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

3 **Running Title: Histological benefit of aerobic exercise in MAFLD**

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27

1 **Authorship Statement**

2 **Guarantor of article:** Suzanne Norris

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4 **Study concept:** Philip O’Gorman, Sara Naimimohasses, Ann Monaghan, John Gormley and
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12 **All authors approved the final version of the manuscript, including the authorship list.**

13

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15

16 **Abbreviations:** ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass
17 index; CAP, controlled attenuation parameter; CRP, c-reactive protein; CVD, cardiovascular disease;
18 ESR, erythrocyte sedimentation rate; GLUF, fasting plasma glucose; HbA_{1c}, glycated haemoglobin; IL-
19 1 β , interleukin 1 β ; IL-6, interleukin 6; LFTs, liver function tests; MAFLD, metabolic (dysfunction)
20 associated fatty liver disease; MAS, MAFLD activity score; MASH, metabolic (dysfunction) associated
21 steatohepatitis; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; T0,
22 baseline assessment; T1, week 13 follow-up assessment; T2, week 24 follow-up assessment; T3, week
23 52 follow-up assessment; T2DM, type 2 diabetes mellitus; TNF- α , tumour necrosis factor α ; VAT,
24 visceral adipose tissue; $\dot{V}O_{2max}/\dot{V}O_{2peak}$, maximal oxygen consumption/peak oxygen consumption

25

1 **Summary**

2 **Background**

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

6 **Aims**

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

9 **Methods**

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

14 **Results**

15 Twenty-four patients completed the exercise intervention (exercise group n=16,
16 control group n=8). In the exercise group, 12 weeks of aerobic exercise reduced
17 fibrosis and hepatocyte ballooning by one stage in 58% ($P=0.034$) and 67% ($P=0.020$)
18 of patients, with no changes in steatosis ($P=1.000$), lobular inflammation ($P=0.739$) or
19 MAFLD activity score ($P=0.172$). Estimated $\dot{V}O_{2max}$ increased by 17% compared to
20 the control group ($P=0.027$) but this level of improvement was not maintained at 12 or
21 52 weeks after the intervention. Patients with fibrosis and ballooning improvement
22 increased estimated $\dot{V}O_{2max}$ by 25% ($P=0.020$) and 26% ($P=0.010$), respectively.
23 Anthropometric reductions including body mass ($P=0.038$), waist circumference

1 ($P=0.015$) and fat mass ($P=0.007$) were also observed, but no patient achieved 7-10%
2 weight loss.

3 **Conclusion**

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

7 **Keywords**

8 MAFLD; Aerobic exercise; Exercise intervention; Histological; Cardiorespiratory
9 fitness; NAFLD

10

1 **Introduction**

2 Metabolic (dysfunction) associated fatty liver disease (MAFLD) is now the most
3 common cause of chronic liver disease worldwide with a global estimated prevalence
4 of 25%¹; this is linked to the increasing global incidence of type 2 diabetes mellitus
5 (T2DM) and obesity¹⁻³. MAFLD comprises a spectrum of disease that ranges from
6 simple steatosis to metabolic (dysfunction) associated steatohepatitis (MASH) and is
7 increasingly becoming the leading cause of liver cirrhosis^{2,4} and hepatocellular
8 carcinoma in liver transplant candidates⁵. Patients with MAFLD are also at a high risk
9 of cardiometabolic comorbidities including central obesity, insulin resistance and
10 cardiovascular disease (CVD)^{2,6}, to the extent that a recent consensus statement has
11 proposed the term 'MAFLD' to be used rather than 'non-alcoholic fatty liver disease'
12 (NAFLD)^{7,8}. In the absence of approved pharmacological therapies, lifestyle
13 interventions remain the cornerstone of treatment of MAFLD, with current guidelines
14 recommending a weight loss of 7-10% to achieve optimum histological benefit⁹.

15 Exercise is known to be beneficial for the treatment and prevention of many chronic
16 inflammatory diseases such as cancer, T2DM, arthritis and CVD¹⁰⁻¹². However, the
17 independent role of exercise in the treatment of MAFLD remains unclear. A recent
18 meta-analysis in patients with established MAFLD reported that both aerobic and
19 resistance exercise training, without significant weight loss, produces a 20-30%
20 reduction in intrahepatic lipid content, as assessed by non-invasive methodologies¹³.
21 However, the optimal dose, frequency and type of exercise for improving histological
22 endpoints of MAFLD remains unknown¹⁴. Hickman et al. reported no histological
23 improvements following a six-month resistance exercise intervention¹⁵ while Eckard et
24 al. reported no histological improvements following a six-month combined aerobic and
25 resistance exercise intervention¹⁶, but no other exercise-alone trials using histological

1 endpoints have substantiated these findings. However, cross-sectional studies
2 suggest that moderate-to-vigorous intensity physical activity may be required for
3 histological improvements^{17,18}, and have highlighted the potential role of
4 cardiorespiratory fitness¹⁹. Cardiorespiratory fitness has been proposed to be a
5 validated, independent predictor of all-cause mortality in MAFLD patients²⁰ and
6 therefore could represent an important clinical endpoint for MAFLD patients.

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

16

1 **Materials and Methods**

2 *Ethics Declaration*

3 The study was approved by the St. James's and the Adelaide and Meath Hospitals,
4 Dublin, Ireland, Research Ethics Committee. Written informed consent was obtained
5 from all patients and the study was conducted in accordance with the guidelines
6 outlined in the Declaration of Helsinki, 2013²¹. Recruitment and follow-up occurred
7 between January 2018 and June 2019.

8 *Participants*

9 Twenty-four patients with biopsy-confirmed MAFLD (median age: 61 ± 16 yrs,
10 male/female n: 7/17, mean body mass index [BMI]: 35.7 ± 6.4 kg/m²) attending the
11 hepatology outpatient clinic at St James's Hospital, Dublin, Ireland completed the
12 intervention (exercise group, n=16, control group, n=8). Prior to enrolment, eligible
13 patients had a medical screen to exclude uncontrolled cardiopulmonary disease or
14 other contra-indications to exercise testing or prescription as outlined in the American
15 College of Sports Medicine guidelines¹⁰. Inclusion criteria were: aged ≥18 years,
16 biopsy-proven MAFLD and the ability to attend bi-weekly exercise classes in St
17 James's Hospital for 12 weeks. Exclusion criteria were: contraindications to exercise
18 testing or prescription¹⁰, significant orthopaedic or neuromuscular limitations,
19 unwillingness to participate, alcohol consumption >40g/day (males) or >20g/day
20 (females), or coexisting liver disease. Participant recruitment and attrition rates are
21 presented in Figure 1.

22 *Study Design*

23 Patients were enrolled in this study using NAFLD diagnostic criteria but the term
24 'MAFLD' rather than 'NAFLD' is used throughout this manuscript⁸. Following baseline

1 assessment (T0), 28 participants were recruited by convenience sampling and
2 allocated to an exercise group (n=18) or control group (n=10), without any prescribed
3 dietary changes, based on participants' individual preference. The exercise
4 intervention comprised 3-5 aerobic exercise sessions per week (2 exercise specialist-
5 led supervised exercise sessions and 1-3 unsupervised exercise sessions) for 12
6 weeks. The control group received standard of care. The aerobic exercise intervention
7 protocol is further detailed in *Supporting Methods and Supporting Table 1*. Following
8 completion of the exercise intervention, all participants (exercise group and control
9 group) were reassessed at week 13 (T1). Participants in the exercise group were then
10 encouraged to continue exercise participation but no formal exercise intervention was
11 prescribed or monitored. Both exercise group and control group participants were
12 reassessed at week 24 (T2) and exercise group participants alone were reassessed
13 at week 52 (T3) to determine if the benefits of the exercise intervention were sustained
14 longitudinally. For each assessment timepoint (T0-T3), participants were requested to
15 avoid strenuous physical activity, caffeine and alcohol intake for 24 hours prior to each
16 assessment and fast for 12 hours prior to each assessment to ensure standardisation
17 of each assessment timepoint.

18 *Dietary Assessment*

19 Dietary intakes were assessed at T0 and T1 as previously described²², both by four-
20 day diet diaries returned by mail and by a food frequency questionnaire administered
21 via a 20-min interview by a trained nutritionist. The dietary assessment is further
22 detailed in *Supporting Methods*.

23

24

1 *Histological Analysis of Liver Biopsies*

2 Liver biopsies were performed on all participants (exercise group and control group)
3 at T0 and the exercise group had repeat biopsies at T1. All liver biopsy specimens
4 were reviewed and scored by a single, blinded histopathologist. Hepatic steatosis was
5 scored based on the proportion of hepatocytes affected and subsequently classed into
6 four grades (0-3). The severity of liver injury was assessed and scored using the non-
7 alcoholic steatohepatitis (NASH) Clinical Research Network criteria²³. The MAFLD
8 activity score (MAS) was graded between 0 and 8 and hepatic fibrosis was staged
9 between 0 and 4²⁴.

10 *Transient Elastography Assessment*

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

14 *Cardiorespiratory Fitness and Physical Activity Assessment*

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

24 *Supporting Methods.*

1 *Cardiometabolic Analysis*

2 Standing height was assessed using a wall-mounted vertical stadiometer and body
3 mass was measured using a digital scale. Measures of fat mass and skeletal muscle
4 mass were assessed using bioimpedance analysis (Seca mBCA 515, Seca,
5 Germany). Participants were requested to void their bladder and bowels prior to
6 bioimpedance analysis to ensure standardisation of measurements. To determine the
7 degree of central obesity, waist circumference and hip circumference were measured
8 using a non-stretch measuring tape around the bare abdomen and widest part of the
9 hips, respectively, and waist-to-hip ratio was subsequently calculated. Vascular health
10 was assessed using a Mobil-O-Graph[®] pulse wave analysis monitor (IEM, GmbH,
11 Germany). Fasting venous blood samples were collected to measure liver function
12 tests (LFTs), lipid profiles, fasting plasma glucose (GLUF), glycated haemoglobin
13 (HbA_{1c}) and circulating inflammatory markers (c-reactive protein, CRP; erythrocyte
14 sedimentation rate, ESR; tumour necrosis factor-alpha, TNF- α ; interleukin 6, IL-6 and
15 interleukin 1 β , IL-1 β). TNF- α , IL-6 and IL-1 β concentrations were measured using
16 DuoSet ELISA kits (R&D Systems, USA) and plates were read spectrophotometrically
17 at 450nm using a VersaMax plate reader. All cardiometabolic assessments were
18 assessed at all timepoints (T0-T3).

19 *Statistical Analysis*

20 All statistical analyses were performed using the Statistical Package for the Social
21 Sciences software version 25. Data were assessed for normality using the Shapiro-
22 Wilk test. Baseline between-group differences were assessed using independent *t*-
23 tests or Mann-Whitney *u*-tests for normal and non-normal data, respectively. Paired *t*-
24 tests or Wilcoxon signed-rank tests were used to assess within-group differences for

1 repeated measures for normal and non-normal continuous data, respectively.
2 McNemar's test was used to assess within-group differences for repeated measures
3 for categorical data. Where appropriate, time by group interactions were assessed
4 using a two-way repeated-measures analysis of variance. Measures of effect size
5 were calculated using partial eta² (η^2) and defined as small (0.01), medium (0.06) or
6 large (0.14)²⁵. Pearson's and Spearman's correlation were used to assess
7 associations between normal and non-normal variables, respectively. Where
8 appropriate, missing data is noted on each respective table and figure. Statistical
9 significance for all tests was set at $P \leq 0.05$. Continuous data are displayed as mean
10 (standard deviation) or median (interquartile range) for normal and non-normal data,
11 respectively. Categorical data are displayed as number (percentage).

12

1 **Results**

2 *Baseline characteristics*

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

18 *Changes in cardiorespiratory fitness and physical activity with exercise*

19 At T1, there was a significant time by group interaction in the exercise group, with a
20 large effect size, for estimated $\dot{V}O_{2max}$ (4.7 ± 5.2 mL/min/kg [$17 \pm 18\%$] mean increase,
21 $P=0.027$, partial $\eta^2=0.202$) compared to the control group. There was also a significant
22 within-group improvement in estimated $\dot{V}O_{2max}$ in the exercise group compared to T0
23 ($P=0.003$). At T1, the time spent in sedentary activity, light physical activity and
24 moderate-to-vigorous physical activity was unchanged in both groups. All raw

1 cardiorespiratory fitness and physical activity data between T0 and T1 are detailed in
2 *Supporting Table 2*. At T2, there was no significant time by group interaction in the
3 exercise group for estimated $\dot{V}O_{2\max}$ ($P=0.117$, partial $\eta^2=0.113$) compared to the
4 control group and no significant within-group changes for estimated $\dot{V}O_{2\max}$ ($P=0.437$)
5 in the exercise group compared to T0. At T3, estimated $\dot{V}O_{2\max}$ was not significantly
6 different from T0 ($P=0.354$).

7 *Improvements in cardiometabolic markers with exercise*

8 At T1, there were significant time by group interactions in the exercise group, with
9 large effect sizes, for body mass ($2.1 \pm 2.1\%$ mean reduction, $P=0.038$, partial
10 $\eta^2=0.181$), waist circumference ($4.0 \pm 3.3\%$ mean reduction, $P=0.015$, partial
11 $\eta^2=0.242$) and fat mass ($4.9 \pm 5.2\%$ mean reduction, $P=0.007$, partial $\eta^2=0.289$)
12 compared to the control group. There were also significant within-group reductions in
13 body mass ($P\leq 0.001$), waist circumference ($P\leq 0.001$), waist-to-hip ratio ($2.4 \pm 3.1\%$
14 mean reduction, $P=0.008$) and fat mass ($P\leq 0.001$), in addition to a significant within-
15 group increase in skeletal muscle mass ($3.8 \pm 6.9\%$ mean increase, $P=0.034$) in the
16 exercise group compared to T0, with 3/16 (19%) participants achieving 5% weight loss
17 during the exercise intervention. Anthropometric improvements in the exercise group
18 could be directly attributed to the exercise intervention, as no changes in participants'
19 energy intake or overall dietary quality were observed between T0 and T1 (*Supporting*
20 *Table 3, Supporting Figure 1*). At T1, in the exercise group there were no significant
21 time by group interactions observed compared to the control group, and no significant
22 within-group changes in the exercise group compared to T0 for circulating
23 inflammatory markers, glucose and lipid regulation or measures of vascular health. All
24 raw cardiometabolic data between T0 and T1 are detailed in *Supporting Table 2*. At
25 T2, there was a significant time by group interaction in the exercise group, with a large

1 effect size, for waist circumference ($P=0.029$, partial $\eta^2=0.208$) compared to the
2 control group. There were also significant within-group improvements in waist
3 circumference ($P\leq 0.001$) and BMI ($P\leq 0.001$) in the exercise group compared to T0. At
4 T3, waist circumference ($P=0.211$) and BMI ($P=0.330$) were not significantly different
5 from T0.

6 *Improvements in liver histology with exercise*

7 At baseline, 13/16 (81%) participants in the exercise group had MASH and the
8 remainder had simple steatosis (median MAS: 3.9 ± 1.7). Repeat biopsies were
9 performed on 12/16 (75%) participants in the exercise group within seven days of the
10 completion of the exercise intervention (T1). Four participants refused a repeat biopsy
11 and were excluded from the final histological analysis. At T1, a number of histological
12 changes were observed (Table 3): (i) a significant reduction in fibrosis (Figure 2a.),
13 equating to 7/12 (58%) participants regressing one fibrosis stage (50% net reduction,
14 $P=0.034$); (ii) a significant reduction in hepatocyte ballooning (Figure 2b.), equating to
15 8/12 (67%) participants regressing one hepatocyte ballooning stage (58% net
16 reduction, $P=0.020$); (iii) 2/12 (17%) participants regressed one steatosis stage but
17 2/12 (17%) participants progressed one steatosis stage which led to no significant net
18 changes in steatosis ($P=1.000$); (iv) 3/12 (25%) participants regressed a lobular
19 inflammation stage (one stage $n=2$, two stages $n=1$) but 3/12 (25%) participants
20 progressed one stage, leading to no significant net changes in lobular inflammation
21 ($P=0.739$); and (v) no significant net changes in MAS ($P=0.172$). Improvements in
22 hepatic fibrosis were more strongly associated with improvements in estimated $\dot{V}O_{2\max}$
23 ($r_s = -0.423$, $P=0.171$) than % weight-loss ($r_s = 0.116$, $P=0.720$) or % fat mass loss (r_s
24 $= 0.230$, $P=0.473$) at T1. Similarly, improvements in hepatocyte ballooning were more
25 strongly associated with improvements in estimated $\dot{V}O_{2\max}$ ($r_s = -0.483$, $P=0.111$) than

1 % weight loss ($r_s = 0.160$, $P=0.620$) or % fat mass loss ($r_s = 0.307$, $P=0.473$) at T1.
2 Furthermore, participants who achieved fibrosis regression at T1 ($n=7$) significantly
3 increased estimated $\dot{V}O_{2max}$ by 5.9 ± 5.4 mL/min/kg ($25 \pm 20\%$ increase, $P=0.020$) at
4 this timepoint, while participants without fibrosis regression ($n=5$) demonstrated
5 increased estimated $\dot{V}O_{2max}$ by 2.1 ± 5.7 mL/min/kg ($7 \pm 18\%$ increase, $P=0.590$)
6 (Figure 3a.). Participants with hepatocyte ballooning regression at T1 ($n=8$)
7 significantly increased estimated $\dot{V}O_{2max}$ by 6.5 ± 5.5 mL/min/kg ($26 \pm 20\%$ increase,
8 $P=0.010$) at this timepoint, while participants without hepatocyte ballooning regression
9 ($n=4$) demonstrated increased estimated $\dot{V}O_{2max}$ by 0.04 ± 2.5 mL/min/kg ($2 \pm 12\%$
10 increase, $P=0.980$) (Figure 3b.). There were no significant differences in overall
11 exercise adherence rates between patients with and without fibrosis regression
12 ($P=0.343$) and between patients with and without hepatocyte ballooning regression
13 ($P=0.214$).

14 *Changes in transient elastography measures and liver function tests with exercise*

15 At T1, there was a significant time by group interaction for CAP scores in the exercise
16 group, with a large effect size, compared to the control group ($14.0 \pm 16.7\%$ reduction,
17 $P=0.047$, partial $\eta^2=0.175$). There were no significant time by group interactions for
18 liver stiffness measurements in the exercise group compared to the control group
19 ($P=0.450$, partial $\eta^2=0.029$). There were also significant within-group improvements in
20 CAP scores ($P=0.006$) and liver stiffness measurements ($P=0.028$) in the exercise
21 group compared to T0. There was no significant time by group interactions or within-
22 group changes for LFTs at T1 in either group compared to T0. All raw transient
23 elastography and LFTs data between T0 and T1 are detailed in *Supporting Table 4*.
24 At T2, there were no significant time by group interactions in the exercise for CAP
25 scores ($P=0.233$, partial $\eta^2=0.074$) or liver stiffness measurements ($P=0.872$, partial

1 $\eta^2=0.001$) compared to the control group. There were significant within-group
2 improvements in CAP scores ($P=0.003$) but not liver stiffness measurements
3 ($P=0.056$) in the exercise group compared to T0. At T3, CAP scores ($P=0.182$) and
4 liver stiffness measurements ($P=0.272$) were not significantly different from T0.

5

1 **Discussion**

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

14 Current guidelines state that lifestyle modifications which combine diet and exercise
15 produce significant reductions in MASH and fibrosis, therefore, weight loss is the
16 current primary endpoint for treating MAFLD⁹. The guidelines suggest that weight loss
17 of 7-10% is required for significant improvements in histological endpoints of MAFLD⁹;
18 this was based on one study reporting 90% MASH resolution, 81% fibrosis regression
19 and 100% improvement of steatosis with $\geq 10\%$ weight loss²⁶. Exercise-only
20 interventions have reported reductions in hepatic fat content without significant weight
21 loss, but data assessing the benefits of exercise on histological endpoints in MAFLD
22 patients are limited^{14,27}. In contrast to Hickman et al. and Eckard et al. who reported
23 no significant changes in any histological endpoints following a six-month resistance
24 exercise intervention¹⁵ and six-month combined aerobic and resistance exercise
25 intervention¹⁶, respectively, our study demonstrated statistically significant

1 improvements in hepatic fibrosis and hepatocyte ballooning staging in 58% and 67%
2 of patients following a 12-week moderate-to-vigorous intensity aerobic exercise
3 intervention. This disparity in results may be partially explained by the different study
4 designs employed. Hickman et al. employed moderate intensity resistance exercise
5 training¹⁵ while Eckard et al. employed moderate intensity aerobic and resistance
6 exercise training, but without strict exercise supervision. Aerobic exercise results in
7 relatively higher energy consumption and improves cardiorespiratory fitness, while
8 resistance exercise results in relatively less energy consumption but improves
9 muscular strength and endurance^{12,13}. Furthermore, the review by Kenneally and
10 colleagues reported that exercise supervision provides greater benefits in MAFLD
11 patients during exercise trials²⁷. The increased energy expenditure observed during
12 moderate-to-vigorous intensity aerobic exercise, combined with improvements in
13 cardiorespiratory fitness body composition and exercise supervision in our study may
14 have contributed to histological improvements. While the exact type and intensity of
15 exercise needed for histological benefits in MAFLD remains unclear, moderate-to-
16 vigorous physical activity may be required^{17,18}. Despite the significant regression in
17 hepatic fibrosis and hepatocyte ballooning observed in our study, the benefits did not
18 extend to improvements in histologically measured steatosis and MAS, in line with
19 previous published data^{15,16}.

20 The improvement in estimated $\dot{V}O_{2max}$ observed at T1 indicates that the intensity, type
21 and frequency of exercise was sufficient to induce significant improvements in
22 cardiorespiratory fitness. These improvements in estimated $\dot{V}O_{2max}$ were associated
23 with fibrosis and ballooning regression, suggesting a potential interrelationship.
24 Patients who achieved fibrosis and hepatocyte ballooning regression significantly
25 increased estimated $\dot{V}O_{2max}$ by 25-26%, with minimal body mass reductions (1-2%),

1 suggesting that improvements in cardiorespiratory fitness may be a more sensitive
2 clinical endpoint for histological changes in MAFLD patients during exercise trials
3 rather than weight loss. Cardiorespiratory fitness has previously been demonstrated
4 to be inversely associated with MASH²⁸ and predicts hepatic fat loss during lifestyle
5 interventions²⁹. In addition to these benefits, a 3.5mL/min/kg increase in $\dot{V}O_{2max}$ is
6 associated with a 10-25% reduction in all-cause mortality in the US general
7 population^{30,31} and represents an important clinical modifier for CVD risk, the leading
8 cause of mortality in MAFLD populations^{20,32}.

9 The physiological mechanisms underlying the change in liver fat following exercise
10 training in MAFLD are well described and include changes in energy-balance,
11 circulating lipids and insulin sensitivity¹⁴. However, the exact mechanisms underlying
12 exercise-induced improvements in MASH and fibrosis are unknown but may relate to
13 exercise-induced changes in intrahepatic inflammatory and fibrogenic activity. Hepatic
14 stellate cells are a key mediator in the initiation, progression and regression of hepatic
15 fibrosis³³ and several rodent studies have linked exercise participation with reduced
16 hepatic stellate cell activity, independently of weight loss³⁴⁻³⁶. Exercise training is
17 known to have anti-inflammatory effects³⁷ but whether these anti-inflammatory effects
18 directly lead to improvements in local hepatic inflammatory pathways in MASH
19 patients is unknown. Although our study did not observe significant reductions in
20 circulating inflammatory markers, similar to published data³⁸, reductions in
21 inflammatory mediators may have been specific to hepatic tissue and therefore not
22 detected in circulation³⁹, as reported in rodent studies with significant reductions in
23 intrahepatic immune cell populations following exercise training^{35,40,41}. In the study by
24 Kawanishi et al., obesogenic mice that exercised for 60 min/day, five times/week, for
25 16 weeks demonstrated significant reductions in hepatic TNF- α levels, resident

1 macrophage infiltration, and fibrosis markers (Sirius red and α -smooth muscle actin
2 staining, and tissue inhibition of matrix metalloproteinase-1 mRNA)³⁵. Huber et al.
3 reported significant reductions in TNF-mediated liver injury, intrahepatic CD45 positive
4 leukocyte populations, and inflammatory cytokines following seven weeks of exercise
5 in healthy mice⁴⁰. Similarly, after four weeks of voluntary wheel running in a group of
6 obesogenic mice, Gehrke et al. reported significant reductions in hepatic inflammatory
7 cytokine expression and intrahepatic macrophages infiltration, with improvements in
8 histological steatosis, ballooning and inflammation⁴¹. Interestingly, these intrahepatic
9 immunological changes in these studies occurred without significant weight loss^{35,40,41}.
10 Collectively, these rodent studies indicate the exercise-induced change in intrahepatic
11 anti-inflammatory pathways which may contribute to histological regression in MAFLD
12 patients. Changes in intrahepatic immune cells were not investigated in our study, but
13 reports of changes in circulating immune cell populations in individuals with a higher
14 cardiorespiratory fitness suggest a potential link between exercise-induced changes
15 in cardiorespiratory fitness and histological endpoints⁴²⁻⁴⁴.

16 While our study did not assess the link between hepatic inflammation and fibrosis and
17 visceral adipose tissue (VAT), liver necroinflammation and fibrosis increase
18 significantly with VAT in a dose-dependent manner⁴⁵. VAT can synthesise and secrete
19 cytokines and adipokines, and IL-6 and TNF α are expressed in greater amount in VAT
20 than subcutaneous fat⁴⁶. We were unable to show any significant difference in
21 circulating IL-6 or TNF- α at T1 in patients who demonstrated a significant reduction in
22 waist circumference and waist-to-hip ratio, a clinical surrogate of VAT. One possible
23 explanation may relate to the lack of steatosis regression^{38,45}.

24 The failure to sustain the benefits of the exercise intervention at 12 months post
25 exercise intervention completion (T3) is in keeping with previous exercise interventions

1 in MAFLD⁴⁷, T2DM⁴⁸, and obesity⁴⁹ cohorts, and emphasises the unmet need for
2 exercise maintenance in the unsupervised setting. Following a 16-week exercise
3 intervention in patients with MAFLD⁴⁷, Pugh et al. observed that improvements in liver
4 fat and $\dot{V}O_{2peak}$ were not sustained at a 12-month follow-up reassessment, concluding
5 that effective mechanisms for promoting long-term sustainability of exercise in MAFLD
6 cohorts are urgently required. Studies investigating the use of smart technology for the
7 prescription of exercise in MAFLD cohorts are emerging. Two recent studies which
8 incorporated an eight-week, web-based exercise intervention reported significant
9 improvements in surrogate markers of hepatic fibrosis, $\dot{V}O_{2peak}$ and fat mass upon
10 completion of the exercise intervention and, furthermore, that these benefits were
11 sustained at 12-week follow-up reassessment^{50,51}. The authors concluded that
12 individualisation of the exercise intervention and appropriate patient education are
13 important factors to achieve sustained benefits and continued self-driven exercise.
14 The high adherence rate to exercise during the exercise intervention of 93% in our
15 study indicates that a group training approach may have improved patient motivation,
16 and conversely, once completed, contributed to the attrition of the exercise
17 intervention benefits longitudinally. Furthermore, the implementation of a care bundle
18 approach, where patients have multiple intervention options determined at a patient
19 individual level, may help sustain intervention benefits⁵².

20 *Limitations*

21 This study has limitations: (i) the small sample size (n=24) and lack of liver biopsies at
22 T1 in the control group makes it difficult to draw definitive conclusions on the effects
23 of aerobic exercise on histological endpoints of MAFLD; (ii) the requirement for two
24 liver biopsies proved challenging and limited study recruitment; (iii) the study was not
25 powered to detect significant histological changes and therefore type 2 error cannot

1 be disregarded; (iv) the study was not randomised; patients were allocated to the
2 exercise group or control group based on individual preference, which may indicate a
3 degree of bias; and (v) medication history and dosage was recorded at baseline but
4 not at other timepoints. It is possible that medication dose changes/removal of
5 medications may have occurred during the study which may have influenced
6 outcomes.

7 *Conclusions*

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

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Table 1. Baseline participant characteristics

Variable	Exercise group (n=16)	Control group (n=8)	Between-group <i>p</i> value
Age, years †	61 (15)	58 (23)	0.444 ^a
Gender, n (%n)			0.647 ^b
Female	12 (75)	5 (63)	
Male	4 (25)	3 (37)	
T2DM/IGT, n (%n)	11 (69)	6 (75)	1.000 ^b
Hypoglycaemic medications, n (%n)	9 (56)	5 (63)	1.000 ^b
Hypertension, n (%n)	9 (56)	4 (50)	1.000 ^b
Anti-hypertensive medication, n (%n)	9 (56)	3 (38)	0.667 ^b
Hypercholesteremia, n (%n)	9 (56)	4 (50)	1.000 ^b
Lipid lowering medications, n (%n)	9 (56)	3 (38)	0.667 ^b
Hypertriglyceridemia, n (%n)	6 (38)	3 (38)	1.000 ^b
Polypharmacy, n (%n)	7 (44)	2 (25)	0.657 ^b
MetSyn, n (%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 (%n)			1.000 ^b
Overweight (25.0-29.9kg/m ²)	3 (19)	2 (25)	
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 (%n)			1.000 ^b
Below average	14 (88)	7 (88)	
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

1 Notes: †Non-normal data (median [interquartile range]), ‡Normal data (mean [standard deviation]),
2 ¹n=15, ²n=7, ^aMann-Whitney u-test, ^bFisher's exact test, ^cIndependent t-test, T2DM=Type 2 Diabetes
3 Mellitus, IGT=Impaired Glucose Tolerance, MetSyn=Metabolic Syndrome, BMI=Body Mass Index,
4 $\dot{V}O_{2max}$ =Maximal Oxygen Consumption, ALT=Alanine Aminotransferase, AST=Aspartate
5 Aminotransferase
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Table 2. Baseline liver histology

Variable	Exercise group (n=16)	Control group (n=8)	Between-group <i>P</i> value
MAS †	3.9 (1.7)	4.6 (2.1)	0.360 ^a
MAS components, n (%n)			0.673 ^b
≥5	6 (38)	4 (50)	
<5	10 (63)	4 (50)	
Steatosis, n (%n)			0.282 ^b
<5% (0)	0 (0)	1 (12.5)	
5-33% (1)	8 (50)	2 (25)	
33-66% (2)	4 (25)	4 (50)	
>66% (3)	4 (25)	1 (12.5)	
Lobular inflammation, n (%n)			0.103 ^b
None (0)	3 (19)	0 (0)	
<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 (%n)			0.521 ^b
None (0)	3 (19)	2 (24)	
Few Cells (1)	10 (62)	3 (38)	
Many Cells (2)	3 (19)	3 (38)	
MASH, n (%n)			1.000 ^b
Yes	13 (81)	6 (75)	
No	3 (19)	2 (25)	
Fibrosis, n (%n)			0.281 ^b
Absent (0)	1 (6)	0 (0)	
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)	

1 Notes: † Normal data (mean [standard deviation]), ^aIndependent t-test, ^bFisher's exact test, MAS=MAFLD
 2 Activity Score, MASH=Metabolic (dysfunction) Associated Steatohepatitis

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Table 3. Changes in histological staging between pre-intervention (T0) and post-intervention (T1) timepoints (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	<i>P=0.034*</i>
Hepatic steatosis	
Increased 1 stage	2
Maintained the same stage	8
Decreased 1 stage	2
Net Change	0
Significance	<i>P=1.000</i>
Lobular inflammation	
Increased 1 stage	3
Maintained the same stage	6
Decreased 1 stage	2
Decreased 2 stages	1
Net change	-1
Significance	<i>P=0.739</i>
Hepatocellular ballooning	
Increased 1 stage	1
Maintained the same stage	3
Decreased 1 stage	8
Net change	-7
Significance	<i>P=0.020*</i>
MAS	
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	<i>P=0.172</i>

Notes: MAS=NAFLD Activity Score, * $P \leq 0.05$ (Wilcoxon signed-rank test)

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1 **Statements of Interest**

2 **Declaration of funding interests:** This study was funded, in full, by a grant held by
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5 **Conflicts of Interest:** The authors who have taken part in this study declared that they
6 do not have anything to disclose or any conflicts of interest with respect to this
7 manuscript.

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STROBE Checklist

	Item No.	Recommendation	Page No.	Relevant text from manuscript
Title and abstract	1	(a) Indicate the study's design with a commonly used term in the title or the abstract	1	N/A
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found	3 and 4	<p>“Patients with biopsy confirmed MAFLD participated in a 12-week aerobic exercise intervention. Liver histology, cardiorespiratory fitness (estimated $\dot{V}O_2\text{max}$), physical activity, anthropometry and biochemical markers were assessed at baseline, intervention completion, and 12 and 52 weeks after intervention completion”</p> <p>“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,</p>

with no changes in steatosis (P=1.000), lobular inflammation (P=0.739) or MAFLD activity score (P=0.172). Estimated $\dot{V}O_2\text{max}$ 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 $\dot{V}O_2\text{max}$ 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”

Introduction

Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	5 and 6	“However, the optimal dose, frequency and type of exercise for improving histological endpoints of MAFLD remains unknown ¹⁴ . Hickman et al. reported no histological improvements following a six-month resistance exercise intervention while Eckard et al. reported no histological improvements following a six-month combined aerobic and resistance exercise intervention, 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
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				histological improvements, and have highlighted the potential role of cardiorespiratory fitness.”
Objectives	3	State specific objectives, including any prespecified hypotheses	6	“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.”
Methods				
Study design	4	Present key elements of study design early in the paper	7 and 8	Following baseline assessment (T0), 28 participants were allocated to an exercise group (n=18) or control group (n=10), without any prescribed dietary changes. The exercise intervention comprised 3-5 aerobic exercise sessions per week (2 exercise specialist-led supervised exercise sessions and 1-3 unsupervised exercise sessions) for 12 weeks.
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	7	“Recruitment and follow-up occurred between January 2018 and June 2019.”

			<p>“Twenty-four patients with biopsy-confirmed MAFLD (median age: 61 ± 16 yrs, male/female n: 7/17, mean body mass index [BMI]: 35.7 ± 6.4 kg/m²) attending the hepatology outpatient clinic at St James’s Hospital, Dublin, Ireland completed the intervention (exercise group, n=16, control group, n=8).”</p>	
Participants	6	<p>(a) <i>Cohort study</i>—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up</p> <p><i>Case-control study</i>—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls</p> <p><i>Cross-sectional study</i>—Give the eligibility criteria, and the sources and methods of selection of participants</p>	7	<p>“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 prescription¹⁰, significant orthopaedic or neuromuscular limitations, unwillingness to participate, alcohol consumption >40g/day (males) or</p>

				>20g/day (females), or coexisting liver disease”
		(b) <i>Cohort study</i> —For matched studies, give matching criteria and number of exposed and unexposed	N/A	N/A
		<i>Case-control study</i> —For matched studies, give matching criteria and the number of controls per case		
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	8-10	“Dietary assessment”; “Histological analysis of liver biopsies”; “Transient elastography assessment”; “Cardiorespiratory fitness and physical activity levels assessment”; “Cardiometabolic analysis”; “Statistical analysis”
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	8-10	“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”

“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.”

“A transient elastography
device (FibroScan® touch
502, Echosens, France) was
used to non-invasively
assess hepatic fibrosis (liver
stiffness score) and steatosis
(controlled attenuation
parameter [CAP])

measurements at all timepoints (T0-T3).”

“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, USA).”

“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, Germany). 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 non-stretch 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 (HbA1c) and circulating inflammatory markers (c-reactive protein, CRP; erythrocyte sedimentation rate, ESR; tumour necrosis factor-alpha, TNF- α ; interleukin 6, IL-6 and interleukin 1 β , IL-1 β). TNF- α , IL-6 and IL-1 β concentrations were measured using DuoSet ELISA kits (R&D Systems, USA) and plates were read spectrophotometrically at 450nm using a VersaMax plate reader. All cardiometabolic assessments were assessed at all timepoints (T0-T3).”

Bias	9	Describe any efforts to address potential sources of bias	N/A	Not completed as this study was a pilot study
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Study size	10	Explain how the study size was arrived at	21-22	<p>The study size was based on a convenience sample as it was a pilot study.</p> <p>“the study was not powered to detect significant histological changes and therefore type 2 error cannot be disregarded; and (iv) the study was not randomised and patients were allocated to the exercise group or control group based on individual preference, which may indicate a degree of bias.”</p>
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Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	N/A	N/A
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding	11	“Statistical analysis”
		(b) Describe any methods used to examine subgroups and interactions	11	“Where appropriate, time by group interactions were assessed using a 2-way repeated-measures analysis of variance.”
		(c) Explain how missing data were addressed	11	“Where appropriate, missing data is noted on each respective table and figure.”
		(d) <i>Cohort study</i> —If applicable, explain how loss to follow-up was addressed <i>Case-control study</i> —If applicable, explain how matching of cases and controls was addressed <i>Cross-sectional study</i> —If applicable, describe analytical methods taking account of sampling strategy	N/A	N/A
		(e) Describe any sensitivity analyses	N/A	N/A
Results				
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	7 and Figure 1	“Twenty-four patients with biopsy-confirmed MAFLD (median age: 61 ± 16 yrs, male/female n: 7/17, mean

				body mass index [BMI]: 35.7 ± 6.4 kg/m ²) attending the hepatology outpatient clinic at St James's Hospital, Dublin, Ireland completed the intervention (exercise group, n=16, control group, n=8)."
		(b) Give reasons for non-participation at each stage	12	"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.)."
		(c) Consider use of a flow diagram	Supporting Document Page 1	"Figure 1."
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	11	"Baseline participant characteristics and histological characteristics are detailed in Table 1 and Table 2, respectively. The exercise

group and control group were well matched with no significant differences between baseline or histological characteristics. 79% of the cohort had the diagnostic criteria for MASH. The cohort had coexisting comorbidities: obesity (79%), T2DM (71%), hypertension (56%), metabolic syndrome (63%) and below-average cardiorespiratory fitness (88%).”

(b) Indicate number of participants with missing data for each variable of interest	<p>Table 1 (page 27), Table 2 (page 28), Table 3 (page 29),</p> <p>Supporting Table 1, Supplementary Table 2, Supporting Table 3 and</p>	<p>Example from Table 1: ¹<i>n</i>=15, ²<i>n</i>=7”</p>
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			Supporting Table 4	
		(c) <i>Cohort study</i> —Summarise follow-up time (eg, average and total amount)	N/A	N/A
Outcome data	15*	<i>Cohort study</i> —Report numbers of outcome events or summary measures over time	N/A	N/A
		<i>Case-control study</i> —Report numbers in each exposure category, or summary measures of exposure	N/A	N/A
		<i>Cross-sectional study</i> —Report numbers of outcome events or summary measures	N/A	N/A
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	12-16 and Table 1 (page 25), Table 2 (page 26), Table 3 (page 27), Supporting Table 1, Supplementary Table 2, Supporting Table 3 and Supporting Table 4	All results contain a descriptor of central tendency (mean/median) and precision (standard deviation/interquartile range). Example from cardiorespiratory fitness and physical activity results (page 12-13): “At T1, there was a significant time by group interaction in the exercise group, with a large effect size, for estimated $\dot{V}O_{2\max}$ ($4.7 \pm 5.2\text{mL/min/kg}$ [$17 \pm 18\%$] 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).”

Example from Table 1 (page 25):

“Notes: †Non-normal data (median [interquartile range]), ‡Normal data (mean [standard deviation])”

(b) Report category boundaries when continuous variables were categorized	N/A	N/A
(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	N/A	N/A

Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	15	<p>“Furthermore, participants who achieved fibrosis regression at T1 (n=7) significantly increased $\dot{V}O_{2max}$ by 5.9 ± 5.4 mL/min/kg ($25 \pm 20\%$ increase, $p=0.02$) at this timepoint, while participants without fibrosis regression (n=5) demonstrated increased $\dot{V}O_{2max}$ by 2.1 ± 5.7 mL/min/kg ($7 \pm 18\%$ increase, $p=0.59$) (Figure 3a.). Participants with hepatocyte ballooning regression at T1 (n=8) significantly increased $\dot{V}O_{2max}$ by 6.5 ± 5.5 mL/min/kg ($26 \pm 20\%$ increase, $p=0.01$) at this timepoint, while participants without hepatocyte ballooning regression (n=4) demonstrated increased $\dot{V}O_{2max}$ by 0.04 ± 2.5 mL/min/kg ($2 \pm 12\%$ increase, $p=0.98$) (Figure 3b.).”</p>
Discussion				
Key results	18	Summarise key results with reference to study objectives	17	<p>“This study investigated the effects of a 12-week, moderate-to-vigorous intensity aerobic</p>

exercise intervention, in the absence of dietary change, on histological and cardiometabolic endpoints in patients with biopsy confirmed MAFLD. The main findings were: (i) 12 weeks of aerobic exercise produced significant histological improvements in hepatic fibrosis and hepatocyte ballooning; (ii) 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%; (iii) 12 weeks of aerobic exercise did not produce significant histological changes in steatosis or lobular inflammation grades; (iv) 12 weeks of aerobic exercise did not produce significant changes in vascular health or lipid and glucose regulation; and (v) in the absence of continuous prescribed and monitored

			exercise, the benefits of the 12-week aerobic exercise intervention were not sustained by T3.”	
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	21-22	“This study has limitations: (i) 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; (ii) the requirement for two liver biopsies proved challenging and limited study recruitment; (iii) the study was not powered to detect significant histological changes and therefore type 2 error cannot be disregarded; (iv) 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 (v) medication history

and dosage was recorded at baseline but not at other timepoints. It is possible that medication dose changes/removal of medications may have occurred during the study which may have influenced outcomes.”

Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	22
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“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 one-year follow-up”
Generalisability	21	Discuss the generalisability (external validity) of the study results	22	“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.”
Other information				
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	30	“Declaration of funding interests: This study was funded, in full, by a grant held by

Suzanne Norris from the Health Research Board, Ireland (grant number: HRA-POR-2015-1185). Philip O’Gorman was funded through this grant for his PhD studentship.

Conflicts of Interest: The authors who have taken part in this study declared that they do not have anything to disclose any conflicts of interest with respect to this manuscript.”
