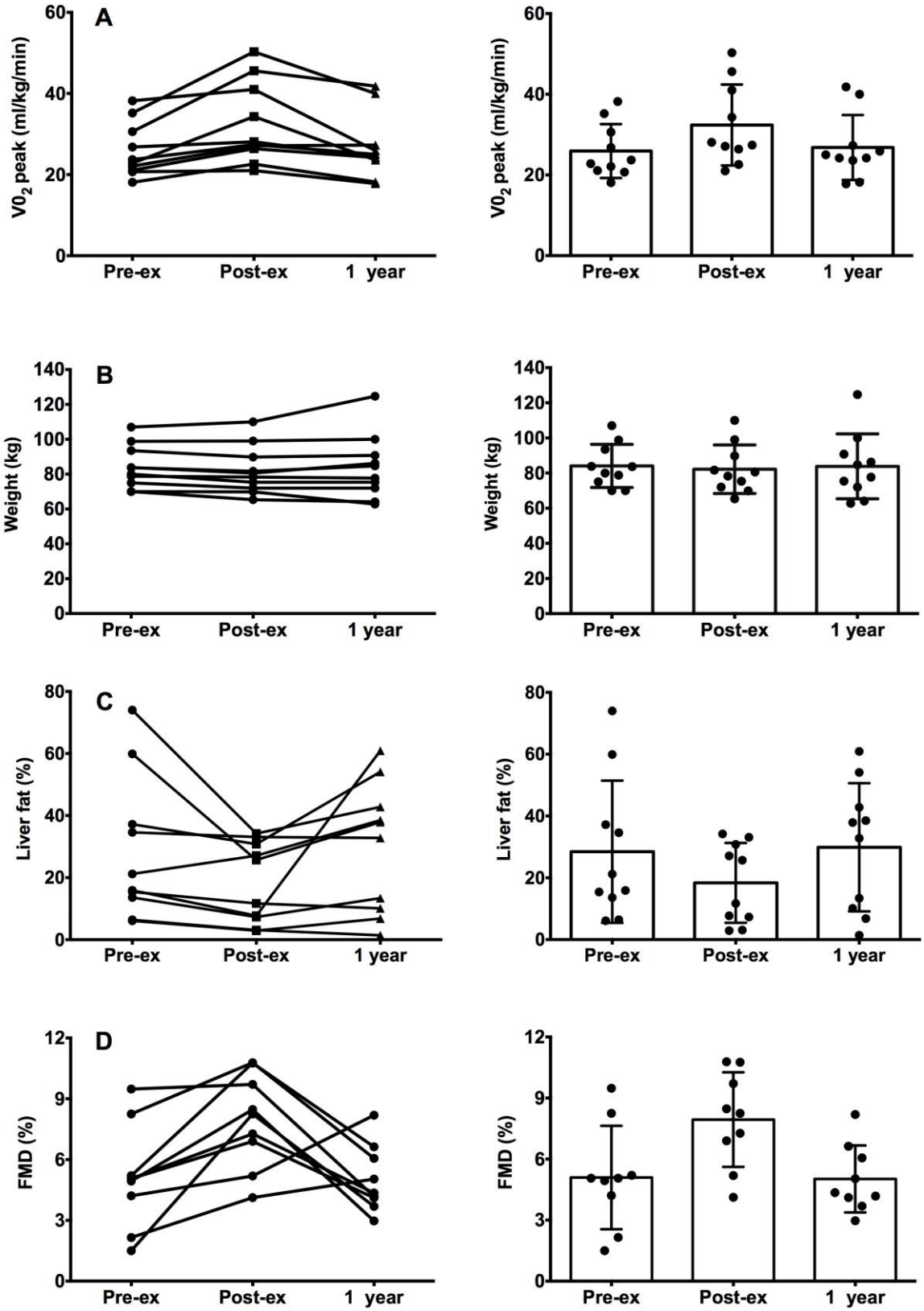

Figure 1 Changes in **A)** cardiorespiratory fitness (VO_{2peak}), **B)** liver fat (%), **C)** flow mediated dilatation (FMD) (%) and **D)** body weight at baseline ('Pre-ex'), following 16 weeks of supervised exercise training ('Post-ex') and 12-months following cessation of exercise supervision ('1 year'). Data are presented as mean (95% CI) and as individual patients' values.

Table 1 Characteristics of NAFLD patients at baseline ('Pre-Ex'), immediately following 16-weeks of supervised exercise training ('Post-ex') and 12 months following ('1 year') the cessation of supervised exercise.

	Pre-Ex	Post-Ex	1 year	*P*
Anthropometrics				
Weight (kg)	84.4(75.6, 93.1)	82.1(72.7, 91.5)	83.8(70.6, 97.0)	0.40
BMI ($kg.m^{-2}$)	30(28, 32)	29(27, 31)	30(27, 33)	0.37
Waist circumference (cm)	103(97, 108)	97(91, 104)†	101(97, 108)‡	0.03
Systolic BP (mmHg)	128(123, 134)	125(120, 130)	129(120,136)	0.23
Diastolic BP (mmHg)	79(74, 85)	76(74, 81)	78(71,85)	0.59
Fitness ($L.min^{-1}$)	2.23 (1.61, 2.85)	2.73 (1.9,3.55)†	2.28 (1.63,2.93)‡	<0.01
Liver Enzymes				
ALT ($u.l^{-1}$)	57(33, 81)	37(25, 48)†	67(40, 94)‡	0.05
AST ($u.l^{-1}$)	39(26, 51)	28(24, 31)†	41(31, 51)‡	0.04
GGT ($u.l^{-1}$)	85(18, 152)	60(18, 103)	68(38, 99)	0.26
Glucose and Lipid Profile				
Glucose ($mmol.l^{-1}$)	5.0(4.6,5.4)	4.9(4.5, 5.3)	5.2(4.7, 5.6)	0.40
Cholesterol ($mmol.l^{-1}$)	5.4(4.6, 6.1)	5.3(4.6, 5.9)	5.7(5.0, 6.5)	0.10
Triglyceride ($mmol.l^{-1}$)	2.0(1.6,2.4)	1.9(1.6,2.2)	1.9(1.4, 2.4)	0.85
HDL ($mmol.l^{-1}$)	1.4(1.2, 1.5)	1.4(1.3, 1.5)	1.5(1.3, 1.7)	0.16
LDL ($mmol.l^{-1}$)	3.1(2.6, 3.6)	3.0(2.4, 3.6)	3.3(2.6, 4.0)	0.12
Chol:HDL ratio	3.8(3.3, 4.4)	3.8(3.1, 4.5)	3.9(3.2, 4.6)	0.89
Adipose tissue deposition				
VAT (l)	5.5(3.9, 7.1)	5.5(4.1, 6.8)	5.0(3.9, 6.0)	0.20
SAT (l)	8.2(6.0, 10.3)	7.7(5.6, 9.8)	7.9(5.0, 10.8)	0.27
Total abdominal AT (l)	13.7(11.3, 16.0)	13.1(11.2, 15.1)	12.8(9.1, 15.5)	0.23
Brachial Artery Function				
GTN-Mediated Dilation (%)	13.5(9.1, 17.8)	14.6(10.1, 19.0)	14.1(10.5, 18.7)	0.74

Data are presented as mean (95% CI). † Significantly different from baseline ($P<0.05$).
‡ Significantly different from immediately following 16 weeks of supervised exercise training ($P<0.05$).
BMI Body mass index, **BP** blood pressure, **ALT** Alanine aminotransferase, **AST** Aspartate aminotransferase, **GGT** Gamma-glutamyltransferase, **HDL** High density lipoprotein, **LDL** Low density lipoprotein, **VAT** Visceral adipose tissue **SAT** Subcutaneous adipose tissue **AT** Adipose tissue **GTN** Glyceryl trinitrate

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