TITLE:

HOME-HIT IMPROVES MUSCLE CAPILLARISATION AND eNOS/NAD(P)HOXIDASE PROTEIN RATIO IN OBESE INDIVIDUALS WITH ELEVATED CARDIOVASCULAR DISEASE RISK

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SHORT TITLE:

HOME-BASED HIT IN OBESE INDIVIDUALS

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AUTHOR PROFILE:

Sam Scott completed his PhD at Liverpool John Moores University and is currently a Post-Doctoral Researcher at York University, Toronto. His research interests focus on the metabolic and hormonal responses to exercise stress in people with type 1 diabetes. Sam is also interested in the effects of exercise on skeletal muscle metabolism and the links to insulin resistance.

Abbreviations

BMI	body mass index
CVD	cardiovascular disease
FA	fibre cross-sectional area
CC	capillary contacts
CD	capillary density
C/Fi	capillary-to-fibre ratio on an individual fibre basis
CFPE	capillary-fibre perimeter exchange index
HIT	high intensity interval training
MICT	moderate intensity continuous training
Home-HIT	Home-based HIT
Home-MICT	home-based MICT
Lab-HIT	laboratory-based HIT
NAD(P)Hox	NAD(P)Hoxidase
NO	nitric oxide
$\cdot O_2^-$	superoxide anion
NOX2	catalytic subunit of the NAD(P)Hox complex
$p47^{phox}$	regulatory subunit of the NAD(P)Hox complex
OGTT	oral glucose tolerance test
$\dot{V}O_{2peak}$	peak oxygen consumption
W_{max}	maximal aerobic power output
HR	heart rate
SMA	smooth muscle actin
Ser^{1177}	$serine^{1177}$
UEA-I FITC	*Ulex europaeus*-FITC conjugated
WGA-350	wheat germ agglutinin-350
ROS	reactive oxygen species
IMTG	intramuscular triglyceride
COX IV	cytochrome c oxidase complex IV
DXA	dual-energy x-ray absorptiometry

FMD	flow-mediated dilation
GLUT4	glucose transporter 4
ISI	insulin sensitivity index
PWV	pulse wave velocity
MHCI	myosin heavy chain I

KEY POINTS SUMMARY

- Obesity and sedentary behaviour are associated with capillary rarefaction and impaired muscle microvascular vasoreactivity, due to reduced nitric oxide bioavailability.
- Low-volume high-intensity interval training (HIT) is a time-efficient alternative to traditional moderate-intensity continuous training (MICT), but its effect on the muscle microvasculature has not been studied.
- The applicability of current lab- and gym-based HIT protocols for obese individuals with low fitness and mobility has been disputed by public health experts, who cite the strenuous nature and complex protocols as major barriers. Therefore, we developed a virtually-supervised HIT protocol targeting this group that can be performed at home without equipment (Home-HIT).
- This study is the first to show that 12-weeks of virtually-supervised Home-HIT in obese individuals with elevated CVD risk leads to similar increases in capillarisation and eNOS/NAD(P)Hoxidase protein ratio within the muscle microvascular endothelium as virtually-supervised home-based MICT and laboratory-based HIT, while reducing many of the major barriers to exercise.

Abstract

This study investigated the effect of a novel virtually-supervised home-based high-intensity interval training (HIT) (Home-HT) intervention in obese individuals with elevated cardiovascular disease (CVD) risk on capillarisation and muscle microvascular eNOS/NAD(P)Hoxidase ratio. Thirty-two adults with elevated CVD risk (age 36±10 years; BMI 34.3±5 $kg \cdot m^{-2}$; $\dot{V}O_{2peak}$ 24.6±5.7 $ml \cdot kg \cdot min^{-1}$), completed one of three 12-week training programmes: Home-HIT (n=9); laboratory-based supervised HIT (Lab-HIT; n=10) or virtually-supervised home-based moderate-intensity continuous training (Home-MICT; n=13). Muscle biopsies were taken pre- and post-training to assess changes in vascular enzymes, capillarisation, mitochondrial density, intramuscular triglyceride content and GLUT4 protein expression using quantitative immunofluorescence microscopy. Training increased $\dot{V}O_{2peak}$ (P<0.001), whole-body insulin sensitivity (P=0.033) and flow-mediated dilation (P<0.001), while aortic pulse wave velocity decreased (P<0.001) in all 3 groups. Immunofluorescence microscopy revealed comparable increases in total eNOS content in terminal arterioles and capillaries (P<0.001) in the 3 conditions. There was no change in eNOS ser^{1177} phosphorylation (arterioles P=0.802; capillaries P=0.311), but eNOS ser^{1177}/eNOS content ratio significantly decreased following training in arterioles and capillaries (P<0.001). Training decreased NOX2 content (arterioles P<0.001; capillaries P<0.001), but there was no change in $p47^{phox}$ content (arterioles P=0.101; capillaries P=0.345). All measures of capillarisation increased (P<0.05). There were no between group differences. Despite having no direct supervision during exercise, virtually-supervised Home-HIT resulted in comparable structural and endothelial enzymatic changes in the skeletal muscle microvessels to the traditional training methods. We provide strong evidence that

Home-HIT is an effective novel strategy to remove barriers to exercise and improve health in an obese population at risk of CVD.

INTRODUCTION

The physically inactive and sedentary lifestyle of industrialised nations combined with the overconsumption of energy dense food has led to a global obesity epidemic with >650 million adults worldwide classified as obese (BMI ≥30 $m \cdot kg^{-2}$; WHO, 2018). Physical inactivity has been identified as one of the leading global risks for premature mortality, and obesity has been shown to double the risk of all-cause mortality due to its association with cardio-metabolic pathologies such as cardiovascular disease (CVD) and type 2 diabetes (Berrington de Gonzalez *et al.*, 2010; Ding *et al.*, 2016).

Obesity and sedentary behaviour lead to reduced capillary density and impaired vasodilation of the skeletal muscle microvasculature in response to meal ingestion and exercise (Wagenmakers *et al.*, 2016). These structural and functional impairments in the skeletal muscle microvasculature significantly reduce the ability of the skeletal muscle to meet its metabolic demands and contribute to the development of insulin resistance and chronic diseases (Wallis *et al.*, 2002; Vincent *et al.*, 2003; Clerk *et al.*, 2006). This decline in skeletal muscle microvascular function has been proposed to precede macrovascular impairments (Krentz *et al.*, 2009). Together these observations suggest that the skeletal muscle microvasculature should be regarded as a primary target for intervention in the increasingly obese population.

Reduced skeletal muscle microvascular nitric oxide (NO) bioavailability is a

central factor contributing to capillary rarefaction and the impaired vasodilatory response seen in obesity (Frisbee, 2007; McAllister & Laughlin, 2006; Olver & Laughlin, 2016). Endothelial nitric oxide synthase (eNOS) is the rate limiting enzyme responsible for NO synthesis, with the ability of eNOS to synthesise NO being determined by its protein content and activity in the endothelial layer of the muscle microvasculature (Cocks & Wagenmakers, 2016). eNOS activation is determined by phosphorylation on multiple sites, with increases in insulin, shear stress and VEGFA leading to eNOS serine^{1177} phosphorylation and vasodilation of the muscle microvasculature (Cocks & Wagenmakers, 2016; Hellsten *et al.*, 2008; Hoier *et al.*, 2013; Mount *et al.*, 2007). Obesity and inactivity have been shown to alter the balance between NO production by eNOS and increased NO quenching by superoxide anions and other reactive oxygen species (ROS) (Frisbee, 2005; McAllister & Laughlin, 2006). The enzyme complex NAD(P)Hoxidase (NAD(P)Hox) has been shown to be a major source of superoxide anion production in obese individuals (Silver *et al.*, 2007; La Favor *et al.*, 2016). As such, the eNOS to NAD(P)Hox protein ratio has been suggested to be a key marker of microvascular function in skeletal muscle (Cocks *et al.*, 2016; Cocks & Wagenmakers, 2016).

Exercise prescription consisting of moderate-intensity continuous exercise in line with the physical activity guidelines is an important first line strategy for the management of obesity and cardio-metabolic disease (Ismail *et al.*, 2013). However, adherence to exercise programmes is poor unless there is adequate supervision (Eriksson & Lindgärde, 1991; Faulkner *et al.*, 2014). Recent work has demonstrated that 4 weeks of sprint interval training leads to similar improvements in capillarisation and eNOS/NAD(P)Hox protein ratio as traditional moderate-intensity continuous training (MICT) in obese males (Cocks *et al.*, 2016). Although the sprint interval

training protocol used by Cocks *et al.* (2016) offers a time-efficient alternative to MICT, the suitability of sprint interval training as a safe and tolerable exercise strategy in obese individuals with elevated CVD risk has been questioned (Levinger *et al.*, 2015). As such, low-volume high-intensity interval training (HIT) protocols, consisting of 60 seconds of intense constant-load cycling at 100% W_{max} interspersed with 60 seconds of active recovery, have been developed as a safe and tolerable alternative (Little *et al.*, 2011). Despite evidence showing low-volume HIT interventions are effective (Hood *et al.*, 2011; Little *et al.*, 2011; Tan *et al.*, 2018) the applicability to sedentary obese individuals has been disputed by health experts (Biddle & Batterham, 2015; Courneya, 2010; Hardcastle *et al.*, 2014), who cite the strenuous nature and complex protocols as major barriers in sedentary, exercise-naïve individuals. Furthermore, most successful HIT interventions are laboratory-based, providing participants optimal conditions with continuous supervision and specialised equipment (Hood *et al.*, 2011; Little *et al.*, 2011; Tjønna *et al.*, 2008). Exercise in laboratory settings do not address additional barriers to exercise including difficulties with access to facilities (including travel distance and cost) and embarrassment due to a perceived negative body image and low exercise self-efficacy in public gyms (Korkiakangas *et al.*, 2009).

Performing body-weight exercises in the home environment at a high intensity to mimic that of laboratory-based low-volume HIT may remove some of the barriers to exercise including lack of time, a requirement for equipment, costs and difficulty with transport (Machado *et al.*, 2017). Only a handful of studies have investigated the efficacy of home-based exercise training programmes (Ong *et al.*, 2009; Halse *et al.*, 2014; Dadgostar *et al.*, 2016; Blackwell *et al.*, 2017). However, no studies to date have made comparisons between Home-HIT and more traditional exercise training

strategies on the microvascular enzymes controlling NO production, and skeletal muscle microvascular density in previously sedentary obese individuals with elevated CVD risk. In addition, although home-based interventions remove traditional barriers to exercise, research suggests that lack of support from exercise specialists can create new barriers to exercise (Morgan *et al.*, 2016). Advances in wearable technology provide the opportunity to facilitate feedback between the exerciser and healthcare provider. As such, the home-based interventions in the current study employed a novel virtual-monitoring system.

The aim of this study was to investigate the effect of a 12-week virtually-supervised Home-HIT intervention on skeletal muscle capillary density and skeletal muscle microvascular enzymes responsible for NO production (eNOS content and ser^{1177} phosphorylation) and NO quenching (NOX2 and p47^{phox} content) in sedentary obese individuals with elevated CVD risk. To further assess the effectiveness of the developed intervention the study also investigated: 1) changes in cardio-metabolic health markers, 2) changes in classical markers of training adaptation within skeletal muscle. The study also provided preliminary data on adherence and compliance (defined as ability to meet prescribed heart rates) to the programme. Two control groups were included: 1) a supervised laboratory-based HIT (Lab-HIT) group, to assess the effect of Home-HIT compared to optimal conditions, and 2) a virtually-supervised home-based moderate-intensity continuous training (Home-MICT) group, to allow comparison to a group achieving the physical activity guidelines (Colberg *et al.*, 2016). The primary hypothesis was that microvascular density and eNOS content would increase to a similar extent in all three groups alongside an increase in $\dot{V}O_{2peak}$ and insulin sensitivity. The secondary hypothesis was that the three training

programmes would reduce the protein content of NOX2 and its activator $p47^{phox}$ to a similar degree in the endothelial layer of terminal arterioles and capillaries.

METHODS

Ethical Approval

All participants provided written informed consent, and the study was approved by the Black Country NHS Research Ethics Committee (approval reference no. 14/WM/1222) and conformed to the *Declaration of Helsinki*. The following clinical trials registration ID was used: NCT03557736.

Participants

Thirty-two sedentary obese adults (BMI >30kg·m^2 or waist/hip ratio of >0.9 in men and >0.85 in women) with at least 2 further CVD risk factors, according to the American Heart Association criteria (Grundy *et al.*, 1999), completed the study (Table 1). Participants were self-allocated to one of three 12-week training groups: Home-HIT (*n*=9); Home-MICT (*n*=13); or Lab-HIT (*n*=10) matched for age, BMI and $\dot{V}O_{2peak}$ (further details of the self-allocation process, and the rationale for this, can be found in the "training protocol" section of the methods). Participants completed a 12 lead ECG to check for contraindications to exercise, and were free of diagnosed cardiovascular and/ or metabolic disease.

Experimental Protocol

Participants performed an incremental exercise test to exhaustion on an electromagnetically-braked cycle ergometer (Corival, Lode, Groningen,

Netherlands), using an online gas collection system (MOXUS, AEI technologies, Pittsburgh, PA) as described previously (Cocks *et al.*, 2016). Waist-to-hip ratio was recorded, and body composition was analysed using Dual-energy X-ray Absorptiometry (DXA). Finally, participants were provided with a physical activity monitor (ActiGraph GT3X+, Fort Walton Beach, FL) and diet diary so that habitual physical activity and diet could be assessed over 7 and 3 days, respectively.

Three to seven days after initial testing participants attended the laboratory following an overnight fast, having abstained from caffeine, alcohol and vigorous exercise the day before testing. Following 20 minutes of supine rest, blood pressure was measured in triplicate using a sphygmomanometer (Dianamap; GE Pro 300V2, Tampa, Florida). Brachial artery endothelial function was measured using flow-mediated dilation (FMD), using the previously described method (Thijssen *et al.*, 2011; Cocking *et al.*, 2018). Aortic pulse wave velocity (PWV) was then measured in triplicate using a SphygmoCor (AtCor Medical, Sydney, Australia) (Cocks *et al.*, 2013). A resting muscle biopsy was then taken from the lateral portion of the *m. vastus lateralis* under local anaesthesia (0.5% Marcaine), using the Weil-Blakesley conchotome technique (Baczynska *et al.*, 2016). Finally, insulin sensitivity was measured using an oral glucose tolerance test (OGTT) (Matsuda & DeFronzo, 1999). A cannula was inserted into an antecubital vein and a baseline 10 ml blood sample was taken before consumption of a 25% glucose beverage containing 75g of glucose and 225 ml of water. Further 5 ml blood samples were collected at 30, 60, 90 and 120 minutes after glucose ingestion. Muscle and blood samples were then stored at -80°C until analysis.

Assessments of FMD, aortic PWV and $\dot{V}O_{2peak}$ were repeated after 4 weeks and at the end of the 12 weeks of training using identical procedures to pre-training. Post-training assessment of $\dot{V}O_{2peak}$ was performed instead of the 35th training session. ~72h following the final training session post-training testing was conducted with procedures, methods and timings identical in all respects to pre-training.

Training Protocols

Participants trained for 12 weeks in one of three groups:

1. Home-HIT: repeated 1-minute bouts of exercise interspersed with 1 minute of rest. Participants were advised to achieve ≥80% of predicted heart rate maximum (HR_{max}; 220–age) during the intervals. The 1-minute intervals were composed of two different 30-second bodyweight exercises with no rest in between. Participants were provided with 9 exercise pairs, detailed in an exercise pack, and were free to choose which exercises they completed (supplemental material). During weeks 1-4 participants were advised to complete 4 intervals, which increased by one interval each fortnight up to a maximum of 8 intervals.

2. Home-MICT: participants performed continuous exercise of their choosing (swimming, cycling or walking/running), at an advised exercise intensity of ~65% predicted HR_{max}. During weeks 1-4 participants were asked to exercise for 30 minutes which increased by 5 minutes each fortnight up to 50 minutes.

3. Lab-HIT: participants completed the same protocol as Home-HIT, but on a cycle ergometer at the University laboratory. During the intervals, participants exercised at an intensity of 100% W_{max} (Little *et al.*, 2011) in order to elicit a HR of ≥80% HR_{max}. The number of intervals was identical to Home-HIT. The training sessions were supervised, and participants were given strong encouragement throughout. Participants were excluded if ≥80% of sessions were not completed.

Participants were allowed to choose their training group, based on which fitted their current lifestyle best. To minimise potential allocation bias all participants were provided detailed (written and verbal) information on the three programmes before choosing their training group. In addition, recruitment to the groups was not restricted at any point; i.e. groups were left open for recruitment until appropriate participant numbers were achieved in all three groups. Self-allocation was chosen to increase the real-world translation of the findings.

Lab-HIT participants completed all of their training within the exercise laboratories of Liverpool John Moores University. Sessions were scheduled by the research team for participants, and all sessions were supervised with researchers providing strong encouragement throughout. Participants trained 3x/week and were excluded if ≤80 of sessions were completed. In contrast, participants in the home-based interventions trained in a place of their choosing outside the laboratory. Participants were responsible for scheduling their own training sessions. Although participants were monitored virtually throughout the intervention (see below), training sessions were

completed without supervision or encouragement from the research team. Participants were advised to train 3x/week, but unlike Lab-HIT this was not enforced.

Home-based participants were virtually monitored using a HR monitor which connected via Bluetooth to their smart phone (Polar Beat; www.polar.com/beat/uk-en). During exercise this allowed participants to monitor their HR and provided immediate feedback on exercise intensity. To guide participants they were given a target HR to achieve during the sessions based on their predicted HR_{max} (220-age; Home-HIT >80% HR_{max}, Home-MICT ~65% HR_{max}). The rationale for predicted HR_{max} over actual HR_{max} (obtained on the $\dot{V}O_{2max}$ test) was to increase the real world translation of the study, as the research team do not envisage, or deem it feasible, that all individuals engaging in home-based training should complete a maximal exercise test before commencing training. Following each training session HR data was automatically uploaded to a cloud storage site (www.flow.polar.com), which allowed participants to monitor their progression. The website was also available to the research team to monitor if the programme was being completed as advised. The research team used this data to contact participants by text/email every 2 weeks to enquire about training progress and to provide support if required. If participants missed consecutive sessions, the text/email enquired as to whether there was a specific reason for this. The monitoring system was also used to provide an objective measure of adherence (number of sessions completed) and compliance (whether HR thresholds and correct number of intervals were achieved during each session).

Immunofluorescence Microscopy

Details of the specific quantification techniques can be found below and all techniques have been described in detail previously, including antibody specificity

experiments (Cocks *et al.*, 2013; Shepherd *et al.*, 2013; Bradley *et al.*, 2014; Shepherd *et al.*, 2017). All techniques used frozen muscle biopsy samples cryosectioned to a thickness of 5μm mounted onto uncoated glass microscope slides so that transverse orientated samples could be used for analysis.

For eNOS content, eNOS ser^{1177} phosphorylation, GLUT4 content and capillarisation measures sections were fixed in acetone and ethanol (3:1) for 5 minutes. For mitochondrial density and intramuscular triglyceride (IMTG) analysis sections were fixed in 3.7% formaldehyde for 1 hour, rinsed briefly (3 x 30s) in deionized water, and permeabilized in 0.5% Triton-X 100 for 5 minutes. Subsequently, slides underwent incubation with appropriate primary antibodies against OXPhos Complex IV (Invitrogen, Paisley, UK), eNOS (Transduction Laboratories, Lexington, KY, USA), eNOS ser^{1177} (Cell Signalling Technology, Beverly, MA, USA) or GLUT4 (Abcam, Cambridge, UK). Muscle fibre type (used during analysis of mitochondrial density, IMTG content and capillarisation) was determined using an anti-myosin antibody for slow twitch fibres (A4.840-c, DSHB, developed by Dr Blau). Following primary antibody incubation sections were incubated in appropriate secondary antibodies and UEA-I-FITC (Sigma-Aldrich, UK) (eNOS content and phosphorylation and capillarisation) and/ or wheat germ agglutinin-350 (WGA-350; Invitrogen) (mitochondrial density, GLUT4 content, IMTG content and capillarisation) as markers of the endothelium and plasma membrane, respectively. Finally, for IMTG visualisation sections were incubated with Bodipy (Sigma-Aldrich).

Image Capture

Images for mitochondrial density and capillarisation were acquired using a Lecia DM6000FS widefield microscope and 40x 0.6 numerical aperture (NA) objective. Images for GLUT4 and IMTG content were acquired using an inverted confocal microscope (Zeiss LSM-710, Carl Zeiss, Germany) with a 63x 1.4NA oil immersion objective and the images for the vascular enzymes were captured using a 40x 1.3NA oil immersion objective. Alexa Fluor 405 was excited using the 405 nm line of the diode laser and detected with 371-422 nm emission. FITC fluorescence was excited with a 488 nm line of the argon laser and detected with 493–559 nm emission. Alexa Fluor 546 and 633 fluorophores were excited with 543 nm and 633 nm lines of the helium–neon laser and 548–623 nm and 638–747 nm emission, respectively. The images were acquired at a resolution of 1,024 X 1,024 pixels and stored in 24-bit tagged image format file format. No image processing was carried out prior to intensity analysis and identical settings were used for all image capture for each variable within each participant.

Image Analysis

All image analysis was performed using ImagePro Plus 5.1 (Media Cybernetics Inc, Bethesda, MD, USA).

Mitochondrial density and GLUT4 content

Mitochondrial density and GLUT4 content were assessed using the methods described by Shepherd *et al.* (2013) and Bradley *et al.* (2014), respectively. Briefly, fluorescence intensity was quantified by measuring the signal intensity within the

intracellular regions of a mask created by the dystrophin stain in a fibre type specific manner.

IMTG Analysis

Fibre type specific IMTG analysis to assess peripheral and central regions of the myocyte was assessed using the method described in (Shepherd *et al.*, 2017). This method was adapted in order to assess IMTG content, lipid droplet size and number in the peripheral and central regions of the myocyte. The peripheral region was defined as the 5μm below the plasma membrane. Briefly, an intensity threshold was uniformly selected to represent a positive signal for IMTG. IMTG content was expressed as the positively stained area fraction relative to the total area of each muscle fibre. IMTG density was calculated as the number of IMTG objects relative to area. The mean area of individual IMTG (lipid droplets) objects was used as a measure of lipid droplet size.

Vascular Enzymes

NOX2 and $p47^{phox}$ protein content in the skeletal muscle microvascular endothelium and sarcolemma were assessed using the previously developed immunofluorescence staining protocol and quantification technique (Cocks *et al.*, 2012; Cocks *et al.*, 2013), adapted to allow for differentiation between capillaries and terminal arterioles (Cocks *et al.*, 2016). Capillary and terminal arteriole specific eNOS content and eNOS ser^{1177} phosphorylation were also assessed using previously established methods (Cocks *et al.*, 2016); however, the method was adapted to allow for assessment of individual vessel eNOS ser^{1177}/eNOS ratio to be calculated. Briefly, blood vessels were divided into either capillaries or arterioles

using the αSMA image. The endothelial (UEA-I-FITC) outline was then overlaid onto the corresponding vascular enzyme image. Fluorescence intensity of the vascular enzyme signal was then quantified within the endothelial specific area. Diameter of the arterioles was determined on calibrated images. Vessels larger than 20μm in diameter were excluded to remove 3rd and 4th order arterioles (Wu *et al.*, 2011) from the analysis, which rarely appear in muscle cross-sections. eNOS and eNOS ser^{1177} phosphorylation were stained on the same sections, as such, it was possible to establish eNOS ser^{1177}/eNOS ratio on an individual vessel basis, as the same endothelial outline could be placed over both eNOS and eNOS ser^{1177} images. Cell membrane specific fluorescence for NOX2 and p47^{phox} was determined using the WGA-633 stain to create an outline of the cell membrane. This mask was then overlaid onto the corresponding image to determine membrane specific fluorescence intensity for NOX2 or p47^{phox}.

Capillarisation

Capillaries were quantified in a fibre type specific manner manually, using the UEA-I, WGA-633 and myosin heavy chain images. The following indexes were measured (Hepple *et al.*, 1997): 1) the number of capillaries around a fibre (capillary contacts), 2) capillary-to-fibre ratio on an individual fibre basis and 3) capillary-fibre perimeter exchange (CFPE) index. In addition, overall capillary density was determined. Quantification of capillarisation was performed only on transverse fibres. In line with previous studies assessing capillarisation, at least 50 complete fibres were included in each analysis (Porter *et al.*, 2002). Fibre cross-sectional area and perimeter were measured on calibrated images using ImagePro Plus 5.1 software.

Statistical Analysis

The primary aim of the study was to compare the effects of training on muscle microvascular eNOS protein content. The study was powered to detect between-group differences in this variable in response to training. G*Power 3.1 software (G*Power Software Inc., Kiel, Germany) was used to calculate the required sample size. The study was designed to detect a between-group effect of f = 0.35, representative of a medium-sized effect (Cohen, 1992), adopting an alpha of 0.05 and power of 0.80. This was deemed to be a physiologically relevant difference, as the authors have previously observed a medium effect size difference following 6 weeks of SIT and MICT in sedentary individuals (Cocks *et al.*, 2013). Measures taken pre, mid and post-training and measures assessing a fibre type differences were analysed using a two- or three-way mixed design ANOVA with the within group factors 'training' (pre vs. mid vs. post) and 'fibre type' (type I vs. type II) and the between group factor 'group' (Home-HIT vs. Home-MICT vs. Lab-HIT). All other variables taken pre and post-training were analysed using a 2-way mixed design ANOVA with between factor 'group' (Home-HIT vs. Home-MICT vs. Lab-HIT) and within group factor 'training status' (pre vs. post). In the case of a significant interaction, a Bonferroni post-hoc test was applied. Eight muscle biopsies were taken and analysed pre and post training in each training group. Matsuda Index values are missing in one Lab-HIT participant and three Home-MICT participants because it was not possible to get blood samples. All analyses were performed using IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp. Significance was set at $P \leq 0.05$ and data are presented as mean ± SD.

RESULTS

Adherence and Compliance

Figure 2 shows a flow chart of participant recruitment and any reasons for study drop out. Although not significant, adherence tended to be different between groups (*P*=0.053; Figure 1A). Post-hoc analysis revealed a trend for greater adherence to Lab-HIT compared to Home-MICT (*P*=0.081), but no significant differences were observed between Home-HIT and Lab-HIT (*P*=1.000) or Home-MICT (*P*=0.195). Training compliance showed no differences between groups (*P*= 0.420; Figure 1B).

General Characteristics

Baseline data showed no differences in age, BMI or $\dot{V}O_{2peak}$ between groups (*P*=0.369; 0.455 and 0.898, respectively). Training increased $\dot{V}O_{2peak}$ (main effect, *P*<0.001), but no difference between groups was observed. Post-hoc analysis revealed that $\dot{V}O_{2peak}$ increased following 4 weeks of training (Home-HIT 9%, Home-MICT 6%, Lab-HIT 8%; *P*<0.001) and continued to increase further after 12 weeks (Home-HIT 16%, Home-MICT 12%, Lab-HIT 20%; *P*<0.001). Training decreased body mass and BMI (main effect, *P*=0.003 and *P*=0.005, respectively), with no differences between groups. Post-hoc analysis revealed both body mass and BMI decreased following 4 weeks of training (*P*=0.004 and *P*=0.007, respectively), and this decrease in body mass and BMI was retained after 12 weeks (*P*=0.014 and *P*=0.022, respectively). There was a 4% decrease in body fat percentage in all three groups (main effect, *P*<0.01), with no difference between groups (*P*=0.468). Visceral fat mass was also significantly reduced in all groups (Home-HIT -27%; Home-MICT -12%; Lab-HIT -3%; main effect, *P*=0.025), with no difference between groups (*P*=0.304). There was no change in lean mass following training (*P*=0.387).

Blood Variables

Insulin AUC decreased with training (Home-HIT -24%, Home-MICT -20%, Lab-HIT -18%; main effect, $P<0.001$), but there was no change in glucose AUC ($P>0.05$), with no difference between groups for either variable. The Matsuda ISI was significantly increased by 12 weeks of training (Home-HIT 39%, Home-MICT 18%, Lab-HIT 13%; main effect, $P=0.032$), with no difference between groups ($P=0.609$). There was no change in fasting plasma glucose, insulin, cholesterol, triglycerides, HDL or LDL concentrations ($P>0.05$). Data are presented in Table 2.

Vascular Measures

There was a significant increase in FMD (main effect, $P<0.001$), with no difference between groups ($P=0.246$) or interaction. Post-hoc analysis revealed that FMD was unchanged following 4 weeks of training ($P=1.000$), but significantly increased following 12 weeks of training (Home-HIT 30%, Home-MICT 43%, Lab-HIT 49%; $P<0.001$). Baseline artery diameter was unchanged by training ($P=0.334$). There was no change in aortic PWV following 4 weeks of training ($P=1.000$), but a significant decrease following 12 weeks of training (Home-HIT -17%, Home-MICT -14%, Lab-HIT -4%; $P=0.04$), with no difference between groups ($P=0.417$) or interaction ($P=0.327$). There was no difference in any of the blood pressure variables ($P>0.05$).

Quantitative Immunofluorescence

Mean diameter of arterioles assessed for analysis of the vascular enzymes were 10.2 ± 0.8 μm which is consistent with the interpretation that only terminal or 5^{th}

order arterioles were analysed (Wu *et al.*, 2011). The mean number of arterioles analysed was 9 ± 2 at each time point per participant.

eNOS Content and Phosphorylation

Terminal arteriole eNOS content increased with training (Home-HIT = 20%; Home-MICT = 18%; Lab-HIT = 15%; main effect of training, $P<0.001$; Figure 4). There was also an increase in capillary eNOS content (Home-HIT = 21%; Home-MICT = 7%; Lab-HIT = 9%; main effect of training, $P=0.001$; Figure 4). Training did not change eNOS ser^{1177} phosphorylation in the terminal arterioles (training effect, $P=0.802$) or capillaries (training effect, $P=0.311$; Figure 4). When eNOS ser^{1177} phosphorylation was normalised to eNOS content (eNOS ser^{117}/eNOS ratio) on an individual vessel basis there were significant decreases with training in the arterioles and capillaries (main effect of training, $P=0.001$ and $P<0.001$, respectively; Figure 4). There were no between group differences for any of the variables.

NAD(P)Hox Subunits

Terminal arteriole NOX2 (catalytic subunit of NAD(P)Hoxidase) content was significantly reduced with training (Home-HIT = -22%; Home-MICT = -21%; Lab-HIT = -24%; main effect of training, $P<0.001$). Training also reduced skeletal muscle capillary NOX2 content (Home-HIT = -18%; Home-MICT = -14%; Lab-HIT = -24%; main effect of training, $P<0.001$; Figure 5). There was no change in $p47^{phox}$ (regulatory subunit of the NAD(P)Hox complex) content in the terminal arterioles ($P=0.101$) or capillaries ($P=0.345$) following training. Sarcolemma-associated NOX2 and $p47^{phox}$ content were unaltered by training (main effect of training, $P=0.897$ and

P=0.561, respectively). There were no between group differences in any of the variables (*P*>0.05).

Mitochondrial Density

COX IV protein expression (fluorescence intensity), a marker of mitochondrial density, was greater in type I fibres than type II fibres (main effect *P*<0.001; Figure 7). Mitochondrial density increased in both type I (Home-HIT 14%, Home-MICT 6%, Lab-HIT 22%) and type II fibres (Home-HIT 34%, Home-MICT 11%, Lab-HIT 33%) following training (main effect; *P*<0.001), with no differences between groups.

Muscle GLUT4 Content

Total muscle fibre GLUT4 content was increased by training (main effect, *P*=0.005), with no difference between groups (Figure 7). There was also a strong trend towards a significant training x fibre type interaction (*P*=0.061), and although not significant this trend was explored further. This analysis revealed training increased GLUT4 content in type II fibres (*P*=0.005), but not type I fibres (*P*=0.089). Post-training GLUT4 content was higher in type II fibres than type I fibres (*P*=0.02), while there was not a fibre type difference before training (*P*=0.983).

Intramuscular Triglyceride Content

IMTG content was significantly greater in type I fibres compared to type II fibres (*P*<0.001; Figure 7). IMTG content increased following training in both type I and II fibres (main effect, *P*<0.01), with no difference between groups. The increase in IMTG content was due to an increase in lipid droplet (LD) density (*P*=0.034) following

training. LD size did not change with training (*P*=1.000). Total and central IMTG content increased following 12 weeks of training (main effect of training, total *P*=0.006; central *P*=0.026). There was a non-significant trend towards an increase in peripheral IMTG content (*P*=0.06). The increase in IMTG content was due to an increase in central LD density (*P*=0.034) following training, and non-significant trends towards increased total (*P*=0.069) and peripheral (*P*=0.082) LD density. LD size was unchanged by training (*P*=1.000).

Capillarisation

Table 3 summarises the capillarisation results. Capillary density was increased by training (Home-HIT = 15%; Home-MICT = 33%; Lab-HIT = 16%; main effect of training *P*<0.001), with no differences between groups (*P*=0.850). Capillary-to-fibre ratio, capillary-fibre perimeter exchange index and capillary contacts were all higher in type I fibres than type II fibres irrespective of training status (main effect of fibre type, *P*<0.05). Capillary-to-fibre ratio on an individual fibre basis (*C/Fi*) increased with training (Home-HIT = 16%; Home-MICT = 25%; Lab-HIT = 10%; main effect of training, *P*<0.001), with no difference between groups (*P*=0.774). Capillary-fibre perimeter exchange increased with training (Home-HIT = 14%; Home-MICT = 19%; Lab-HIT = 5%; main effect of training, *P*<0.001), with no differences between groups (*P*=0.378). Capillary contacts increased with training (Home-HIT = 15%; Home-MICT = 33%; Lab-HIT = 16%; main effect of training, *P*<0.001), with no difference between groups (*P*=0.706). There was a trend towards an effect of fibre type on fibre cross-sectional area (P=0.077), but there was no effect of fibre type on fibre perimeter (*P*=0.242). Training had no effect on fibre cross-sectional area (*P*=0.190) or perimeter (*P*=0.394).

DISCUSSION

The most important and novel findings of the present study are that virtually-monitored Home-HIT and Home-MICT and low-volume Lab-HIT in obese individuals with elevated CVD risk: 1) increased skeletal muscle endothelial eNOS protein content in both terminal arterioles and capillaries, 2) reduced eNOS ser^{1177} phosphorylation when normalised to the increase in eNOS content, 3) decreased endothelial NOX2 protein content in skeletal muscle terminal arterioles and capillaries, and 4) increased skeletal muscle capillarisation. Importantly, these microvascular adaptations coincided with improvements in $\dot{V}O_{2peak}$ and whole-body insulin sensitivity. In addition, all three training modes caused significant improvements in brachial artery endothelial dependent dilation and aortic stiffness. Finally, muscle biopsy data revealed skeletal muscle adaptations typically observed following endurance training in all three groups. Despite the training sessions being completed at home without direct supervision, participants in both the Home-HIT and Home-MICT groups had high adherence at the prescribed exercise intensity, similar to fully supervised Lab-HIT, which provides initial support for home-based training interventions using virtual monitoring. This study suggests virtually monitored Home-HIT is an effective and practical training strategy capable of producing metabolic and functional adaptations in the skeletal muscle microvasculature in a direction consistent with substantial health benefits in obese individuals with elevated CVD risk. Therefore, Home-HIT may be an effective public health intervention for sedentary obese individuals, and future research should now investigate the applicability of these home-based training programmes in a larger cohort.

Skeletal Muscle Endothelial Enzymes Regulating NO Bioavailability

Here we demonstrate for the first time that low-volume Lab-HIT increased terminal arteriole and capillary eNOS expression. These findings are similar to previous work from our group demonstrating that lab-based sprint interval training increased skeletal muscle microvascular eNOS content in lean (Cocks *et al.*, 2013) and obese (Cocks *et al.*, 2016) individuals. However, the low-volume HIT protocol used in the current study was developed as a more suitable training method than sprint interval training for the obese population studied due to the lower workload (Gibala *et al.*, 2012). This study is also the first to demonstrate that the two "real world" home-based exercise programmes, performed with virtual supervision, produced similar increases in eNOS content as the highly controlled Lab-HIT protocol. The current study found comparable increases in eNOS content in the two HIT groups and the MICT group, confirming our observations in a previous study comparing sprint interval training and MICT in obese individuals (Cocks *et al.*, 2016). However, an earlier study in sedentary young lean men demonstrated that the increase in eNOS was significantly greater following six weeks of “all-out” sprint interval training than MICT (Cocks *et al.*, 2013). This may suggest differences in the training stimulus (30-second “all out” sprints vs. 1-minute submaximal exercise in the current study) and/or fitness differences between populations may influence eNOS expression in response to training.

There was no change in basal eNOS ser^{1177} phosphorylation in the microvascular endothelium following 12 weeks of training in all three groups. However, when normalised to eNOS content, eNOS ser^{1177} phosphorylation was reduced. This is different to previous studies investigating the effect of training on basal eNOS ser^{1177} phosphorylation. Our group has previously found that 6 weeks of

sprint interval training or MICT in sedentary lean individuals reduced eNOS ser^{1177} phosphorylation expressed by itself and when normalised to eNOS content (Cocks *et al.*, 2013). However, eNOS ser^{1177} phosphorylation was shown to increase and eNOS ser^{1177}/eNOS content ratio was unchanged in obese individuals following 4 weeks or sprint interval training or MICT (Cocks *et al.*, 2016). The data produced by these studies indicate that the response of eNOS ser^{1177} phosphorylation to training is affected by obesity and temporal differences. In combination, the studies suggest that in obesity eNOS ser^{1177} phosphorylation initially increases in response to training before reducing over time. This reduction in obese individuals may continue with training to eventually reduce eNOS ser^{1177} phosphorylation irrespective of eNOS content as observed in the lean individuals (Cocks *et al.*, 2013). The decrease in eNOS ser^{1177} phosphorylation following training has been attributed to a decrease in shear stress due to the increased capillary density (Cocks *et al.*, 2013), however Gliemann *et al.* (2014) suggested it may be a reflection of increased NO bioavailability as a result of less NO being scavenged by NOX2 and therefore less activation of eNOS is needed.

Expression of the catalytic subunit of the NAD(P)Hox complex NOX2 was reduced in terminal arterioles and capillaries following Home-HIT to a similar degree as Home-MICT and Lab-HIT. This adds to previous work that found 4 weeks of laboratory-based sprint interval training and MICT reduced mixed microvascular NOX2 content in obese individuals (Cocks *et al.*, 2016). Conversely, when investigating sedentary lean individuals, Cocks *et al.* (2013) found no change in mixed microvascular NOX2 protein content following sprint interval training or MICT, presumably because the lean individuals have a very low NOX2 protein content at baseline. There was no change in arteriole or capillary content of the regulatory of

the NAD(P)Hox complex $p47^{phox}$ following training. These findings agree with those of La Favor *et al.* (2016), who used Western blots on whole tissue homogenates to show that 8 weeks of aerobic interval training did not alter the expression of $p47^{phox}$ in obese individuals, despite elevated baseline levels. Together the current study and that of La Favor *et al.* (2016) demonstrate the importance of measuring multiple NAD(P)Hox subunits to gain full insight into the effect of training on $\cdot O_2^-$ production.

The increase in eNOS content and reduced NOX2 content following training indicates an altered balance between NO formation and quenching by $\cdot O_2^-$ anions and other ROS leading to increased skeletal muscle microvascular NO bioavailability. Previous work has shown that NO-mediated increases in skeletal muscle perfusion are essential for optimal glucose uptake (Vincent *et al.*, 2003, 2004) and that this mechanism is impaired in obesity, contributing to impaired glucose disposal in this population (Clerk *et al.*, 2006; Keske *et al.*, 2009). As such, the improved eNOS/NAD(P)Hox ratio observed in the current study likely contributed to the improved insulin sensitivity observed following training. The metabolic importance of elevated endothelial eNOS content was highlighted by Kubota *et al.* (2011) who observed that an increase in endothelial eNOS content, through administration of bera-prost sodium (a prostaglandin I2 analogue that stimulates eNOS mRNA expression and protein synthesis), increased skeletal muscle capillary perfusion and glucose uptake in IRS-2 knockout and high-fat fed mice. In addition, obesity is associated with elevated oxidative stress in skeletal muscle due to elevated NOX-mediated ROS production, which leads to microvascular endothelial dysfunction (Weseler & Bast, 2010; La Favor *et al.*, 2016). La Favor *et al.* (2016) found that 8 weeks of aerobic interval training in obese individuals decreased expression of NAD(P)Hox subunits which coincided with reduced ROS production

and reversed microvascular endothelial dysfunction. The observations from these previous studies combined with the results of the present study suggest that the increased eNOS/NAD(P)Hox ratio in obese individuals following exercise training will result in increased NO bioavailability upon insulin stimulation and a more metabolically healthy phenotype.

Capillarisation

This is the first study to demonstrate that two home-based exercise interventions performed with virtual supervision and no equipment improve capillary density, capillary contacts and capillary-fibre perimeter exchange index. The findings also extend the previous work of Tan *et al.* (2018), which demonstrated that 6 weeks of low-volume HIT increased capillary contacts in overweight/obese women, while we show that 12 weeks of low-volume HIT increased capillary density and capillary-fibre perimeter exchange index. The similar increases in capillarisation with Home-HIT and supervised Lab-HIT suggest Home-HIT is an effective strategy to increase capillarisation while simultaneously reducing the major barriers to exercise. The findings also provide support for previous shorter duration sprint interval training (Cocks *et al.*, 2013, 2016) and HIT studies (Tan *et al.*, 2018), which show no difference in fibre type specific angiogenesis in response to interval training, which is in contrast to previous work in rats showing fibre type difference in response to interval training and MICT (Gute *et al.*, 1994).

The increase in skeletal muscle capillarisation, as shown here, is an established adaptation to exercise training that is likely to be a key contributing factor to improved $\dot{V}O_{2peak}$ (Andersen & Henriksson, 1977; Saltin, 1988; Bassett & Howley,

2000; Hellsten & Nyberg, 2015), due to prolonged mean erythrocyte transit time and decreased diffusion distance to allow increased delivery and extraction of oxygen. Increased capillarisation is also likely to contribute to the improved insulin sensitivity observed, which would improve glucose tolerance and delay progression to type 2 diabetes in obese individuals with elevated metabolic disease risk. This is supported by Akerstrom *et al.* (2014), who directly investigated the effects of capillarisation on insulin sensitivity by treating sedentary rats with Prazosin (an α_1-adrenergic receptor antagonist). The ~20% increase in capillary density following 3 weeks of Prazosin treatment resulted in a ~30% increase in insulin-stimulated skeletal muscle glucose disposal, despite no change in skeletal muscle insulin signalling. This suggests that increased capillarisation with exercise training has a direct effect on insulin sensitivity, independent of other metabolic adaptations.

Vascular Measures

FMD increased by 2% after 12 weeks of Home-HIT. Brachial artery FMD is an independent predictor of CVD (Gokce *et al.*, 2002; Green *et al.*, 2011) and is a surrogate of coronary artery endothelial function (Anderson *et al.*, 1995). Indeed, there is a 9% decrease in risk of cardiovascular events with each 1% increase in FMD (Green *et al.*, 2011). Improved FMD has been suggested to be the result of elevated nitric oxide (NO) bioavailability following training (McAllister & Laughlin, 2006).

This is the first study to investigate the time course of brachial artery FMD in response to MICT and low volume HIT in obese individuals with elevated CVD risk. Sawyer *et al.* (2016) investigated the effect of low-volume HIT (10x1 min intervals on

a cycle ergometer at 90-95% HR_{max}) and MICT (30 min at 70-75% HR_{max} on a cycle ergometer) on brachial artery FMD after 4 and 8 weeks of training in obese adults. Similar to the current study no change in brachial artery FMD was observed after 4 weeks in either group. However, unlike the current study differences in the response to MICT and low-volume HIT were observed after 8 weeks of training, with FMD being unchanged following MICT and increased following low-volume HIT. In addition, differences in baseline artery diameter were observed, with MICT inducing significant increases following 8 weeks of training, and no difference following 8 weeks of low-volume HIT. The reason for the differences between studies is unclear, but it may be due to participants (obese vs. obese with at least 2 further CVD risk factors) or duration of training (8 vs. 12 weeks). Importantly, studies in healthy young volunteers have demonstrated that endothelial function is increased following 2-4 weeks of training, but that function is normalised after prolonged training (>6-weeks) due to structural adaptation i.e. increased brachial artery diameter (Green *et al.*, 2017). The current results and those of Sawyer *et al.* (2016) differ from this paradigm as endothelial function was increased only after 8 or 12-weeks, and there was no suggestion of arterial remodelling in the current study. These data are in line with previous work investigating the time-course of arterial adaptations in individuals with chronic heart failure and coronary artery disease (Maiorana *et al.*, 2000; Walsh *et al.*, 2003). This may be due to the impact of oxidative stress or inflammation on NO bioavailability, which are known to be elevated in obese individuals with increased CVD risk (Silver *et al.*, 2007; La Favor *et al.*, 2016).

This is the first investigation to study the effects of exercise training on aortic PWV over 12 weeks in obese individuals with elevated CVD risk. The results mirror the changes in FMD with no change in aortic PWV following 4 weeks, but a

significant improvement after 12 weeks. Obesity results in increased central artery stiffness even in young individuals, with subsequent negative cardiovascular outcomes (Zebekakis *et al.*, 2005). Therefore, the improved PWV may be related to improved cardiovascular risk.

Myocyte Adaptations

The myocyte adaptations investigated were selected because they are classical markers of training adaptation associated with improved health (Hawley & Lessard, 2008). Previous research has shown that HIT increases GLUT4 content (Bradley *et al.*, 2014), IMTG content (Shepherd *et al.*, 2013, 2017) and mitochondrial density (Shepherd *et al.*, 2017; Tan *et al.*, 2018). The increase in mitochondrial content likely underpins the improved $\dot{V}O_{2peak}$ as mitochondrial biogenesis is a major training adaptation that increases lipid and glucose fuel handling. High IMTG content is associated with insulin resistance in sedentary individuals, as excess IMTG content in obesity leads to accumulation of lipid metabolites such as ceramides, LCFA-CoA and diacylglycerol that can impair insulin signalling via serine phosphorylation at the insulin receptor 1, eventually leading to insulin resistance (Shulman, 2014). However, it is important to note that athletes combine high IMTG content with high insulin sensitivity (Goodpaster *et al.*, 2001), due to their greater capacity to oxidise IMTG. Here, training increased total IMTG content in all three groups, which was driven by an increase in central LD density (larger number of small LDs). This training adaptation is in line with the observations of Shepherd *et al.* (2017) showing that 4 weeks of SIT and MICT reduced ceramide concentrations and increased the number of lipid droplets in contact with the mitochondria in obese males. The present study provides further evidence that an increase in central IMTG content is an

important training adaptation that means the muscle is more efficient at using fatty acids released by the IMTG pool for oxidation. The increase in mitochondrial density alongside increased LD density will lead to greater IMTG utilisation during exercise and has been suggested to improve insulin sensitivity (Shepherd *et al.*, 2013).

GLUT4 is the primary insulin-responsive glucose transporter in skeletal muscle, and experimental increases in skeletal muscle GLUT4 in animal models have been shown to increase whole-body insulin sensitivity (Ren *et al.*, 1995; Hansen *et al.*, 1995; Tsao *et al.*, 1996). As such, the increase in GLUT4 expression likely contributed to the increased insulin sensitivity.

An Effective 'Virtually Monitored' Training Programme

A number of groups have shown HIT to be effective at improving a range of cardio-metabolic health parameters (Hood *et al.*, 2011; Little *et al.*, 2011; Cocks *et al.*, 2013, 2016). However, most of these studies are highly controlled laboratory-based interventions (Weston *et al.*, 2014), or field-based work with high levels of participant supervision (Ong *et al.*, 2009; Lunt *et al.*, 2014; Shepherd *et al.*, 2015). Therefore, health researchers have argued that although effective under optimal conditions, HIT cannot become an effective public health intervention when targeted at exercise-naïve populations. In addition, many of the current protocols require exercise equipment (ergometers or treadmills), introducing additional barriers to exercise such as difficulties with access to equipment or facilities (including distance and cost) and potential embarrassment due to negative body image within a gym setting (Trost *et al.*, 2002).

Despite previous concerns (Frazao *et al.*, 2016), our data suggest obese participants with elevated CVD risk were able to adhere to the Home-HIT

programme, and exercise at sufficiently high exercise intensities to elicit health benefits without close supervision. The training intervention in the current study was designed for individuals with low fitness and mobility, and to remove the need for costly equipment or facilities (Trost *et al.*, 2002; Morgan *et al.*, 2016). To achieve this, participants were provided with a number of exercises which ranged from simple low-impact exercises to complex movements with higher impact. This allowed participants to modify exercise sessions, choosing exercises that elicited the desired HR response, but were suitable for their level of mobility and fitness. In addition, recent studies have suggested that sedentary individuals report pleasant feelings during the first 3-4 bouts of low-volume HIT (Frazao *et al.*, 2016), but importantly enjoyment of HIT increases as participants progress through a training programme (Heisz *et al.*, 2016). Therefore, unlike previous low-volume HIT studies (Little *et al.*, 2011; Tan *et al.*, 2018), the current intervention started with a low number of intervals and progressed training volume throughout (4 intervals during week 1-2 to 8 intervals during week 11-12).

Interestingly, we observed high adherence levels in both home-based interventions (MICT and HIT), which was higher than two studies investigating supervised gym-based and outdoor MICT and HIT programmes (Lunt *et al.*, 2014; Shepherd *et al.*, 2015). Whilst home-based exercise programmes have a number of benefits over supervised programmes, lack of support and supervision from exercise professionals presents a significant barrier. Indeed, a recent meta-analysis suggested that supervision from exercise professionals was needed to build and maintain motivation to exercise (Morgan *et al.*, 2016). As such, the novel virtual-monitoring system may have positively influenced the findings of this study, contributing to the high adherence observed. Previous work in sedentary individuals

has shown that immediate feedback illustrating HR can positivity influence participant motivation (Kinnafick *et al.*, 2018). Allowing the research team to provide feedback to participants throughout the intervention also likely improved motivation by creating a supportive environment (Petit & Cambon, 2016). In addition, knowing the research team were monitoring exercise may have increased motivation to adhere to the programme. Such extrinsic motivation can facilitate adoption to exercise and result in adaptive outcomes when accompanied by more autonomous motivation (e.g., facilitated through self-monitoring) (Thøgersen-Ntoumani & Ntoumanis, 2006). As such, future studies should investigate the potential of novel monitoring systems to increase adherence to exercise interventions.

Limitations

The study was powered to detect a medium effect size between groups for skeletal muscle capillarisation and microvascular eNOS content, and as a result the sample size was not high enough to detect between-group differences in other variables displaying a larger variability (e.g. increases in $\dot{V}O_{2peak}$ and whole-body insulin sensitivity). Using the data generated in this study a power calculation suggests 69 participants per group would be required to detect a between group difference in $\dot{V}O_{2peak}$ of 1.5 $ml \cdot kg^{-1} \cdot min^{-1}$ between Home-HIT and Home-MICT. Therefore, based on this promising data, future trials should investigate virtually-monitored Home-HIT in larger cohorts to investigate its true effectiveness compared to traditional training interventions. We decided not to include an untrained control group in this study. Although this would have strengthened the design, it would have reduced the feasibility of completing the study due to costs, difficulty with recruitment and the invasive nature of some of the measures. It is also important to acknowledge the

non-exclusion of females using hormonal contraceptives and that not all females were measured in days 1-7 of their menstrual cycle. Self-allocation of participants to the training groups may be deemed as a limitation as this does not conform to the traditional randomisation approach. However, it is known that offering patients choice and actively involving them in decision making about treatment strategies can improve health outcomes (Redfern *et al.*, 2009). As such, self-allocation of exercise training should be encouraged and was used to improve the real world translation of the findings. Importantly, there were no between group differences before training started, suggesting the groups included similar individuals, based on the health measures included. However, future large scale studies should investigate if there are differences in exercise choice between individuals based on health status. In addition, future work should investigate the specific demographics of the groups, for example, what is the previous exercise experience of participants, what is the participants' knowledge of the training programmes on offer, to see if these variables influence choice of training mode and training adherence and compliance.

Conclusions

This study provides novel evidence that 12 weeks of virtually-monitored Home-HIT, and Home-MICT and Lab-HIT in obese individuals with elevated CVD risk leads to similar skeletal muscle microvascular adaptations that likely underpin the functional improvements in insulin sensitivity and $\dot{V}O_{2peak}$. All three interventions induced similar improvements in endothelial enzyme balance as indicted by increased eNOS protein content, reduced eNOS ser^{1177}/eNOS ratio and reduced expression of the catalytic subunit of the NAD(P)Hox subunit of NOX2. All three training modes also induced similar improvements in capillarisation, mitochondrial density, IMTG and

GLUT4 content which occurred alongside increased $\dot{V}O_{2peak}$ and whole-body insulin sensitivity. The virtually-monitored Home-HIT intervention used in this study was time-efficient and reduced many of the other traditional barriers to exercise. Therefore, this study is an important first step that suggests Home-HIT may be an effective strategy to improve cardio-metabolic health by increasing physical activity participation in the obese population most in need. Future research should investigate how home-based exercise removes barriers to exercise and its applicability to larger cohorts.

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Additional Information

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Author contributions – SNS, SOS, JAS, PK, AJMW & MC conception or design of the work; SNS, SOS, NH, EAD, JAS, DJW, RGC, PK, AJMW & MC acquisition, analysis or interpretation of data for the work. SNS, SOS, NH, EAD, JAS, DJW, RGC, PK, AJMW & MC drafting the work or revising it critically for important intellectual content. All authors approved the final version of the manuscript. All authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and

resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

Authors' Translational Perspective

Laboratory-based high-intensity interval training (HIT) is a time-efficient alternative to moderate-intensity continuous training (MICT). However, the currently open question is whether data produced in highly controlled laboratory environments can be translated to the real world. Therefore, this study tested the hypothesis that a novel, virtually-supervised home-based HIT (Home-HIT) intervention could be used for prevention and management of cardio-metabolic diseases. We investigated whether HOME-HIT would have similar values for adherence and compliance and for recognised metabolic and functional outcome measures as home-based moderate-intensity continuous training (MICT) and laboratory-based HIT in obese individuals with elevated cardiovascular disease risk. This is the first study to show that home-based exercise modes (either HIT or MICT), which do not require any equipment or direct supervision, can induce favourable adaptations in skeletal muscle and its microvasculature that likely underpin the observed functional improvements observed ($\dot{V}O_{2peak}$, insulin sensitivity, brachial artery endothelial function (FMD) and aortic stiffness). This combination of metabolic/structural measurements with whole-body functional measures has not previously been made in real-life home-based training modes. The virtually-monitored Home-HIT intervention was time-efficient and reduced many of the traditional barriers to exercise including travel time and gym membership costs. Therefore, these data may underpin future studies aiming to improve exercise adherence using home-based exercise, resulting in increased acceptance of exercise as a therapy for the prevention and management of disease. Future research should investigate how home-based exercise removes barriers to exercise and its applicability to larger cohorts.

Table 1. Participant characteristics and overview of the number of patients that met American Heart Association coronary heart disease risk factor thresholds

	Home-HIT	Home-MICT	Lab-HIT
Age (yrs)	32 ± 8	38 ± 9	37 ± 13
Sex (male/female)	4/5	4/9	5/5
Height (cm)	168 ± 12	172 ± 8	172 ± 8
BMI ($kg \cdot min^{-2}$)	35.9 ± 4.1	33.3 ± 5.2	34.2 ± 4.2
$\dot{V}O_{2peak}$ ($ml \cdot kg^{-1} \cdot min^{-1}$)	23.8 ± 2.5	24.9 ± 6.8	24.8 ± 6.4
Medication	1/9	8/13	4/10
Smoker/previous smoker	1/9	3/13	5/10
Family history	5/9	6/13	4/10
Obesity	9/9	13/13	10/10
Sedentary lifestyle	9/9	13/13	10/10
Impaired fasting glucose	1/9	1/13	1/10
Dyslipidaemia	9/9	12/13	7/10
Hypertension	4/9	7/13	1/10
Mean number of risk factors per participant	4 ± 1	4 ± 2	4 ± 2
Range of risk factors	3-5	3-6	3-6

Medication included blood pressure medication (e.g. ramipril, felodipine, losartan, amilodipine, indipamide), metformin or statins. Family history included diabetes and/or cardiovascular disease in an immediate family member. Obesity was classified as a BMI >30 $kg \cdot m^2$ or waist/hip ratio of >0.9 in men and >0.85 in women. Dyslipidaemia was defined as total cholesterol >11.1 $mmol.L^{-1}$, HDL <2.2 $mmol.L^{-1}$ or LDL >7.2 $mmol.L^{-1}$. Hypertension was classified as >140/90 mmHg or on antihypertensive medication and impaired fasting glucose was defined as fasting blood glucose >6.1 $mmol.L^{-1}$. Sedentary lifestyle was defined as persons not participating in a regular exercise programme or accumulating 30 minutes or more of moderate physical activity on most days of the week. Data are presented as mean±SD when appropriate.

Table 2. Participant characteristics at pre, week 4 and post training

	Home-HIT			Home-MICT			Lab-HIT		
	Pre	Week 4	Post	Pre	Week 4	Post	Pre	Week 4	Post
Body mass (kg)	101.5±21.6	100.2±22.0*	100.1±22.4*	98.3±16.0	96.7±16.1*	95.5±16.4*	101.1±15	99.6±14*	99.2±15*
BMI ($m \cdot kg^2$)	35.9±4.1	35.4±4.3*	35.4±4.6*	33.3±5.2	32.8±5.1*	32.3±5.1*	34.2±4.2	33.7±3.5*	33.6±3.5*
Body fat (%)	40.1±5.8	-	38.4±5.6*	35.8±8.4	-	34.4±8.7*	38.1±7.6	-	36.6±7.9*
Visceral fat (g)	523.1±198.9	-	412.0±183.2*	645.4±250.9	-	557.2±192.1*	626.7±243.3	-	611.0±216.3*
Lean mass (kg)	56.7±14.3	-	58.1±14.9	57.9±9.5	-	57.5±10.6	58.1±12.5	-	58.8±12.5
$\dot{V}O_{2peak}$ ($ml \cdot kg^{-1} \cdot min^{-1}$)	23.8±2.5	25.9±2.5*	27.6±4.7*#	24.9±6.8	26.4±6.6*	28.0±8.1*#	24.8±6.4	26.9±7.0*	29.8±8.2*#
$\dot{V}O_{2peak}$(L/min^{-1})	2.4 ± 0.6	2.6 ± 0.6*	2.8 ± 0.8*#	2.5±0.8	2.5±0.8*	2.7±1.0*#	2.5±0.8	2.7±0.9*	3.0±1.0*#
W_{max} (W)	180±34	202±35*	213±34*#	182±53	188±55*	208±60*#	174±43	198±54*	221±49*#
W/H ratio	0.93±0.13	0.92±0.13	0.92±0.14	0.92±0.11	0.92±0.09	0.92±0.08	0.94±0.09	0.92±0.08	0.91±0.11
ISI Matsuda	2.8±2.2	-	3.9±3.6*	2.8±1.6	-	3.4±1.5*	2.2±1.0	-	2.5±0.9*
Glucose AUC ($mmol.L^{-1}.120min^{-1}$)	15551±1562	-	15155±2744	13979±7098	-	14868±5313	18401±4273	-	17840±3970
Insulin AUC ($mmol.L^{-1}.120min^{-1}$)	13740±7750	-	10442±5518*	12556±7341	-	10043±5684*#	11914±3964	-	9723±2777*

Fasting glucose ($mmol.L^{-1}$)	5.4±0.7	-	5.0±0.8	5.2±0.7	-	5.5±1.0	5.2±0.6	-	5.6±0.7
Cholesterol ($mmol.L^{-1}$)	4.2±0.8	-	4.1±1.0	4.4±0.9	-	4.5±0.9	5.3±1.3	-	5.3±1.1
Triglycerides ($mmol.L^{-1}$)	1.0±0.3	-	1.0±0.6	1.1±0.6	-	1.3±0.7	1.6±1.0	-	1.4±0.5
HDL ($mmol.L^{-1}$)	0.8±0.2	-	0.9±0.3	1.0±0.2	-	1.0±0.2	1.1±0.2	-	1.1±0.2
LDL ($mmol.L^{-1}$)	3.7±0.8	-	3.7±1.0	3.7±1.3	-	3.8±1.1	4.4±1.5	-	4.4±1.2
MAP (mmHg)	86±10	82±9	83±9	91±12	90±11	90±11	86±6	87±4	85±7
SBP (mmHg)	119±12	115±13	115±12	127±17	124±15	125±14	122±8	123±6	121±9
DBP (mmHg)	70±10	66±8	66±10	73±11	73±9	72±10	68±6	69±4	67±8
Calorie intake (kcal)	1838±479	-	2216±439	1952±565	-	2043±402	1849±332	-	1696±473
Energy expenditure (kcal)	447±164	-	499±137	471±184	-	538±279	304±165	-	339±220

Values are means ± SD. #Denotes a significant difference with training from baseline and *indicates a difference between week 4 and 12 (P<0.05). At baseline there were no differences in age, BMI or $\dot{V}O_{2peak}$ between groups (P>0.05). Matsuda Index values are reported for 9 Home-HIT, 10 Home-MICT and 9 Lab-HIT participants as it was not possible to obtain blood from all participants.

Table 3. Capillarisation pre and post training

	Home-HIT		Home-MICT		Lab-HIT	
Variable	Pre	Post	Pre	Post	Pre	Post

Overall FA (mm^2)	3732±1294	4758±3482	3054±966	3210±968	3912±2025	4569±3411
Type I FA (mm^2)	4041±1449	5658±5840	3397±809	3180±752	4061±2154	4698±3646
Type II FA (mm^2)	3550±1317	4277±2488	2852±1150	3192±1226	3901±2134	4509±3378
Overall perimeter (mm^2)	292.2±81.4	307.5±93.6	253.0±56.0	263.7±63.7	324.7±104.9	333.2±118.0
Type I perimeter (mm^2)	300.9±87.2	318.0±120.8	261.4±46.4	267.7±73.9	329.3±103.1	327.1±106.1
Type II perimeter (mm^2)	287.2±81.7	301.3±89.2	249.1±66.3	259.9±67.4	323.1±109.0	334.4±122.7
Overall CC*	3.97±0.61	4.56±0.86	3.52±1.17	4.68±0.45	3.93±0.83	4.56±0.94
Type I CC*	4.33±0.99	4.84±1.13	3.78±1.20	4.99±0.56	4.35±0.60	4.76±1.00
Type II CC*	3.70±0.57	4.27±0.69	3.38±1.18	4.48±0.70	3.69±1.03	4.44±0.96
Overall *C/FI**	1.54±0.32	1.79±0.35	1.41±0.37	1.77±0.26	1.66±0.42	1.83±0.48
Type I *C/FI**	1.63±0.43	1.86±0.41	1.57±0.37	1.89±0.34	1.85±0.42	1.92±0.49
Type II *C/FI**	1.47±0.35	1.70±0.37	1.30±0.32	1.69±0.35	1.54±0.42	1.76±0.49
Overall CFPE*	5.60±1.11	6.38±1.91	5.79±1.50	6.90±1.11	5.63±1.91	5.92±1.24
Type I CFPE*	5.80±1.37	6.60±2.23	6.20±1.63	7.25±1.36	6.39±2.29	6.42±1.82
Type II CFPE*	5.38±0.94	6.10±1.69	5.52±1.42	6.68±1.09	5.10±1.55	5.65±0.92

CD (caps mm^{-2})*	682.6±183.5	812.7±226.8	806.4±223.2	955.7±192	675.8±238.2	836.6±144.4

Values are mean ± SD. *Indicates $P<0.05$, main effect of training. FA = fibre cross-sectional area, CD = capillary density, CC = capillary contacts, C/F_I = capillary-to-fibre ratio on an individual fibre basis, CFPE = capillary-fibre perimeter exchange.

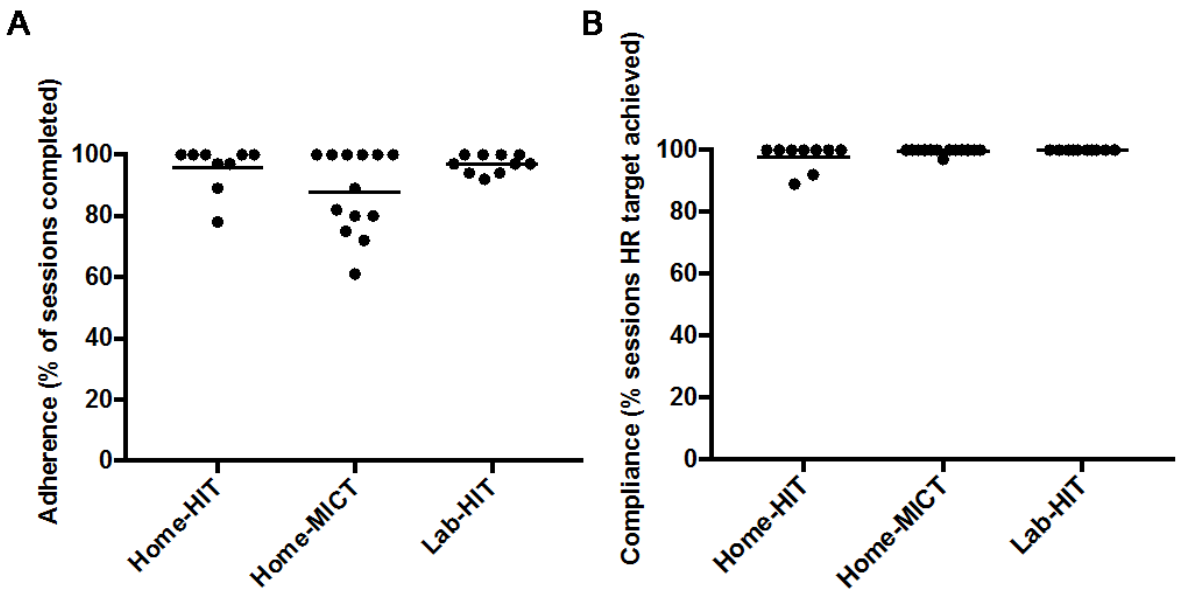


Figure 1. Adherence and compliance to home-based high intensity interval training (Home-HIT), home-based moderate intensity continuous training (Home-MICT) and lab-based HIT (Lab-HIT).

A) Adherence is defined as the percentage of scheduled training sessions completed. B) Compliance, defined as the percentage of training sessions where target HR threshold was met (80% HR_{max} in the HIT groups and 65% in Home-MICT).

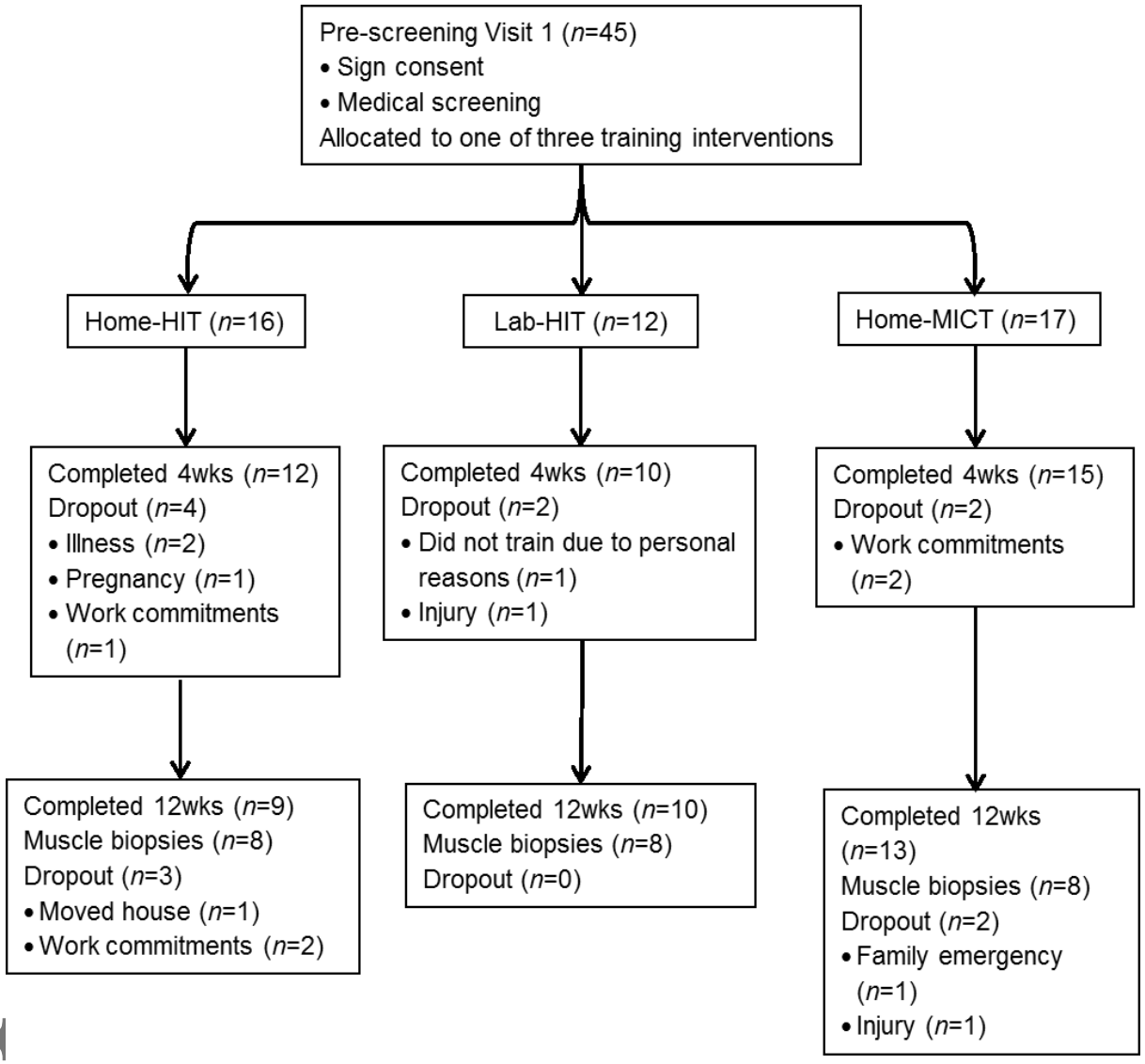


Figure 2. Flow chart of study design

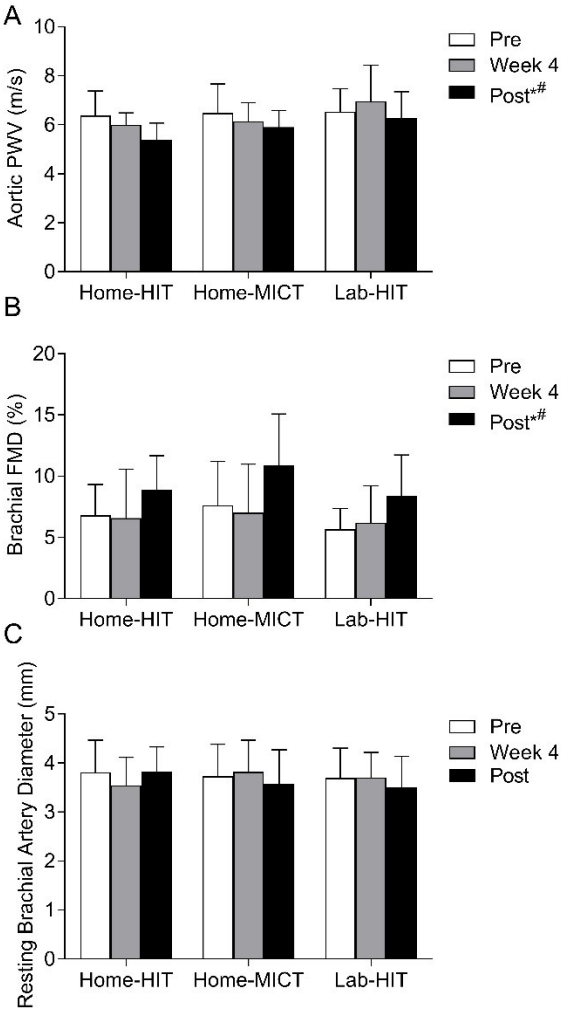
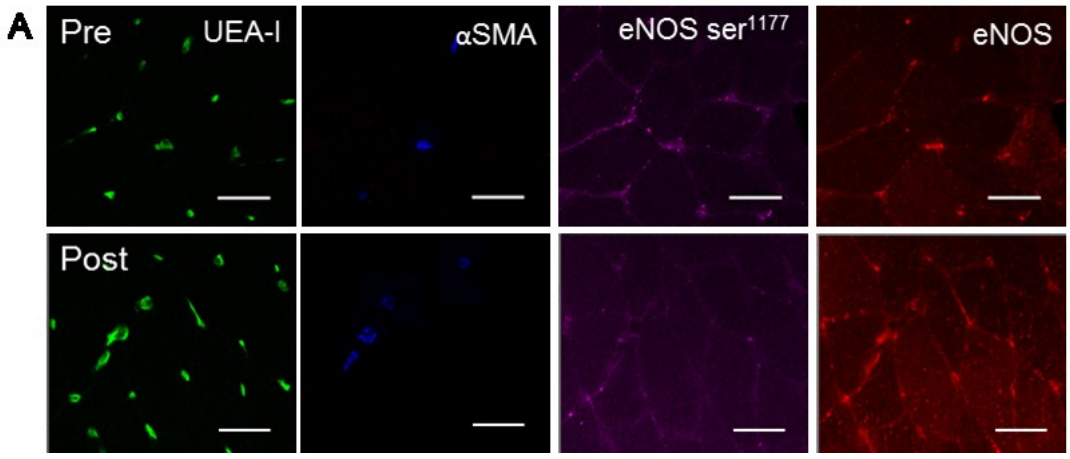


Figure 3. Effect of Home-based high intensity interval training (Home-HIT), home-based moderate intensity continuous training (Home-MICT) and lab-based HIT (Lab-HIT) on aortic pulse wave velocity (PWV; A), brachial artery flow mediated dilation (FMD; B) and resting brachial artery diameter (C).

*indicates a significant difference from baseline (P<0.05) and #indicates a significant difference from week 4 (P<0.05). There were no significant differences in any of the variables between the groups. Data are presented as mean ± SD. Aortic PWV was recorded in 8 Home-HIT, 6 Home-MICT and 9 Lab-HIT participants due to difficulty scanning some participants. Original data is presented for FMD as the same findings were reported when baseline diameter was analysed using allometric scaling (Atkinson & Batterham, 2013).



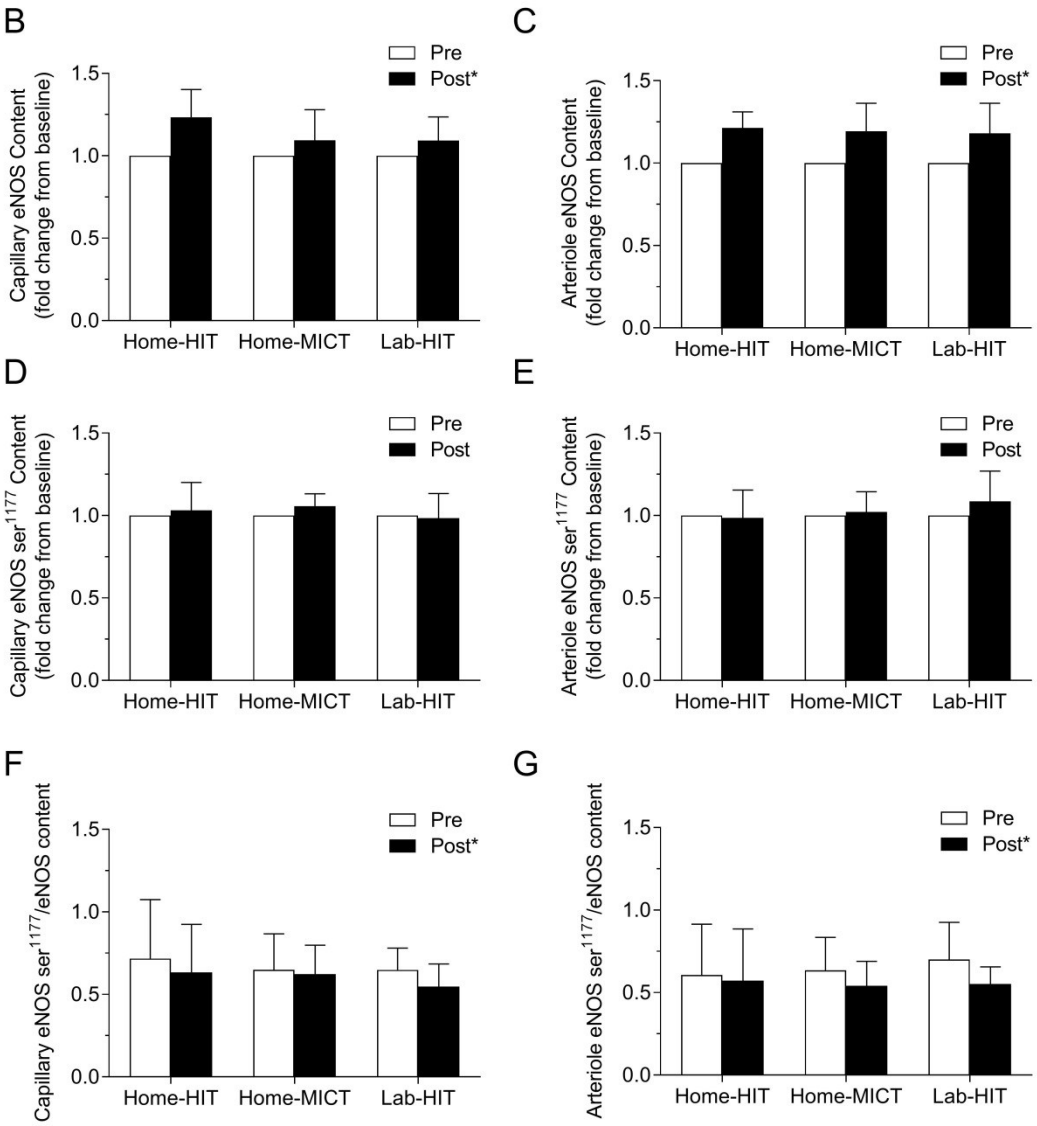


Figure 4. Effect of 12 weeks of home-based high-intensity interval training (Home-HIT), home-based moderate-intensity continuous training (Home-MICT) and lab-based HIT (Lab-HIT) on eNOS content and eNOS ser^{1177} phosphorylation in capillaries and terminal arterioles

(A) Representative confocal microscopy images of skeletal muscle from pre (top) and post (bottom). The skeletal muscle microvascular endothelium was revealed using *Ulex* europaeus-FITC conjugated lectin (green). Arterioles and capillaries were differentiated using anti-α-smooth muscle actin (αSMA) in combination with Alexa Fluor 405 conjugated secondary antibody (blue). Skeletal muscle eNOS ser^{1177} phosphorylation was revealed using Alexa Fluor 633 conjugated secondary antibody (purple). Skeletal muscle eNOS expression was revealed using Alexa Fluor 546 conjugated secondary antibody (red). (B) and (C) show mean fold change in eNOS content in capillaries and arterioles with training; (D) and (E) show mean fold change in eNOS ser^{1177} phosphorylation in capillaries and arterioles with training and (F) and (G) show change in eNOS/PeNOS ser^{1177} ratio with training. *Indicates a significant main effect of training (P<0.05). White bar = 50 μm.

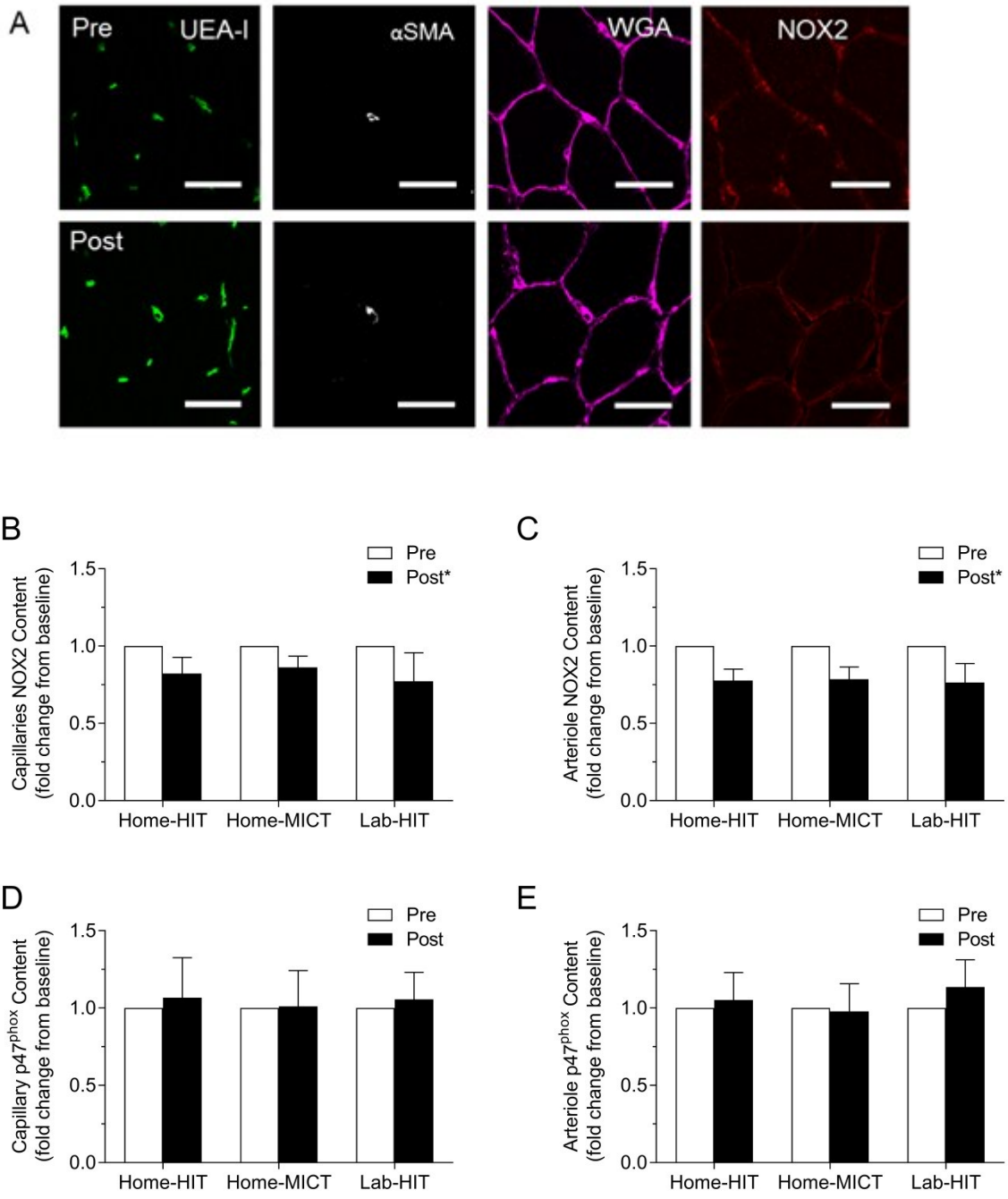


Figure 5. Effect of 12 weeks of home-based high intensity interval training (Home-HIT), home-based moderate intensity continuous training (Home-MICT) and lab-based HIT (Lab-HIT) on NOX2 and p47^{phox} content.

(A) Representative confocal microscopy images of skeletal muscle from pre (top) and post (bottom) training on NOX2 content. The skeletal muscle microvascular endothelium was revealed using *Ulex* europaeus-FITC conjugated lectin (green). Arterioles and capillaries were differentiated using anti-α-smooth muscle actin (αSMA) in combination with Alexa Fluor 405 conjugated secondary antibody (greyscale). Wheat germ

agglutinin-633 (WGA-633; Invitrogen, Paisley, UK) was used as a plasma marker membrane (pink). Skeletal muscle NOX2 expression was revealed using Alexa Fluor 546 conjugated secondary antibody (red). White bar = 50 μm. (B) and (C) show mean fold change in NOX2 content in capillaries and arterioles with training; (D) and (E) show mean fold change in $p47^{phox}$ expression in capillaries and arterioles with training. *Indicates a significant main effect of training (P<0.05). White bar = 50 μm.

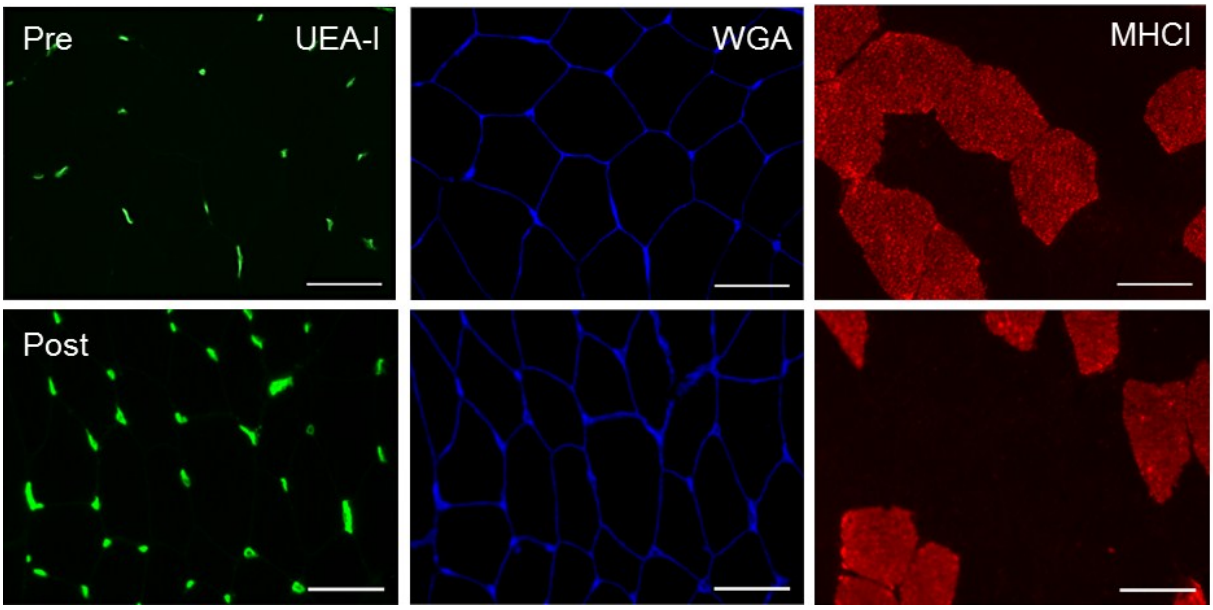


Figure 6. Effect of 12 weeks of home-based high intensity interval training (Home-HIT), home-based moderate intensity continuous training (Home-MICT) and lab-based HIT (Lab-HIT) on skeletal muscle capillarisation

Representative widefield microscopy images of skeletal muscle pre (top) and post (bottom) training. Capillarisation was revealed using *Ulex* europaeus-FITC conjugated lectin (UEA-I, green). The skeletal muscle membrane was revealed using wheat germ agglutinin-633 (WGA, blue). Fibre type was revealed using anti-myosin I (MHC-I, red). White bar = 50 μm.

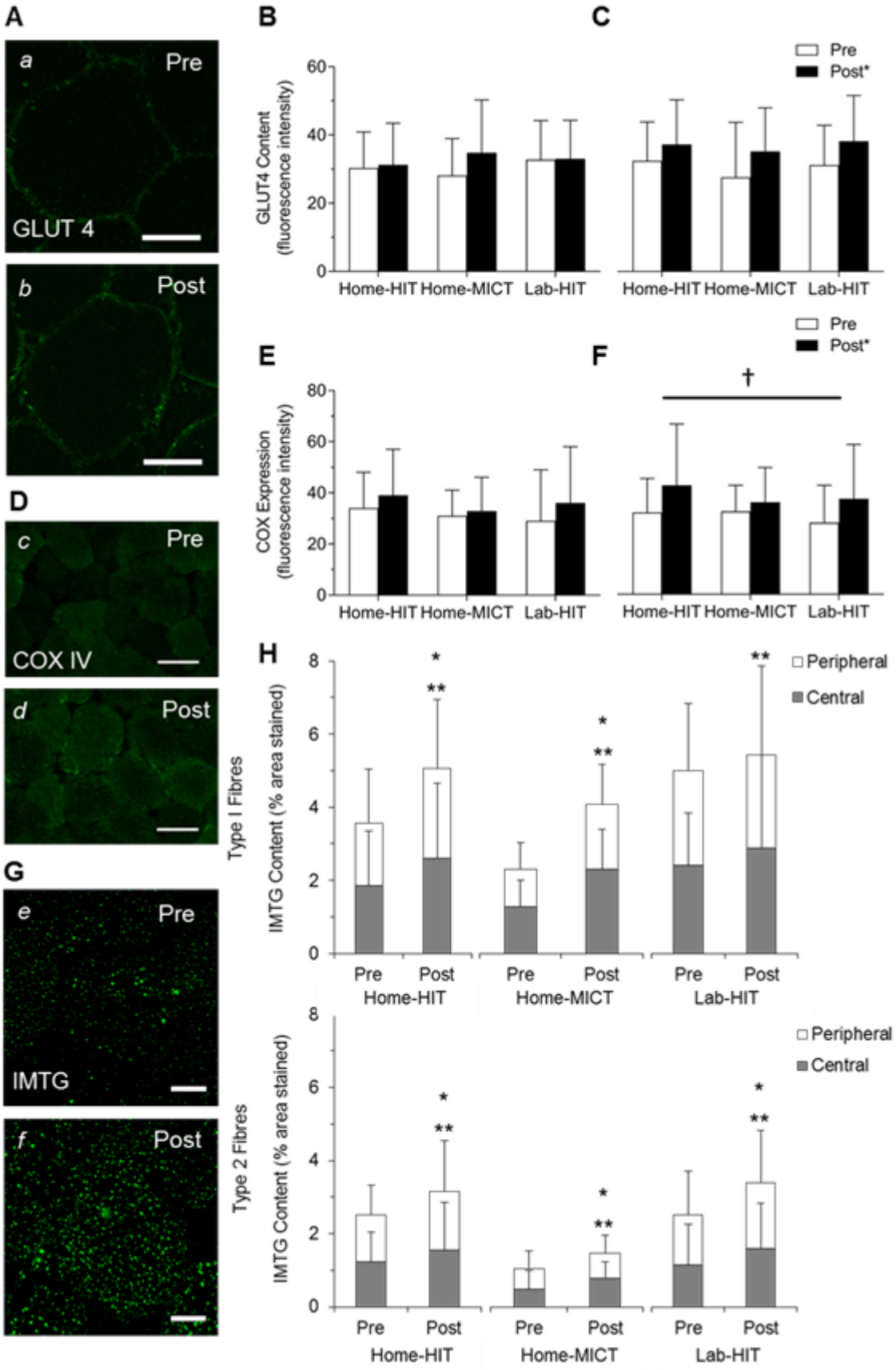


Figure 7. Effect of 12 weeks of home-based high intensity interval training (Home-HIT), home-based moderate intensity continuous training (Home-MICT) and lab-based HIT (Lab-HIT) on fibre type specific GLUT4 content, mitochondria density indicated by COX IV fluorescence intensity and intramuscular triglyceride (IMTG) content.

(A) Representative confocal microscope images of skeletal muscle GLUT4 fluorescence intensity pre (*a*) and post-training (*b*); white bar = 50 μm. Change in GLUT4 pre-post training is shown in type I fibres (B) and type II fibres (C). (D) Representative widefield microscopy images of COX IV fluorescence intensity pre (*c*) and post-training (*d*). Change in COX expression pre-post training is shown in type I fibres (E) and type II fibres (F). *Indicates main effect of training (P<0.05); white bar = 50 μm. †Indicates a main effect of fibre type (P<0.05). (G) Representative confocal microscope images of IMTG pre (*e*) and post (*f*) training. Analysis was performed in both the peripheral (5μm border from the plasma membrane) and central (remainder of the cell) regions of each fibre. White bar = 20 μm. (H) Shows change in IMTG content pre and post training in type I and type II fibres in the central and peripheral region of the cells. *Indicates main effect of training in total IMTG content; **indicates a change in central IMTG content (P<0.05). GLUT4 protein expression, mitochondrial density and IMTG content were analysed using a three-way mixed ANOVA, with the between-group factor being 'training group' and within-group factors 'training status' (pre vs. post) and 'fibre type' (type I vs. type II). Eight muscle biopsies were taken and analysed pre- and post-training in each group.