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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

3 **Specific author contributions:**

4 **Study concept:** Philip O’Gorman, Sara Naimimohasses, Ann Monaghan, John Gormley and  
5 Suzanne Norris

6 **Study design:** Philip O’Gorman, Sara Naimimohasses, Ann Monaghan, Derek Doherty, John  
7 Gormley and Suzanne Norris

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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<sub>1c</sub>, glycated haemoglobin; IL-  
19 1 $\beta$ , interleukin 1 $\beta$ ; 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- $\alpha$ , tumour necrosis factor  $\alpha$ ; 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%<sup>1</sup>; this is linked to the increasing global incidence of type 2 diabetes mellitus  
5 (T2DM) and obesity<sup>1-3</sup>. 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<sup>2,4</sup> and hepatocellular  
8 carcinoma in liver transplant candidates<sup>5</sup>. Patients with MAFLD are also at a high risk  
9 of cardiometabolic comorbidities including central obesity, insulin resistance and  
10 cardiovascular disease (CVD)<sup>2,6</sup>, 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)<sup>7,8</sup>. 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 benefit<sup>9</sup>.

15 Exercise is known to be beneficial for the treatment and prevention of many chronic  
16 inflammatory diseases such as cancer, T2DM, arthritis and CVD<sup>10-12</sup>. 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 methodologies<sup>13</sup>.  
21 However, the optimal dose, frequency and type of exercise for improving histological  
22 endpoints of MAFLD remains unknown<sup>14</sup>. Hickman et al. reported no histological  
23 improvements following a six-month resistance exercise intervention<sup>15</sup> while Eckard et  
24 al. reported no histological improvements following a six-month combined aerobic and  
25 resistance exercise intervention<sup>16</sup>, 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<sup>17,18</sup>, and have highlighted the potential role of  
4 cardiorespiratory fitness<sup>19</sup>. Cardiorespiratory fitness has been proposed to be a  
5 validated, independent predictor of all-cause mortality in MAFLD patients<sup>20</sup> 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, 2013<sup>21</sup>. 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/m<sup>2</sup>) 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 guidelines<sup>10</sup>. 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 prescription<sup>10</sup>, 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 manuscript<sup>8</sup>. 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 described<sup>22</sup>, 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 criteria<sup>23</sup>. The MAFLD  
8 activity score (MAS) was graded between 0 and 8 and hepatic fibrosis was staged  
9 between 0 and 4<sup>24</sup>.

## 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)<sup>12</sup> 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<sup>®</sup> 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<sub>1c</sub>) and circulating inflammatory markers (c-reactive protein, CRP; erythrocyte  
14 sedimentation rate, ESR; tumour necrosis factor-alpha, TNF- $\alpha$ ; interleukin 6, IL-6 and  
15 interleukin 1 $\beta$ , IL-1 $\beta$ ). TNF- $\alpha$ , IL-6 and IL-1 $\beta$  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 eta<sup>2</sup> ( $\eta^2$ ) and defined as small (0.01), medium (0.06) or  
6 large (0.14)<sup>25</sup>. 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  $\dot{V}O_{2max}$  ( $4.7 \pm 5.2$  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:  $3.9 \pm 1.7$ ). 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  $5.9 \pm 5.4$  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  $2.1 \pm 5.7$  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  $6.5 \pm 5.5$  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  $0.04 \pm 2.5$  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%<sup>9</sup>; (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 MAFLD<sup>9</sup>. The guidelines suggest that weight loss  
17 of 7-10% is required for significant improvements in histological endpoints of MAFLD<sup>9</sup>;  
18 this was based on one study reporting 90% MASH resolution, 81% fibrosis regression  
19 and 100% improvement of steatosis with  $\geq 10\%$  weight loss<sup>26</sup>. 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<sup>14,27</sup>. 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 intervention<sup>15</sup> and six-month combined aerobic and resistance exercise  
25 intervention<sup>16</sup>, 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 training<sup>15</sup> 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<sup>12,13</sup>. Furthermore, the review by Kenneally and  
10 colleagues reported that exercise supervision provides greater benefits in MAFLD  
11 patients during exercise trials<sup>27</sup>. 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<sup>17,18</sup>. 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<sup>15,16</sup>.

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 MASH<sup>28</sup> and predicts hepatic fat loss during lifestyle  
5 interventions<sup>29</sup>. 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<sup>30,31</sup> and represents an important clinical modifier for CVD risk, the leading  
8 cause of mortality in MAFLD populations<sup>20,32</sup>.

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 sensitivity<sup>14</sup>. 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 fibrosis<sup>33</sup> and several rodent studies have linked exercise participation with reduced  
16 hepatic stellate cell activity, independently of weight loss<sup>34-36</sup>. Exercise training is  
17 known to have anti-inflammatory effects<sup>37</sup> 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 data<sup>38</sup>, reductions in  
21 inflammatory mediators may have been specific to hepatic tissue and therefore not  
22 detected in circulation<sup>39</sup>, as reported in rodent studies with significant reductions in  
23 intrahepatic immune cell populations following exercise training<sup>35,40,41</sup>. 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- $\alpha$  levels, resident

1 macrophage infiltration, and fibrosis markers (Sirius red and  $\alpha$ -smooth muscle actin  
2 staining, and tissue inhibition of matrix metalloproteinase-1 mRNA)<sup>35</sup>. 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 mice<sup>40</sup>. 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 inflammation<sup>41</sup>. Interestingly, these intrahepatic  
9 immunological changes in these studies occurred without significant weight loss<sup>35,40,41</sup>.  
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 endpoints<sup>42-44</sup>.

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 manner<sup>45</sup>. VAT can synthesise and secrete  
19 cytokines and adipokines, and IL-6 and TNF $\alpha$  are expressed in greater amount in VAT  
20 than subcutaneous fat<sup>46</sup>. We were unable to show any significant difference in  
21 circulating IL-6 or TNF- $\alpha$  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<sup>38,45</sup>.

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 MAFLD<sup>47</sup>, T2DM<sup>48</sup>, and obesity<sup>49</sup> cohorts, and emphasises the unmet need for  
2 exercise maintenance in the unsupervised setting. Following a 16-week exercise  
3 intervention in patients with MAFLD<sup>47</sup>, Pugh et al. observed that improvements in liver  
4 fat and  $\dot{V}O_{2\text{peak}}$  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,  $\dot{V}O_{2\text{peak}}$  and fat mass upon  
10 completion of the exercise intervention and, furthermore, that these benefits were  
11 sustained at 12-week follow-up reassessment<sup>50,51</sup>. 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 benefits<sup>52</sup>.

## 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<sup>19,20,28,31</sup>. 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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## 1 References

- 2 1 Younossi, Z. *et al.* Global burden of NAFLD and NASH: trends, predictions, risk  
3 factors and prevention. *Nat Rev Gastroenterol Hepatol* **15**, 11-20,  
4 doi:10.1038/nrgastro.2017.109 (2018).
- 5 2 Diehl, A. M. & Day, C. Cause, Pathogenesis, and Treatment of Nonalcoholic  
6 Steatohepatitis. *N Engl J Med* **377**, 2063-2072, doi:10.1056/NEJMra1503519  
7 (2017).
- 8 3 Moore, J. B. From sugar to liver fat and public health: systems biology driven  
9 studies in understanding non-alcoholic fatty liver disease pathogenesis. *Proc*  
10 *Nutr Soc* **78**, 290-304, doi:10.1017/s0029665119000570 (2019).
- 11 4 Li, B., Zhang, C. & Zhan, Y. T. Nonalcoholic Fatty Liver Disease Cirrhosis: A  
12 Review of Its Epidemiology, Risk Factors, Clinical Presentation, Diagnosis,  
13 Management, and Prognosis. *Can J Gastroenterol Hepatol* **2018**, 2784537,  
14 doi:10.1155/2018/2784537 (2018).
- 15 5 Younossi, Z. *et al.* Nonalcoholic Steatohepatitis Is the Fastest Growing Cause  
16 of Hepatocellular Carcinoma in Liver Transplant Candidates. *Clin Gastroenterol*  
17 *Hepatol* **17**, 748-755.e743, doi:10.1016/j.cgh.2018.05.057 (2019).
- 18 6 Armstrong, M. J., Adams, L. A., Canbay, A. & Syn, W.-K. Extrahepatic  
19 complications of nonalcoholic fatty liver disease. *Hepatology* **59**, 1174-1197,  
20 doi:10.1002/hep.26717 (2014).
- 21 7 Eslam, M., Sanyal, A. J. & George, J. MAFLD: A Consensus-Driven Proposed  
22 Nomenclature for Metabolic Associated Fatty Liver Disease. *Gastroenterology*  
23 **158**, 1999-2014.e1991, doi:10.1053/j.gastro.2019.11.312 (2020).
- 24 8 The Lancet Gastroenterology Hepatology. Redefining non-alcoholic fatty liver  
25 disease: what's in a name? *Lancet Gastroenterol Hepatol* **5**, 419,  
26 doi:10.1016/s2468-1253(20)30091-1 (2020).
- 27 9 EASL. EASL-EASD-EASO Clinical Practice Guidelines for the management of  
28 non-alcoholic fatty liver disease. *J Hepatol* **64**, 1388-1402,  
29 doi:10.1016/j.jhep.2015.11.004 (2016).
- 30 10 American College of Sports Medicine. *ACSM's guidelines for exercise testing*  
31 *and prescription*. (2018).
- 32 11 World Health Organisation. *Global Recommendations on Physical Activity for*  
33 *Health*. (2010).
- 34 12 Heyward, V. H. *Advanced fitness assessment and exercise prescription*.  
35 Seventh edition. edn, (Champaign, IL : Human Kinetics, 2014).
- 36 13 Hashida, R. *et al.* Aerobic vs. resistance exercise in non-alcoholic fatty liver  
37 disease: A systematic review. *J Hepatol* **66**, 142-152,  
38 doi:10.1016/j.jhep.2016.08.023 (2017).
- 39 14 Romero-Gomez, M., Zelber-Sagi, S. & Trenell, M. Treatment of NAFLD with  
40 diet, physical activity and exercise. *J Hepatol* **67**, 829-846,  
41 doi:10.1016/j.jhep.2017.05.016 (2017).
- 42 15 Hickman, I. *et al.* A Pilot Randomised Study of the Metabolic and Histological  
43 Effects of Exercise in Non-alcoholic Steatohepatitis. *Journal of Diabetes &*  
44 *Metabolism* **4** (2013).
- 45 16 Eckard, C. *et al.* Prospective histopathologic evaluation of lifestyle modification  
46 in nonalcoholic fatty liver disease: a randomized trial. *Therap Adv Gastroenterol*  
47 **6**, 249-259, doi:10.1177/1756283x13484078 (2013).

1 17 Kistler, K. D. *et al.* Physical activity recommendations, exercise intensity, and  
2 histological severity of nonalcoholic fatty liver disease. *Am J Gastroenterol* **106**,  
3 460-468; quiz 469, doi:10.1038/ajg.2010.488 (2011).

4 18 Cho, J., Kim, S., Lee, S. & Kang, H. Effect of Training Intensity on Nonalcoholic  
5 Fatty Liver Disease. *Med Sci Sports Exerc* **47**, 1624-1634,  
6 doi:10.1249/mss.0000000000000595 (2015).

7 19 Johnson, N. A. & George, J. Fitness versus fatness: moving beyond weight loss  
8 in nonalcoholic fatty liver disease. *Hepatology* **52**, 370-381,  
9 doi:10.1002/hep.23711 (2010).

10 20 Croci, I. *et al.* Non-alcoholic fatty liver disease: Prevalence and all-cause  
11 mortality according to sedentary behaviour and cardiorespiratory fitness. The  
12 HUNT Study. *Progress in Cardiovascular Diseases* **62**, 127-134,  
13 doi:<https://doi.org/10.1016/j.pcad.2019.01.005> (2019).

14 21 World Medical Association. World Medical Association Declaration of Helsinki:  
15 ethical principles for medical research involving human subjects. *Jama* **310**,  
16 2191-2194, doi:10.1001/jama.2013.281053 (2013).

17 22 Bredin, C. *et al.* Development and relative validation of a short food frequency  
18 questionnaire for assessing dietary intakes of non-alcoholic fatty liver disease  
19 patients. *Eur J Nutr* **59**, 571-580, doi:10.1007/s00394-019-01926-5 (2020).

20 23 Kleiner, D. E. *et al.* Design and validation of a histological scoring system for  
21 nonalcoholic fatty liver disease. *Hepatology* **41**, 1313-1321,  
22 doi:10.1002/hep.20701 (2005).

23 24 Brunt, E. M., Janney, C. G., Di Bisceglie, A. M., Neuschwander-Tetri, B. A. &  
24 Bacon, B. R. Nonalcoholic steatohepatitis: a proposal for grading and staging  
25 the histological lesions. *Am J Gastroenterol* **94**, 2467-2474, doi:10.1111/j.1572-  
26 0241.1999.01377.x (1999).

27 25 Richardson, J. T. E. Eta squared and partial eta squared as measures of effect  
28 size in educational research. *Educational Research Review* **6**, 135-147,  
29 doi:<https://doi.org/10.1016/j.edurev.2010.12.001> (2011).

30 26 Vilar-Gomez, E. *et al.* Weight Loss Through Lifestyle Modification Significantly  
31 Reduces Features of Nonalcoholic Steatohepatitis. *Gastroenterology* **149**, 367-  
32 378.e365; quiz e314-365, doi:10.1053/j.gastro.2015.04.005 (2015).

33 27 Kenneally, S., Sier, J. H. & Moore, J. B. Efficacy of dietary and physical activity  
34 intervention in non-alcoholic fatty liver disease: a systematic review. *BMJ Open*  
35 *Gastroenterol* **4**, e000139, doi:10.1136/bmjgast-2017-000139 (2017).

36 28 Krasnoff, J. B., Painter, P. L., Wallace, J. P., Bass, N. M. & Merriman, R. B.  
37 Health-related fitness and physical activity in patients with nonalcoholic fatty  
38 liver disease. *Hepatology* **47**, 1158-1166, doi:10.1002/hep.22137 (2008).

39 29 Kantartzis, K. *et al.* High cardiorespiratory fitness is an independent predictor  
40 of the reduction in liver fat during a lifestyle intervention in non-alcoholic fatty  
41 liver disease. *Gut* **58**, 1281-1288, doi:10.1136/gut.2008.151977 (2009).

42 30 Kaminsky, L. A. *et al.* The importance of cardiorespiratory fitness in the United  
43 States: the need for a national registry: a policy statement from the American  
44 Heart Association. *Circulation* **127**, 652-662,  
45 doi:10.1161/CIR.0b013e31827ee100 (2013).

46 31 Ross, R. *et al.* Importance of Assessing Cardiorespiratory Fitness in Clinical  
47 Practice: A Case for Fitness as a Clinical Vital Sign: A Scientific Statement  
48 From the American Heart Association. *Circulation* **134**, e653-e699,  
49 doi:doi:10.1161/CIR.0000000000000461 (2016).

- 1 32 Francque, S. M., van der Graaff, D. & Kwanten, W. J. Non-alcoholic fatty liver  
2 disease and cardiovascular risk: Pathophysiological mechanisms and  
3 implications. *J Hepatol* **65**, 425-443, doi:10.1016/j.jhep.2016.04.005 (2016).
- 4 33 Zhang, C.-Y., Yuan, W.-G., He, P., Lei, J.-H. & Wang, C.-X. Liver fibrosis and  
5 hepatic stellate cells: Etiology, pathological hallmarks and therapeutic targets.  
6 *World journal of gastroenterology* **22**, 10512-10522,  
7 doi:10.3748/wjg.v22.i48.10512 (2016).
- 8 34 Albano, E. *et al.* Immune response towards lipid peroxidation products as a  
9 predictor of progression of non-alcoholic fatty liver disease to advanced fibrosis.  
10 *Gut* **54**, 987-993, doi:10.1136/gut.2004.057968 (2005).
- 11 35 Kawanishi, N. *et al.* Exercise training attenuates hepatic inflammation, fibrosis  
12 and macrophage infiltration during diet induced-obesity in mice. *Brain,*  
13 *behavior, and immunity* **26**, 931-941,  
14 doi:<https://doi.org/10.1016/j.bbi.2012.04.006> (2012).
- 15 36 Linden, M. A. *et al.* Aerobic exercise training in the treatment of non-alcoholic  
16 fatty liver disease related fibrosis. *J Physiol* **594**, 5271-5284,  
17 doi:10.1113/jp272235 (2016).
- 18 37 Gleeson, M. *et al.* The anti-inflammatory effects of exercise: mechanisms and  
19 implications for the prevention and treatment of disease. *Nature Reviews*  
20 *Immunology* **11**, 607, doi:10.1038/nri3041 (2011).
- 21 38 Houghton, D. *et al.* Exercise Reduces Liver Lipids and Visceral Adiposity in  
22 Patients With Nonalcoholic Steatohepatitis in a Randomized Controlled Trial.  
23 *Clin Gastroenterol Hepatol* **15**, 96-102.e103, doi:10.1016/j.cgh.2016.07.031  
24 (2017).
- 25 39 Amsen, D., de Visser, K. E. & Town, T. Approaches to determine expression of  
26 inflammatory cytokines. *Methods in molecular biology (Clifton, N.J.)* **511**, 107-  
27 142, doi:10.1007/978-1-59745-447-6\_5 (2009).
- 28 40 Huber, Y. *et al.* Voluntary distance running prevents TNF-mediated liver injury  
29 in mice through alterations of the intrahepatic immune milieu. *Cell death &*  
30 *disease* **8**, e2893, doi:10.1038/cddis.2017.266 (2017).
- 31 41 Gehrke, N. *et al.* Voluntary exercise in mice fed an obesogenic diet alters the  
32 hepatic immune phenotype and improves metabolic parameters – an animal  
33 model of life style intervention in NAFLD. *Scientific Reports* **9**, 4007,  
34 doi:10.1038/s41598-018-38321-9 (2019).
- 35 42 Spielmann, G. *et al.* Aerobic fitness is associated with lower proportions of  
36 senescent blood T-cells in man. *Brain, behavior, and immunity* **25**, 1521-1529,  
37 doi:10.1016/j.bbi.2011.07.226 (2011).
- 38 43 Nieman, D. C. & Wentz, L. M. The compelling link between physical activity and  
39 the body's defense system. *Journal of Sport and Health Science* **8**, 201-217,  
40 doi:<https://doi.org/10.1016/j.jshs.2018.09.009> (2019).
- 41 44 Gustafson, M. P. *et al.* A systems biology approach to investigating the  
42 influence of exercise and fitness on the composition of leukocytes in peripheral  
43 blood. *J Immunother Cancer* **5**, 30, doi:10.1186/s40425-017-0231-8 (2017).
- 44 45 van der Poorten, D. *et al.* Visceral fat: a key mediator of steatohepatitis in  
45 metabolic liver disease. *Hepatology* **48**, 449-457, doi:10.1002/hep.22350  
46 (2008).
- 47 46 Fenkci, S. *et al.* Relationship of serum interleukin-6 and tumor necrosis factor  
48 alpha levels with abdominal fat distribution evaluated by ultrasonography in  
49 overweight or obese postmenopausal women. *J Investig Med* **54**, 455-460,  
50 doi:10.2310/6650.2006.06010 (2006).

- 1 47 Pugh, C. J. *et al.* Exercise-induced improvements in liver fat and endothelial  
2 function are not sustained 12 months following cessation of exercise  
3 supervision in nonalcoholic fatty liver disease. *Int J Obes (Lond)* **40**, 1927-1930,  
4 doi:10.1038/ijo.2016.123 (2016).
- 5 48 Haw, J. S. *et al.* Long-term Sustainability of Diabetes Prevention Approaches:  
6 A Systematic Review and Meta-analysis of Randomized Clinical Trials. *JAMA*  
7 *Internal Medicine* **177**, 1808-1817, doi:10.1001/jamainternmed.2017.6040  
8 (2017).
- 9 49 Wu, T., Gao, X., Chen, M. & Van Dam, R. M. Long-term effectiveness of diet-  
10 plus-exercise interventions vs. diet-only interventions for weight loss: a meta-  
11 analysis. *Obesity Reviews* **10**, 313-323, doi:10.1111/j.1467-  
12 789X.2008.00547.x (2009).
- 13 50 Pfirrmann, D., Huber, Y., Schattenberg, J. M. & Simon, P. Web-Based Exercise  
14 as an Effective Complementary Treatment for Patients With Nonalcoholic Fatty  
15 Liver Disease: Intervention Study. *Journal of medical Internet research* **21**,  
16 e11250, doi:10.2196/11250 (2019).
- 17 51 Huber, Y. *et al.* Improvement of non-invasive markers of NAFLD from an  
18 individualised, web-based exercise program. *Aliment Pharmacol Ther*,  
19 doi:10.1111/apt.15427 (2019).
- 20 52 Lavalley, J. F., Gray, T. A., Dumville, J., Russell, W. & Cullum, N. The effects  
21 of care bundles on patient outcomes: a systematic review and meta-analysis.  
22 *Implement Sci* **12**, 142, doi:10.1186/s13012-017-0670-0 (2017).

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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 <sup>a</sup>
Gender, n (%n)			0.647 <sup>b</sup>
Female	12 (75)	5 (63)	
Male	4 (25)	3 (37)	
T2DM/IGT, n (%n)	11 (69)	6 (75)	1.000 <sup>b</sup>
Hypoglycaemic medications, n (%n)	9 (56)	5 (63)	1.000 <sup>b</sup>
Hypertension, n (%n)	9 (56)	4 (50)	1.000 <sup>b</sup>
Anti-hypertensive medication, n (%n)	9 (56)	3 (38)	0.667 <sup>b</sup>
Hypercholesteremia, n (%n)	9 (56)	4 (50)	1.000 <sup>b</sup>
Lipid lowering medications, n (%n)	9 (56)	3 (38)	0.667 <sup>b</sup>
Hypertriglyceridemia, n (%n)	6 (38)	3 (38)	1.000 <sup>b</sup>
Polypharmacy, n (%n)	7 (44)	2 (25)	0.657 <sup>b</sup>
MetSyn, n (%n)	9 (56)	6 (75)	0.657 <sup>b</sup>
BMI, kg/m <sup>2</sup> ‡	36.7 (9.1)	33.6 (6.3)	0.490 <sup>b</sup>
BMI category, n (%n)			1.000 <sup>b</sup>
Overweight (25.0-29.9kg/m <sup>2</sup> )	3 (19)	2 (25)	
Obese (≥30kg/m <sup>2</sup> )	13 (81)	6 (75)	
Estimated $\dot{V}O_{2max}$ , mL/min/kg ‡	26.9 (10.1)	27.0 (9.3)	0.340 <sup>c</sup>
Cardiorespiratory fitness level, n (%n)			1.000 <sup>b</sup>
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 <sup>a</sup>
AST (IU/L) ‡	36 (14)	47 (16)	0.094 <sup>c</sup>
Hepatic CAP (dB/m) ‡	337 (46)	330 (44) <sup>2</sup>	0.759 <sup>c</sup>
Hepatic stiffness (kPa) ‡	11.9 (4.8) <sup>1</sup>	14.9 (8.7) <sup>2</sup>	0.431 <sup>c</sup>

1 Notes: †Non-normal data (median [interquartile range]), ‡Normal data (mean [standard deviation]),  
2 <sup>1</sup>n=15, <sup>2</sup>n=7, <sup>a</sup>Mann-Whitney u-test, <sup>b</sup>Fisher's exact test, <sup>c</sup>Independent 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  
6  
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**Table 2. Baseline liver histology**

Variable	Exercise group (n=16)	Control group (n=8)	Between-group <i>P</i> value
<b>MAS †</b>	3.9 (1.7)	4.6 (2.1)	0.360 <sup>a</sup>
<b>MAS components, n (%n)</b>			0.673 <sup>b</sup>
≥5	6 (38)	4 (50)	
<5	10 (63)	4 (50)	
<b>Steatosis, n (%n)</b>			0.282 <sup>b</sup>
<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)	
<b>Lobular inflammation, n (%n)</b>			0.103 <sup>b</sup>
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)	
<b>Hepatocyte ballooning, n (%n)</b>			0.521 <sup>b</sup>
None (0)	3 (19)	2 (24)	
Few Cells (1)	10 (62)	3 (38)	
Many Cells (2)	3 (19)	3 (38)	
<b>MASH, n (%n)</b>			1.000 <sup>b</sup>
Yes	13 (81)	6 (75)	
No	3 (19)	2 (25)	
<b>Fibrosis, n (%n)</b>			0.281 <sup>b</sup>
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]), <sup>a</sup>Independent t-test, <sup>b</sup>Fisher'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)
<b>Hepatic fibrosis</b>	
Increased 1 stage	1
Maintained the same stage	4
Decreased 1 stage	7
<b>Net change</b>	-6
<b>Significance</b>	<b><i>P=0.034*</i></b>
<b>Hepatic steatosis</b>	
Increased 1 stage	2
Maintained the same stage	8
Decreased 1 stage	2
<b>Net Change</b>	0
<b>Significance</b>	<i>P=1.000</i>
<b>Lobular inflammation</b>	
Increased 1 stage	3
Maintained the same stage	6
Decreased 1 stage	2
Decreased 2 stages	1
<b>Net change</b>	-1
<b>Significance</b>	<i>P=0.739</i>
<b>Hepatocellular ballooning</b>	
Increased 1 stage	1
Maintained the same stage	3
Decreased 1 stage	8
<b>Net change</b>	-7
<b>Significance</b>	<b><i>P=0.020*</i></b>
<b>MAS</b>	
Increased 3 scores	1
Maintained the same score	5
Decreased 1 score	3
Decreased 2 scores	2
Decreased 4 scores	1
<b>Net change</b>	-8
<b>Significance</b>	<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  
3 Suzanne Norris from the Health Research Board, Ireland (grant number: HRA-POR-  
4 2015-1185). Philip O’Gorman was funded through this grant for his PhD studentship.

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

	<b>Item No.</b>	<b>Recommendation</b>	<b>Page No.</b>	<b>Relevant text from manuscript</b>
<b>Title and abstract</b>	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 <math>\dot{V}O_2\text{max}</math>), 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>

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with no changes in steatosis (P=1.000), lobular inflammation (P=0.739) or MAFLD 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”

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**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 <sup>14</sup> . 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

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				was to determine the sustainability of the exercise intervention at 12 weeks and 52 weeks post exercise intervention completion.”
<b>Methods</b>				
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/m<sup>2</sup>) 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 prescription<sup>10</sup>, significant orthopaedic or neuromuscular limitations, unwillingness to participate, alcohol consumption &gt;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 <sup>22</sup> , both by 4-day diet diaries returned by mail and by a food frequency questionnaire administered via a 20-min

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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])

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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)<sup>12</sup> 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

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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

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				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- $\alpha$ ; interleukin 6, IL-6 and interleukin 1 $\beta$ , IL-1 $\beta$ ). TNF- $\alpha$ , IL-6 and IL-1 $\beta$ 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
<b>Results</b>				
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 <sup>2</sup> ) 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	Table 1 (page 27), Table 2 (page 28), Table 3 (page 29),  Supporting Table 1, Supplementary Table 2, Supporting Table 3 and	Example from Table 1: <sup>1</sup> <i>n</i> =15, <sup>2</sup> <i>n</i> =7”
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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 $\dot{V}O_{2max}$ ( $4.7 \pm 5.2\text{mL}/\text{min}/\text{kg}$ [ $17 \pm 18\%$ ] mean increase, $P=0.027$ , partial $\eta^2=0.202$ )

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compared to the control group. There was also a significant within-group improvement in estimated  $\dot{V}O_{2\max}$  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])”

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(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

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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 <math>\dot{V}O_{2max}</math> by <math>5.9 \pm 5.4</math> mL/min/kg (<math>25 \pm 20\%</math> increase, <math>p=0.02</math>) at this timepoint, while participants without fibrosis regression (n=5) demonstrated increased <math>\dot{V}O_{2max}</math> by <math>2.1 \pm 5.7</math> mL/min/kg (<math>7 \pm 18\%</math> increase, <math>p=0.59</math>) (Figure 3a.). Participants with hepatocyte ballooning regression at T1 (n=8) significantly increased <math>\dot{V}O_{2max}</math> by <math>6.5 \pm 5.5</math> mL/min/kg (<math>26 \pm 20\%</math> increase, <math>p=0.01</math>) at this timepoint, while participants without hepatocyte ballooning regression (n=4) demonstrated increased <math>\dot{V}O_{2max}</math> by <math>0.04 \pm 2.5</math> mL/min/kg (<math>2 \pm 12\%</math> increase, <math>p=0.98</math>) (Figure 3b.).”</p>
<b>Discussion</b>				
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>

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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

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			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

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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.”

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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

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				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 <sup>19,20,28,31</sup> . 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.”
<b>Other information</b>				
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	<b>“Declaration of funding interests:</b> This study was funded, in full, by a grant held by

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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.”

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