



## Research Article

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## A Pilot Randomised Study of the Metabolic and Histological Effects of Exercise in Non-alcoholic Steatohepatitis

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## Abstract

**Aims:** Type 2 diabetes is a risk factor for the development and progression of non-alcoholic fatty liver disease (NAFLD). Lifestyle intervention is the principal treatment for NAFLD however the effects of exercise alone on the histological and metabolic severity of NAFLD are unclear. This study assessed the effects of 6 months exercise training and diet-induced weight loss on insulin resistance and liver histology in overweight patients with NAFLD.

**Methods:** 21 patients were randomised to circuit exercise (EX) training (60 min×3/week) or dietary induced weight loss (DIWL) (-500 kcal/d). Insulin sensitivity (euglycaemic-hyperinsulinemic clamp with tracer), adiposity (CT scan) and histology (liver biopsy) were assessed at 0 and 6 months.

**Results:** Weight decreased by  $9.7 \pm 4.6\%$  ( $-6.7 \pm 6.3$  kg  $p=0.02$ ) with DIWL but was unchanged after EX. Both groups equivalently reduced visceral fat (DIWL  $-22 \pm 24\%$   $p=0.06$  and EX  $-18 \pm 18\%$   $p<0.05$ ) while only EX increased lean mass ( $+3\%$   $p<0.01$ ). DIWL markedly reduced steatosis ( $73 \pm 36\%$  to  $23 \pm 32\%$ ,  $p<0.05$ ) and NAFLD activity score NAS (median (range) 5 (1-7) to 1 (0-5),  $p<0.05$ ). After EX, there was no change in steatosis or NAS. A decrease in steatosis was associated with weight loss ( $r_s=0.82$ ,  $p<0.0001$ ). An improvement in fibrosis was associated with a decrease in steatosis ( $r_s=0.64$ ,  $p=0.02$ ). Small improvements in fasting hepatic insulin resistance were similar in both groups while changes in muscle insulin resistance were not significant.

**Conclusions:** Circuit exercise is safe and efficacious for improving cardiometabolic risk factors in patients with NAFLD, however this dose of circuit training, without concomitant weight loss, was insufficient for histological improvements in NAFLD. The pilot study outcomes should stimulate further development of different exercise protocols (type, frequency and intensity) to address disease-specific conditions in those with severe insulin resistance.

**Keywords:** Exercise; Physical exercise; Weight loss; Metabolic syndrome; Insulin resistance

**Abbreviations:** Adipo-IR: Adipose Tissue Insulin Resistance Index; ALT: Alanine Transaminase; BMI: Body Mass Index; DEXA: Dual-Energy X-ray Absorptiometry; DI: Dietary Intervention; EGP: Endogenous Glucose Production; EX: Exercise Intervention; FFM: Fat Free Mass; GDR: Glucose Disposal Rate; Hepatic-IR: Hepatic Insulin Resistance Index; HDL-cholesterol: High Density Lipoprotein Cholesterol; HMW: High Molecular Weight; IR: Insulin Resistance; NAFLD: Non-alcoholic Fatty Liver Disease; NAS: NAFLD Activity Score; NASH: Non-alcoholic Steatohepatitis; NEFA: Non-esterified Fatty Acids; MRS: Magnetic Resonance Spectroscopy; Ra: Rate of Appearance;  $S_A$ : Ratio of High Molecular Weight Adiponectin to Total Adiponectin; SAT: Subcutaneous Adipose Tissue; TGD: Total Glucose Disposal; VAT: Visceral Adipose Tissue;  $VO_{2\text{peak}}$ : Peak Oxygen Consumption

## Introduction

Non-alcoholic fatty liver disease (NAFLD) is characterised by central obesity and insulin resistance and comprises a spectrum of disease severity from steatosis, to hepatic necro-inflammation and fibrosis termed non-alcoholic steatohepatitis (NASH). NASH is the third most common indication for liver transplantation in the United

States and is predicted to overtake hepatitis C as the most common indication for liver transplantation by 2025 [1]. Patients with NASH are also at high risk for cardiometabolic complications such as type 2 diabetes and cardiovascular disease [2].

Attempts at pharmacotherapy have been disappointing [3] and there is general consensus that lifestyle intervention remains the principal approach to treatment [4,5], but our understanding of the distinct role of exercise on liver injury remains unclear. A recent meta-analysis of exercise protocols in patients with established NAFLD,

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demonstrated that even low-intensity exercise may reduce hepatic steatosis [6]. However, all exercise interventions to date have been short duration ( $\leq 4$  months) and rely on magnetic resonance spectroscopy (MRS) assessment of total hepatic triglyceride content, so effects on disease severity remain unknown [7].

Visceral fat and altered adipokines may contribute to insulin resistance and steatosis [8,9]. Exercise that includes both resistance and aerobic components, such as circuit training, reduce visceral fat while maintaining muscle mass [10]. However, the effect of circuit training on sites of insulin resistance and lipid metabolism is not clear. In addition, if physical activity is shown to benefit NASH, its uptake in this population will depend on acceptance of the type of exercise prescription. Low- to moderate-intensity circuit training may be more appealing to obese patients with limited physical functioning [11].

We hypothesize that circuit exercise training without weight loss will have beneficial effects on liver histology, insulin resistance and body composition. The primary outcome measure is steatosis assessed by histology and the secondary outcome measures include peripheral and hepatic insulin resistance, visceral adiposity and adipokines. This study assessed the histological and metabolic effects of 6 months circuit exercise training (EX) compared with diet-induced weight loss (DIWL) in overweight patients with biopsy-proven NAFLD.

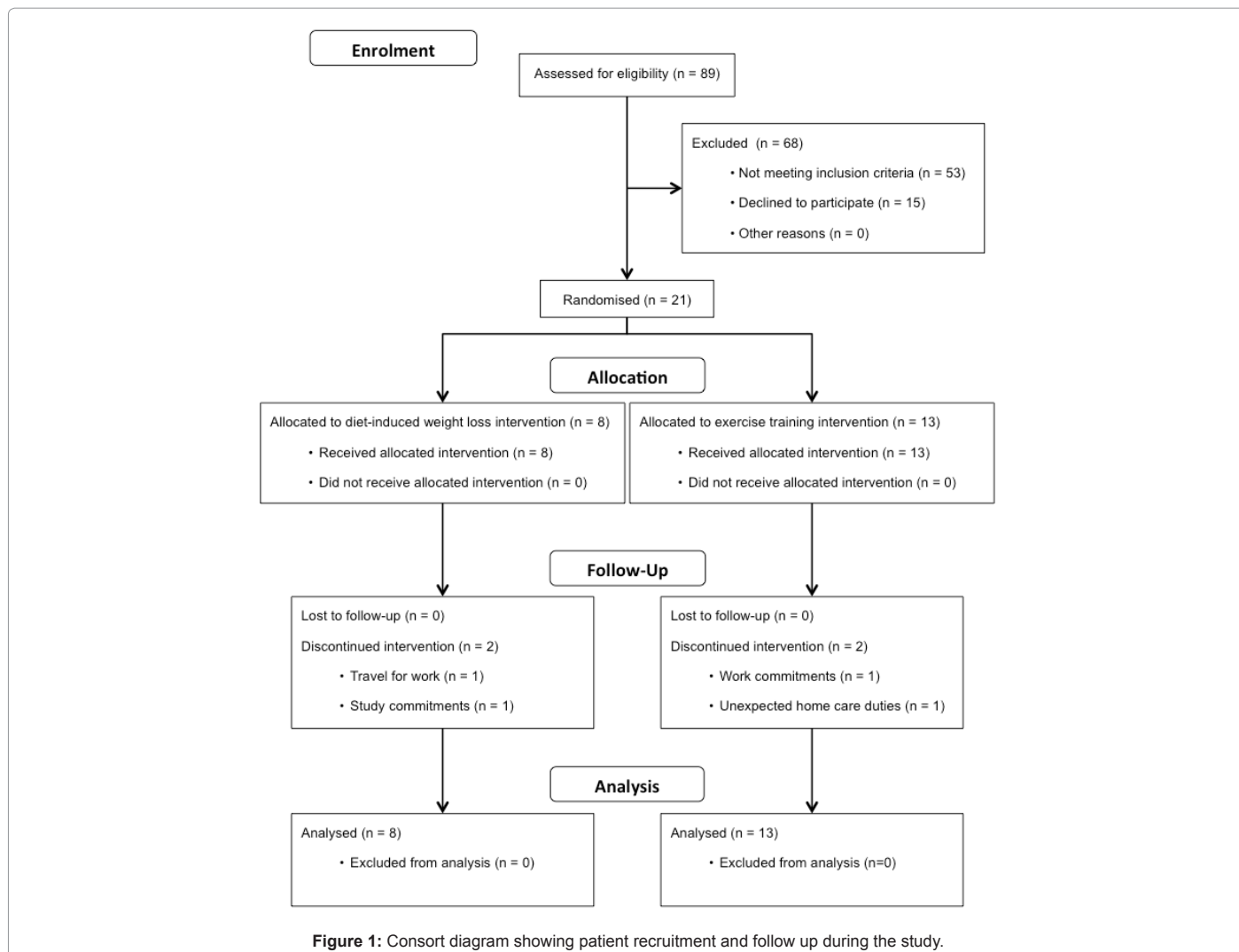
## Patients and Methods

### Ethics statement

The study was approved by the Human Research Ethics Committees of the Princess Alexandra Hospital and the University of Queensland and registered through the Australian and New Zealand Clinical Trials Registry, ACTRN12612001087842. Written informed consent was obtained from all participants and the study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki. Recruitment and follow up occurred between 2009 and 2010. The CONSORT diagram is presented as Figure 1.

### Participants

Twenty-one patients with NAFLD participated in the study, while 16 healthy control subjects acted as a comparator group at baseline only. Patients were recruited from outpatient clinics. Exclusion criteria included the presence of other liver diseases or evidence of cirrhosis, alcohol consumption  $>40$  g/day (males) or  $>20$  g/day (females), and diabetes. Controls were non-smoking, non-obese adults with minimal alcohol intake, normal liver enzymes, no evidence of liver disease and no features of the metabolic syndrome [12]. In individuals meeting these same criteria, the prevalence of steatosis has been shown to be



<5% [13]. Control subjects did not undergo liver biopsy as this was deemed unethical.

### General design

At baseline 21 patients with NAFLD were randomised by the study co-ordinator, using random number generator, into one of two groups; dietary induced weight loss (DIWL) (without changes to physical activity) or a circuit exercise training program (EX) without changes to dietary intake. The exercise program involved 3 sessions per week of circuit exercise training for 6 months, without dietary changes, with the aim to improve physical fitness and muscle strength without significant body weight loss. Exercise was conducted using pneumatic resistance training equipment (AbHurOy, Kokkola, Finland). Each circuit consisted of 15 moderate-intensity resistance exercises covering the main muscular groups. Some machines were bi-functional, allowing participants to exercise two different muscular groups, usually antagonistic. On the bi-functional machines, participants exercised alternatively the agonist or the antagonist muscle. The training program consisted of 30 seconds exercise intervals and 30 seconds rest periods, during which participants moved to the next station and prepared themselves for the following exercise period. A digital timed audio signal was used to indicate the start and the end of the 30 seconds exercise or rest period. Training intensity over the whole duration of the training program was fixed at 50% of 1 repetition maximum (1-RM). Number of circuits, and consequently session duration, was progressively increased from one circuit (12 min) in week one to 5 circuits in week 12 (60 minutes) and was kept constant from week 16 to the end of the training program (week 24). A 1-RM was completed on each of the 15 exercises the week prior to the beginning of the program, and was then reassessed every four weeks to account for any strength adaptations over the course of the training period.

The 6 month dietary-induced weight loss intervention was individually tailored to induce an energy restriction calculated (based on Harris-Benedict predicted energy requirements and sedentary activity factors) for 5 to 10% body weight loss over a 16-week intensive phase whereby patients were reviewed weekly by a Dietician. This was followed by an 8-week weight maintenance phase involving dietetic review every two weeks. Participants were instructed not to change their usual physical activity habits. The intervention was supported by an educational manual, which included information about energy content of food portions, macro- and micronutrient content of individual foods and combination dishes, food label reading, shopping, cooking and eating-out guidelines, motivational tools and goal setting skills and activities. A dietary composition of 40% carbohydrate, 40% fat and 20% protein sources of energy was encouraged, with specific advice on reduced saturated fat, lowering sugar (including reduced sugary drinks), avoiding micronutrient poor/energy dense food options and aiming for regular meal patterns. Weekly weight and waist measures and 24-hour diet recall interviewing assisted compliance and encouraged ongoing self-monitoring. Motivational interviewing and behavioural management techniques were used throughout the program.

### Physical activity and fitness

Physical activity was measured using RT3 accelerometers (StayHealthy Inc., Monrovia, CA) worn for 7 days at months 0, 3 and 6. Activity was categorised according to a vector magnitude of  $\leq 1000$  counts/min (sedentary); 1001-2000 counts/min (moderate) or  $> 2000$  counts/min (vigorous). Peak oxygen consumption ( $VO_{2peak}$ ) was determined with a graded exercise test on cycle ergometer (Excalibur Sport, Lode, Groningen, Netherlands).

### Histological analysis of liver biopsy

Liver biopsy specimens were fixed in 10% neutral buffered formalin, embedded in paraffin, and reviewed and scored in a blinded fashion by an expert hepatopathologist (AC). The proportion of hepatocytes affected by steatosis was estimated as a percentage. The severity of liver injury was assessed using the Clinical Research Network NAFLD activity score (NAS) and fibrosis stage [14]. A diagnosis of steatosis alone or NASH was made using conventional histologic criteria, independent of NAS [15].

### Body composition

Fat mass and fat-free mass (FFM) were measured by dual-energy X-ray absorptiometry (GE Lunar Prodigy enCore 2005, General Electric, Madison, WI). Distribution of abdominal fat (visceral adipose tissue, VAT and subcutaneous adipose tissue, SAT) was determined by computed tomography scan (Philips Brilliance 16, Cleveland, OH) as previously described [16].

### Hyperinsulinemic-euglycemic clamp

Insulin sensitivity was evaluated by a 2-hour hyperinsulinemic-euglycemic clamp [17], with a protocol mirroring that of Bugianesi et al. [18] (detailed in supporting material). All experiments were performed after an overnight fast (8-10 hours) with a minimum of 72 hours since any exercise training and weight stable for minimum of 2 weeks. Basal endogenous glucose production (EGP) was calculated as the glucose rate of appearance (Ra) with a steady-state equation for stable isotope tracers. During the clamp, total glucose Ra was calculated using non steady-state Steele's equation [19]. EGP during the clamp was estimated as the difference between total Ra and the exogenous glucose infusion rate, and percent suppression from basal calculated. Fasting hepatic insulin resistance index (Hepatic-IR) was calculated [20]. Total glucose disposal (TGD), was calculated by adding the mean rate of EGP during hyperinsulinemia to the steady state glucose infusion rate. The peripheral insulin sensitivity index (TGD/I), was calculated by dividing TGD by steady state 2 hr insulin concentrations, multiplied by 100 [17].

### Biochemical analysis

Insulin was assayed using an immunoenzymatic assay with chemiluminescence detection (Beckman Coulter, Brea, CA). Cholesterols and triglycerides were assayed by an enzymatic colorimetric assay (Roche, SanFrancisco CA). Plasma non-esterified fatty acids (NEFA) were measured with an *in vitro* enzymatic colorimetric method (Wako chemicals, Richmond, VA). An index of adipocyte insulin resistance (Adipo-IR) was calculated [21]. Plasma  $\beta$ -hydroxybutyrate, an index of hepatic lipid oxidation [22], was measured enzymatically (Stanbio, Boerne, TX). TNF-alpha was measured by high sensitivity milliplex kit (Millipore, Billerica, MA, USA). HsCRP was measured using an immunoturbidimetric method (Kamiya, Seattle, WA, USA). Total and high molecular weight (HMW) adiponectin was assessed by ELISA (ALPCO (multimeric) kit, ALPCO, Salem, NH, USA).

### Indirect calorimetry

Indirect calorimetry (TrueOne 2400 Metabolic Measurement System, Parvo Medics, UT) was performed under fasted and insulin-stimulated conditions. Substrate oxidation rates were calculated using stoichiometric equations [23].

### Statistical analysis

Sample size calculation based on our previous study of weight loss

(paired biopsies from 10 patients after diet and exercise combined) in patients with chronic liver disease [24], a sample size of 8 subjects will have 80% power to detect a difference in mean steatosis of 1 point with a standard deviation of 0.91, at  $p < 0.05$ . A 25% difference in glucose disposal rate requires 5-8 subjects for within group analysis [25]. All data were expressed as means  $\pm$  standard deviation, or median (range) if not normally distributed. Data were analyzed as intention to treat for all variables except histology, whereby only those undergoing a second biopsy were included in paired analysis. Unpaired t-test or Mann-Whitney U test was used to compare groups at baseline; proportions were compared by the Fischer's exact test. Within group difference after intervention was assessed using a paired t-test or a Wilcoxon matched-pair signed rank test (2 samples) if not normally distributed. Pearson's correlation was used to test for associations between normally distributed variables, while Spearman's rank test was used for variables not normally distributed. A treatment effect was assessed by 2 (group)  $\times$  2 (time) RMANOVA and/or independent t-test of each within group variable change. Multiple linear regressions were used to adjust for confounding variables. P values less than 0.05 were considered statistically significant. All analyses performed using IBM SPSS for Macintosh, version 20.

## Results

### Subject compliance and adverse events

Four patients did not complete the study: two each from DIWL and EX arms due to work, study commitments and unexpected care responsibilities. Attendance to EX was  $>90\%$  with no adverse events. Participants undertook little physical activity at baseline with  $98 \pm 2\%$  of the day spent in sedentary or no activity. The proportion of the day (excluding exercise training) spent in sedentary, moderate or vigorous activity did not change during the study.

### Baseline characteristics

Baseline characteristics (Table 1) of DIWL and EX groups were similar, with marked central obesity and cardiovascular risk factors. Patients demonstrated severe insulin resistance with fasting insulin levels seven times higher, hepatic-IR five times higher and peripheral glucose disposal less than half that of the lean control group. More than 85% of patients met diagnostic criteria for NASH.

### Metabolic response

Aerobic fitness ( $VO_{2peak}$ ) improved by 17% with EX ( $p < 0.0001$ ) but

	Healthy control (n=16) <sup>‡</sup>	NAFLD diet (n=8)		NAFLD exercise (n=13)	
		Pre	Post	Pre	Post
Age (years)	40 $\pm$ 12	45 $\pm$ 14	-	50 $\pm$ 9	-
Gender (M/F)	11/5	4/4	-	9/4	-
BMI (kg/m <sup>2</sup> )	23 $\pm$ 3 <sup>‡</sup>	35 $\pm$ 8	32 $\pm$ 7 *	33 $\pm$ 6	33 $\pm$ 5
Waist (cm)	82 $\pm$ 8 <sup>‡</sup>	111 $\pm$ 17	104 $\pm$ 20 *	114 $\pm$ 18	109 $\pm$ 16**
Body fat (%)	23 $\pm$ 6	38 $\pm$ 8	35 $\pm$ 11	39 $\pm$ 8	34 $\pm$ 7**
Diastolic BP (mmHg)	72 $\pm$ 7 <sup>‡</sup>	80 $\pm$ 9	75 $\pm$ 12	88 $\pm$ 9	84 $\pm$ 10
Systolic BP (mmHg)	115 $\pm$ 8 <sup>‡</sup>	126 $\pm$ 13	117 $\pm$ 13 *	140 $\pm$ 19	138 $\pm$ 18
Fasting glucose (mg/dl)	90 $\pm$ 7 <sup>‡</sup>	95 $\pm$ 7	94 $\pm$ 13	99 $\pm$ 9	95 $\pm$ 7
Fasting insulin ( $\mu$ U/ml)	3 $\pm$ 2 <sup>‡</sup>	19 $\pm$ 15	13 $\pm$ 7	24 $\pm$ 22	15 $\pm$ 10
Fasting triglycerides (mg/dl)	44 $\pm$ 18 <sup>‡</sup>	142 $\pm$ 71	124 $\pm$ 78	177 $\pm$ 97	177 $\pm$ 115
Fasting HDL cholesterol (mg/dl)	53 $\pm$ 12 <sup>‡</sup>	37 $\pm$ 12	40 $\pm$ 11	35 $\pm$ 10	40 $\pm$ 10 **
ALT (U/L)	23 $\pm$ 12 <sup>‡</sup>	70 $\pm$ 58	51 $\pm$ 47	61 $\pm$ 30	53 $\pm$ 34
Patients with the metabolic syndrome n (%)	0 (0)	5 (63)	5 (63)	8 (62)	8 (62)
TNF $\alpha$ (pg/ml)	4.1(1.6-18.3)	5.0(2.1-12.0)	4.5(2.1-6.5)	5.1(3.8-17.3)	5.7(3.5-17.3)
hsCRP (mg/L)	0.6(0.1-3.7) <sup>‡</sup>	2.7(0.5-9.7)	1.7(0.5-9.0)	1.5(0.7-4.8)	1.0(0.2-4.8)
Total adiponectin ( $\mu$ g/ml)	5.6 $\pm$ 3.1 <sup>‡</sup>	3.1 $\pm$ 1.3	3.8 $\pm$ 1.4*	3.6 $\pm$ 1.6	3.4 $\pm$ 1.8
HMMW adiponectin ( $\mu$ g/ml)	2.7 $\pm$ 1.8 <sup>‡</sup>	1.1 $\pm$ 0.8	1.7 $\pm$ 1.1*	1.5 $\pm$ 1.0	1.2 $\pm$ 1.0
<b>Glucose metabolism</b>					
Clamp plasma insulin ( $\mu$ U/ml)	49 $\pm$ 10 <sup>‡</sup>	76 $\pm$ 28	70 $\pm$ 22	81 $\pm$ 26	79 $\pm$ 18
Clamp plasma glucose (mg/dl)	79.2 $\pm$ 7.2	79.2 $\pm$ 7.2	84.6 $\pm$ 7.2	84.6 $\pm$ 7.2	81.9 $\pm$ 7.2
<b>Muscle</b>					
TGD (mg/kg <sub>FFM</sub> /min)	9.1 $\pm$ 2.3 <sup>‡</sup>	4.3 $\pm$ 1.4	5.2 $\pm$ 1.6	4.5 $\pm$ 1.7	5.3 $\pm$ 1.6*
TGD/I (mg/kg <sub>FFM</sub> /min)/(mU/L)	19.8 $\pm$ 7.8 <sup>‡</sup>	6.2 $\pm$ 2.7	7.8 $\pm$ 2.6	6.5 $\pm$ 4.3	7.3 $\pm$ 4.0
Oxidative GDR (mg/kg <sub>FFM</sub> /min)	3.7 $\pm$ 0.7	3.2 $\pm$ 0.9	3.3 $\pm$ 0.8	3.6 $\pm$ 1.7	2.9 $\pm$ 1.1
Non-oxidative GDR (mg/kg <sub>FFM</sub> /min)	5.3 $\pm$ 2.7 <sup>‡</sup>	1.0 $\pm$ 1.0	1.9 $\pm$ 1.4	0.9 $\pm$ 2.3	2.4 $\pm$ 1.3*
<b>Liver</b>					
Fasting EGP ( $\mu$ mol/kg/min)	12.2 $\pm$ 2.7	10.1 $\pm$ 1.0	10.9 $\pm$ 2.0	10.9 $\pm$ 1.7	10.9 $\pm$ 2.3
Hepatic-IR ( $\mu$ mol/kg <sub>FFM</sub> /min/pmol/L)	5.4 $\pm$ 3.4 <sup>‡</sup>	21.1 $\pm$ 16.1	15.6 $\pm$ 8.2	26.2 $\pm$ 21.5	16.4 $\pm$ 11.8
Suppression EGP (%)	94 $\pm$ 11	91 $\pm$ 11	95 $\pm$ 6	86 $\pm$ 12	98 $\pm$ 4*
<b>Lipid metabolism</b>					
Fasting NEFA (mmol/L)	0.61 $\pm$ 0.19	0.62 $\pm$ 0.16	0.64 $\pm$ 0.21	0.56 $\pm$ 0.18	0.60 $\pm$ 0.23
Adipose tissue-IR (mmol/pmol/L)	15.2 $\pm$ 8.3 <sup>‡</sup>	84.3 $\pm$ 69.4	47.4 $\pm$ 24.1	94.3 $\pm$ 90.4	60.3 $\pm$ 42.9
$\beta$ -Hydroxybutyrate (mmol/L)	0.14 $\pm$ 0.07 <sup>‡</sup>	0.08 $\pm$ 0.02	0.10 $\pm$ 0.04*	0.10 $\pm$ 0.03	0.11 $\pm$ 0.06
Fasting whole body fat oxidation (mg/kg/min)	1.01 $\pm$ 0.34 <sup>‡</sup>	0.73 $\pm$ 0.24	0.85 $\pm$ 0.32	0.77 $\pm$ 0.15	0.88 $\pm$ 0.26

Values are mean  $\pm$  SD or median (range) for non-parametric variables. There was no difference between NAFLD groups in any variable at baseline. Intention-to-treat analysis performed for pre- and post-analysis.

<sup>‡</sup>P < 0.01 for independent t-test (or Wilcoxon matched pairs test) comparisons with total NAFLD group at baseline

\*p < 0.05, \*\*p < 0.01 paired t-test for change within group

<sup>†</sup>p < 0.05 for treatment effect between diet and exercise groups assessed by RMANOVA (time  $\times$  group) or independent t-test of delta at 6 months

**Table 1:** Characteristics of subjects at baseline (Pre) and after 6 months intervention (Post).

did not change with DIWL ( $p=0.52$ , Figure 2A). DIWL led to weight loss of  $9.7 \pm 4.6\%$  (Figure 2B), with clinically significant decreases in SAT ( $-15 \pm 15\%$ ,  $p=0.06$ ) and VAT ( $-22 \pm 24\%$ ,  $p=0.06$ ) and a minor decrease in lean body mass ( $-2 \pm 0.4\%$ ,  $p=0.06$ , Figure 2C and D). Weight did not change after EX (Figure 2B), however both SAT ( $-7 \pm 8\%$ ,  $p<0.05$ ) and VAT ( $-18 \pm 18\%$ ,  $p=0.04$ ) decreased while lean mass increased ( $+3 \pm 2\%$ ,  $p<0.01$  Figure 2C and D). The two interventions had differential effects on features of the metabolic syndrome (Table 1): DIWL lowered systolic blood pressure, while EX increased HDL cholesterol. Fasting triglycerides and ALT remained unchanged with both interventions (Table 1).

### Liver histology

At baseline, 18 of the 21 patients had a diagnosis of NASH (median NAS=5, range 1-8). Repeat liver biopsy was performed after DIWL ( $n=5$ ) and EX ( $n=9$ ) (All raw data in Table 2). At baseline, fibrosis correlated with severity of insulin resistance in adipose tissue ( $r=0.59$ ,  $p=0.006$ ) and muscle (TGD  $r=-0.71$ ,  $p=0.001$ ), but not in liver ( $p=0.41$ ).

There was no baseline difference in any clinical variable between those who did and did not have a repeat biopsy. In the subset of patients with repeat biopsy, within group changes in clinical variables pre and post intervention were no different to the full cohort reported except that the reduction in VAT after DIWL became statistically significant ( $-36 \pm 18\%$ ,  $p<0.05$ ) but was not significantly greater than decrease in VAT in patients with a repeat biopsy after EX ( $p=0.23$ ). DIWL was associated with a remarkable improvement in steatosis ( $73 \pm 36\%$  to  $23 \pm 32\%$   $p<0.05$  Figure 3A) with 4 out of 5 (80%) decreasing steatosis by  $\geq 2$  scores. All five (100%) patients with re-biopsy showed improvements in lobular inflammation, while four (80%) showed reduced hepatocyte ballooning (Table 2). The net effect of these changes was a significant reduction in NAS (median (range) 5 (1-7) to 1 (0-5),  $p=0.05$ , Figure 3B). In contrast, the histological response to EX was variable with only small or no improvements in steatosis ( $71 \pm 32\%$  to  $54 \pm 36\%$ ,  $p=0.12$ , Figure 3A), only one out of nine (11%) patients improving steatosis by

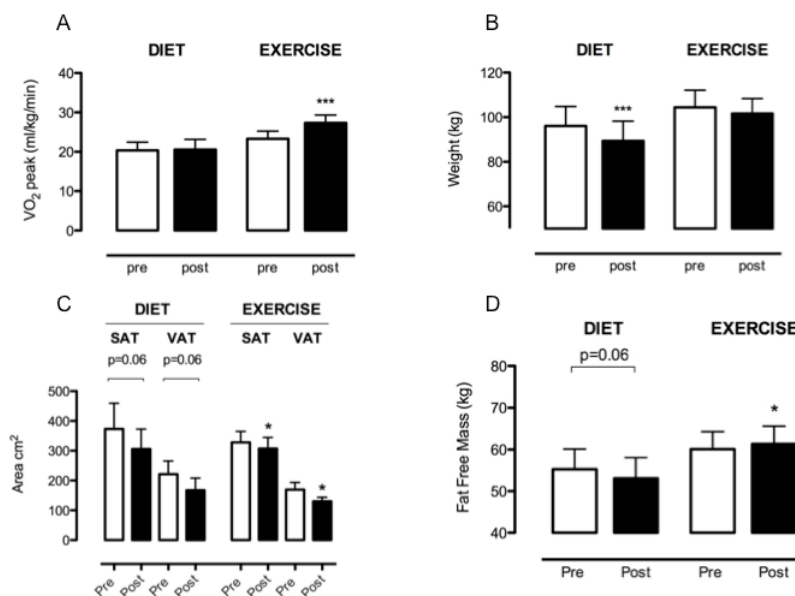
$\geq 2$  scores, only two of the nine patients improving lobular inflammation and therefore no net improvement in the NAS (Figure 3B). The greater proportion of patients improving lobular inflammation after DIWL compared to EX was statistically significant (Figure 3C and Table 2). There were subtle improvements in Brunt fibrosis staging in 60-70% of patients that did not reach statistical significance in either group (Figure 3D). Overall, an improvement in steatosis correlated with degree of weight loss ( $r_s=0.82$ ,  $p<0.0001$ ; Figure 4A). Improvements in fibrosis were associated with the reduction in steatosis ( $r_s=0.64$ ,  $p=0.02$ ; Figure 4B). This persisted after correcting for weight and body fat loss (standardized  $\beta=0.88$ ,  $p=0.04$ ). An improvement (decrease) in hepatic lobular inflammation was correlated with an increase in HMW adiponectin ( $r_s=-0.66$ ,  $p=0.01$ ) and an improvement (decrease) in hepatic-IR ( $r_s=0.56$ ,  $p=0.04$ ).

### Glucose metabolism: Muscle

Total glucose disposal (TGD) was severely impaired in participants with NAFLD, but there was considerable variability, ranging from the lower end of normal through to profoundly impaired (range 2.63 to 8.53  $\text{mg/kg}_{\text{DFM}}/\text{min}$ ). When corrected for circulating plasma insulin levels achieved during the clamp (TGD/I), a variable, non-significant peripheral response to intervention was observed with an improvement in some patients with severe baseline insulin resistance, but a paradoxical worsening of insulin resistance in others (Figure 5). Muscle insulin sensitivity in NAFLD remained almost one third that of lean controls after intervention. Any modest improvement in TGD/I gained after intervention was due to an increase in non-oxidative glucose disposal (glycogen storage), as oxidative glucose disposal was unchanged (Table 1).

### Glucose metabolism: Liver

Fasting EGP were within normal ranges in this non-diabetic NAFLD group, as was the ability to suppress hepatic glucose under insulin-stimulation, although these processes occurred with considerably



**Figure 2:** Changes in fitness, weight and body composition after the diet and exercise interventions. Fitness assessed by  $\text{VO}_{2\text{peak}}$  (Panel A), total body weight (Panel B), subcutaneous (SAT) and visceral (VAT) adipose tissue assessed by CT scan (Panel C) and fat free mass assessed by DEXA (Panel D), pre and post 6-month DIWL or EX. Bars represent mean  $\pm$  standard error of the mean. \* $p<0.05$ ; \*\*\* $p<0.001$ .

Histological Feature	Diet (n=5)			Exercise (n=9)			P Value DIWL vs EX*
	Pre	Post	P Value	Pre	Post	P Value	
<b>Steatosis</b>							
% hepatocytes affected	73 ± 36	23 ± 32	0.04	71 ± 32	54 ± 36	0.12	0.05
Score – no. of subjects							
0 (<5%)	0	1		0	0		
1 (5-33%)	1	3		2	3		
2 (>33% - 66%)	0	0	0.08	1	1	0.59	
3 (>66%)	4	1		6	5		
No. pts with improvement - %		80%			22%		
Pts with a reduction in score of ≥2* – no./total no. (%)		4/5(80)			1/9(11)		0.02
<b>Hepatocyte Ballooning</b>							
Score – no. of subjects							
0 (None)	2	4		1	3		
1 (Few)	2	1		4	3		
2 (Many)	1	0	0.50	4	3	0.34	0.06
No. pts with improvement - %		4/5(80)			4/9(44)		0.30
<b>Lobular Inflammation</b>							
Score – no. of subjects							
0 (0 or 1 focus)	2	4		0	1		
1 (2-4 foci per 200x field)	1	1		5	4		
2 (>4 foci per 200x field)	2	0	0.17	4	4	0.77	0.005
No. pts with improvement - %		5/5(100)			2/9(22)		0.02
<b>NAS</b>							
Median (range)	5 (1-7)	2 (0-5)	0.05	5 (2-8)	5 (1-7)	0.29	0.02
Pts with a reduction in score of ≥ 2* – no./total no. (%)		4/5(80)			2/9(22)		0.09
<b>Fibrosis</b>							
Score – no. of subjects							
0 (None)	2	4		3	5		
1 (Perisinusoidal or periportal)	2	1		2	1		
2 (Perisinusoidal and portal or periportal)	1	0		2	0		
3 (Bridging fibrosis)	0	0		2	1		
4 (Cirrhosis)	0	0	0.50	0	2	1.0	0.13
No. pts with improvement - %		4/5(80)			6/9(67)		1.0
Pts with a reduction in score of ≥ 2* – no./total no. (%)		4/5(80)			5/9(56)		0.58
<b>NASH<sup>§</sup></b>							
Pts with resolution of NASH –no./total no. (%)		2/3(67)			2/8(25)		0.49

\* Reduction in score of ≥ 2 or ≥ 2 but normalised tissue or was normal at baseline and remained normal

\*p<0.05 RMANOVA for time x treatment difference between groups at 6 months

DIWL: Diet-induced Weight Loss Intervention; EX: Exercise Intervention; NAS: NAFLD Activity Score; Pts: Patients; NASH: Nonalcoholic Steatohepatitis

§Number of patients with diagnosed NASH at baseline: n=3 in diet group and n=8 in exercise group

**Table 2:** Raw data of hepatic histologic scores pre and 6 months post-diet and exercise intervention in those patients with a repeat liver biopsy.

higher serum insulin concentrations. The fasting hepatic-IR index was thus significantly higher in NAFLD at baseline compared to lean controls (Table 1). Improvement in fasting hepatic-IR was similar in both groups (Table 1: DIWL 26% decrease, p=0.08; EX 37% decrease, p=0.06).

### Lipid metabolism

Adipose tissue IR index (Adipo-IR) was significantly worse, six times higher, in patients with NAFLD compared to lean controls (Table 1) and was associated with both lower muscle insulin sensitivity ( $r=-0.6$ ,  $p<0.001$ ) and higher hepatic-IR ( $r=0.9$ ,  $p<0.001$ ). In the whole patient group, an improvement in Adipo-IR correlated with improvements in hepatic-IR ( $r=0.67$ ,  $p=0.004$ ) but not changes in VAT ( $p=0.12$ ) or weight loss ( $p=0.28$ ).  $\beta$ -hydroxybutyrate was significantly lower in patients with NAFLD compared with lean controls ( $0.09 \pm 0.03$  vs.  $0.14 \pm 0.07$ ,  $p=0.018$ ), indicating there was reduced hepatic fat oxidation. Only DIWL resulted in an increase in  $\beta$ -hydroxybutyrate (increase

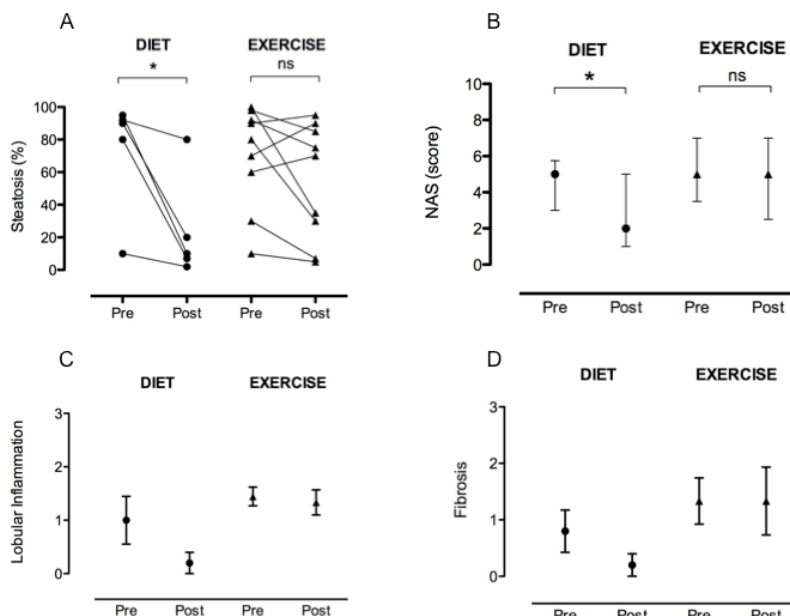
by 25%). In contrast, whole body fasting lipid oxidation increased irrespective of intervention type ( $0.75 \pm 0.19$  to  $0.87 \pm 0.28$  mg/kg/min,  $p=0.02$ ) and correlated with improved hepatic-IR ( $r_s=-0.55$ ,  $p=0.03$ ).

### Adipokine response

Total serum adiponectin was 40% lower in patients compared to lean controls ( $p=0.008$ ) mainly due to reduced HMW fraction (Table 1). Changes in total adiponectin levels were not detected after EX, although there was a trend for a selective decrease in the HMW fraction that resulted in a significant decrease in the ratio of HMW to total adiponectin ( $S_A$ ;  $0.37 \pm 0.13$  to  $0.29 \pm 0.11$ ,  $p=0.004$ ). DIWL was associated with a significant increase in total adiponectin and increase in the ratio of HMW to total adiponectin ( $S_A$ ;  $0.34 \pm 0.11$  to  $0.42 \pm 0.15$ ,  $p<0.05$ ).

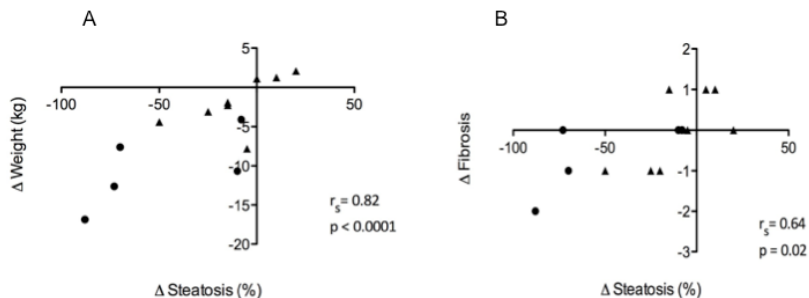
### Discussion

Therapies that target both liver disease and cardiometabolic risk factors are important for NASH. Patients who undertook circuit



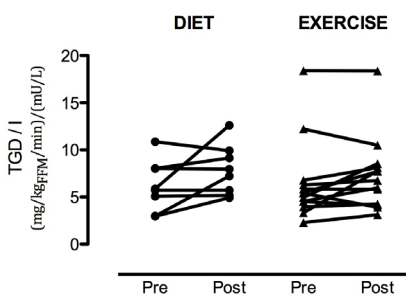
**Figure 3:** Effect of diet and exercise interventions on steatosis and disease severity.

Individual change in steatosis (Panel A,  $p < 0.05$  Wilcoxon signed rank test) and median score change in NAFLD activity Score (NAS) (Panel B,  $p < 0.05$  Wilcoxon signed rank test), Lobular inflammation (Panel C,  $p < 0.05$  RMANOVA for time x treatment difference between groups at 6 months) and Fibrosis (Panel D,  $p = \text{NS}$ ) assessed by liver biopsy pre and post intervention. Symbols • depicts DIWL and ▲ depicts EX group.



**Figure 4:** Factors associated with change in steatosis after intervention.

Change in steatosis was correlated with overall weight loss  $r_s = 0.82$   $p < 0.0001$  (Panel A) and change in fibrosis assessed by Brunt score  $r_s = 0.64$ ,  $p = 0.02$  (Panel B). Symbols • depicts DIWL and ▲ depicts EX group.



**Figure 5:** Effect of diet and exercise interventions on peripheral insulin sensitivity.

Individual response of peripheral (muscle) insulin sensitivity index to DIWL and EX, illustrated by total glucose disposal (TGD) rate/steady state plasma insulin levels, during the hyperinsulinemic-euglycaemic clamp. Pre- and post-comparison using paired Wilcoxon signed rank test was not significantly different after either intervention.

exercise training for six months showed cardiometabolic improvements including reduced visceral adiposity, increased aerobic fitness and improvements in HDL cholesterol. But in the absence of weight loss, histological improvement in liver disease was not achieved. In contrast, dietary induced weight reduction, achieved while still maintaining a very sedentary lifestyle, led to improvements in blood pressure, HMW adiponectin and histological features of NASH. Both interventions achieved an equivalent improvement in fasting hepatic insulin sensitivity but showed a variable or absent peripheral (muscle) response in insulin sensitivity.

The histological results of this study are unique and were chosen as the primary outcome variable due to the lack of data regarding exercise prescription that may prevent or delay progression of NASH [7]. The lack of improvement in steatosis after circuit training contrasts earlier studies of exercise in NAFLD, which have typically used shorter exercise prescriptions and different techniques to assess steatosis. These previous studies have shown a 10-20% relative reduction in hepatic fat measured by MRS [26-28]. This is a real, yet small effect compared to the ~70% relative reduction in MRS assessed hepatic fat seen after 10% body weight loss [29]. While MRS and histological assessment of steatosis are correlated [30], they are not equivalent or comparable units of measure. MRS measures absolute hepatic triglyceride content whereas histological assessment reflects the proportion of hepatocytes with visible triglyceride droplets and modest changes in total triglyceride content assessed by MRS may not be reflected in histological quantitation of steatosis. The additional value of histological assessment is the ability to assess features of NASH including inflammation, hepatocyte ballooning and fibrosis, all of which cannot be distinguished by MRS and have not been reported in any previous study.

In the current study, improvements in steatosis correlated with overall weight loss, rather than specific fat loss, perhaps reflecting the need for a calorie deficit in order to mobilize steatosis. The greater improvement in hepatic inflammation after DIWL compared to EX may in part be due to the reduction/mobilisation of steatosis and a healthier adipokine profile as suggested by the relationship between improved lobular inflammation and HMW adiponectin. Patients in the DIWL group were weight stable for at least two weeks prior to re-biopsy. Some patients remained considerably overweight (despite significant weight loss) and the long-term histological consequences of ceasing calorie restriction while maintaining a lower, yet still overweight body mass index is unknown. The only variable associated with improvement in fibrosis was a decrease in steatosis and this was independent of weight and fat loss. This suggests that a reduction in steatosis (in this study achieved via calorie restriction) is necessary for an improvement in liver disease severity, irrespective of improvements in other metabolic risk factors.

Improvement in muscle insulin sensitivity was modest at best, with a smaller change in TGD compared to other studies of weight loss in obese patients without NASH [31]. This small to no effect of exercise alone on markers of insulin resistance in NAFLD has been reported by others [27,28] and suggests the type and intensity of exercise prescription (in the absence of weight loss) still requires further investigation to determine what prescription will best target the profound muscle insulin resistance associated with NASH. A similar phenomenon was seen when aerobic exercise without weight loss did not change lipid kinetics (VLDL secretion) in NAFLD [26]. The plausible role of genetic, inflammatory or disease specific influences on insulin resistance in NASH [32] may need to be considered when prescribing lifestyle advice (dose and intensity of exercise and/or amount of weight loss)

to this patient population. The weight loss experienced after DIWL involved losses from both fat and lean mass. Given that muscle insulin resistance is likely the primary driver of progression to type 2 diabetes in this patient population [33], the independent and combined effects of DIWL and different types of EX on body composition and muscle metabolism is clinically relevant and warrants further exploration.

While there is a paucity of well-controlled studies investigating the benefits of exercise alone on hepatic insulin resistance, it seems that any effect may be independent of obesity, change in weight, energy balance, or presence of steatosis. The amelioration of steatosis in the DIWL did not produce a significantly greater improvement in hepatic-IR to that seen with EX where severe steatosis remained. Equivalent improvements in hepatic insulin sensitivity have been demonstrated in both lean and obese study participants after 12 weeks of a high intensity exercise program [31]. Our study supports the observation that improvements in hepatic-IR can be distinct from muscle and can be demonstrated after both diet-induced weight loss and EX training without weight loss. This suggests DIWL and EX may have either a shared mechanism (such as an increase in whole body fat oxidation) or equally effective separate mechanism for improving hepatic-IR, independent of steatosis.

Visceral fat increases metabolic and cardiovascular risk but the most effective method for targeting reductions in visceral adiposity while maintaining muscle mass is unclear. Evidence exists to support both aerobic training alone [34] and resistance training [35] as a means to reduce visceral fat, but most studies are in conjunction with some weight reduction. In this study, both DIWL and EX reduced visceral adiposity. The potential role of adipose tissue in the progression of liver disease has been highlighted [36,37] and our data supports the existence of cross-talk between fat and liver, firstly by demonstrating correlations at baseline between Adipo-IR and both hepatic-IR and fibrosis; and secondly by demonstrating that improvements in Adipo-IR and whole body fat oxidation are linked to beneficial changes in hepatic-IR. Adiponectin is an insulin sensitising adipokine, with the HMW isomer having a particular effect on the liver [38]. Adiponectin has been proposed as a key contributor to the histological benefits of thiazolidinedione therapy of NASH [39]. The increase in HMW adiponectin after DIWL and its association with improved lobular inflammation supports a liver specific benefit associated with the HMW adiponectin isomer. Improving the metabolic "fitness" of fat tissue appears to be an important target for obese populations.

When proposing a therapeutic option for a chronic condition it is important to consider its acceptability for the target population. The circuit EX training was offered in a supervised environment and prescribed at a moderate intensity deemed safe for obese patients. Attendance was high and no adverse events were reported. However, sustaining behaviour change in unsupervised environments has not yet been established. All participants in the study maintained a very sedentary lifestyle outside of the study with no measurable aerobic activity above the intensity of slow walking. If similar weight losses to DIWL were to be achieved through exercise alone, a minimum of brisk walking for >1.5 hours every day would be required. A cross-sectional study by Kistler et al. [40] supports the hypothesis that low to moderate physical activity may not be protective for the development of NASH, whereas vigorous activity greater than 150 minutes per week is associated with lower risk for severe fibrosis. As that amount of vigorous physical activity is well above that recommended for general health, this raises important clinical questions about what may be the



most appropriate intensity of exercise prescription for the prevention of NAFLD and the treatment of established NASH given the patient is likely to be overweight with little previous experience with intense physical activity. Further investigations of various combinations of diet, exercise and combined/simultaneous weight loss and exercise prescriptions are needed in this patient population.

## Limitations

Requirement for liver biopsy made recruitment difficult and performing biopsies in a NAFLD control group with no intervention is ethically challenging, therefore the DIWL acted as a 'standard care' control group. However, even with natural fluctuation in histological features over time presumed, clear differences between the groups were still observed. The pilot study was small and powered for within group change in steatosis and glucose disposal rate only, therefore a type 2 error is possible for the remaining outcomes. The variability in peripheral metabolic response to intervention was not anticipated and larger numbers are recommended to confirm our observations. The study excluded patients with type 2 diabetes and so may not be representative of the proportion of patients with NAFLD and comorbid diabetes, which may be common in a clinical setting. The six-month time frame may not have been sufficient to detect changes in some clinical variables.

## Conclusions

These results demonstrate that dietary induced weight loss and exercise alone have distinct and complementary benefits for NAFLD. Circuit exercise is safe, well accepted and efficacious for improving cardiometabolic risk in patients with NAFLD. However this dose of exercise, without concomitant weight loss, was inadequate for histological improvements in liver disease severity or substantial benefit to peripheral insulin sensitivity after 6 months of therapy. The pilot study outcomes should stimulate further development of different exercise protocols (type, frequency and intensity) to address these disease-specific conditions and add new insight to the practice guidelines for the management of NAFLD.

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Australian and New Zealand Clinical Trials Registry <http://www.anzctr.org.au/ACTRN12612001087842>

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