

**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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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.

Materials and Methods. Ten NAFLD patients [5/5 males/females, age 51 ± 13 years, BMI $31 \pm 3 \text{ kg.m}^2$ (mean \pm SD)] underwent a 16-week supervised moderate-intensity exercise intervention. Biochemical markers, cardiorespiratory fitness ($\text{VO}_{2\text{peak}}$), 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 $\text{VO}_{2\text{peak}}$ [$6.5 \text{ ml.kg}^{-1}.\text{min}^{-1}$ (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, $\text{VO}_{2\text{peak}}$ [$0.9 \text{ ml.kg}^{-1}.\text{min}^{-1}$ (-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 mortality¹, yet cardiovascular disease (CVD) is the leading cause of its mortality². 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 NAFLD³. 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 function⁶ 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 ($\geq 5.5\%$) by magnetic resonance spectroscopy (¹H 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 ± 13 y; BMI

31±3kg.m⁻²) underwent repeat assessments 12-months later. All participants remained with similar alcohol intake and as normotensive, normoglycaemic non-smokers. Liverpool Central Research Ethics Committee approved the study, and all participants gave written informed consent.

Measurements were performed fasted at baseline, after 16-weeks supervised exercise training and 12-months after its end⁵. Anthropometric measurements were taken and blood samples collected for plasma glucose, lipid profiles and liver enzymes.

Magnetic resonance scanning at 1.5T was as previously described⁵. Abdominal visceral (VAT) and subcutaneous adipose tissue (SAT) were calculated from whole-body axial T1-weighted fast spin echo scans. Total abdominal adipose tissue (AT) = VAT + SAT. Liver fat was measured using ¹H MRS and expressed as % CH₂ lipid amplitude relative to water signal.

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

Cardiorespiratory fitness⁵ was assessed on a treadmill ergometer, initially 2.7 km.h⁻¹ at 5° gradient, with step-wise increments every minute. VO_{2peak} was calculated from expired gas (Oxycon Pro, Jaegar, Germany) as the highest consecutive 15s periods of oxygen uptake in the last minute before exhaustion. No self-reported or objective assessment of physical activity and/or exercise was made following the cessation the 16-week structured exercise 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 6.0⁸ 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].

$\text{VO}_{2\text{peak}}$ 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.

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Duality of interest Nil

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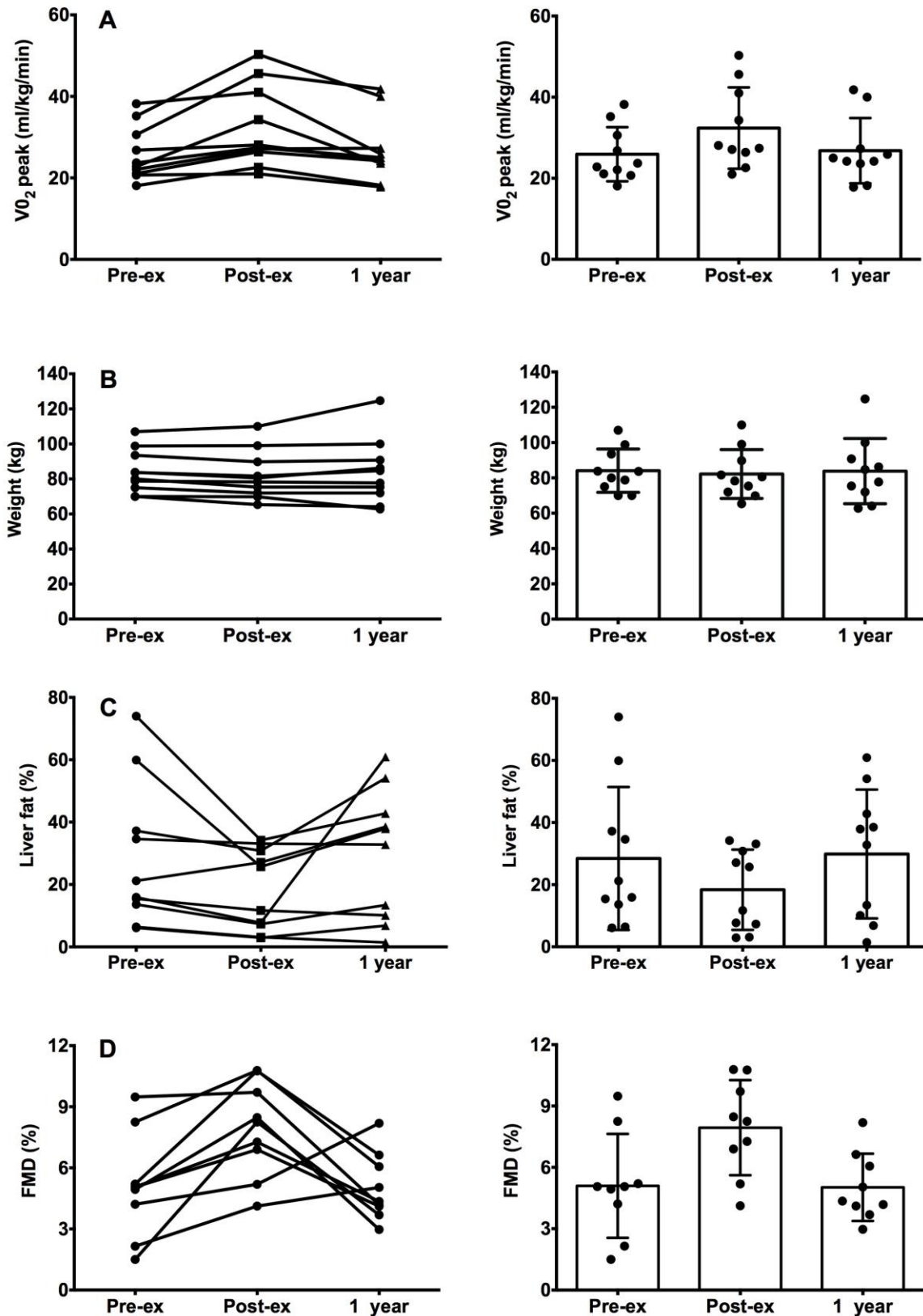


Figure 1 Changes in **A)** cardiorespiratory fitness ($VO_{2\text{peak}}$), **B)** liver fat (%), **C)** flow mediated dilatation (FMD) (%) and **D)** body weight at baseline ('Pre-ex'), following 16 weeks of supervised exercise training ('Post-ex') and 12-months following cessation of exercise supervision ('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

References

1. Adams LA, Lymp Jf Fau - St Sauver J, St Sauver J Fau - Sanderson SO, Sanderson So Fau - Lindor KD, Lindor Kd Fau - Feldstein A, Feldstein A Fau - Angulo P *et al.* The natural history of nonalcoholic fatty liver disease: a population-based cohort study. *Gastroenterology* 2005; **129**(1): 113-21.
2. Targher G, Day CP, Bonora E. Risk of cardiovascular disease in patients with nonalcoholic fatty liver disease. *N Engl J Med* 2010; **363**(14): 1341-50.
3. Harrison SA, Day CP. Benefits of lifestyle modification in NAFLD. *Gut* 2007; **56**(12): 1760-1769.
4. Pugh CJ, Spring VS, Kemp GJ, Richardson P, Shojaee-Moradie F, Umpoleby AM *et al.* Exercise training reverses endothelial dysfunction in nonalcoholic fatty liver disease. *Am J Physiol Heart Circ Physiol* 2014; **307**(9): H1298-306.
5. Cuthbertson DJ, Shojaee-Moradie F, Sprung VS, Jones H, Pugh CJ, Richardson P *et al.* 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. *Clin Sci (Lond)* 2015.
6. Hallsworth K, Thoma C, Hollingsworth KG, Cassidy S, Anstee QM, Day CP *et al.* Modified high-intensity interval training reduces liver fat and improves cardiac function in non-alcoholic fatty liver disease: A randomised controlled trial. *Clin Sci (Lond)* 2015.
7. Sprung VS, Cuthbertson DJ, Pugh CJ, Aziz N, Kemp GJ, Daousi C *et al.* Exercise training in polycystic ovarian syndrome enhances flow-mediated dilation in the absence of changes in fatness. *Med Sci Sports Exerc* 2013; **45**(12): 2234-42.

8. Thomas EL, Hamilton G, Patel N, O'Dwyer R, Dore CJ, Goldin RD *et al.* Hepatic triglyceride content and its relation to body adiposity: a magnetic resonance imaging and proton magnetic resonance spectroscopy study. *Gut* 2005; **54**(1): 122-7.
9. Woodman RJ, Playford DA, Watts GF, Cheetham C, Reed C, Taylor RR *et al.* Improved analysis of brachial artery ultrasound using a novel edge-detection software system. *J Appl Physiol* 2001; **91**(2): 929-37.
10. Lesser IA, Dick T, Gasevic D, Mackey DC, Leipsic JA, Lear SA. The association between physical activity and liver fat after five years of follow-up in a primary prevention multi-ethnic cohort. *Prev Med* 2014; **67**: 199-203.
11. Keating SE, Hackett DA, Parker HM, O'Connor HT, Geroft JA, Sainsbury A *et al.* Effect of aerobic exercise training dose on liver fat and visceral adiposity. *J Hepatol* 2015; **63**(1): 174-82.
12. Inaba Y, Chen JA, Bergmann SR. Prediction of future cardiovascular outcomes by flow-mediated vasodilatation of brachial artery: a meta-analysis. *The international journal of cardiovascular imaging* 2010; **26**(6): 631-40.
13. Haufe S, Haas V, Utz W, Birkenfeld AL, Jeran S, Böhnke J *et al.* Long-lasting improvements in liver fat and metabolism despite body weight regain after dietary weight loss. *Diabetes Care* 2013; **36**(11): 3786-92.
14. Ong JP, Pitts A, Younossi ZM. Increased overall mortality and liver-related mortality in non-alcoholic fatty liver disease. *J Hepatol* 2008; **49**(4): 608-12.
15. Haus JM, Solomon TP, Kelly KR, Fealy CE, Kullman EL, Scelsi AR *et al.* Improved hepatic lipid composition following short-term exercise in nonalcoholic fatty liver disease. *J Clin Endocrinol Metab* 2013; **98**(7): E1181-8.

