Effect of aerobic exercise training dose on liver fat and visceral adiposity

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Background & Aims: Aerobic exercise reduces liver fat and visceral adipose tissue (VAT). However, there is limited data from randomized trials to inform exercise programming recommendations. This study examined the efficacy of commonly prescribed exercise doses for reducing liver fat and VAT using a randomized placebo-controlled design.

Methods: Inactive and overweight/obese adults received 8 weeks of either: i) low to moderate intensity, high volume aerobic exercise (LO:HI, 50% VO₂peak, 60 min, 4 d/week); ii) high intensity, low volume aerobic exercise (HI:LO, 70% VO₂peak, 45 min, 3 d/week); iii) low to moderate intensity, low volume aerobic exercise (LO:LO, 50% VO₂peak, 45 min, 3 d/week); or iv) placebo (PLA).

Results: Forty-seven of the 48 (n = 12 in each group) participants completed the trial. There were no serious adverse events. There was a significant change in group × time interaction in liver fat, which reduced in HI:LO by 2.38 ± 0.73%, in LO:HI by 2.62 ± 1.00%, and in LO:LO by 0.84 ± 0.47% but not in PLA (increase of 1.10 ± 0.62%) (p = 0.04). There was a significant reduction in VAT in HI:LO (-258.38 ± 87.78 cm³), in LO:HI (-386.80 ± 119.5 cm³), and in LO:LO (-212.96 ± 105.54 cm³), but not in PLA (92.64 ± 83.46 cm³) (p = 0.03). There were no significant differences between the dose or intensity of the exercise regimen and reductions in liver fat or VAT (p > 0.05).

Conclusion: The study found no difference in efficacy of liver fat reduction by either aerobic exercise dose or intensity. All of the aerobic exercise regimens employed reduced liver fat and VAT by a small amount without clinically significant weight loss.

Introduction

It is now recognized that the topography of body fat distribution predicts the adverse cardiovascular and metabolic consequences of obesity independent of fat quantity. For instance, high levels of visceral adipose tissue (VAT) are an independent predictor of hypertension [1], myocardial infarction [2], and insulin resistance [3,4]. Likewise, excess liver fat, as seen in non-alcoholic fatty liver disease (NAFLD), has emerged as an independent risk factor for cardiometabolic disease that may be more strongly associated with risk than VAT [5,6]. Hence, reducing ectopic fat deposits is a suitable target for interventions to prevent disease.

Since there are limited pharmacological agents to specifically reduce ectopic fat, the efficacy of lifestyle therapies has become a focus of research. In this regard, weight loss via diet and exercise reduces VAT [7,8] and liver fat [9–11] but reductions of ≥3% of body weight are generally required for hepatic benefit, with greater weight loss (5–10%) producing superior benefits [12]. However, these levels of weight loss are inherently difficult to sustain [13]. Given that NAFLD afflicts one third of adults [14] and most obese individuals [15], the efficacy of lifestyle interventions that do not rely on weight loss (such as moderate exercise) warrants investigation.

Several trials have examined the effect of exercise interventions on liver fat, but their interpretation is limited by small sample sizes, lack of non-exercise control groups and heterogeneity in exercise modality and dose. These studies employed exercise...
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doses ranging from 2 to 6 days per week, lasting for 20–60 min per session, with intensities ranging between low (45% VO\textsubscript{2peak}) and strenuous (85% of VO\textsubscript{2peak}) \cite{9,10,16–22}. Therefore, establishing practical recommendations for exercise prescription is difficult based on the current evidence.

The purpose of this study was to investigate the efficacy of regular aerobic exercise at one of three doses requiring different levels of effort and time commitment vs. a sham exercise placebo control (PLA) in reducing liver fat and VAT in overweight/obese adults using gold standard, double-blind, randomized placebo-controlled design. We also measured the effects of these interventions on other markers of cardiometabolic risk in order to advise practical recommendations.

Subjects and methods

Experimental protocol

Intrahepatic lipid (IHL) and abdominal adiposity, including VAT, were assessed before and after 8 weeks of exercise training or PLA intervention. Cardiorespiratory fitness/work capacity, anthropometric measurements (body weight, BMI, and waist circumference), blood lipids, serum aminotransferases,hs-CRP and fasting blood pressure were also measured at baseline and post-intervention. The primary aim of the study was assessed by comparing the mean change in liver fat and VAT between the PLA group and each of the exercise groups: low to moderate intensity, high volume aerobic exercise (LO:HI); high intensity, low volume aerobic exercise (HI:LO); and low to moderate intensity, low volume aerobic exercise (LO:LO).

Volunteers were required to abstain from alcohol, over-the-counter medication and strenuous exercise for 24 h prior to baseline and post-intervention testing. Randomization was undertaken after baseline assessments by equally distributed pre-generated lists (www.randomization.com) of permuted blocks with participants given sealed opaque envelopes containing group allocation (by SK).

Participants

Inactive (exercising <3 days/week) and overweight or obese (BMI >25 kg/m\textsuperscript{2}) adult (29 to 59 year-old); men and women randomized into one of four arms involving either 8 weeks of: HI:LO, LO:HI, LO:LO, or PLA exercise intervention from August 2011 to October 2013.

The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki and the study was approved by the Human Research Ethics Committee of The University of Sydney and was retrospectively registered with the ANZCTR (ACTRN12614000723684). Participants provided written informed consent after obtaining medical clearance to undertake the study. Volunteers were excluded if taking lipid-lowering or insulin sensitizing medication, reported a high alcohol intake (>20 g/day), had secondary causes of steatohepatitis, alcoholic liver disease or viral hepatitis.

Primary outcomes

Intrahepatic lipid

Liver fat, as IHL was measured by proton magnetic resonance spectroscopy (\textsuperscript{1}H-MRS) using a 1.5 Tesla Achieva whole-body system (Philips Medical Systems; Best, The Netherlands) and performed by technicians blinded to intervention group allocation and the purpose of the study. Intrahepatic lipid concentration and composition were measured as previously outlined \cite{28}. Briefly, image-guided, localized \textsuperscript{1}H-MRS were acquired from a voxel of 3.0 × 2.0 cm × 2.0 cm using the whole-body (Q body) coil and a torso coil (flex M multi-channel surface), with volumes of interest centred within the right lobe of the liver. Subjects lay supine, with spectra acquired during respiratory gating. Spectra were acquired using the PRESS (point resolved spectroscopy) technique (TR = 5000 ms, TE = 34 ms, 32 measurements, 1024 sample points). A similar voxel placement was facilitated between measures within-individual, guided by image capture at the baseline measurement which was subsequently replicated as closely as possible. Whilst there are inherent limitations with this technique, variation was further minimized by the use of a large voxel, and we have previously shown that the coefficient of variation for this technique is approximately 7% \cite{28}. Fully automated high-order shimming was performed on the volume of interest to ensure maximum field homogeneity. Unsuppressed water spectra were acquired in vivo for use as the internal standard. Excitation water suppression was also used to suppress the water signal during data acquisition.

Spectral data were post-processed by magnetic resonance user interface software (MIRUI version 3.0, El Projet). For hepatic lipid concentration and composition, a five resonance model was employed \cite{18,28}. Hepatic water signal amplitudes were measured from the non-water suppressed spectrum using Hankel Lanczos Squares Singular Values Decomposition.

Abdominal (including visceral) fat

Abdominal (including visceral) fat volumes were measured by magnetic resonance imaging (MRI). All MRI assessments were undertaken with the patient supine. A sagittal localizing image was used to position transverse slices from the diaphragm to pelvis. Axial T1-weighted fast field echo images were acquired during suspended end-expiration (TR = 11 ms, TE = 4.5 ms, flip angle = 40°), with slice thickness of 10 mm and an inter-slice gap of 10 mm.

Cross-sectional areas of both abdominal VAT and subcutaneous adipose tissue (SAT) were measured by automated software (Hippo Fat\textsuperscript{TM}) \cite{29}. Volumes of VAT and SAT were calculated by summation of VAT and SAT area from the total abdominal slices and adjustment for slice thickness and inter-slice gap.

\textsuperscript{1}H-MRS and MRI processing were performed by an experimenter blinded to treatment allocation.

Secondary outcomes

Cardiorespiratory fitness/work capacity

Cardiorespiratory fitness/work capacity was assessed by a graded maximal exercise test on an electronically-braked cycle ergometer (Lode Corval, Netherlands) under the supervision of the study physician. All tests commenced with a three minute warm up at 35W for women and 65W for men. The test comprised an increase of 25W every 150 s until volitional fatigue. Heart rate, blood pressure and 12-lead ECG were obtained at each stage of exercise, with ratings of perceived exertion (RPE) measured using the Borg scale \cite{30}. The test was terminated when the pedalling rate fell below 50 revolutions per minute or the participant ceased exercise. Peak work capacity (W\textsubscript{peak}) was measured \cite{31} and peak oxygen consumption (VO\textsubscript{2peak}) estimated \cite{32}.

Anthropometrics and blood pressure

Stature was measured by stadiometer (Tanita Best Weight, Seca Model 220, Germany). Waist circumference was measured in the horizontal plane, midway between the inferior margin of the ribs and the superior border of the iliac crest during deep expiration. Blood pressure was measured manually on each arm after 10–15 min of quiet sitting, with a second reading on each arm taken, when there was a difference >10 mmHg between the highest reading recorded was taken.

Blood sampling and analysis

After an overnight fast (>10 h) venous blood was collected from the antecubital vein. Blood analysis was performed on the same day as collection for serum glucose, insulin and lipids (including triglycerides (TAG), total cholesterol (TC), high density lipoprotein cholesterol (HDL-C), and low density lipoprotein cholesterol (LDL-C), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and high sensitivity C-reactive protein (hs-CRP). Serum free fatty acids (FFAs) were assessed on stored plasma.

Habitual physical activity and dietary control

Participants were asked to maintain their habitual activity and eating behaviours for the duration of the intervention. A tri-axial accelerometer which also measures energy expenditure (SenseWear\textsuperscript{TM}, BodyMedia Inc., PA, USA) was worn on the left upper arm for three non-exercising days (two weekdays and one weekend day) during weeks 1 and 8 of the study for 24 h/day, except during water-based activities. Mean daily time spent in sedentary time, physical activity, daily steps and energy expenditure were analyzed by an assessor blinded to group allocation. Data were omitted from analysis if the monitor was worn for <90% of a 24 h period. Participants completed a diet diary and subjective physical activity questionnaire \cite{33} during this period. Diet diaries were analyzed by a dietitian blinded to group allocation. Average daily intake of energy and macronutrients were quantified by Foodworks\textsuperscript{TM} (Foodworks 2009, Xyris Software, v6.0.6502).

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Exercise intervention
All exercise training was supervised by an Accredited Exercise Physiologist with heart rate, RPE and blood pressure continuously monitored. Participants were blinded to the primary purpose of the study and the PLA group were instructed that the stretching/ massage/fitball intervention was intended to reduce inflammation and abdominal fat.

High intensity: Low volume aerobic exercise training. Participants in HI:LO performed continuous cycling on the ergometer at an intensity of 60–70% of VO2peak for two days per week, and performed and recorded an additional brisk walk at the same intensity at home one day per week. Training progressed from 30 min at 50% VO2peak week one to 45 min at 70% VO2peak by the third week, totalling 90–135 min per week.

Low to moderate intensity: High volume aerobic exercise training. Participants in LO:HI performed continuous cycling on the ergometer at an intensity of 50% of VO2peak for three days per week, and performed and recorded an additional brisk walk at the same intensity at home one day per week. Training progressed from 30 min in week one to 60 min by the third week, totalling 180–240 min per week.

Low to moderate intensity: Low volume aerobic exercise training. Participants in LO:LO performed continuous cycling on the ergometer at an intensity of 50% of VO2peak for two days per week, and performed and recorded an additional brisk walk at the same intensity at home one day per week. Training progressed from 30 min in week one to 45 min by the third week, totalling 90–135 min per week.

Placebo. Participants in the PLA group were prescribed a stretching, self-massage and fitball program. Participants received one fortnightly supervised session which involved instructions of new exercises and a 5 min-cycle at very low intensity (30W) to maintain familiarity with the cycle ergometer. When combined with home-based sessions, participants in PLA undertook the sham exercise on three days per week. Sessions were recorded in a logbook to ensure compliance. The PLA intervention was designed to elicit no cardiometabolic improvements but to control for factors such as attention and participation in a lifestyle intervention.

Statistical analysis
Power calculation
An a priori, two-tailed power calculation at an alpha of 0.05 and beta of 0.20 gave an actual power of 0.81 for a sample size of 116 in each group (G-Power software, University of Trier, Trier, Germany). This calculation was based on the pooled effect size (−0.37) from the limited existing data pool [23] to detect a benefit of a specific exercise regimen on liver fat within groups. Given the logistic constraints of such an undertaking using magnetic resonance spectroscopy in over 450 subjects, the lack of overall data to inform effect size, and emerging findings of significant benefits of exercise interventions from small cohort studies (n = 7–25) [18,24–27], we recruited 48 subjects with the goal of determining the efficacy of commonly prescribed aerobic exercise doses vs. PLA on liver fat and VAT.

Data analysis
Compliance with the exercise interventions was calculated as (total number of sessions attended/total number of sessions available) × 100. An intention-to-treat (ITT) analysis was employed with group mean change scores imputed for dropouts [34] (imputations are depicted in relevant Table legends). The primary analyses explored effects between the control (PLA) and the exercise groups. The group × time interaction for the change score of all outcome measures was assessed by analysis of covariance (ANCOVA) using the baseline value as the covariate (SPSS version 17.0), with LSD post hoc comparison used to locate significant treatment differences. Whilst the study was underpowered to do so, planned a priori secondary analyses (ANCOVA) were also performed to examine differences between mean change in outcomes between exercise groups. Effect size was calculated using Hedges g corrected for bias with 95% confidence intervals. Analyses of relationships between change in liver fat and VAT with change in other variables, and potential confounders (diet or non-exercise physical activity time) during treatment were determined by Pearson correlation coefficients. Statistical significance was accepted at p < 0.05. Values are reported as means ± SE.

Results
One hundred and twenty-two individuals were screened by telephone interview, with 48 eligible volunteers (17 men and 31 women) undergoing initial assessment and randomization (Fig. 1). The study population had an average BMI of 33.4 ± 1.3 kg/m2 and mean age of 43.6 ± 3.0 years. Baseline participant characteristics are described in Table 1. All participants completed the training and PLA interventions. Post-intervention dietary and habitual physical activity data were unavailable for some participants (Table 3) and one participant in LO:HI did not complete the final fasting blood test or MRI/MRS scan. Compliance with the exercise intervention was 94%, 90%, 96%, and 82% in HI:LO, LO:HI, LO:LO, and PLA groups, respectively. There were no adverse events during exercise testing or training in PLA and LO:LO groups, and there was one syncopal episode during exercise testing in each of the HI:LO and LO:HI groups.

Primary outcomes
Intrahepatic lipid and abdominal fat (including VAT)
There were no significant difference between the dose or intensity of the exercise regimen in reducing IHL (p > 0.05). However, there was a significant effect of aerobic exercise training on IHL across all groups compared with the PLA (Fig. 2A). The HI:LO group exhibited a reduction by 2.38 ± 0.73% in IHL (p = 0.02, effect size (ES) 1.42), the LO:HI group demonstrated a reduction by 2.62 ± 1.00% (p < 0.01, ES 1.23), and the LO:LO group a reduction by 0.84 ± 0.47% (p = 0.03, ES 0.96). There was a non-significant increase in IHL in PLA group (1.10 ± 0.62%) (Fig. 2A). These data are shown in Table 2A.

There were also no significant difference between the dose or intensity of the exercise regimen in reducing VAT (p > 0.05). However, a significant effect was observed across all groups compared with PLA for the reduction in VAT (p = 0.026) (Fig. 2B), with reductions seen in HI:LO (by 259.38 ± 87.78 cm2, p = 0.039), LO:HI (by 386.8 ± 119.50 cm2, p < 0.01), and the LO:LO group (by 212.96 ± 105.54 cm3, p = 0.049) compared with PLA (gain of 92.67 ± 83.96 cm3). There was a significant group × time interaction for reduction in SAT (p < 0.01), with SAT reducing in HI:LO by 567.80 ± 139.33 cm3, in LO:HI by 596.68 ± 201.74 cm3, and in the LO:LO group by 165.56 ± 141.22 cm3, but not in PLA in which SAT increased by 319.69 ± 156.42 cm3.

Secondary outcomes
Cardiorespiratory fitness
There was a significant group × time interaction for change in fitness (p < 0.001), which increased in HI:LO by 2.99 ± 0.48 ml/kg/min (p < 0.01), in LO:HI by 2.29 ± 0.77 ml/kg/min (p < 0.01), and in the LO:LO group by 2.24 ± 0.54 ml/kg/min (p < 0.01), but not in PLA (−0.37 ± 0.32 ml/kg/min) (Table 2A). There was no significant difference between the dose or intensity of exercise regimen in improving fitness (p > 0.05).

Anthropometrics and blood pressure
There was a significant group × time interaction for change in body mass, with a small but significant reduction observed in HI:LO (−1.25 ± 0.50 kg) and LO:HI (−1.41 ± 0.67 kg) but not in the LO:LO or PLA groups (gain of 0.16 ± 0.53 kg and 0.78 ± 0.31 kg respectively). There was also a significant effect
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Responded to advertisement: n = 262

Responders potentially eligible and screened by telephone interview: n = 122

Eligible: n = 64

Eligible but declined participation: n = 16

Time commitment: n = 7

Location: n = 5

No reason given: n = 4

Undertook initial assessment and randomized n = 48 (M = 17; F = 31)

Table 1. Baseline participant characteristics.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>PLA (n = 12)</th>
<th>HI:LO (n = 12)</th>
<th>LO:HI (n = 12)</th>
<th>LO:LO (n = 12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Demographics</td>
<td></td>
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</tr>
<tr>
<td>Age (years)</td>
<td>39.1 (2.9)</td>
<td>44.2 (2.8)</td>
<td>45.5 (2.3)</td>
<td>45.6 (3.6)</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>3/9</td>
<td>6/6</td>
<td>5/7</td>
<td>3/9</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>32.2 (1.4)</td>
<td>36.3 (1.7)</td>
<td>33.9 (0.9)</td>
<td>31.3 (0.8)</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>93.7 (1.5)</td>
<td>106.6 (4.7)</td>
<td>104.5 (2.9)</td>
<td>95.0 (2.9)</td>
</tr>
<tr>
<td>Baseline habitual physical activity (Bouchard (kJ/kg/day))</td>
<td>42.4 (1.1)</td>
<td>38.7 (0.8)</td>
<td>43.3 (1.6)</td>
<td>41.8 (1.2)</td>
</tr>
</tbody>
</table>

Presented as mean (± SE).

PLA, placebo; HI:LO, high intensity, low volume aerobic exercise; LO:HI, low to moderate intensity, high volume aerobic exercise; LO:LO, low to moderate intensity, low volume aerobic exercise; M, male; F, female; BMI, body mass index.

Fig. 1. Flowchart showing the study process. M, male; F, female; HI:LO, high intensity, low volume aerobic exercise; LO:HI, low to moderate intensity, high volume aerobic exercise; LO:LO, low to moderate intensity, low volume aerobic exercise; PLA, Placebo control group; MRI, magnetic resonance imaging; MRS, magnetic resonance spectroscopy.

Fig. 2. Effect of 8 weeks of exercise regimen on intrahepatic fat and visceral adipose tissue. Participants followed 8 weeks of high intensity, low volume aerobic exercise (HI:LO), or low to moderate intensity, high volume aerobic exercise (LO:HI), or low to moderate intensity, low volume aerobic exercise (LO:LO), or control (PLA), which resulted in significant changes in (A) Intrahepatic lipid (IHL) and (B) visceral adipose tissue (VAT). Circles show individual percentage change from baseline and horizontal bars show mean group percentage change from baseline. *Significant treatment × time interaction (p < 0.05).

Table 2A. Systolic blood pressure (SBP) exhibited a significant group × time interaction (p < 0.01), reducing significantly in LO:HI (reduction of 5.60 ± 2.04 mmHg, p < 0.01) but not in HI:LO (reduction of 4.83 ± 2.49 mmHg, p = 0.11) or LO:LO (gain of 5.76 ± 1.75 mmHg, p = 0.17) compared with PLA (gain of 1.33 ± 1.99 mmHg). Diastolic blood pressure (DBP) reflected the changes observed with SBP, exhibiting a significant group × time interaction (p = 0.02), with DBP reducing significantly in LO:HI (reduction of 8.05 ± 2.03 mmHg, p < 0.01) but not in HI:LO (reduction of 3.83 ± 1.80 mmHg, p = 0.60) or LO:LO (reduction of 1.67 ± 2.42 mmHg, p = 0.53) compared with PLA (reduction of 0.17 ± 1.60 mmHg) (Table 2B). There was no significant difference between the dose or intensity of exercise regimen for change in anthropometry or blood pressure (p > 0.05).

Blood lipids and biochemistry

There were no significant group × time interactions for change in fasting serum levels of ALT, hs-CRP, triglycerides, total cholesterol, HDL-C, LDL-C, FFA, insulin or glucose (p > 0.05 for all). There was a significant group × time interaction for fasting serum AST (p < 0.05), with values of this parameter reducing significantly in HI:LO (by 2.64 ± 1.52 U/L, p < 0.01) but not in the LO:HI (increase of 2.48 ± 1.55 U/L, p = 0.17) or LO:LO (change of 0.00 ± 0.98 U/L, p = 0.84) compared with PLA (increase of 2.83 ± 1.72 U/L) (Table 2B).

Habitual physical activity and dietary control

Accelerometry data for four participants were omitted from baseline analysis and five from post-intervention analysis due to their wearing the accelerometer for insufficient time. Another
participant refused to wear the accelerometer (Table 3). Total energy expenditure, steps taken per day, time spent in sedentary behaviour, time spent in moderate physical activity and self-reported physical activity levels were not different between groups over time (p > 0.05) (Table 3). Diet and physical activity diary data were unavailable for two participants at baseline in PLA and three participants at post-intervention in PLA and LO:LO due to non-compliance with the questionnaires. There was no significant group × time interaction for any measure of energy or macronutrient intake as determined by diet diaries or self-reported physical activity (p > 0.05), except for a slight reduction in CHO intake in LO:LO compared with PLA being large (ES = 1.42, 1.23, and 0.96 for the HI:LO, LO:HI, and LO:LO groups, respectively). These benefits were observed in the absence of meaningful weight loss. We did not detect any difference between the exercise interventions.

Excess visceral and liver adipose tissue is known to be independently associated with the cardiometabolic risk in obesity. Weight loss via lifestyle intervention can reduce both VAT and liver fat, but weight loss of ~5–15% is desirable for clinically significant reductions in VAT (10–40%) [35] and for histological improvements in the liver (e.g. 65% improvement in NAS score with ≥9% weight loss) [36,37]. However, a major limitation is that these benefits are only maintained if weight loss is sustained, an unlikely outcome of lifestyle intervention [13]. On the other hand, emerging evidence demonstrates that exercise training programs reduce liver fat [18,38] and VAT [7,8] even in the absence of weight loss [7,8,18,23,38].

Our results extend this by demonstrating that aerobic exercise reduces liver fat and that this can occur with programs that emphasize intensity over volume or, volume over intensity. A significant reduction can also be seen with even minimal engagement in exercise (i.e. LO:LO), although not in all individuals in our study.

The sophistication of measurement techniques for the analysis of VAT and liver fat, including MRI and 1H-MRS, has aided longitudinal research in this area. Whilst there are emerging MRI based techniques to measure hepatic fat fraction that are more widely available and may be less time consuming [39],

### Discussion

We investigated the effectiveness of commonly prescribed doses of aerobic exercise training compared with a PLA group, independent of dietary intervention or weight loss, on fat distribution including liver fat and VAT in previously inactive adults. We observed a significant reduction in liver fat in all aerobic exercise intervention groups, with the effect size when compared with PLA being large (ES = 1.42, 1.23, and 0.96 for the HI:LO, LO:HI, and LO:LO groups, respectively). These benefits were observed in the absence of meaningful weight loss. We did not detect any difference between the exercise interventions.

## Table 2A. Outcome measures: Body composition and fitness.

<table>
<thead>
<tr>
<th></th>
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<th>HI:LO</th>
<th>LO:HI</th>
<th>LO:LO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)</td>
<td>90.7 (4.9)</td>
<td>91.5 (5.0)</td>
<td>101.8 (5.7)</td>
<td>1.36 (0.47, 2.25)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>32.2 (1.4)</td>
<td>32.9 (4.8)</td>
<td>36.3 (1.7)</td>
<td>35.8 (1.7)</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>93.7 (1.5)</td>
<td>94.5 (4.7)</td>
<td>104.1 (4.5)</td>
<td>1.87 (0.91, 2.83)</td>
</tr>
<tr>
<td>Intrahepatic lipid</td>
<td>7.7 (2.6)</td>
<td>8.8 (3.2)</td>
<td>8.4 (1.5)</td>
<td>6.0 (1.0)</td>
</tr>
<tr>
<td>Hepatic saturation index</td>
<td>0.97 (0.01)</td>
<td>0.95 (0.01)</td>
<td>0.97 (0.01)</td>
<td>0.96 (0.01)</td>
</tr>
<tr>
<td>Subcutaneous adipose tissue (cm²)</td>
<td>12,067.6 (1157.3)</td>
<td>12,416.4 (1192.6)</td>
<td>11,272.5 (1130.1)</td>
<td>10,704.7 (1077.5)</td>
</tr>
<tr>
<td>Visceral adipose tissue (cm²)</td>
<td>2700.9 (514.0)</td>
<td>2793.5 (551.3)</td>
<td>3580.6 (393.8)</td>
<td>3322.2 (371.7)</td>
</tr>
<tr>
<td>VO₂peak (ml/kg/min)**</td>
<td>21.7 (1.8)</td>
<td>21.3 (1.7)</td>
<td>21.9 (1.4)</td>
<td>24.9 (1.6)</td>
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</tbody>
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Presented as mean (± SE). PLA, placebo; HI:LO, high intensity, low volume aerobic exercise; LO:HI, low to moderate intensity, high volume aerobic exercise; LO:LO, low to moderate intensity, low volume aerobic exercise; VO₂peak, peak aerobic capacity; ES, Hedges Bias Corrected Effect Size; CI, Confidence Interval.

*Estimated from W₀peak, †Significant group × time interaction (p < 0.05).

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We investigated the effectiveness of commonly prescribed doses of aerobic exercise training compared with a PLA group, independent of dietary intervention or weight loss, on fat distribution including liver fat and VAT in previously inactive adults. We observed a significant reduction in liver fat in all aerobic exercise intervention groups, with the effect size when compared with PLA being large (ES = 1.42, 1.23, and 0.96 for the HI:LO, LO:HI, and LO:LO groups, respectively). These benefits were observed in the absence of meaningful weight loss. We did not detect any difference between the exercise interventions.

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<td>90.7 (4.9)</td>
<td>91.5 (5.0)</td>
<td>101.8 (5.7)</td>
<td>1.36 (0.47, 2.25)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>32.2 (1.4)</td>
<td>32.9 (4.8)</td>
<td>36.3 (1.7)</td>
<td>35.8 (1.7)</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>93.7 (1.5)</td>
<td>94.5 (4.7)</td>
<td>104.1 (4.5)</td>
<td>1.87 (0.91, 2.83)</td>
</tr>
<tr>
<td>Intrahepatic lipid</td>
<td>7.7 (2.6)</td>
<td>8.8 (3.2)</td>
<td>8.4 (1.5)</td>
<td>6.0 (1.0)</td>
</tr>
<tr>
<td>Hepatic saturation index</td>
<td>0.97 (0.01)</td>
<td>0.95 (0.01)</td>
<td>0.97 (0.01)</td>
<td>0.96 (0.01)</td>
</tr>
<tr>
<td>Subcutaneous adipose tissue (cm²)</td>
<td>12,067.6 (1157.3)</td>
<td>12,416.4 (1192.6)</td>
<td>11,272.5 (1130.1)</td>
<td>10,704.7 (1077.5)</td>
</tr>
<tr>
<td>Visceral adipose tissue (cm²)</td>
<td>2700.9 (514.0)</td>
<td>2793.5 (551.3)</td>
<td>3580.6 (393.8)</td>
<td>3322.2 (371.7)</td>
</tr>
<tr>
<td>VO₂peak (ml/kg/min)**</td>
<td>21.7 (1.8)</td>
<td>21.3 (1.7)</td>
<td>21.9 (1.4)</td>
<td>24.9 (1.6)</td>
</tr>
</tbody>
</table>

Presented as mean (± SE). PLA, placebo; HI:LO, high intensity, low volume aerobic exercise; LO:HI, low to moderate intensity, high volume aerobic exercise; LO:LO, low to moderate intensity, low volume aerobic exercise; VO₂peak, peak aerobic capacity; ES, Hedges Bias Corrected Effect Size; CI, Confidence Interval.

*Estimated from W₀peak, †Significant group × time interaction (p < 0.05).
we employed $^1$H-MRS because it is established and validated and is still considered to be the most accurate noninvasive approach [39,40]. The PRESS sequence employed was selected for its good 1H quantitation in the current study was based on the methylene interval.

### Lipids

<table>
<thead>
<tr>
<th>Total cholesterol (mmol/L)</th>
<th>5.6 (0.4)</th>
<th>5.5 (0.3)</th>
<th>5.5 (0.4)</th>
<th>5.6 (-1.24, 0.38)</th>
<th>5.5 (0.3)</th>
<th>5.4 (-0.32, 0.78)</th>
<th>5.7 (0.4)</th>
<th>5.7 (-0.91, 0.89)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HDL (mmol/L)</td>
<td>1.4 (0.1)</td>
<td>1.4 (0.1)</td>
<td>1.3 (0.1)</td>
<td>-0.21 (0.1)</td>
<td>1.5 (0.1)</td>
<td>1.4 (0.1)</td>
<td>1.3 (0.1)</td>
<td>1.3 (0.1)</td>
</tr>
<tr>
<td>LDL (mmol/L)</td>
<td>3.6 (0.3)</td>
<td>3.5 (0.3)</td>
<td>3.6 (0.3)</td>
<td>-0.97 (0.2)</td>
<td>3.6 (0.3)</td>
<td>3.5 (0.3)</td>
<td>3.6 (0.3)</td>
<td>0.2 (0.3)</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>1.3 (0.2)</td>
<td>1.5 (0.3)</td>
<td>1.5 (0.2)</td>
<td>-0.18 (-0.12, 0.12)</td>
<td>1.4 (0.3)</td>
<td>1.4 (0.3)</td>
<td>1.8 (0.3)</td>
<td>0.3 (0.1)</td>
</tr>
<tr>
<td>Free fatty acids (umol/L)</td>
<td>334.7 (43.2)</td>
<td>401.1 (45.8)</td>
<td>364.9 (35.7)</td>
<td>385.1 (42.1)</td>
<td>380.9 (41.21)</td>
<td>290.8 (43.0)</td>
<td>459.8 (43.0)</td>
<td>366.5 (43.6)</td>
</tr>
</tbody>
</table>

### Blood pressure

<table>
<thead>
<tr>
<th>SBP (mmHg)</th>
<th>120.6 (4.2)</th>
<th>118.4 (13.0)</th>
<th>123.2 (4.6)</th>
<th>0.76 (3.6)</th>
<th>122.0 (2.4)</th>
<th>116.4 (4.0)</th>
<th>125.0 (4.5)</th>
<th>123.2 (4.3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DBP (mmHg)</td>
<td>77.5 (2.1)</td>
<td>75.6 (8.3)</td>
<td>79.5 (2.2)</td>
<td>0.60 (1.4)</td>
<td>77.8 (1.4)</td>
<td>69.8 (2.5)</td>
<td>77.2 (2.5)</td>
<td>75.5 (2.5)</td>
</tr>
</tbody>
</table>

Presented as mean (± SE). PLA, placebo; HI:LO, high to low intensity; low volume aerobic exercise; LO:HI, low to moderate intensity, low volume aerobic exercise; AST, aspartate aminotransferase; ALT, alanine aminotransferase; hs-CRP, high sensitivity C-reactive protein; HDL-C, low density lipoprotein cholesterol; LDL-C, high density lipoprotein cholesterol; SBP, systolic blood pressure; DBP, diastolic blood pressure; ES, Hedges Bias Corrected Effect Size; CI, Confidence Interval.

Significant group *x* time interaction ($p < 0.05$).
preferences, and barriers of adopting an exercise regimen, such as fear of falling [49] or time constraints [50]. For high-risk populations where adherence to exercise programs may be challenging, even minimal engagement in exercise (i.e. LO:LO) will provide fitness, body composition, and hepatic benefits, although this may not be effective in all people.

Based on a pooled effect size from a paucity of available data on the topic [23], our a priori power calculation recommended a sample size of n = 116 per group. Whilst the study was apparently underpowered to detect changes in the primary outcome the results show that, compared with PLA control, each commonly prescribed aerobic exercise program was effective in reducing liver fat and VAT. Nevertheless, we are not able to recommend an “optimal” dose of aerobic exercise for improving liver fat on the basis of these findings. Based on our current results, a sample size of 2454 in each group would be required to detect differences between HI:LO and LO:HI.

In conclusion, the results from this study show that all exercise doses irrespective of volume or intensity were efficacious in reducing liver fat and VAT by a small, but potentially clinically important amount in previously inactive, overweight or obese adults compared with PLA. These changes were observed without clinically significant weight loss. We found no difference between exercise regimens for these benefits.

Financial support

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Conflict of interest

S.E. Keating, D.A. Hackett, H.M. Parker, M.K Baker, V.H. Chuter, H.T. O’Connor, J.A. Gerofi, and A. Sainsbury declare no conflict of interest. N.A. Johnson: has received honoraria for speaking engagements for Merck Sharp & Dohme. I.D. Caterson: has performed and still performs clinical trials of obesity treatment and prevention some of which have been funded by government, but others by the pharmaceutical industry. Current trials are funded by the NHMRC [3], NovoNordisk, Amylin Corporation, the Egg Board. He serves on the steering committees of international trials (SCOUT and EXSEL) and has received honoraria for this. He has given talks for NovoNordisk, Servier Laboratories, Pfizer and iNova pharmaceuticals in the last 3 years. He serves

Table 3. Habitual energy intake and energy expenditure.

<table>
<thead>
<tr>
<th>Energy intake</th>
<th>PLA</th>
<th>HI:LO</th>
<th>LO:HI</th>
<th>LO:LO</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat (g/day)</td>
<td>77.8(7.6)</td>
<td>75.1(6.9)</td>
<td>86.8(6.9)</td>
<td>89.9(11.2)</td>
<td>10.1(14.9)</td>
</tr>
<tr>
<td>% intake</td>
<td>30.0(1.7)</td>
<td>33.5(1.6)</td>
<td>33.5(1.3)</td>
<td>33.7(2.4)</td>
<td>36.1(3.0)</td>
</tr>
<tr>
<td>Carbohydrate (g/day)</td>
<td>261.1(22.9)</td>
<td>178.5(19.8)</td>
<td>181.8(31.5)</td>
<td>248.0(35.9)</td>
<td>223.9(29.3)</td>
</tr>
<tr>
<td>% intake</td>
<td>44.6(2.4)</td>
<td>40.1(1.9)</td>
<td>39.4(2.6)</td>
<td>39.8(3.1)</td>
<td>34.9(3.2)</td>
</tr>
<tr>
<td>Protein (g/day)</td>
<td>102.7(8.4)</td>
<td>106.3(12.6)</td>
<td>114.4(9.9)</td>
<td>115.4(8.0)</td>
<td>109.4(7.9)</td>
</tr>
<tr>
<td>% intake</td>
<td>18.7(1.4)</td>
<td>21.5(1.3)</td>
<td>20.8(1.3)</td>
<td>21.2(1.8)</td>
<td>20.8(1.4)</td>
</tr>
<tr>
<td>Total energy intake (kJ/day)</td>
<td>9529.1(752.0)</td>
<td>8337.7(679.5)</td>
<td>9756.2(863.1)</td>
<td>9852.7(983.8)</td>
<td>10,132.7(1183.7)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Energy expenditure</th>
<th>PLA</th>
<th>HI:LO</th>
<th>LO:HI</th>
<th>LO:LO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total energy expenditure (kJ/kg/day)</td>
<td>11,152(407)</td>
<td>10,770(360)</td>
<td>11,828(771)</td>
<td>12,341(836)</td>
</tr>
<tr>
<td>Steps/day</td>
<td>10,015(764)</td>
<td>8553(757)</td>
<td>7384(636)</td>
<td>8111(930)</td>
</tr>
<tr>
<td>Sedentary time, &lt;3 METS (hours/day)</td>
<td>21:26(0:21)</td>
<td>21:58(0:16)</td>
<td>21:59(0:19)</td>
<td>22:00(0:14)</td>
</tr>
<tr>
<td>Moderate activity, 3-6 METS (hours/day)</td>
<td>1:17(0:15)</td>
<td>0:58(0:16)</td>
<td>0:58(0:09)</td>
<td>1:03(0:10)</td>
</tr>
<tr>
<td>Total self-reported energy expenditure (Bouchard score, kJ/kg/day)</td>
<td>42.4(1.1)</td>
<td>39.0(2.3)</td>
<td>38.7(0.8)</td>
<td>37.1(1.6)</td>
</tr>
</tbody>
</table>

Presented as mean (± SE).

PLA, placebo; HI:LO, high intensity, low volume aerobic exercise; LO:HI, low intensity, high volume aerobic exercise; LO:LO, low intensity, low volume aerobic exercise; METS, metabolic equivalents.

Habitual energy intake and energy expenditure.

Table 3.
on the scientific advisory board of the Sansom Institute for Health Research, University of SA, the board of the Children’s Medical Research Institute, and chairs the Executive Management Committee of the bariatric surgical registry for the Obesity Surgery Society of Australia and New Zealand. JG has no conflicts to declare in relation to this submission.

Authors’ contributions

Study concept and design (JGeorge, NJ), acquisition of data (SK, DH), analysis and interpretation of data (SK, NJ, JGeorge and HP), drafting and critical revision of manuscript (all authors), statistical analysis (SK, NJ). All authors approved the final draft for submission.

References


