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Effects of sprint interval training on ectopic lipids and tissue-specific insulin sensitivity in men with non-alcoholic fatty liver disease

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Short title: Sprint interval training in men with NAFLD

Abstract

Purpose: This study examined the feasibility of sprint interval exercise training (SIT) for men with non-alcoholic fatty liver disease (NAFLD) and its effects on intrahepatic triglyceride (IHTG), insulin sensitivity (hepatic and peripheral), visceral (VAT) and subcutaneous adipose tissue (ScAT).

Methods: Nine men with NAFLD (age 41±8 years; BMI 31.7±3.1 $kg \cdot m^{-2}$; IHTG 15.6±8.3%) were assessed at: 1) baseline 2) after a control phase of no intervention (pre-training) and 3) after six weeks of SIT (4-6 maximal 30 s cycling intervals, three times per week). IHTG, VAT and ScAT were measured using magnetic resonance spectroscopy or imaging and insulin sensitivity was assessed via dual-step hyperinsulinaemic-euglycaemic clamp with [6,6-D2] glucose tracer.

Results: Participants adhered to SIT, completing ≥96.7% of prescribed intervals. SIT increased peak oxygen uptake ($\dot{V}O_2$ peak: +13.6% [95% CI: 8.8 to 18.2%]) and elicited a relative reduction in IHTG (-12.4% [-31.6 to 6.7%]) and VAT (-16.9% [-24.4 to -9.4%]; *n*=8), with no change in body weight or ScAT. Peripheral insulin sensitivity increased throughout the study (*n*=8; significant main effect of phase) but changes from pre- to post-training were highly variable (range: -18.5 to +58.7%) and not significant (*P*=0.09), despite a moderate effect size (*g**=0.63). Hepatic insulin sensitivity was not influenced by SIT.

Conclusions: SIT is feasible for men with NAFLD in a controlled laboratory setting and is able to reduce IHTG and VAT in the absence of weight loss.

Key words: Exercise, NAFLD, hepatic steatosis, insulin sensitivity

List of abbreviations:

NAFLD – non-alcoholic fatty liver disease

SIT – sprint interval exercise training

IHTG – intrahepatic triglyceride

VAT – visceral adipose tissue

ScAT – subcutaneous adipose tissue

BMI – body mass index

T2DM – type 2 diabetes mellitus

HIIT – high intensity intermittent exercise training

MR – magnetic resonance

^{1}H-MRS – proton magnetic resonance spectroscopy

EGP – endogenous glucose production

HISI – hepatic insulin sensitivity index

%EGP_{supp} – percentage suppression of EGP by low-dose insulin infusion

$\dot{V}O_2$ peak – peak oxygen uptake

HDL – high-density lipoprotein

LDL – low-density lipoprotein

TG – triglyceride

NEFA – non-esterified fatty acids

HOMA-IR – homeostatic model assessment of insulin resistance

Adipo-IR – adipose tissue insulin resistance index

Author contribution statement

JAK, GPA, MAN, IAM and PAG generated the study idea and designed the protocol. JAS, EJS, SB, JAK, JC, KS, MCT and JLD collected the study data and performed the analyses. JAS, JAK, SB, JC, KS and GPA led the manuscript preparation. All authors read, edited and approved the final version of the manuscript.

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Conflicts of interest

IAM is on the scientific advisory boards of Ikea, Nestlé and Mars Inc. All other authors have no conflicts of interest to declare.

Introduction

Non-alcoholic fatty liver disease (NAFLD) is a common complication of obesity that is integrated in the pathogenesis of extra-hepatic comorbidities such as type 2 diabetes (T2DM) and cardiovascular disease (Byrne and Targher 2015). Insulin resistance is a central pathophysiological feature of NAFLD with associations between intrahepatic triglyceride content (IHTG) and insulin action in skeletal muscle, adipose tissue and the liver (Bril et al. 2017). The prominence of these metabolic defects within the development and progression of NAFLD makes them priority targets for intervention.

Lifestyle interventions, incorporating diet and physical activity, remain the cornerstone of treatment for NAFLD (Marchesini et al. 2016) and the importance of structured exercise within such interventions is underscored by both hepatic and extra-hepatic benefits. Continuous moderate-to-vigorous intensity exercise interventions increase cardiorespiratory fitness, reduce adiposity, improve peripheral insulin sensitivity, enhance cardiovascular function and improve circulating markers of metabolic health (Pugh et al. 2014; Hallsworth et al. 2015; Keating et al. 2015; Cuthbertson et al. 2016; Zhang et al. 2016).

Guidelines for the management of NAFLD suggest that individuals undertake 150-200 min of moderate-intensity aerobic or resistance exercise each week, spread over three to five sessions (Marchesini et al. 2016). Observational evidence (Kistler et al. 2011) and experimental data (Cho et al. 2015; Oh et al. 2017) suggest that high-intensity exercise may be more potent in attenuating IHTG accumulation and NAFLD progression than moderate-intensity exercise. This evidence is consistent with exercise intensity-dependent improvements in wider cardiometabolic outcomes, including indices of insulin sensitivity (Tjønna et al. 2008; Weston et al. 2013). Moderate-intensity exercise improves peripheral insulin sensitivity in patients with NAFLD (Cuthbertson et al. 2016) but evidence of its

impact on hepatic insulin sensitivity is unclear (Shojaee-Moradie et al. 2007; Cuthbertson et al. 2016). The effects of high-intensity exercise on hepatic and peripheral insulin sensitivity in individuals with NAFLD have not been assessed. A better understanding of these outcomes is important, given the link between IHTG, glycaemic control and metabolic disease (Byrne and Targher 2015; Marchesini et al. 2016; Bril et al. 2017).

High-intensity intermittent exercise training (HIIT), which is characterised by repeated intervals of high-intensity exercise interspersed with periods of rest or low-intensity active recovery, has emerged as a form of exercise capable of providing many health benefits for individuals with, or at risk of, chronic disease (Gibala et al. 2012). Sprint interval training (SIT) is a version of HIIT consisting of brief bursts (30 s) of maximal-intensity exercise (Little et al. 2011). SIT induces adaptations in skeletal muscle which improve oxidative metabolism (Gibala et al. 2012) and, in some studies, enhances whole-body insulin sensitivity and glycaemic control (Richards et al. 2010; Cocks et al. 2015). These adaptations are likely to be of benefit for individuals with NAFLD, but the influence of SIT on IHTG and tissue-specific (muscle, adipose tissue, liver) insulin sensitivity remains unknown.

This study investigated the feasibility and efficacy of SIT as a therapeutic strategy in overweight or obese men with NAFLD. We sought to determine the effect of six weeks of SIT on IHTG, visceral (VAT) and subcutaneous adipose tissue (ScAT), as well as hepatic and peripheral (skeletal muscle and adipose tissue) insulin sensitivity. We hypothesised that SIT would reduce IHTG, VAT and ScAT, and increase insulin sensitivity.

Participant and Methods

Ethical approval

This study was approved by the research ethics committees of Loughborough University and the University of Nottingham and was conducted in accordance with the Declaration of Helsinki (World Health Organisation 2013).

Participants

Nine white European men were recruited from the general population and gave informed, written consent to participate. Although no power calculation was performed for this study, this sample size was chosen based on studies reporting significant improvements in cardiorespiratory fitness and indices of glycaemic control with HIIT (Little et al. 2011; Cocks et al. 2015). Eligibility criteria included inactive but weight-stable individuals aged 25 to 55 years with a body mass index (BMI) between 27 and 40 $kg \cdot m^{-2}$ and a waist circumference ≥ 94 cm. Participants were considered inactive if they did not complete any form of regular structured exercise. Participants were identified as exhibiting NAFLD in that they had IHTG ≥ 5.56%, determined during screening using magnetic resonance (MR) spectroscopy (^{1}H-MRS), and did not report excessive alcohol consumption (>21 $units \cdot week^{-1}$) or other secondary causes of hepatic steatosis (Marchesini et al. 2016). Participants were excluded if they: a) had any form of diagnosed chronic metabolic disease, b) were taking prescribed medications for hypertension, dyslipidaemia or glucose regulation or c) had contraindications to exercise or MR procedures.

Study design

This study utilised a repeated measures longitudinal design in which, following screening, participants completed two consecutive six week phases (control and SIT). The control phase

acted to monitor the variation in study outcomes over a similar period to that of the exercise intervention, but with participants maintaining their usual lifestyle. All participants completed the control phase followed by the exercise intervention, in order to avoid the confounding effects of detraining during the control phase. All study assessments were performed on consecutive days at baseline, pre-training and post-training: day 1) IHTG, VAT and ScAT; day 2) hepatic and peripheral insulin sensitivity and systemic metabolic biomarkers; day 3) cardiorespiratory fitness. Post-training assessments began 48 h after the final SIT session to eliminate the confounding influence of the final exercise training session on insulin sensitivity (Sylow et al. 2017). Dietary intake was standardised for 24 h before metabolic assessments through provision of all food and energy-containing drinks. This diet provided a balanced macronutrient profile and was tailored to each participant's estimated energy requirement (Mifflin et al. 1990) using a multiplication factor of 1.45 to account for the physical activity level of an inactive group (FAO et al. 2001). Participants were instructed and regularly reminded to maintain their usual lifestyle habits throughout both the control and SIT phases of the study. This included instructions to maintain dietary habits. Energy intake was not recorded due to concerns that monitoring may prompt dietary changes and given documented concerns regarding the accuracy of self-reported energy intake data (Dhurandhar et al. 2015).

Imaging and Metabolic Assessments

All metabolic assessments were performed after an overnight fast. MR measurements were performed on a Philips Achieva 3T system with 32 channel XL-Torso coil. IHTG was measured from a 20x20x20 mm voxel within the right lobe of the liver using ^{1}H-MRS with Stimulated Echo Acquisition Mode (STEAM) localization (repetition time = 2046 ms) (Stephenson et al. 2013; Bawden et al. 2017). VAT and ScAT were assessed using a two-

point modified Dixon technique (Philips) (Nakai et al. 2010) and an in-house algorithm to generate fat boundaries of visceral and subcutaneous regions.

Hepatic and peripheral insulin sensitivity were assessed using a modified version of the hyperinsulinaemic, euglycaemic clamp with two stages of insulin infusion, low- (20 $mU \cdot m^{-2} \cdot min^{-1}$) and high-dose (50 $mU \cdot m^{-2} \cdot min^{-1}$), each lasting 120 min. A continuous infusion of [6,6-D2] glucose tracer was initiated 120 min before the first hyperinsulinaemic stage and continued throughout for the quantification of endogenous glucose production (EGP) (Johnston et al. 2013). Blood glucose was clamped at 4.5 $mmol \cdot L^{-1}$ (coefficient of variation: mean (± SD) = 1.6 ± 0.9 and 2.7 ± 1.1 % at steady-state low- and high-dose insulin infusion, respectively).

The hepatic insulin sensitivity index (HISI) (Matsuda and DeFronzo 1999) and the percentage suppression of EGP by low-dose insulin infusion ($\%EGP_{supp}$) were calculated as indices of hepatic insulin sensitivity in the fasted and insulin-stimulated states, respectively. Peripheral insulin sensitivity was assessed as whole-body glucose uptake, which was assumed to be equal to the exogenous glucose infusion rate required to maintain euglycaemia at high-dose insulin infusion, during which EGP was negligible.

Assessment of cardiorespiratory fitness and habitual physical activity

Peak oxygen uptake ($\dot{V}O_2$ peak) and peak power output were measured using a ramped (+16 $Watt \cdot min^{-1}$) cycling test on an electromagnetically-braked cycle ergometer (Excalibur Sport, Lode BV, The Netherlands) during which participants exercised until they were unable to maintain a pedalling cadence of 80 revolutions per min. $\dot{V}O_2$ was measured throughout (Metalyser 3B, Cortex Biophysik GmbH, Germany) and $\dot{V}O_2$ peak was determined as the highest value achieved across 15 s epochs (Robergs et al. 2010). $\dot{V}O_2$ peak and peak power output are presented as both absolute units ($L \cdot min^{-1}$ and W) and relative to the participant's

body weight ($mL \cdot kg^{-1} \cdot min^{-1}$ and $W \cdot kg^{-1}$). Participants were familiarised with this test one week before baseline assessments.

To assess the impact of SIT on habitual physical activity levels, participants wore a tri-axial accelerometer (GT3x, Actigraph LLC, USA) for seven consecutive days before each assessment (baseline, pre-training and post-training). Data were analysed using computer software (Kinesoft 3.3.80, USA) (Troiano et al. 2008) and are presented as absolute minutes per day in each activity domain (sedentary time, light, moderate and vigorous physical activity) as well as percentages of accelerometer wear time.

Exercise training

Participants completed a SIT program consisting of three exercise sessions per week for six weeks. Sessions consisted of a low-intensity warm-up (5 min cycling at 50W), followed by 30 s intervals of maximal sprint cycling on a stationary ergometer (Ergomedic 894E, Monark Exercise AB, Sweden), which was separated by periods of active recovery (4.5 min of low intensity cycling at 50W). The braking resistance of the ergometer was increased during intervals through the application of a load equivalent to 6.5% of lean body mass, determined using bioelectrical impedance analysis (BC-418, TANITA Europe BV, Amsterdam, The Netherlands). Participants were instructed to cycle 'all-out' during intervals whilst members of the research team provided verbal encouragement (Whyte et al. 2010). Four intervals were completed per session in the first two weeks with an additional interval added to each session every two weeks. Participants therefore completed 90 intervals over the six weeks of supervised training.

Biochemical analyses

Plasma glucose isotope enrichment (atoms percent excess) was quantified as the oxime/trimethylsilyl derivative via gas chromatography mass spectrometry (GC-MS; 7890B, MSD 5977A; Agilent Technologies, UK) using selected ion monitoring of the ions at m/z 319 and 321 (CV = 6.4%). Fasted circulating concentrations of total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), triglyceride (TG) and non-esterified fatty acids (NEFA) were analysed in plasma aliquots, collected before the start of hyperinsulinaemic euglycaemic clamps, by enzymatic colorimetric methods using a benchtop analyser (Pentra 400, HORIBA ABX Diagnostics, France) (All CV ≤ 1.5%). Serum insulin was quantified using radioimmunoassay (Millipore, USA) (CV = 7.3%), and HOMA-IR and Adipo-IR were calculated (Matthews et al. 1985; Gastaldelli et al. 2007).

Tracer calculations

Rates of EGP in the basal state and at low-dose insulin infusion were calculated (Wolfe and Chinkes 2005; Vella and Rizza 2009) accounting for non-steady-state during low-dose insulin infusion and assuming a fractional volume of distribution of 160 $mL \cdot kg^{-1}$.

Statistical analyses

Statistical analyses were performed using software (SPSS version 23.0, SPSS Inc., USA). All data were checked for normality of distribution prior to analysis. Normally distributed data are presented as mean with standard deviation (SD) and one-way repeated measures ANOVA was used to assess changes in outcomes across assessment visits (main effect of phase). The homogeneity of variance between data collected at each visit was assessed and a Greenhouse-Geisser or Huynh-Feldt correction was applied, where appropriate. Statistically significant main effects were explored *post-hoc* using paired samples *t*-tests. Non-normally distributed data are presented as the median with interquartile range (IQR) and Friedman tests were used to assess the main effect of phase. Wilcoxon matched pairs tests were used for *post-hoc*

pairwise comparisons on non-normally distributed data. Probability (*P*-) values for *post-hoc* tests were adjusted using the Holm-Bonferroni correction (Holm 1979) to account for multiple comparisons. In text, the changes from pre- to post-training are presented as relative percentage change along with 95% confidence interval [CI] and effect size (adjusted Hedges' *g**). Cohen's descriptors were used to interpret the magnitude of effect (Cohen 1988). For clarity, the change in IHTG is presented as both the absolute and relative percentage change. The association between changes in IHTG and whole-body glucose uptake from pre- to post-SIT was assessed using Pearson's bivariate correlation analysis. Statistical significance was accepted at $P \leq 0.05$.

Results

Participant characteristics and exercise training compliance

All assessments made at baseline, pre- and post-training can be found in Table 1. The median self-reported alcohol intake of recruited participants was four units per week (range: 1 to 14). One participant was unable to attend hyperinsulinaemic, euglycaemic clamp assessments. All participants completed exercise training and attended all 18 sessions. Due to fatigue, one participant failed to complete two intervals in their first session and one interval in session two, but completed all prescribed intervals thereafter (total intervals: 87 = 96.7%).

There were no significant differences in any measured outcome between baseline and pre-training assessments, determined as a non-significant main effect of phase or, where appropriate, post-hoc comparison. However, from baseline to pre-training assessments, there was a tendency for increased fasted serum insulin and HOMA-IR, and reduced relative $\dot{V}O_2$ peak (uncorrected P = 0.06, 0.07 and 0.07, respectively; all other outcomes $P \geq 0.13$).

Insert Table 1 here

Effects of SIT on cardiorespiratory fitness and habitual physical activity

Training improved absolute and relative $\dot{V}O_2$ peak by 11.2% [95% CI: 6.4 to 16.0%] (g* = 0.83) and 13.6% [8.8 to 18.2%] (g* = 0.78; Figure 1a), respectively ($P \leq 0.001$). This was alongside improvements in absolute (14.7% [10.7 to 18.7%], g* = 0.75) and relative (16.2% [11.1 to 21.2%], g* = 0.64; Figure 1b) peak power output ($P \leq 0.001$). As outlined in Table 2, there were no differences in sedentary time or light, moderate or vigorous physical activity

throughout the duration of the study when analysed either as minutes per day or as a percentage of accelerometer wear time (main effect of phase: $P \geq 0.24$).

Insert Figure 1 here

Effects of SIT on ectopic fat and systemic metabolic biomarkers

Despite no change in body weight across study visits (main effect of phase: $P = 0.17$; Figure 2a), SIT elicited a reduction in IHTG ($P = 0.03$; Figure 2b). From pre- to post-training, the mean absolute reduction was 2.1% [-3.4 to 0.8%], which equated to a relative reduction of 12.4% [-31.6 to 6.7%] ($g^* = -0.23$). One MR-image was found to be corrupted at the point of analysis so changes in VAT and ScAT are presented for n=8. These data do not correspond to the same eight individuals who completed the hyperinsulinaemic, euglycaemic clamp assessments. Training reduced VAT by 16.9% [-24.4 to -9.4%] ($g^* = -0.62$, $P = 0.02$; Figure 2c), but there were no changes in ScAT (main effect; $P = 0.16$; Figure 2d). Training increased circulating HDL by 8.4% [4.6 to 12.2%] ($g^* = 0.44$, $P = 0.02$) but total cholesterol, LDL and triglycerides were unchanged throughout the study ($P \geq 0.19$).

Insert Figure 2 here

Effects of SIT on peripheral and hepatic insulin sensitivity

There was a significant main effect of phase for the increase in whole-body glucose uptake across the three study visits ($P = 0.02$; Figure 3a). However, responses to SIT were highly

variable between individuals (range: -18.5% to +58.7%; Figure 3b) and, despite a medium effect size (g^* = 0.63), the change from pre- to post-training (18.1% [-3.0 to 39.2%]) was not statistically significant (unadjusted P = 0.09). There was an association between the change in IHTG and the change whole-body glucose uptake from pre- to post-training (r = -0.83, P = 0.01). Basal EGP, HISI (Figure 3c) and $\%EGP_{supp}$ (Figure 3d) did not differ across study visits ($P \geq 0.37$).

Fasted blood glucose, plasma NEFA and Adipo-IR remained unchanged across the study visits ($P \geq 0.13$). From pre- to post-training, fasted serum insulin (-13.9% [-24.9 to -2.9%], g^* = -0.48, P = 0.04) and HOMA-IR (-16.6% [-27.5 to -5.6%], g^* = -0.56, P = 0.02) were reduced. However, these reductions were similar in magnitude to the increases from baseline to pre-training (unadjusted P = 0.06 and P = 0.07, respectively) such that post-training values were no different from those measured at baseline ($P \geq 0.68$).

Insert Figure 3 here

Discussion

The principal findings of this study are that a six-week SIT intervention is feasible for individuals with NAFLD and is well adhered to in a controlled laboratory setting. Furthermore, six weeks of SIT reduces IHTG and VAT in the absence of body weight change and, whilst hepatic insulin sensitivity appears to be unaffected, changes in peripheral insulin sensitivity are highly variable between individuals.

This study reports almost perfect adherence to a six-week SIT intervention in nine individuals. Specifically, every participant completed the exercise programme, attending all 18 training sessions. Eight participants completed all 90 of the prescribed intervals whilst the remaining participant completed 87 intervals, with the three missing intervals contained within the first two training sessions. The implication is that individuals with NAFLD are able and willing to perform exercise training sessions composed of bursts of maximal exercise. This is important because observational evidence (Kistler et al. 2011) and experimental data (Cho et al. 2015; Oh et al. 2017) suggest that high-intensity exercise may be more potent in attenuating IHTG accumulation and NAFLD progression than moderate-intensity exercise. This SIT intervention may, therefore, represent a model of exercise that facilitates the completion of more intense exercise in individuals with NAFLD. This intervention was performed in a tightly controlled laboratory setting, with specialist equipment and where participants were individually supported by the research team. The participants recruited to this study were also screened thoroughly to ensure the absence of advanced cardiometabolic disease and may, therefore, not be representative of the majority of individuals with NAFLD. Given that the risk of an acute cardiac event during exercise is elevated in previously inactive individuals with established cardiometabolic disease (Thompson et al. 2007), the implementation of SIT requires additional scrutiny. The necessity for medical clearance and acclimatisation to exercise must be considered in this context (Riebe et al. 2015).

This study reports a mean absolute reduction in IHTG of 2.1% after six weeks of SIT in individuals with elevated IHTG (relative change from baseline: -12.4%). Previous studies employing aerobic and resistance exercise, combined or in isolation, report a reduction of similar magnitude (10 to 21%) in the absence of significant weight loss (Johnson et al. 2009; Hallsworth et al. 2011; Sullivan et al. 2012; Keating et al. 2015; Pugh et al. 2016; Houghton et al. 2017). However, greater reductions in IHTG (27 to 42%) have been reported when significant weight loss occurs as a result of exercise training (Hallsworth et al. 2015; Keating et al. 2015; Cuthbertson et al. 2016; Zhang et al. 2016). Therefore, whilst the independent effects of exercise on IHTG are recognised (Brouwers et al. 2016), the greatest benefits occur when exercise contributes to a negative energy balance.

It was beyond the scope of this study to examine the mechanisms through which SIT reduced IHTG. However, habitual physical activity was consistent throughout and the energy expenditure elicited by SIT is low (Deighton et al. 2013). Therefore, whilst we did not measure energy intake, the absence of significant weight loss suggests that substantial energy restriction is unlikely to have occurred. Consequently, metabolic factors likely underpin the reported change in IHTG (Brouwers et al. 2016). Neither fasted circulating NEFA nor Adipo-IR differed throughout the current study, suggesting that an improvement in adipose tissue insulin sensitivity in the fasted state is unlikely to be responsible for the reduction in IHTG with training. However, the possibility that changes in postprandial adipose tissue insulin sensitivity occurred cannot be dismissed (Brouwers et al. 2016). Circulating glucose stimulates hepatic *de novo* lipogenesis (Ameer et al. 2014) and, although fasted blood glucose was unchanged throughout the study, post-prandial glucose is likely to have been reduced in those with improved peripheral insulin sensitivity. Therefore, particularly in individuals who displayed improvements in whole-body glucose uptake, a reduction in hepatic *de novo* lipogenesis may have contributed to post-training reductions in IHTG (Linden et al. 2015).

Lastly, altered very low density lipoprotein metabolism is unlikely to be responsible for exercise-induced reductions in IHTG (Sullivan et al. 2012) but enhanced capacity to oxidise hepatic lipid is possible (Linden et al. 2015).

Peripheral insulin sensitivity increased throughout this study but individual changes from pre- to post-SIT were highly variable and, despite a mean relative increase of 18.1% and a moderate effect size, this change was not significant. In obese but otherwise healthy men, four weeks (Cocks et al. 2015), but not two weeks (Whyte et al. 2010), of SIT increased peripheral insulin sensitivity. However, 15 to 20% of individuals may display minimal, or even adverse, responses after exercise training in outcomes related to glucose homeostasis (Stephens and Sparks 2015) and insulin sensitivity improved in only 10 out of 12 healthy individuals who completed a two-week SIT intervention, remaining unchanged in one and decreasing in another (Richards et al. 2010). This degree of variation in response to exercise is consistent with our findings where peripheral insulin sensitivity improved in 75% of participants after SIT, yet was reduced in 25%. Given this variation, a greater sample size may be required to detect significant differences in peripheral insulin sensitivity following SIT. A number of factors, including genetic polymorphisms, epigenetics and baseline participant characteristics, may impact on individual responses (Böhm et al. 2016).

Neither basal nor insulin-stimulated hepatic insulin sensitivity are changed after six weeks of SIT. Our findings agree with data showing no change in hepatic insulin sensitivity after 12 weeks of aerobic training in patients with NAFLD, despite significant reductions in body weight (Cuthbertson et al. 2016). EGP during low dose insulin infusion is reduced after aerobic or combined aerobic-plus-resistance exercise training in sedentary, healthy individuals and in patients with T2DM (Shojaee-Moradie et al. 2007; Meex et al. 2010). However, neither of these studies report changes in basal EGP or HISI, and the change at low dose insulin infusion reported by Meex *et al.* was no longer statistically significant when

presented as $\%EGP_{supp}$. EGP in both the basal state and during low dose insulin infusion was reduced in overweight, older women after a 9-month moderate-intensity aerobic exercise intervention (DiPietro et al. 2006). However, this may have been due to notably higher rates of EGP in this group at baseline compared to both the control group, and a separate group completing a higher-intensity exercise programme. The high-intensity exercise training had no effect on EGP in either the basal or insulin-stimulated states (DiPietro et al. 2006). The lack of improvements in hepatic insulin sensitivity in the current study may be due to insufficient intervention duration or because IHTG at the end of the intervention remained elevated (Cuthbertson et al. 2016). Hepatic insulin sensitivity, assessed as $\%EGP_{supp}$, may be impaired with as little as 1.5% IHTG, with no further deterioration as IHTG increases (Bril et al. 2017). Post-training IHTG values in the present study ranged from 4.3 to 25.9% (mean 12.4%).

The favourable changes in IHTG, VAT, HDL and cardiorespiratory fitness in response to SIT are important for individuals with NAFLD. NAFLD is intricately related to the metabolic syndrome and associated with an elevated risk of T2DM, cardiovascular and renal disease (Byrne and Targher 2015). Ectopic lipid and dyslipidaemia are components of the metabolic syndrome and improvements in these risk factors are important in the treatment of NAFLD. Additionally, cardiorespiratory fitness is a marker of metabolic health and inversely associated with all-cause and cardiovascular mortality (Blair et al. 1989; Kodama et al. 2009). The large increase following SIT most likely reflects metabolic adaptations within skeletal muscle (Gibala et al. 2012), which may provide benefit via improved substrate metabolism (Rabøl et al. 2011; Brouwers et al. 2016). Collectively, the present study demonstrates the potential of SIT to elicit relevant metabolic improvements in men with NAFLD, but it is notable that the magnitude of response over this intervention was insufficient to re-establish values in an optimal range.

A strength of this study is the use of the most precise techniques available to assess key outcomes. Conversely, this study was conducted in a relatively small and homogenous sample of white European men with no other chronic metabolic disease. We may have lacked statistical power to detect differences in some of our outcomes following training and the findings of this study may not be generalisable to women, individuals of different ethnicity or those with metabolic co-morbidities. Furthermore, participants did not have a formal diagnosis of NAFLD so we have no information regarding disease severity. Whilst a randomised controlled trial design would be preferred, the inclusion of a control phase was chosen to monitor variation in study outcomes over a period of no intervention, whilst avoiding the additional recruitment of a non-exercise control group.

In this study we have shown that men with NAFLD are compliant with SIT which, over six weeks, improves cardiorespiratory fitness and reduces IHTG and VAT, without altering body weight. Furthermore, changes in peripheral insulin sensitivity with training are highly variable between individuals, whilst hepatic insulin sensitivity remains unchanged. These results support the potential for interval-based, high-intensity exercise as an alternative to continuous moderate-intensity exercise in the management of NAFLD. However, larger RCTs are required to test the effectiveness of SIT in diverse populations, as well as its applicability in a clinical setting, sustainability over time and efficacy in individuals with advanced NAFLD.

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Figure Legends

Figure 1 – a) Peak oxygen uptake ($\dot{V}O_2$ peak) and b) peak power output measured at baseline, pre- and post-training. Data presented as mean ± SD (*n*=9). Outcomes presented relative to participant body weight. † indicates the difference between baseline and pre-training values approached statistical significance (unadjusted $P = 0.07$); * indicates significantly different from pre-training value ($P \leq 0.001$).

Figure 2 – a) Body weight, b) intrahepatic triglyceride (IHTG), c) visceral adipose tissue (VAT) and d) subcutaneous abdominal adipose tissue (ScAT) measured at baseline, pre- and post-training. Data presented as mean ± SD. Data for body weight and IHTG are *n*=9. Data for VAT and ScAT are *n*=8. * indicates significantly different from pre-training value ($P \leq 0.03$).

Figure 3 – a-b) Peripheral and c) basal and d) insulin-stimulated hepatic insulin sensitivity measured at baseline, pre- and post-training. Data in 'A' and 'D' presented as mean ± SD (*n*=8). [#]Data in 'C' are not normally distributed and thus presented as median (IQR). Data in 'B' are % change from pre- to post-training measurements for each participant.

Figure 1

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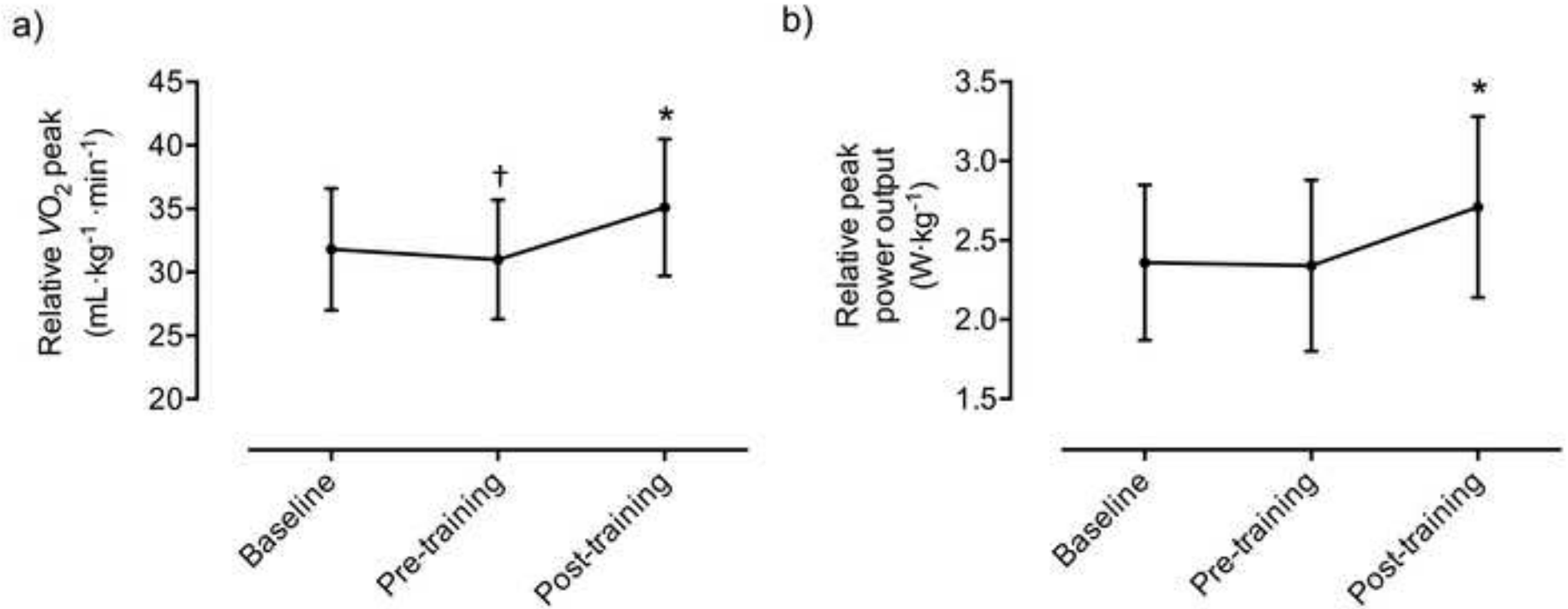


Figure 2

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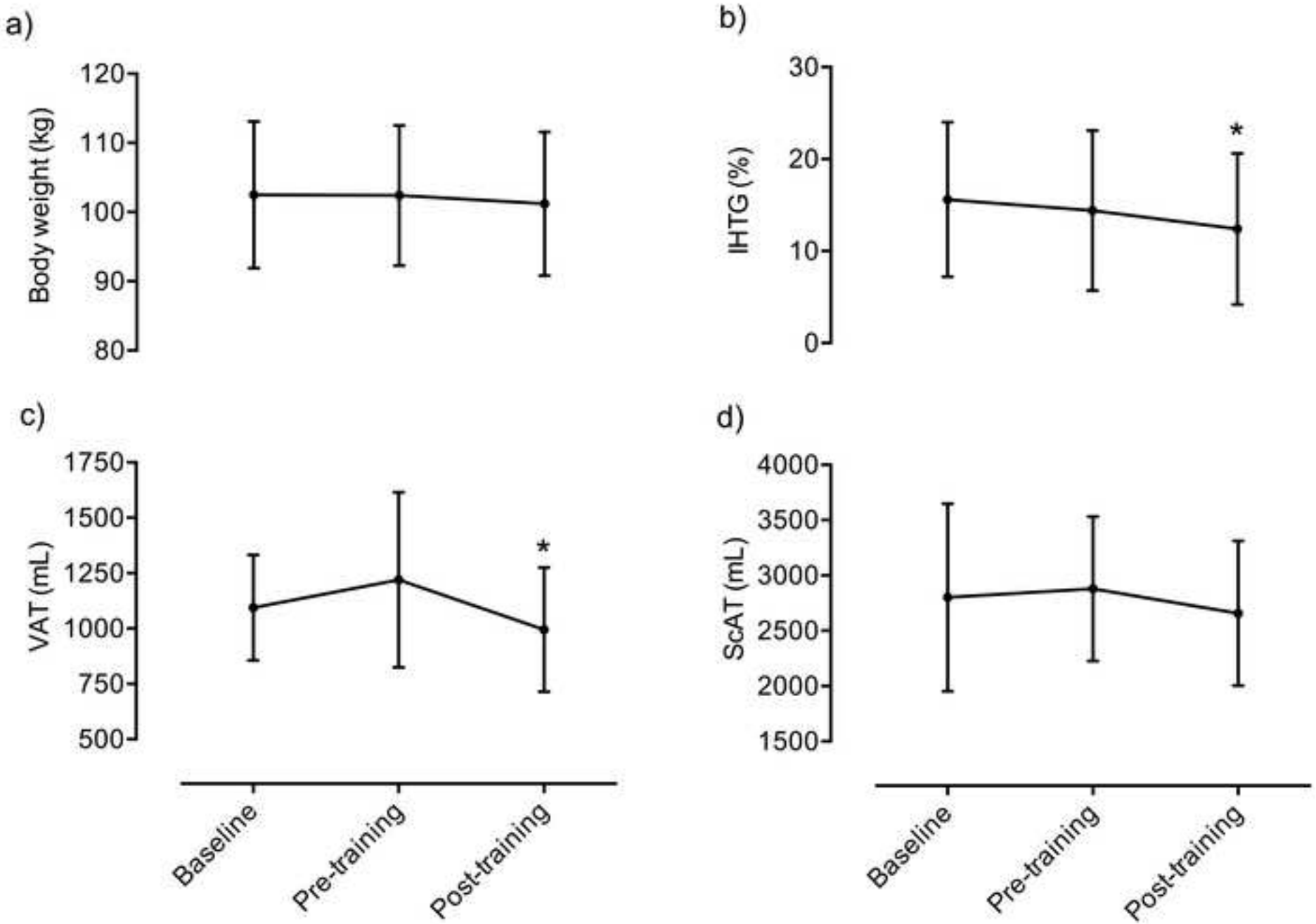


Figure 3

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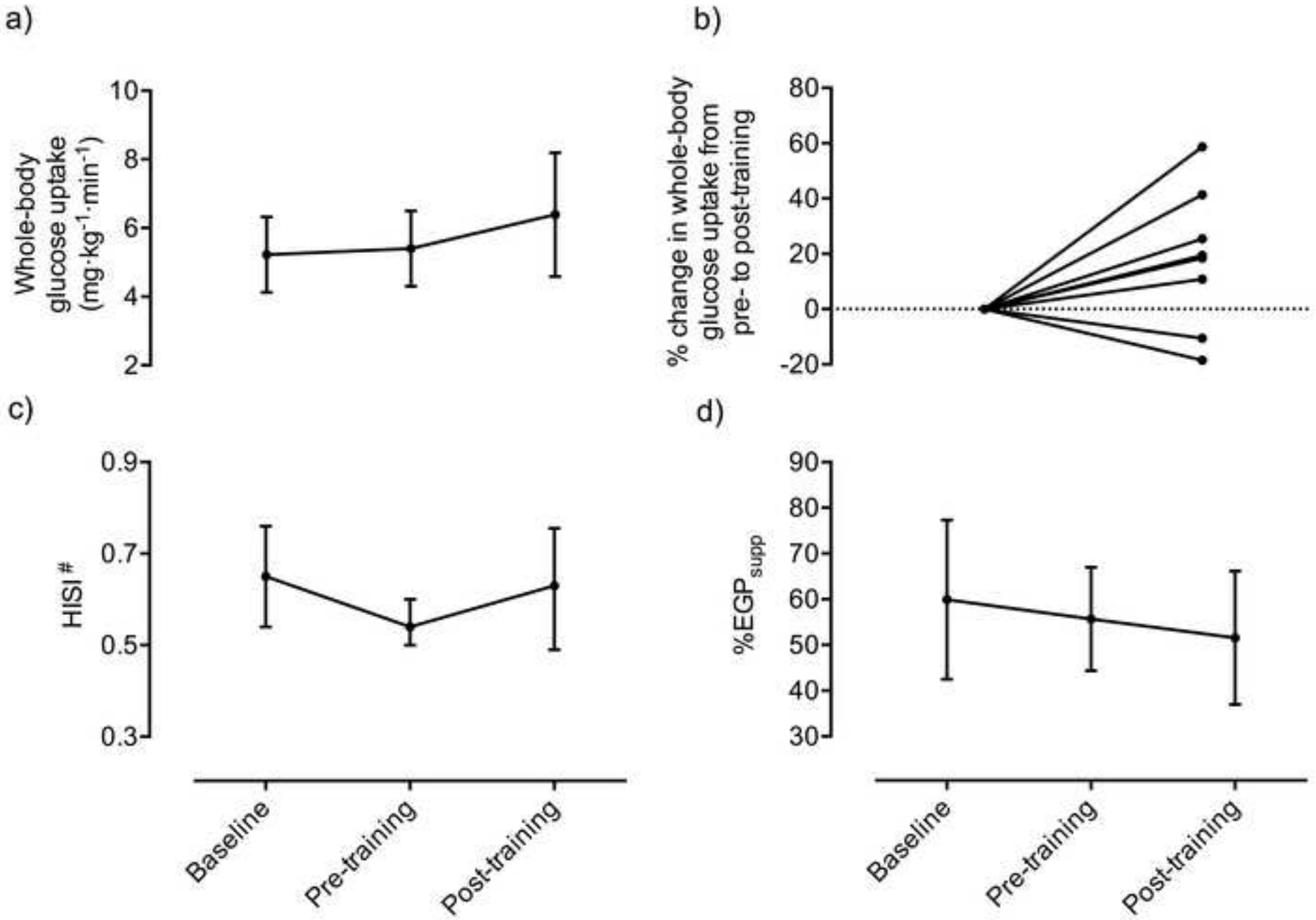


Table 1

Table 1 - Participant characteristics and study outcomes

	Baseline	*Pre-training*	*Post-training*	*P-value (main effect of phase)*	*Effect size (Hedges' g*)*	
					Baseline-Pre-Training	*Pre- to Post-Training*
Anthropometry						
Age (years)	41 ± 8					
BMI (kg·m^{-2})	31.7 ± 3.1					
Waist circumference (cm)	111.3 ± 7.5					
Body weight (kg)	102.5 ± 10.6	102.4 ± 10.1	101.2 ± 10.4	0.17	Trivial	Trivial
VAT (mL) (*n=8*)	1094 ± 238	1220 ± 395	995 ± 281	**0.01**	0.36	-0.62
ScAT (mL) (*n=8*)	2801 ± 847	2800 ± 653	2658 ± 655	0.16	Trivial	-0.21
IHTG (%)	15.6 ± 8.4	14.4 ± 8.7	12.4 ± 8.2	**0.001**	Trivial	-0.23
Cardiorespiratory Fitness						
Absolute $\dot{V}O_2$ peak (L·min^{-1})	3.23 ± 0.41	3.18 ± 0.41	3.53 ± 0.45[c]	**<0.001**	Trivial	0.83
Relative $\dot{V}O_2$ peak (mL·kg^{-1}·min^{-1})	31.8 ± 4.8	31.0 ± 4.7[a]	35.1 ± 5.4[c]	**<0.001**	Trivial	0.78
Absolute peak power output (W)	239 ± 42	237 ± 42	270 ± 43[d]	**<0.001**	Trivial	0.75
Relative peak power output (W·kg^{-1})	2.36 ± 0.49	2.34 ± 0.54	2.71 ± 0.57[d]	**<0.001**	Trivial	0.64
Insulin Sensitivity (n=8)						
Fasted serum insulin (mU·L^{-1})	17.6 ± 4.5	21.8 ± 8.1[a]	18.2 ± 6.0[b]	**0.04**	0.60	-0.48
Fasted blood glucose (mmol·L^{-1})	4.7 ± 0.3	4.7 ± 0.4	4.5 ± 0.5	0.13	Trivial	-0.32
HOMA-IR	3.7 ± 1.0	4.5 ± 1.7[a]	3.7 ± 1.2[b]	**0.03**	0.59	-0.56
Whole-body glucose uptake (mg·kg^{-1}·min^{-1})	5.2 ± 1.1	5.4 ± 1.1	6.4 ± 1.8[e]	**0.02**	Trivial	0.63
Fasted plasma NEFA (mmol·L^{-1})	0.59 ± 0.15	0.55 ± 0.12	0.58 ± 0.19	0.83	-0.41	0.29

Adipo-IR*	52.7 (45.0 – 80.1)	69.3 (51.0 – 81.9)	55.4 (46.5 – 71.5)	>0.99	0.38	-0.24
Basal EGP ($\mu mol \cdot kg^{-1} \cdot min^{-1}$)	10.8 ± 1.5	10.7 ± 2.2	11.2 ± 2.1	0.70	Trivial	0.25
HISI* ($mg \cdot m^{-2} \cdot min^{-1}$ per $mU \cdot L^{-1}$)	0.65 (0.56 – 0.74)	0.54 (0.52 – 0.60)	0.62 (0.58 – 0.69)	0.42	-0.47	0.34
$\%EGP_{supp}$ (%)	59.9 ± 17.4	55.7 ± 11.3	51.6 ± 14.6	0.37	-0.28	-0.30
Circulating Lipids						
Triacylglycerol ($mmol \cdot L^{-1}$)	2.2 ± 0.9	2.2 ± 0.7	1.9 ± 0.7	0.50	Trivial	-0.35
Total Cholesterol ($mmol \cdot L^{-1}$)	4.88 ± 0.59	4.80 ± 0.54	4.62 ± 0.75	0.19	Trivial	-0.26
HDL ($mmol \cdot L^{-1}$)*	0.90 (0.84 – 1.06)	0.88 (0.85 – 1.01)	0.95 (0.90 – 1.16)[b]	**0.01**	Trivial	0.44
LDL ($mmol \cdot L^{-1}$)	2.85 ± 0.61	2.89 ± 0.54	2.77 ± 0.60	0.48	Trivial	-0.21

Data presented as mean ± SD and for n=9 unless otherwise stated. * indicates that data are not normally distributed and thus presented as median (IQR). [a] the difference between baseline and pre-training values approached statistical significance (uncorrected P-values; FPI P=0.06, HOMA-IR P=0.07, Relative $\dot{V}O_2$ peak P=0.07), [b] significantly different from pre-training values (P<0.05), [c] significantly different from pre-training values (P<0.01), [d] significantly different from pre-training values (P<0.001), [e] the difference between pre-training and post-training values approached statistical significance (uncorrected P=0.09); Trivial effect sizes were considered those of magnitude < 0.2; BMI: Body mass index; VAT: visceral adipose tissue; ScAT: subcutaneous abdominal adipose tissue; IHTG: intrahepatic triglyceride; $\dot{V}O_2$ peak: peak oxygen uptake; HOMA-IR: Homeostatic model assessment of insulin resistance; NEFA: Non-esterified fatty acids; Adipo-IR: Adipose tissue insulin resistance index; EGP: Endogenous glucose production; HISI: Hepatic insulin sensitivity index; $\%EGP_{supp}$: Percentage suppression of EGP during low dose insulin infusion ($20mU \cdot m^{-2} \cdot min^{-1}$); HDL: High density lipoprotein; LDL: Low density lipoprotein.

Table 2 – Habitual sedentary time and physical activity during baseline, pre- and post-training assessment periods

	Baseline	*Pre-training*	*Post-training*	*P-value (main effect of phase)*
General				
Device wear time (minutes per day)	1027 ± 131	989 ± 119	1027 ± 166	0.68
Counts per wear minute	293 ± 111	316 ± 156	294 ± 92	0.34
Sedentary Behaviour				
Minutes per day	719 ± 92	693 ± 139	726 ± 155	0.77
% of wear time[#]	70.3 (64.2 – 74.8)	74.9 (59.6 – 76.6)	66.5 (65.6 – 77.3)	0.37
Light Activity				
Minutes per day	270 ± 74	256 ± 82	262 ± 59	0.60
% of wear time	26.1 ± 5.5	26.0 ± 8.1	25.8 ± 5.5	0.93
Moderate Activity				
Minutes per day[#]	23.0 (18.0 – 50.5)	31.0 (19.5 – 63.0)	30.0 (13.5 – 52.5)	0.89
% of wear time[#]	2.4 (1.6 – 5.5)	2.7 (1.9 – 7.2)	2.5 (1.3 – 6.3)	0.24
Vigorous Activity				
Minutes per day[#]	0.0 (0.0 – 2.0)	0.0 (0.0 – 1.5)	0.0 (0.0 – 1.5)	0.94
% of wear time[#]	0.03 (0.00 – 0.18)	0.01 (0.00 – 0.13)	0.03 (0.00 – 0.13)	0.71
Moderate-Vigorous Activity (MVPA)				
Minutes per day[#]	23.0 (18.5 – 55.0)	31.0 (19.5 – 67.0)	32.0 (13.5 – 59.0)	0.92
% of wear time[#]	2.4 (1.7 – 6.1)	2.7 (1.9 – 7.8)	2.7 (1.3 – 7.1)	0.40

Data presented as mean ± SD, unless otherwise stated. *n*=9. [#]indicates that data are not normally distributed and thus presented as median (IQR). *P*-values represent the main effect of phase.

Author contribution statement

JAK, GPA, MAN, IAM and PAG generated the study idea and designed the protocol. JAS, EJS, SB, JAK, JC, KS, MCT and JLD collected the study data and performed the analyses. JAS, JAK, SB, JC, KS and GPA led the manuscript preparation. All authors read, edited and approved the final version of the manuscript.