

# **Effect of resistance training on microvascular density and eNOS content in skeletal muscle of sedentary men**

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**Running title:** Vascular adaptations to resistance training**Abstract**

The effects of resistance training (RT) on muscle mass, strength and insulin sensitivity are well established, but the underlying mechanisms are only partially understood. The main aim of this study is to investigate whether RT induces changes in endothelial enzymes of the muscle microvasculature, which would increase NO bioavailability and could contribute to improved insulin sensitivity. Eight previously sedentary males (age  $20 \pm 0.4$  y, BMI  $24.5 \pm 0.9$  kg.m<sup>-2</sup>) completed 6wk of RT 3x/week. Muscle biopsies were taken from the *m. vastus lateralis* and microvascular density and endothelial specific eNOS content, eNOS Ser<sup>1177</sup> phosphorylation and NOX2 content were assessed pre- and post-RT using quantitative immunofluorescence microscopy. Whole body insulin sensitivity (measured as Matsuda Index), microvascular filtration capacity (functional measure of the total available endothelial surface area) and arterial stiffness (augmentation index, central and peripheral pulse wave velocity) were also measured. Measures of microvascular density, microvascular filtration capacity, microvascular eNOS content, basal eNOS phosphorylation and endothelial NOX2 content did not change from pre-RT to post-RT. RT increased insulin sensitivity ( $P < 0.05$ ) and reduced resting blood pressure and augmentation index ( $P < 0.05$ ), but did not change central or peripheral pulse wave velocity. In conclusion RT did not change any measure of muscle microvascular structure or function.

**Key words**

Insulin sensitivity, muscle microvascular endothelial function, nitric oxide, NAD(P)H oxidase

## Abbreviations

AIx	Augmentation index
AIx@75bpm	Augmentation index normalised to 75 beats per minute
AUC	Area under the curve
CC	Capillary contacts
CD	Capillary density
CFPE	Capillary-fibre perimeter exchange
cPWV	Central pulse wave velocity
DBP	Diastolic blood pressure
eNOS	Endothelial nitric oxide synthase
eNOS ser <sup>1177</sup>	eNOS phosphorylated at serine <sup>1177</sup>
ET	Endurance training
Kf	Filtration capacity
NAD(P)Hox	Nicotinamide adenine dinucleotide phosphate-oxidase
NOX2	Subunit of the NAD(P)Hox complex
NO	Nitric oxide
MAP	Mean arterial pressure
O <sub>2</sub> <sup>-</sup>	Superoxide anion
pPWV	Peripheral pulse wave velocity
PWV	Pulse wave velocity
UEA-I FITC	Ulex Europaeus-FITC conjugated
RT	Resistance training
SBP	Systolic blood pressure
SIT	Sprint interval training
WGA-350	Wheat germ agglutinin-350

1RM

1 repetition maximum

## Introduction

There a number of publications providing compelling evidence that impairments in endothelial nitric oxide (NO) production in the skeletal muscle microvasculature are implicated in the development of skeletal muscle insulin resistance (1, 3, 33), anabolic resistance leading to sarcopenia (30) and potentially in capillary rarefaction seen in ageing, obesity and the metabolic syndrome (15, 24, 42). An attenuated endothelial NO production has also been implicated in the reduction in exercise hyperaemia known to occur in elderly humans (12, 35). As such, interventions which increase skeletal muscle microvascular NO bioavailability should be regarded as primary therapeutic strategies in sedentary and insulin resistant populations.

NO bioavailability is determined by the balance between NO synthesis and scavenging by reactive oxygen species. The rate limiting enzyme for endothelial NO synthesis has been shown to be endothelial nitric oxide synthase (eNOS), with eNOS protein content and serine<sup>1177</sup> (ser<sup>1177</sup>) phosphorylation state together determining eNOS activity and NO production. The main signals that activate eNOS via serine<sup>1177</sup> phosphorylation are meal induced increases in plasma insulin and exercise induced increases in blood shear stress and VEGF production (42). A major source of reactive oxygen species production and NO scavenging in obesity, cardiovascular pathology and ageing is the enzyme NAD(P)H oxidase (NAD(P)Hox) (4, 37). However, the physiological role of NAD(P)Hox in young healthy individuals is not entirely clear. Reactive oxygen species are likely to be important in intracellular signalling leading to mitochondrial biogenesis and angiogenesis (14), but may also reduce NO bioavailability via NO scavenging (15).

A recent study by Cocks et al. (9) has shown that endurance training (ET) and sprint interval training (SIT) are effective means to increase skeletal muscle microvascular eNOS content in healthy young individuals, suggesting that both of these exercise interventions may increase skeletal muscle microvascular NO bioavailability. However to date, no studies have investigated the effect of resistance training (RT) on skeletal muscle microvascular eNOS content and eNOS ser<sup>1177</sup> phosphorylation state. RT forms a fundamental component of the American College of Sports Medicine and American Heart Association guidelines for physical activity and public health (18), due to its established effects on insulin sensitivity (whole body and muscle), muscle mass and muscular strength, variables that are strongly related to promotion and maintenance of independence and health (18).

Due to the paucity of data regarding the effect of RT on the skeletal muscle microvasculature we first sought to determine the effects of 6wk RT on the protein content of microvascular enzymes (eNOS and NOX2) and eNOS ser<sup>1177</sup> phosphorylation, specifically in the endothelial layer of the muscle microvasculature using quantitative immunofluorescence microscopy (10). We also investigated the effect of RT on capillary-fibre-perimeter exchange (CFPE) index, a measure of microvascular density, and filtration capacity (Kf), a functional measure of microvascular density, which have not previously been measured in young individuals following RT. Thirdly, we aimed to investigate the effect of 6wk RT on microvascular eNOS ser<sup>1177</sup> phosphorylation in the resting fasted state. Finally, the effects of 6wk RT on arterial stiffness and blood pressure were measured to investigate how different vascular beds respond to RT training and investigate earlier claims that RT may have a negative effect on arterial stiffness (31). We hypothesized that microvascular density and eNOS content and ser<sup>1177</sup> phosphorylation state would not increase in response to 6wk RT.

## **Materials and Methods**

The percutaneous muscle biopsies taken in this study have been used both for the measurements described in this manuscript and for the measurement of intramuscular triglycerides (IMTG) and perilipin-2 and perilipin-5 content. The latter results are reported in a separate manuscript (36). Measures such as whole body insulin sensitivity are relevant for the interpretation of both studies and are presented in both manuscripts.

## **Participants**

Eight healthy sedentary males participated in the study (Table 1). The sedentary state was defined as performing <1h/wk of structured physical activity (e.g. sports club, commercial gym or exercise class) per week. All participants provided written informed consent and the protocol adhered to the Declaration of Helsinki and was approved by the Black Country NHS Research Ethics Committee. The functional measurements and training intervention were performed at the University of Birmingham and analytical measurements were completed at the University of Birmingham and Liverpool John Moores University.

## **Familiarisation and 1RM**

Participants were first familiarised with all the resistance training equipment and instructed on correct lifting technique. Eight motion guided resistance exercise machines (Cybex International Inc., MA, USA) targeting both the upper and lower body were used (leg press, leg extension, seated leg curls, chest press, lat pull down, shoulder press, arm curls, and arm extensions). In order to determine the initial load for the training period, 1 repetition maximum (1RM, maximal load that can be lifted on a given exercise) was determined on all eight machines, using the method of Kraemer and Fry (25).

## **Experimental protocol**

Experimental testing took place over 2 days and included measures of vascular function, insulin sensitivity (day 1) and a muscle biopsy (day 2). Pre- and post-training testing was identical in all respects and all testing procedures were conducted at least 48 hours after the last exercise bout to minimise the acute effects of exercise. On all occasions testing was performed following 24 hours of a standardised diet and following an overnight fast. Standard diets were matched to the participant's average daily energy intake, calculated using a 3 day diet diary. The composition of the standard diet was 50% carbohydrate, 35% fat and 15% protein.

## **Experimental procedures**

### **Arterial stiffness**

Supine blood pressure was measured using an automated sphygmomanometer (Omron 7051T, Omron Corporation, Kyoto, Japan) following 15 minutes of supine rest. Systemic wave reflection was then investigated using pulse wave analysis, conducted using a semi-automated device and software (SphygmoCor, AtCor Medical, Sydney, Australia), using this augmentation index (AIx) was calculated as described previously (9). To control for the influence of heart rate on AIx, AIx was normalised to a heart rate of 75 beats per minute (AIx@75) (43). Central (carotid- femoral, cPWV) and peripheral (carotid- radial, pPWV) artery stiffness were investigated by pulse wave velocity, assessed using a semi-automated device and software (SphygmoCor, AtCor Medical, Sydney, Australia) as previously described (9). All measurements were made in triplicate.

### **Venous occlusion plethysmography**



Microvascular filtration capacity (Kf) was measured through venous occlusion plethysmography, using the principles described by Gamble et al. (16). The measurements were made in a quiet temperature controlled room following a supine rest of at least 30 minutes. Measurements were made with the left calf elevated to the level of the heart and a congestion pressure cuff placed around the left thigh. Changes in calf circumference were measured using a passive inductive transducer with an accuracy of  $\pm 5 \mu\text{m}$ , in response to five 10 mmHg cumulative congestion pressure steps. The maximum pressure never exceeded the participant's diastolic pressure.

### **Oral glucose tolerance test and Matsuda insulin sensitivity index**

A baseline 25 ml blood sample was taken, an oral glucose tolerance test was then conducted, following the procedure originally proposed by Matsuda & DeFronzo (28) for the assessment of whole body insulin sensitivity. Plasma was separated by centrifugation (10 minutes at 3000 rpm) and stored at  $-80^{\circ}\text{C}$  until analysis. Plasma insulin concentrations were determined by enzyme linked immuno-sorbent assay (ELISA) using a commercially available kit (Invitrogen, UK). Plasma glucose concentrations were analysed using an automated analyzer (IL ILab 650 Chemistry Analyzer, Diamond Diagnostics, USA).

Area under the curve (AUC) for insulin and glucose during the oral glucose tolerance test was calculated using the conventional trapezoid rule. The Matsuda index was used to provide a measure of whole body insulin sensitivity (28).

### **Muscle biopsy**

On day 2 following an overnight fast a muscle biopsy was taken from the lateral portion of the *m. vastus lateralis* under local anaesthesia (1% lidocaine) using the percutaneous needle

biopsy technique, as recently described (39). Excess blood and visible collagen or fat were removed before samples were embedded in Tissue-Tek OCT Compound (Sakura Finetek Europe, Zoeterwoude, Netherlands) and immediately frozen in liquid nitrogen cooled isopentane (Sigma-Aldrich, Dorset, UK). Samples were then stored at -80°C until analysis was performed.

## **Training**

Participants trained three times per week completing a minimum of 16 and maximum of 18 sessions during the 6wk. Participants completed the training sessions in the School of Sport, Exercise and Rehabilitation Sciences at the University of Birmingham under the instruction of the research team. Eight motion guided resistance exercise machines (discussed above) targeting both the upper and lower body were used. During the first 3 sessions, participants completed 3 sets of 10-12 repetitions at 50%, 60% and finally 70% 1RM. Following the first week participants completed 2 sets of 10-12 repetitions, the third set was then performed to volitional fatigue. Loads of 80% 1RM were used. To ensure progression, load was increased by 2.27Kg following successful completion of 3 sets of 12 lifts.

## **Quantitative immunofluorescence microscopy**

The immunofluorescence staining protocol and quantification has previously been described in detail by Cocks et al. (10). The overall coefficient of variation for 5-6 duplicate measurements of one muscle sample for eNOS content, eNOS ser<sup>1177</sup> phosphorylation and NOX2 content of the endothelium and membrane were 7%, 7% and 9.5% 6.5%, respectively (10). Briefly, samples orientated to provide muscle fibre cross-sections were cut (5µm) and placed on glass slides. Both pre- and post-training samples within an individual were placed on the same slide. Sections were fixed in acetone and ethanol (3:1). Following fixation

section were incubated overnight with antibodies for the following: NOX2 (Santa Cruz Biotechnology, Santa Cruz, CA, gp91-phox/NOX2 (C-15), cat No. sc-5827), eNOS (Transduction laboratories, Lexington, KY, cat No. 610297) or p-eNOS ser<sup>1177</sup> (Cell Signalling Technology Beverly, MA, p-eNOS ser<sup>1177</sup>, cat No. 9570L). Sections were then incubated with appropriately labelled secondary antibodies (Invitrogen, Paisley, UK), the endothelial marker Ulex Europaeus-FITC conjugated (UEA-I-FITC; Sigma-Aldrich, UK) and the cell membrane marker wheat germ agglutinin-350 (WGA-350; Invitrogen, UK). For image capture, muscle sections were viewed using a Nikon E600 microscope using a 40x 0.75 numerical aperture objective, illuminated with a 170W Xenon light source. Images were captured using a SPOT RT KE colour three shot camera (Diagnostic Instrument Inc., MI, USA) coupled to the microscope.

Once captured images were analysed using Image Pro Plus 5.1 software (Media Cybernetics Inc, Bethesda, MD, USA). Endothelial specific fluorescence was determined using the UEA-I FITC (endothelial marker) image, which was extracted and overlaid onto the corresponding eNOS, p-eNOS ser<sup>1177</sup> or NOX2 image. Cell membrane specific fluorescence for NOX2 was determined using WGA-350 as a stain to create an outline of the cell membrane. The latter was extracted and overlaid onto the corresponding NOX2 image. Fluorescence intensity of the eNOS, p-eNOS ser<sup>1177</sup> or NOX2 signal was quantified within the endothelium or cell membrane specific area. Values were normalised to pre-training values.

### **Capillarization**

Muscle sections were incubated with anti-myosin type I (developed by Dr Blau DSHB) followed by goat anti-mouse IgM 350 (Invitrogen, Paisley, UK) to identify type I muscle fibres. This was performed in combination with UEA-I-FITC (Sigma-Aldrich, UK) and

wheat germ agglutinin-350 (WGA-350; Invitrogen, UK) as markers of the endothelium and plasma membrane, respectively. Capillaries were manually quantified in a fibre type specific manner, using the UEA-I, WGA-350 and myosin heavy chain images. The following indexes were measured as previously described (21): 1) number of capillaries around a fibre (capillary contacts (CC)), 2) capillary density (CD) and 3) capillary-fibre-perimeter exchange (CFPE) index. Fibre cross sectional area and perimeter were measured using ImagePro Plus 5.1.

## **Statistics**

Statistical analyses were performed using SPSS for windows version 16.0 (SPSS, Chicago, IL)). Capillary contacts, capillary-to-fibre ratio on an individual-fibre basis, capillary-fibre-perimeter exchange, fibre cross sectional area and perimeter were analyzed using a factorial ANOVA, with the factors 'training' (pre versus post) and 'fibre type' (type I versus type II). All other variables were analysed using paired samples t-tests for comparison. Significance was set at  $P \leq 0.05$ . Data is presented as means  $\pm$  S.E.M. The primary aim of the study was to compare the effects of RT on muscle microvascular eNOS content. 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 an effect size of  $d_z=0.98$ , representative of a large sized effect (11) adopting an alpha of 0.05 and power of 0.80. We deemed a  $d_z$  of 0.98 to be a physiologically relevant difference, as we previously observed an effect of this size following 6wk of ET and sprint interval training (SIT) in sedentary individuals (11).

## **Results**

### **Training effects**

As expected participants exhibited significant gains in strength following RT. 1RM increased by 43%, 33%, 32% and 38% for the leg press, leg extension, chest press and shoulder press, respectively (all variables  $P < 0.05$ ; Table 1). Resting heart rate was unchanged by training ( $P = 0.119$ ; Table 1), while brachial systolic blood pressure (SBP), diastolic blood pressure (DBP) and mean arterial pressure (MAP) were all reduced following training ( $P < 0.05$ ; Table 1).

### **Insulin sensitivity**

The Matsuda insulin sensitivity index was significantly increased by 31% following RT ( $P < 0.05$ ; Table 1). Both glucose and insulin AUC were reduced following training ( $P < 0.05$ ; Table 1).

### **Microvascular enzymes**

Skeletal muscle endothelial specific eNOS content and ser<sup>1177</sup> phosphorylation were not significantly different pre- and post-RT (eNOS content:  $P = 0.091$ , Fig. 1; eNOS ser<sup>1177</sup> phosphorylation:  $P = 0.075$ ; Fig. 2). Skeletal muscle endothelial specific and membrane specific NOX2 content were also not changed by RT (endothelial specific:  $P = 0.319$ ; membrane specific  $P = 0.164$ ; Fig.3).

### **Filtration capacity and capillarization**

$K_f$  was unaltered by training (pre  $3.16 \pm 0.41 \text{ mL min}^{-1} 100\text{mL}^{-1} \text{ mmHg}^{-1} \times 10^{-3}$  vs. post  $3.71 \pm 0.52 \text{ mL min}^{-1} 100\text{mL}^{-1} \text{ mmHg}^{-1} \times 10^{-3}$ ;  $P = 0.333$ ). Fibre cross sectional area and perimeter were not changed by RT in either type I or II fibres (fibre cross sectional area:  $P = 0.827$ ;

perimeter:  $P = 0.625$ ). No significant difference was found in capillary density following RT ( $P = 0.715$ ). Type I fibres had significantly higher capillary contacts and capillary-fibre-perimeter exchange than type II fibres both before and after training ( $P < 0.05$ ). However, no parameter of capillarization was significantly changed as a result of RT (CC:  $P = 0.716$ ; CFPE,  $P = 0.654$ ; Table 2).

### **Arterial stiffness**

AIx@75bpm was significantly decreased following training ( $P < 0.05$ ; Fig. 4c), but neither cPWV nor pPWV were affected by training (cPWV  $P = 0.934$ , pPWV  $P = 0.708$ ; Fig. 4a and b).

## **Discussion**

This study demonstrates that significant improvements in insulin sensitivity following 6wk of resistance training in sedentary males occurs in the absence of changes in skeletal muscle capillary density, in the content and ser<sup>1177</sup> phosphorylation of skeletal muscle microvascular endothelial eNOS or in the protein content of NOX2. In addition, our results contrast previous suggestions that RT increases central arterial stiffness (31) and show that AIx a systemic index of arterial stiffness was reduced by 28% ( $P < 0.05$ ) following RT.

### **Capillary density, eNOS content and phosphorylation**

In the current study, we did not observe a significant change in capillary density (CD) in response to RT. This finding is in agreement with several previous studies (17, 29) confirming that RT does not increase skeletal muscle capillary density. However, in contrast to the present study RT has previously been shown to increase the number of capillaries per fibre (17, 29). This rise in number of capillaries following RT was suggested to be proportional to fibre growth, to maintain equal diffusion distances in the enlarged fibres. The lack of change in number of capillaries per fibre in the current study might be explained by the fact that the 6wk RT intervention did not lead to significant muscle fibre hypertrophy in the sampled muscle (as shown by no change in muscle fibre cross-sectional area, Table 2). The lack of significant change in muscle fibre cross sectional area is probably due to the relatively short duration of our RT intervention in comparison to previous studies (10 to 24 weeks) which have demonstrated muscle fibre hypertrophy (2, 7, 13, 17, 27, 29). Further confirmation of the lack of change in capillary density is offered by the absence of an increase in microvascular  $K_f$ , a functional measure of capillary surface area available for diffusion of plasma water, known to correlate with capillary density (6). The results regarding  $K_f$  support previous findings from a cross-sectional comparison of strength trained athletes,

showing no elevation in  $K_f$  in this population compared to sedentary individuals (5). This is the first study to measure capillary fibre perimeter exchange (CPFE) index in young males (17, 29, 40). Again RT did not alter CPFE index. CPFE index is regarded to be a valuable measure of microvascular density as it may provide more information regarding the capacity for oxygen flux, and the transport of substances that rely on receptor or transporter-mediated processes (i.e., glucose and insulin) than traditional measures such as CD (20).

The current RT intervention did not increase skeletal muscle endothelial specific eNOS content. This contrasts with the effects of both ET and SIT which were both effective at increasing skeletal muscle endothelial specific eNOS content (9). An increase in eNOS content may potentially lead to increases in NO production upon stimulation by insulin, exercise induced shear stress and exercise induced VEGF production (23, 38, 41, 42). As such, combined ET and RT may prove to be the most effective training approach to achieve a favourable adaptation to the skeletal muscle microvasculature, while the addition of RT will increase muscle mass and strength, adaptations that are particularly relevant to maintain an independent lifestyle in the rapidly growing ageing population. However, future studies making parallel measurements of eNOS content, eNOS ser<sup>1177</sup> phosphorylation and muscle microvascular blood volume and flow will be required to confirm that RT does not result in favourable functional adaptations for muscle microvascular blood flow regulation via independent mechanisms that have not been investigated in the current study.

RT did not change basal eNOS ser<sup>1177</sup> phosphorylation, although there was a trend towards a decrease ( $P = 0.075$ ). As there is no change in capillary density following RT, the absence of an effect on eNOS ser<sup>1177</sup> phosphorylation is as expected. Cocks et al. (8) have previously observed a reduction in eNOS ser<sup>1177</sup> phosphorylation in sedentary individuals following 6wk



ET and SIT. This decrease was seen both in the resting state and following 1 h of exercise at 65% VO<sub>2</sub>max. The decrease was attributed to a reduction in shear stress resulting from the training-induced increase in capillary density after the ET and SIT training.

The study was designed to detect a large effect size (11) difference in muscle microvascular eNOS content. The sample size was based on previously observed responses following 6wk of ET and SIT in sedentary individuals (9). It should therefore be noted that it is not possible to exclude differences which are smaller than the study was powered to detect. Indeed both eNOS content and eNOS ser<sup>1177</sup> phosphorylation display trends to significance, which given a larger sample size may have resulted in significant differences.

## **NOX2**

No change was seen in the protein content of the membrane bound subunit of the NAD(P)Hox complex NOX2, in either the skeletal muscle membrane or in the muscle microvascular endothelium. This finding is in support of previous work from our lab following ET and SIT (9), where again, no change in NOX2 content was seen following 6wks of training in young lean sedentary males. As the subjects in this study were young sedentary but healthy lean individuals, the assumption is that the protein expression was low before RT and did not change following RT. However, much more research is needed into how oxidative enzymes (including NAD(P)oxidase) respond to training. As such, the findings of the present study provide a building block for future research, which should focus on how oxidative enzymes respond to training in pathology and aging.

## **Arterial stiffness**

In contrast to a recent meta-analysis which suggested that high intensity RT is associated with increased central artery stiffness (31) the current study showed no change in aortic or peripheral artery stiffness following 6wk of high intensity RT. The discrepancy between studies is likely the result of different RT protocols performed (concentric lifting protocols, high volume and high intensity RT versus eccentric lifting protocols). Studies using similar high intensity RT protocols to the current study have also observed no change in arterial stiffness (5, 32, 34). This suggests that when the guidelines for high intensity RT are followed arterial stiffness is not adversely affected; however, unlike exercise modes such as ET and SIT (9), RT does not have a beneficial effect on arterial stiffness.

Unlike local artery stiffness (aorta and brachial artery), AIx was reduced following training. AIx is a measure of the contribution wave reflections make to the arterial pressure waveform (44). As such, AIx is a measure of systemic stiffness, as the amplitude and timing of wave reflections depend on small and large arteries. Systemic stiffness is an important measure as it partly determines left ventricular workload, and is therefore of significant clinical importance (44). The current finding however is in contrast with two previous studies investigating RT in young healthy participants, which both suggested that RT has no effect on systemic stiffness (5, 19). An explanation for these differences is unclear as the current study uses the same protocol to measure AIx and a similar RT protocol (5, 19). Because of the potential clinical implications more detailed future studies are required in young healthy adults, elderly individuals and patient populations.

## **Perspective**

This study generates novel information that 6wk RT in previously sedentary young males does not increase microvascular density or eNOS content and eNOS ser<sup>1177</sup> phosphorylation

state. Together with previous reports investigating the effect of ET (8) and RT (22, 26, 45), we suggest that the combination of both training modes may lead to optimal metabolic and health benefits. ET in addition will be a powerful means to increase muscle oxidative capacity, capillary density and microvascular responsiveness to insulin, VEGF and shear stress, while RT has unique effects on muscle mass and strength.

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## Tables

**Table 1. Subject characteristics, insulin sensitivity and hemodynamics pre and post 6 weeks of resistance training.**

Values are means  $\pm$  S.E.M. \*  $P < 0.05$ . 1RM, 1 repetition maximum

**Table 2. Capillarization pre and post resistance training.** Values are means  $\pm$  S.E.M. FA, fiber cross sectional area; CD, capillary density; CC, capillary contacts; CFPE, capillary-fibre-perimeter exchange.

## **Figure legends**

### **Figure 1. Effect of resistance training (RT) on eNOS content.**

A. Widefield microscopy images of skeletal muscle pre- (left) and post-RT (right). Skeletal muscle eNOS expression was revealed using Alexa-Fluor 594 conjugated secondary antibody (red). Bar = 50µm. B Mean fluorescence intensity of eNOS is indicated. The mean level of eNOS pre-training was assigned a value of 1, and the relative intensity of eNOS post-training was calculated.

### **Figure 2. Effect of resistance training (RT) on basal eNOS serine<sup>1177</sup> phosphorylation.**

A. Widefield microscopy images of skeletal muscle pre- (left) and post-RT (right). Skeletal muscle eNOS serine<sup>1177</sup> phosphorylation was revealed using Alexa-Fluor 594 conjugated secondary antibody (red). Bar = 5µm. B Mean fluorescence intensity of eNOS serine<sup>1177</sup> is indicated. The mean level of eNOS serine<sup>1177</sup> pre-training was assigned a value of 1, and the relative intensity of eNOS serine<sup>1177</sup> post training was calculated.

### **Figure 3. Effects of resistance training (RT) on NOX2 content.**

A. Widefield microscopy images of skeletal muscle pre- (left) and post-RT (right). Skeletal muscle NOX2 content was revealed using Alexa-Fluor 594 conjugated secondary antibody (red). Bar = 50µm. B Mean fluorescence intensity of NOX2 within the muscle membrane is summarized. C Mean fluorescence intensity of NOX2 within the endothelium is indicated. The mean level of NOX2 pre-training was assigned a value of 1, and the relative intensity of NOX2 post-training was calculated.

### **Figure 4. Effect of resistance training (RT) on augmentation index and central and peripheral artery stiffness.**



A. systemic stiffness measured through augmentation index normalized to 75 bpm following RT. B. Central artery (aortic) stiffness measured using pulse wave velocity (PWV) following RT. C. Peripheral artery (brachial artery) stiffness measured using pulse wave velocity following RT. \*  $P < 0.05$ .