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Improvement in histological endpoints of MAFLD following a 12-week aerobic exercise intervention

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Summary

Background: Lifestyle interventions are the primary treatment for metabolic (dysfunction) associated fatty liver disease (MAFLD). However, the histological and cardiometabolic effects of aerobic exercise in MAFLD remain unclear.

Aims: To assess the effects of a 12-week aerobic exercise intervention on histological and cardiometabolic endpoints in MAFLD.

Methods: Patients with biopsy-confirmed MAFLD participated in a 12-week aerobic exercise intervention. Liver histology, cardiorespiratory fitness (estimated $\dot{V}O_{2max}$), physical activity, anthropometry and biochemical markers were assessed at baseline, intervention completion, and 12 and 52 weeks after intervention completion.

Results: Twenty-four patients completed the exercise intervention (exercise group n = 16, control group n = 8). In the exercise group, 12 weeks of aerobic exercise reduced fibrosis and hepatocyte ballooning by one stage in 58% ($P = 0.034$) and 67% ($P = 0.020$) of patients, with no changes in steatosis ($P = 1.000$), lobular inflammation ($P = 0.739$) or NAFLD activity score ($P = 0.172$). Estimated $\dot{V}O_{2max}$ increased by 17% compared to the control group ($P = 0.027$) but this level of improvement was not maintained at 12 or 52 weeks after the intervention. Patients with fibrosis and ballooning improvement increased estimated $\dot{V}O_{2max}$ by 25% ($P = 0.020$) and 26% ($P = 0.010$), respectively. Anthropometric reductions including body mass ($P = 0.038$), waist circumference ($P = 0.015$) and fat mass ($P = 0.007$) were also observed, but no patient achieved 7%-10% weight loss.

Conclusion: This study highlights the potential benefits of a 12-week aerobic exercise intervention in improving histological endpoints of MAFLD. The development of strategies to ensure continued engagement in aerobic exercise in MAFLD are needed.

Philip O'Gorman and Sara Naimimohasses should be considered joint first authors and contributed equally to the study's concept, design and experimental procedures.

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1 | INTRODUCTION

Metabolic (dysfunction) associated fatty liver disease (MAFLD) is now the most common cause of chronic liver disease worldwide with a global estimated prevalence of 25%[1]; this is linked to the increasing global incidence of type 2 diabetes mellitus (T2DM) and obesity.[1-3] MAFLD comprises a spectrum of disease that ranges from simple steatosis to non-alcoholic steatohepatitis (NASH) and is increasingly becoming the leading cause of liver cirrhosis[2,4] and hepatocellular carcinoma in liver transplant candidates.[5] Patients with MAFLD are also at a high risk of cardiometabolic comorbidities including central obesity, insulin resistance and cardiovascular disease (CVD),[2,6] to the extent that a recent consensus statement has proposed the term "MAFLD" to be used rather than "non-alcoholic fatty liver disease" (NAFLD).[7,8] In the absence of approved pharmacological therapies, lifestyle interventions remain the cornerstone of treatment of MAFLD, with current guidelines recommending a weight loss of 7%-10% to achieve optimum histological benefit.[9]

Exercise is known to be beneficial for the treatment and prevention of many chronic inflammatory diseases such as cancer, T2DM, arthritis and CVD.[10-12] However, the independent role of exercise in the treatment of MAFLD remains unclear. A recent meta-analysis in patients with established MAFLD reported that both aerobic and resistance exercise training, without significant weight loss, produces a 20%-30% reduction in intrahepatic lipid content, as assessed by noninvasive methodologies.[13] However, the optimal dose, frequency and type of exercise for improving histological endpoints of MAFLD remain unknown.[14] Hickman et al reported no histological improvements following a 6-month resistance exercise intervention[15] while Eckard et al reported no histological improvements following a 6-month combined aerobic and resistance exercise intervention,[16] but no other exercise alone trials using histological endpoints have substantiated these findings. However, cross-sectional studies suggest that moderate-to-vigorous intensity physical activity may be required for histological improvements,[17,18] and have highlighted the potential role of cardiorespiratory fitness.[19] Cardiorespiratory fitness has been proposed to be a validated, independent predictor of all-cause mortality in MAFLD patients,[20] and therefore could represent an important clinical endpoint for MAFLD patients.

The primary objective of this study was to determine the independent effects of exercise alone, specifically 12 weeks of moderate-to-vigorous intensity aerobic exercise, without prescribed dietary modifications, on histological endpoints of MAFLD. Secondary objectives included: determining the impact of the exercise intervention on cardiorespiratory fitness, physical activity levels and measures of cardiometabolic health including body composition, vascular health, glucose and lipid metabolism and circulating inflammatory markers. The final objective was to determine the sustainability of the exercise intervention at 12 weeks and 52 weeks post-exercise intervention completion.

2 | MATERIALS AND METHODS

2.1 | Ethics declaration

The study was approved by the St. James's and the Adelaide and Meath Hospitals, Dublin, Ireland, Research Ethics Committee. Written informed consent was obtained from all patients and the study was conducted in accordance with the guidelines outlined in the Declaration of Helsinki, 2013.[21] Recruitment and follow-up occurred between January 2018 and June 2019.

2.2 | Participants

Twenty-four patients with biopsy-confirmed MAFLD (median age: 61 ± 16 years, male/female n: 7/17, mean body mass index [BMI]: 35.7 ± 6.4 kg/m^2) attending the hepatology out-patient clinic at St James's Hospital, Dublin, Ireland completed the intervention (exercise group, n = 16, control group, n = 8). Prior to enrolment, eligible patients had a medical screen to exclude uncontrolled cardiopulmonary disease or other contraindications to exercise testing or prescription as outlined in the American College of Sports Medicine guidelines.[10] Inclusion criteria were: aged ≥18 years, biopsy-proven MAFLD and the ability to attend bi-weekly exercise classes in St James's Hospital for 12 weeks. Exclusion criteria were: contraindications to exercise testing or prescription,[10] significant orthopaedic or neuromuscular limitations, unwillingness to participate, alcohol consumption >40 g/day (males) or >20 g/day (females) or coexisting liver disease. Participant recruitment and attrition rates are presented in Figure 1.

2.3 | Study Design

Patients were enrolled in this study using NAFLD diagnostic criteria but the term "MAFLD" rather than "NAFLD" is used throughout this manuscript.[8] Following baseline assessment (T0), 28 participants were recruited by convenience sampling and allocated to an exercise group (n = 18) or control group (n = 10), without any prescribed dietary changes, based on participants' individual preference. The exercise intervention comprised three to five aerobic exercise sessions per week (two exercise specialist-led supervised exercise sessions and one to three unsupervised exercise sessions) for 12 weeks. The control group received standard of care. The aerobic exercise intervention protocol is further detailed in Supporting Methods and Table S1. Following completion of the exercise intervention, all participants (exercise group and control group) were reassessed at week 13 (T1). Participants in the exercise group were then encouraged to continue exercise participation but no formal exercise intervention was prescribed or monitored. Both exercise group and control group participants were reassessed 12 weeks after intervention

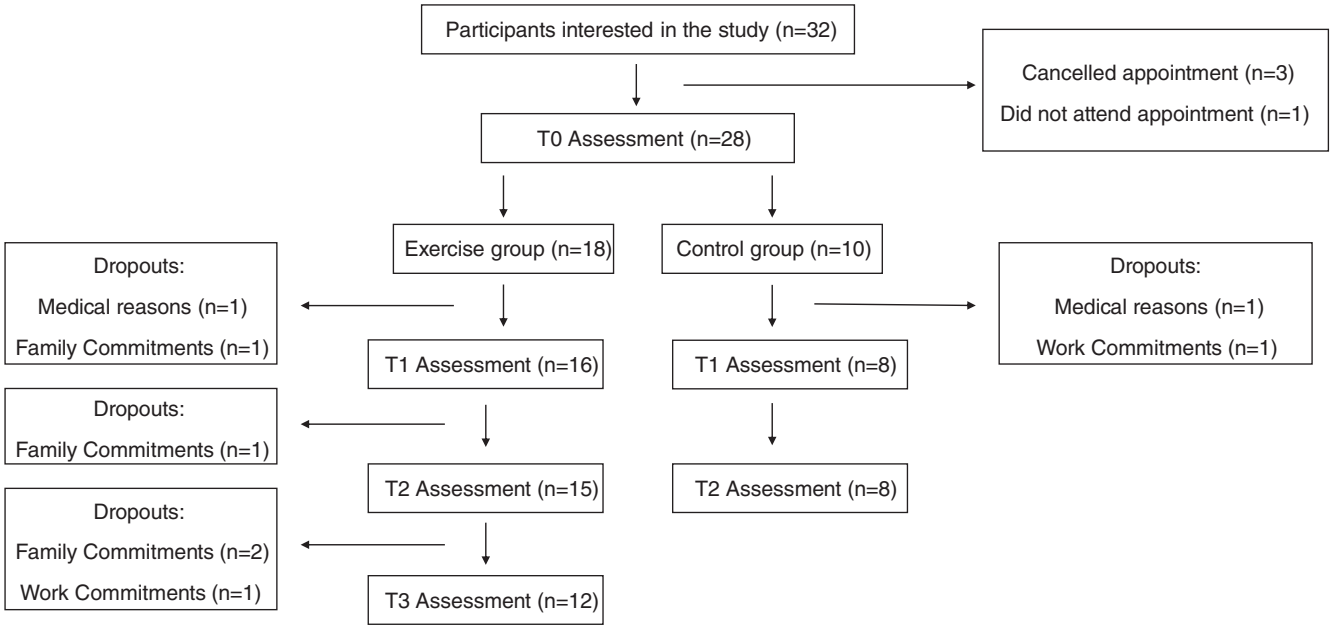

FIGURE 1 Participant recruitment and attrition. Notes: T0 = Baseline assessment, T1 = Week 13 (intervention completion) assessment, T2 = 12-week follow-up assessment, T3 = 52-week follow-up assessment

completion (T2) and exercise group participants alone were reassessed 52 weeks after intervention completion (T3) to determine if the benefits of the exercise intervention were sustained longitudinally. For each assessment time point (T0-T3), participants were requested to avoid strenuous physical activity, caffeine and alcohol intake for 24 hours prior to each assessment and fast for 12 hours prior to each assessment to ensure standardisation of each assessment time point.

2.4 | Dietary Assessment

Dietary intakes were assessed at T0 and T1 as previously described,[22] both by 4-day diet diaries returned by mail and by a food frequency questionnaire administered via a 20-min interview by a trained nutritionist. The dietary assessment is further detailed in Methods S1.

2.5 | Histological analysis of liver biopsies

Liver biopsies were performed on all participants (exercise group and control group) at T0 and the exercise group had repeat biopsies at T1. All liver biopsy specimens were reviewed and scored by a single, blinded histopathologist. Hepatic steatosis was scored based on the proportion of hepatocytes affected and subsequently classed into four grades (0-3). The severity of liver injury was assessed and scored using the NASH Clinical Research Network criteria.[23] The NAFLD activity score (NAS) was graded between 0 and 8 and hepatic fibrosis was staged between 0 and 4.[24]

2.6 | Transient elastography assessment

A transient elastography device (FibroScan® touch 502, Echosens, France) was used to noninvasively assess hepatic fibrosis (liver stiffness score) and steatosis (controlled attenuation parameter [CAP]) measurements at all time points (T0-T3).

2.7 | Cardiorespiratory fitness and physical activity assessment

Cardiorespiratory fitness was assessed using the Modified Bruce submaximal cardiopulmonary exercise test protocol on an electrically-driven treadmill (COSMED T150, DE)[12] to give estimates of maximal oxygen consumption ($\dot{V}O_{2max}$). Physical activity was assessed using a tri-axial accelerometer (Actigraph GT3X+, Actigraph Corp). The accelerometer recorded data at 30 Hz for 7 consecutive days during participants' waking hours and were worn on the right hip and secured using an elasticated waistband. Cardiorespiratory fitness and physical activity levels were assessed at all time points (T0-T3). The cardiopulmonary exercise test protocol, estimated $\dot{V}O_{2max}$ calculation and physical activity assessment protocol are detailed in Methods S1.

2.8 | Cardiometabolic Analysis

Standing height was assessed using a wall-mounted vertical stadiometer and body mass was measured using a digital scale. Measures of fat mass and skeletal muscle mass were assessed using

bioimpedance analysis (Seca mBCA 515, Seca). Participants were requested to void their bladder and bowels prior to bioimpedance analysis to ensure standardisation of measurements. To determine the degree of central obesity, waist circumference and hip circumference were measured using a nonstretch measuring tape around the bare abdomen and widest part of the hips, respectively, and waist-to-hip ratio was subsequently calculated. Vascular health was assessed using a Mobil-O-Graph® pulse wave analysis monitor (IEM, GmbH, Germany). Fasting venous blood samples were collected to measure liver function tests (LFTs), lipid profiles, fasting plasma glucose (GLUF), glycated haemoglobin ($HbA1_c$) and circulating inflammatory markers (C-reactive protein, CRP; erythrocyte sedimentation rate, ESR; tumour necrosis factor-alpha, TNF-α; interleukin 6, IL-6 and interleukin 1β, IL-1β). TNF-α, IL-6 and IL-1β concentrations were measured using DuoSet ELISA kits (R&D Systems) and plates were read spectrophotometrically at 450 nm using a VersaMax plate reader. All cardiometabolic assessments were assessed at all time points (T0-T3).

2.9 | Statistical Analysis

All statistical analyses were performed using the Statistical Package for the Social Sciences software version 25. Data were assessed for normality using the Shapiro-Wilk test. Baseline between-group differences were assessed using independent *t* tests or Mann-Whitney U tests for normal and non-normal data, respectively. Paired *t* tests or Wilcoxon signed-rank tests were used to assess within-group differences for repeated measures for normal and non-normal continuous data, respectively. McNemar's test was used to assess within-group differences for repeated measures for categorical data. Where appropriate, time by group interactions were assessed using a two-way repeated measures analysis of variance. Measures of effect size were calculated using partial eta^2 (η^2) and defined as small (0.01), medium (0.06) or large (0.14).[25] Pearson's and Spearman's correlation were used to assess associations between normal and non-normal variables, respectively. Where appropriate, missing data are noted on each respective table and figure. Statistical significance for all tests was set at $P \leq 0.05$. Continuous data are displayed as mean (standard deviation) or median (interquartile range) for normal and non-normal data, respectively. Categorical data are displayed as number (percentage).

3 | RESULTS

3.1 | Baseline characteristics

Four participants (exercise group n = 2, control group n = 2) did not complete the T1 assessment, one participant (exercise group n = 1) did not complete the T2 assessment and three participants (exercise group n = 3) did not complete the T3 assessment (Figure 1). Adherence to the exercise intervention was 93% (supervised sessions = 96%, unsupervised sessions = 89%). During the supervised exercise sessions, all participants sustained their prescribed heart rate intensity and fully completed each exercise session duration. During the unsupervised sessions, all participants self-reported as meeting the required intensity, type and duration prescribed each week. Baseline participant characteristics and histological characteristics are detailed in Tables 1 and 2, respectively. The exercise group and control group were well matched with no significant differences between baseline participant and histological characteristics. Approximately 79% of the cohort had the diagnostic criteria for NASH. The cohort had coexisting comorbidities: obesity (79%), T2DM (71%), hypertension (56%), metabolic syndrome (63%) and below-average cardiorespiratory fitness (88%).

3.2 | Changes in cardiorespiratory fitness and physical activity with exercise

At T1, there was a significant time by group interaction in the exercise group, with a large effect size, for estimated $\dot{V}O_{2max}$ (4.7 ± 5.2mL/min/kg [17 ± 18%] mean increase, $P = 0.027$, partial $\eta^2 = 0.202$) compared to the control group. There was also a significant within-group improvement in estimated $\dot{V}O_{2max}$ in the exercise group compared to T0 ($P = 0.003$). At T1, the time spent in sedentary activity, light physical activity and moderate-to-vigorous physical activity was unchanged in both groups. All raw cardiorespiratory fitness and physical activity data between T0 and T1 are detailed in Table S2. At T2, there was no significant time by group interaction in the exercise group for estimated $\dot{V}O_{2max}$ ($P = 0.117$, partial $\eta^2 = 0.113$) compared to the control group and no significant within-group changes for estimated $\dot{V}O_{2max}$ ($P = 0.437$) in the exercise group compared to T0. At T3, estimated $\dot{V}O_{2max}$ was not significantly different from T0 (P=0.354).

3.3 | Improvements in cardiometabolic markers with exercise

At T1, there were significant time by group interactions in the exercise group, with large effect sizes, for body mass (2.1 ± 2.1% mean reduction, $P = 0.038$, partial $\eta^2 = 0.181$), waist circumference (4.0 ± 3.3% mean reduction, $P = 0.015$, partial $\eta^2 = 0.242$) and fat mass (4.9 ± 5.2% mean reduction, $P = 0.007$, partial $\eta^2 = 0.289$) compared to the control group. There were also significant within-group reductions in body mass ($P \leq 0.001$), waist circumference ($P \leq 0.001$), waist-to-hip ratio (2.4 ± 3.1% mean reduction, $P = 0.008$) and fat mass ($P \leq 0.001$), in addition to a significant within-group increase in skeletal muscle mass (3.8 ± 6.9% mean increase, $P = 0.034$) in the exercise group compared to T0, with 3/16 (19%) participants achieving 5% weight loss during the exercise intervention. Anthropometric improvements in the exercise group could be directly attributed to the

TABLE 1 Baseline participant characteristics

Variable	Exercise group (n = 16)	Control group (n = 8)	Between-group *P* value
Age (y)†	61 (15)	58 (23)	0.444[a]
Gender, n (%)			
Female	12 (75)	5 (63)	0.647[b]
Male	4 (25)	3 (37)	
T2DM/IGT, n (%)	11 (69)	6 (75)	1.000[b]
Hypoglycaemic medications, n (%)	9 (56)	5 (63)	1.000[b]
Hypertension, n (%)	9 (56)	4 (50)	1.000[b]
Anti-hypertensive medication, n (%)	9 (56)	3 (38)	0.667[b]
Hypercholesteremia, n (%)	9 (56)	4 (50)	1.000[b]
Lipid lowering medications, n (%)	9 (56)	3 (38)	0.667[b]
Hypertriglyceridemia, n (%)	6 (38)	3 (38)	1.000[b]
Polypharmacy, n (%)	7 (44)	2 (25)	0.657[b]
MetSyn, n (%)	9 (56)	6 (75)	0.657[b]
BMI, kg/m^2†	36.7 (9.1)	33.6 (6.3)	0.490[b]
BMI category, n (%)			
Overweight (25.0-29.9kg/m^2)	3 (19)	2 (25)	1.000[b]
Obese (≥30kg/m^2)	13 (81)	6 (75)	
Estimated $\dot{V}O_{2max}$, mL/min/kg ‡	26.9 (10.1)	27.0 (9.3)	0.340[c]
Cardiorespiratory fitness level, n (%)			
Below average	14 (88)	7 (88)	1.000[b]
Average	1 (6)	1 (12)	
Above average	1 (6)	0 (0)	
ALT (IU/L)†	47 (26)	61 (32)	0.221[a]
AST (IU/L)‡	36 (14)	47 (16)	0.094[c]
Hepatic CAP (dB/m)‡	337 (46)	330 (44)[2]	0.759[c]
Hepatic stiffness (kPa) ‡	11.9 (4.8)[1]	14.9 (8.7)[2]	0.431[c]

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; IGT, impaired glucose tolerance; MetSyn, metabolic syndrome; T2DM, type 2 diabetes mellitus, $\dot{V}O_{2max}$, maximal oxygen consumption.

[1]n = 15.

[2]n = 7.

[a]Mann-Whitney U test.

[b]Fisher's exact test

[c]Independent t test.

†Non-normal data (median [interquartile range]).

‡Normal data (mean [SD]).

exercise intervention, as no changes in participants' energy intake or overall dietary quality were observed between T0 and T1 (Table S3, Figure S1). At T1, in the exercise group there were no significant time by group interactions observed compared to the control group, and no significant within-group changes in the exercise group compared to T0 for circulating inflammatory markers, glucose and lipid regulation or measures of vascular health. All raw cardiometabolic data between T0 and T1 are detailed in Table S2. At T2, there was a significant time by group interaction in the exercise group, with a large effect size, for waist circumference ($P = 0.029$, partial $\eta^2 = 0.208$) compared to the control group. There were also significant within-group improvements in waist circumference ($P \leq 0.001$) and BMI ($P \leq 0.001$) in the exercise group compared to T0. At T3, waist circumference ($P = 0.211$) and BMI ($P = 0.330$) were not significantly different from T0.

TABLE 2 Baseline liver histology

Variable	Exercise group (n = 16)	Control group (n = 8)	Between-group *P* value
NAS[†]	3.9 (1.7)	4.6 (2.1)	0.360[a]
NAS components, n (%)			
≥5	6 (38)	4 (50)	0.673[b]
<5	10 (63)	4 (50)	
Steatosis, n (%)			
<5% (0)	0 (0)	1 (12.5)	0.282[b]
5%-33% (1)	8 (50)	2 (25)	
33%-66% (2)	4 (25)	4 (50)	
>66% (3)	4 (25)	1 (12.5)	
Lobular inflammation, n (%)			
None (0)	3 (19)	0 (0)	0.103[b]
<2 Foci (1)	9 (56)	2 (25)	
2-4 Foci (2)	3 (19)	5 (63)	
>4 Foci (3)	1 (6)	1 (12)	
Hepatocyte ballooning, n (%)			
None (0)	3 (19)	2 (24)	0.521[b]
Few cells (1)	10 (62)	3 (38)	
Many cells (2)	3 (19)	3 (38)	
NASH, n (%)			
Yes	13 (81)	6 (75)	1.000[b]
No	3 (19)	2 (25)	
Fibrosis, n (%)			
Absent (0)	1 (6)	0 (0)	0.281[b]
Perisinusoidal or portal/periportal only (1)	4 (25)	2 (25)	
Perisinusoidal and periportal (2)	4 (25)	0 (0)	
Bridging fibrosis (3)	5 (31)	2 (25)	
Cirrhosis (4)	2 (13)	4 (50)	

Abbreviations: NAS, NAFLD activity score, NASH, non-alcoholic steatohepatitis.

[†]Normal data (mean [SD]).

[a]Independent t test.

[b]Fisher's exact test.

3.4 | Improvements in liver histology with exercise

At baseline, 13/16 (81%) participants in the exercise group had NASH and the remainder had simple steatosis (median NAS: 3.9 ± 1.7). Repeat biopsies were performed on 12/16 (75%) participants in the exercise group within 7 days of the completion of the exercise intervention (T1). Four participants refused a repeat biopsy and were excluded from the final histological analysis. At T1, a number of histological changes were observed (Table 3): (a) a significant reduction in fibrosis (Figure 2a.), equating to 7/12 (58%) participants regressing one fibrosis stage (50% net reduction, $P = 0.034$); (b) a significant reduction in hepatocyte ballooning (Figure 2b.), equating to 8/12 (67%) participants regressing one hepatocyte ballooning stage (58% net reduction, $P = 0.020$); (c) 2/12 (17%) participants regressed one steatosis stage but 2/12 (17%) participants progressed one steatosis stage which led to no significant net changes in steatosis ($P = 1.000$); (d) 3/12 (25%) participants regressed a lobular inflammation stage (one stage n = 2, two stages n = 1) but 3/12 (25%) participants progressed one stage, leading to no significant net changes in lobular inflammation ($P = 0.739$); and (e) no significant net changes in NAS ($P = 0.172$). Improvements in hepatic fibrosis were more strongly associated with improvements in estimated $\dot{V}O_{2max}$ ($r_s = -0.423$, $P = 0.171$) than % weight loss ($r_s = 0.116$, $P = 0.720$) or % fat mass loss ($r_s = 0.230$, $P = 0.473$) at T1. Similarly, improvements in hepatocyte ballooning were more strongly associated with improvements in estimated $\dot{V}O_{2max}$ ($r_s = -0.483$, $P = 0.111$) than % weight loss ($r_s = 0.160$, $P = 0.620$) or % fat mass loss ($r_s = 0.307$, $P = 0.473$) at T1. Furthermore, participants who achieved fibrosis regression at

TABLE 3 Changes in histological staging between pre-intervention (T0) and post-intervention (T1) time points (exercise group only)

Variable	Change in histological scores (n = 12)
Hepatic fibrosis	
Increased 1 stage	1
Maintained the same stage	4
Decreased 1 stage	7
Net change	−6
Significance	$P = 0.034^{*}$
Hepatic steatosis	
Increased 1 stage	2
Maintained the same stage	8
Decreased 1 stage	2
Net change	0
Significance	$P = 1.000$
Lobular inflammation	
Increased 1 stage	3
Maintained the same stage	6
Decreased 1 stage	2
Decreased 2 stages	1
Net change	−1
Significance	$P = 0.739$
Hepatocellular ballooning	
Increased 1 stage	1
Maintained the same stage	3
Decreased 1 stage	8
Net change	−7
Significance	$P = 0.020^{*}$
NAS	
Increased 3 scores	1
Maintained the same score	5
Decreased 1 score	3
Decreased 2 scores	2
Decreased 4 scores	1
Net change	−8
Significance	$P = 0.172$

Abbreviation: NAS, NAFLD Activity Score.
*$P \leq 0.05$ (Wilcoxon signed-rank test).

T1 (n = 7) significantly increased estimated $\dot{V}O_{2max}$ by 5.9 ± 5.4 mL/min/kg (25 ± 20% increase, $P = 0.020$) at this time point, while participants without fibrosis regression (n = 5) demonstrated increased estimated $\dot{V}O_{2max}$ by 2.1 ± 5.7 mL/min/kg (7 ± 18% increase, $P = 0.590$) (Figure 3a). Participants with hepatocyte ballooning regression at T1 (n = 8) significantly increased estimated $\dot{V}O_{2max}$ by 6.5 ± 5.5 mL/min/kg (26 ± 20% increase, $P = 0.010$) at this time point, while participants without hepatocyte ballooning regression (n = 4) demonstrated increased estimated $\dot{V}O_{2max}$ by 0.04 ± 2.5mL/min/kg (2 ± 12% increase, $P = 0.980$) (Figure 3b.). There were no significant differences in overall exercise adherence rates between patients with and without fibrosis regression ($P = 0.343$) and between patients with and without hepatocyte ballooning regression ($P = 0.214$).

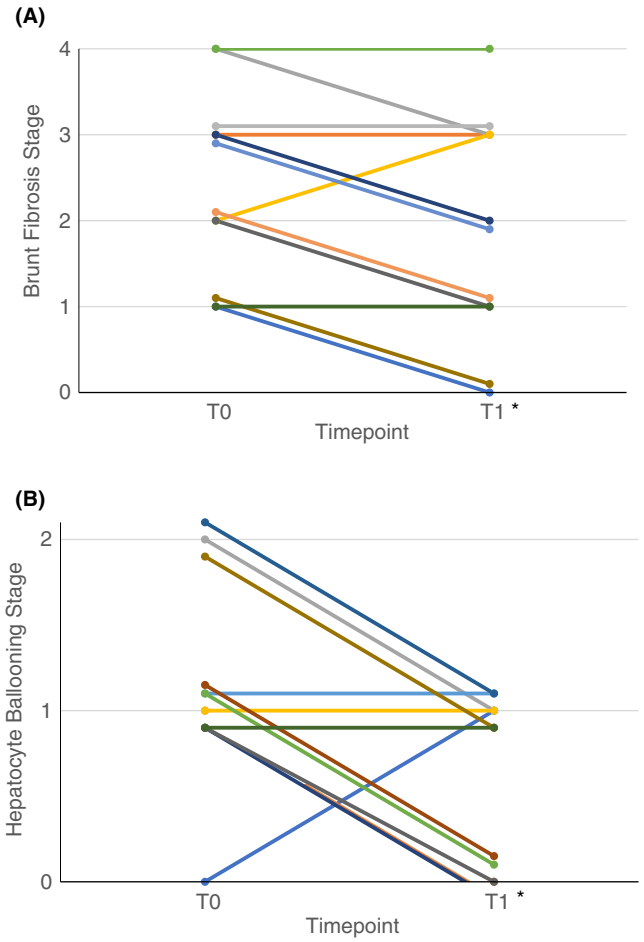

FIGURE 2 Individual histological responses between pre-intervention (T0) and post-intervention (T1) timepoints in the exercise group. (A) Individual changes in hepatic fibrosis staging; (B) Individual changes in hepatocyte ballooning grading. *Significantly different from T0 ($P \leq 0.05$, Wilcoxon signed-rank test)

3.5 | Changes in transient elastography measures and LFTs with exercise

At T1, there was a significant time by group interaction for CAP scores in the exercise group, with a large effect size, compared to the control group (14.0 ± 16.7% mean reduction, $P = 0.047$, partial $\eta^2 = 0.175$). There were no significant time by group interactions for liver stiffness measurements in the exercise group compared to the control group ($P = 0.450$, partial $\eta^2 = 0.029$). There were also significant within-group improvements in CAP scores ($P = 0.006$) and liver stiffness measurements ($P = 0.028$) in the exercise group compared to T0. There was no significant time by group interactions or within-group changes for LFTs at T1 in either group compared to

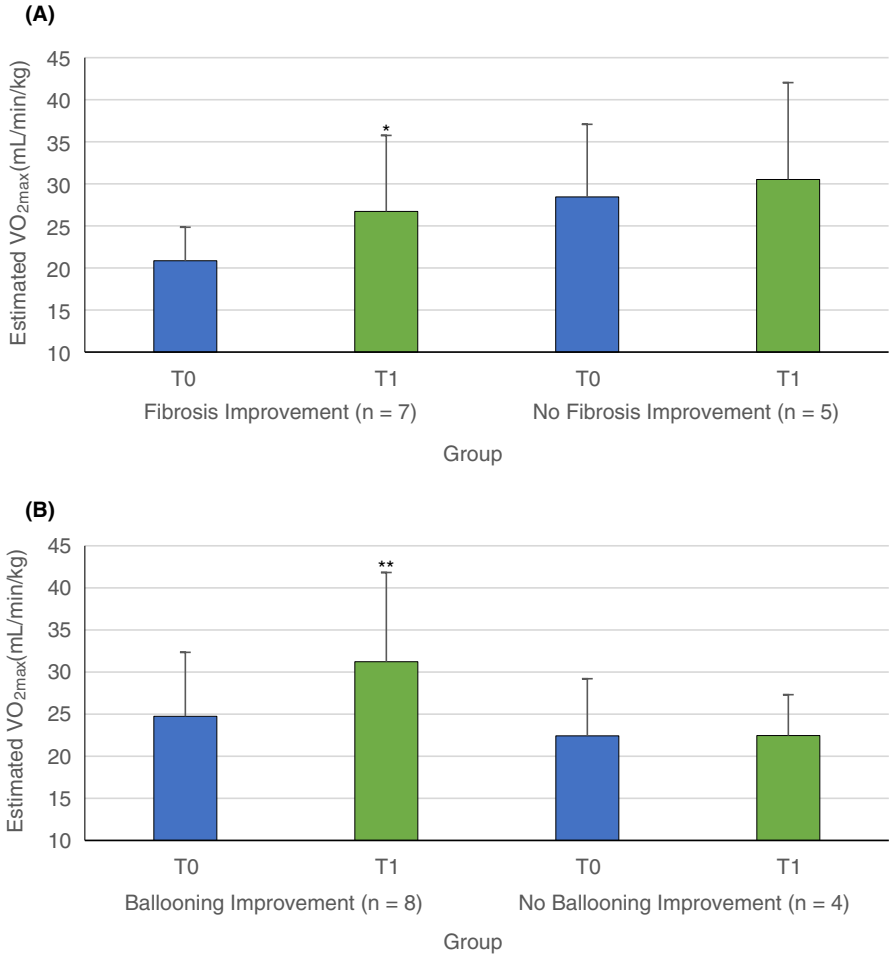

FIGURE 3 Mean estimated $\dot{V}O_{2max}$ in the exercise group based on histological responses between pre-intervention (T0) and post-intervention (T1) timepoints. (A) Mean estimated $\dot{V}O_{2max}$ in participants who had fibrosis improvement (n = 7, $P = 0.020$) and had no fibrosis improvement (n = 5, $P = 0.460$). (B) Mean estimated $\dot{V}O_{2max}$ in participants who had hepatocyte ballooning improvement (n = 8, $P = 0.010$) and had no hepatocyte ballooning improvement (n = 4, $P = 0.980$). Bars represent standard deviation. Abbreviation: $\dot{V}O_{2max}$ = Maximal Oxygen Consumption. *Significantly different from T0 ($P \leq 0.05$, paired t-test). **Significantly different from T0 ($P \leq 0.01$, paired t-test)

T0. All raw transient elastography and LFTs data between T0 and T1 are detailed in Table S4. At T2, there were no significant time by group interactions in the exercise for CAP scores ($P = 0.233$, partial $\eta^2 = 0.074$) or liver stiffness measurements ($P = 0.872$, partial $\eta^2 = 0.001$) compared to the control group. There were significant within-group improvements in CAP scores ($P = 0.003$) but not liver stiffness measurements ($P = 0.056$) in the exercise group compared to T0. At T3, CAP scores ($P = 0.182$) and liver stiffness measurements ($P = 0.272$) were not significantly different from T0.

4 | DISCUSSION

This study investigated the effects of a 12-week, moderate-to-vigorous intensity aerobic exercise intervention, in the absence of dietary change, on histological and cardiometabolic endpoints in patients with biopsy-confirmed MAFLD. The main findings were: (a) 12 weeks of aerobic exercise produced significant histological improvements in hepatic fibrosis and hepatocyte ballooning; (b) 12 weeks of aerobic exercise significantly improved estimated $\dot{V}O_{2max}$, markers of central obesity and fat mass, without the prescribed weight loss target of 7%-10%[9]; (c) 12 weeks of aerobic exercise did not produce significant histological changes in steatosis or lobular inflammation grades; (d) 12 weeks of aerobic exercise did not produce significant changes in vascular health or lipid and glucose regulation; and (e) in the absence of continuous prescribed and monitored exercise, the benefits of the 12-week aerobic exercise intervention were not sustained by T3.

Current guidelines state that lifestyle modifications which combine diet and exercise produce significant reductions in NASH and fibrosis, therefore, weight loss is the current primary endpoint for treating MAFLD.[9] The guidelines suggest that weight loss of 7%-10% is required for significant improvements in histological endpoints of MAFLD[9]; this was based on one study reporting 90% NASH resolution, 81% fibrosis regression and 100% improvement of steatosis with ≥10% weight loss.[26] Exercise-only interventions have reported reductions in hepatic fat content without significant weight loss, but data assessing the benefits of exercise on histological endpoints in MAFLD patients are limited.[14,27] In contrast to Hickman et al and Eckard et al who reported no significant changes in any histological endpoints following a 6-month resistance exercise intervention[15] and 6-month combined aerobic and resistance exercise intervention,[16] respectively, our study demonstrated statistically significant improvements in hepatic fibrosis and hepatocyte ballooning staging in 58% and 67% of patients following a 12-week moderate-to-vigorous intensity aerobic exercise intervention. This disparity in results may be partially explained by the different study designs employed. Hickman et al employed moderate intensity resistance

exercise training[15] while Eckard et al employed moderate intensity aerobic and resistance exercise training, but without strict exercise supervision. Aerobic exercise results in relatively higher energy consumption and improves cardiorespiratory fitness, while resistance exercise results in relatively less energy consumption but improves muscular strength and endurance.[12,13] Furthermore, the review by Kenneally et al reported that exercise supervision provides greater benefits in MAFLD patients during exercise trials.[27] The increased energy expenditure observed during moderate-to-vigorous intensity aerobic exercise, combined with improvements in cardiorespiratory fitness, body composition and exercise supervision in our study may have contributed to histological improvements. While the exact type and intensity of exercise needed for histological benefits in MAFLD remain unclear, moderate-to-vigorous physical activity may be required.[17,18] Despite the significant regression in hepatic fibrosis and hepatocyte ballooning observed in our study, the benefits did not extend to improvements in histologically measured steatosis and NAS, in line with previous published data.[15,16]

The improvement in estimated $\dot{V}O_{2max}$ observed at T1 indicates that the intensity, type and frequency of exercise were sufficient to induce significant improvements in cardiorespiratory fitness. These improvements in estimated $\dot{V}O_{2max}$ were associated with fibrosis and ballooning regression, suggesting a potential interrelationship. Patients who achieved fibrosis and hepatocyte ballooning regression significantly increased estimated $\dot{V}O_{2max}$ by 25%-26%, with minimal body mass reductions (1%-2%), suggesting that improvements in cardiorespiratory fitness may be a more sensitive clinical endpoint for histological changes in MAFLD patients during exercise trials rather than weight loss. Cardiorespiratory fitness has previously been demonstrated to be inversely associated with NASH[28] and predicts hepatic fat loss during lifestyle interventions.[29] In addition to these benefits, a 3.5 mL/min/kg increase in $\dot{V}O_{2max}$ is associated with a 10%-25% reduction in all-cause mortality in the U.S. general population[30,31] and represents an important clinical modifier for CVD risk, the leading cause of mortality in MAFLD populations.[20,32]

The physiological mechanisms underlying the change in liver fat following exercise training in MAFLD are well described and include changes in energy-balance, circulating lipids and insulin sensitivity.[14] However, the exact mechanisms underlying exercise-induced improvements in NASH and fibrosis are unknown but may relate to exercise-induced changes in intrahepatic inflammatory and fibrogenic activity. Hepatic stellate cells are a key mediator in the initiation, progression and regression of hepatic fibrosis[33] and several rodent studies have linked exercise participation with reduced hepatic stellate cell activity, independently of weight loss.[34-36] Exercise training is known to have anti-inflammatory effects[37] but whether these anti-inflammatory effects directly lead to improvements in local hepatic inflammatory pathways in NASH patients is unknown. Although our study did not observe significant reductions in circulating inflammatory markers, which is similar to published data,[38] reductions in inflammatory mediators may have been specific to hepatic tissue, and therefore not detected in circulation,[39] as reported in rodent studies with significant reductions in intrahepatic immune cell populations following exercise training.[35,40,41] In the study by Kawanishi et al, obesogenic mice that exercised for 60 min/day, five times per week, for 16 weeks demonstrated significant reductions in hepatic TNF-α levels, resident macrophage infiltration, and fibrosis markers (Sirius red and α-smooth muscle actin staining and tissue inhibition of matrix metalloproteinase-1 mRNA).[35] Huber et al reported significant reductions in TNF-mediated liver injury, intrahepatic CD45 positive leucocyte populations and inflammatory cytokines following 7 weeks of exercise in healthy mice.[40] Similarly, after 4 weeks of voluntary wheel running in a group of obesogenic mice, Gehrke et al reported significant reductions in hepatic inflammatory cytokine expression and intrahepatic macrophages infiltration, with improvements in histological steatosis, ballooning and inflammation.[41] Interestingly, intrahepatic immunological changes in these studies occurred without significant weight loss.[35,40,41] Collectively, these rodent studies highlight the exercise-induced changes in intrahepatic anti-inflammatory pathways that may contribute to histological improvement in MAFLD patients. Changes in intrahepatic immune cells were not investigated in our study, but reports of changes in circulating immune cell populations in individuals with a higher cardiorespiratory fitness suggest a potential link between exercise-induced changes in cardiorespiratory fitness and histological endpoints.[42-44]

While our study did not assess the link between hepatic inflammation and fibrosis and visceral adipose tissue (VAT), liver necroinflammation and fibrosis increase significantly with VAT in a dose-dependent manner.[45] VAT can synthesise and secrete cytokines and adipokines, and IL-6 and TNF-α are expressed in greater amount in VAT than subcutaneous fat.[46] We were unable to show any significant difference in circulating IL-6 or TNF-α at T1 in patients who demonstrated a significant reduction in waist circumference and waist-to-hip ratio, a clinical surrogate of VAT. One possible explanation may relate to the lack of steatosis regression.[38,45]

The failure to sustain the benefits of the exercise intervention at 12 months post-exercise intervention completion (T3) is in keeping with previous exercise interventions in MAFLD,[47] T2DM[48] and obesity[49] cohorts, and emphasises the unmet need for exercise maintenance in the unsupervised setting. Following a 16-week exercise intervention in patients with MAFLD,[47] Pugh et al observed that improvements in liver fat and $\dot{V}O_{2peak}$ were not sustained at a 12-month follow-up reassessment, concluding that effective mechanisms for promoting long-term sustainability of exercise in MAFLD cohorts are urgently required. Studies investigating the use of smart technology for the prescription of exercise in MAFLD cohorts are emerging. Two recent studies which incorporated an 8-week, web-based exercise intervention reported significant improvements in surrogate markers of hepatic fibrosis, $\dot{V}O_{2peak}$ and fat mass upon completion of the exercise intervention and, furthermore, that these benefits were sustained at 12-week follow-up reassessment.[50,51] The authors concluded that individualisation of the exercise intervention and appropriate patient education are important factors to achieve sustained benefits and continued self-driven exercise. The high adherence rate

to exercise during the exercise intervention of 93% in our study indicates that a group training approach may have improved patient motivation, and conversely, once completed, contributed to the attrition of the exercise intervention benefits longitudinally. Furthermore, the implementation of a care bundle approach, where patients have multiple intervention options determined at a patient individual level, may help sustain intervention benefits.[52]

4.1 | Limitations

This study has limitations: (a) the small sample size (n = 24) and lack of liver biopsies at T1 in the control group makes it difficult to draw definitive conclusions on the effects of aerobic exercise on histological endpoints of MAFLD; (b) the requirement for two liver biopsies proved challenging and limited study recruitment; (c) the study was not powered to detect significant histological changes, and therefore type 2 error cannot be disregarded; (d) the study was not randomised; patients were allocated to the exercise group or control group based on individual preference, which may indicate a degree of bias; and (e) medication history and dosage were recorded at baseline but not at other time points. It is possible that medication dose changes/removal of medications may have occurred during the study which may have influenced outcomes.

5 | CONCLUSIONS

The results of this study demonstrate that 12 weeks of moderate-to-vigorous intensity aerobic exercise significantly improved histological endpoints of MAFLD including fibrosis and hepatocyte ballooning, in the absence of clinically significant weight loss. These improvements were paralleled by significant improvements in cardiorespiratory fitness and measurements of central obesity. The significant histological improvements may relate to improvements in cardiorespiratory fitness, adding to the emerging body of evidence indicating the role for cardiorespiratory fitness as a clinical marker of disease progression/regression in MAFLD patients.[19,20,28,31] In the absence of continued prescribed exercise, the benefits of the exercise intervention were not sustained at 1-year follow-up. This pilot study paves the way for larger randomised controlled trials to investigate the effects of aerobic exercise on histological features of MAFLD, with a particular focus on determining strategies to transition exercise into the community setting in order to promote lifelong adherence to exercise therapy.

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AUTHORSHIP

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SUPPORTING INFORMATION

Additional supporting information will be found online in the Supporting Information section.

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