Effects of aerobic interval training on arterial stiffness and microvascular function of metabolic syndrome subjects

Journal:	*The Journal of Clinical Hypertension*
Manuscript ID	JCH-17-0085.R2
Wiley - Manuscript type:	Original Paper
Date Submitted by the Author:	n/a
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Keywords:	physical fitness, metabolic syndrome X, vascular stiffness, pulse wave velocity, reactive hyperemia
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Running head: Hypertension and metabolic syndrome

Abstract: 245; **Text**: 3139; **References**: 30; **Tables:** 2; **Figures:** 3.

ABSTRACT

We determined the effect of high-intensity aerobic interval training on arterial stiffness and microvascular dysfunction in metabolic syndrome patients (MetS) with hypertension. We used applanation tonometry to measure arterial stiffness and laser Doppler flowmetry to assess microvascular dysfunction before and after 6 months of stationary cycling (TRAIN group; *N*=23) in comparison to a group that remained sedentary (CONT group; *N*=23). While no variable improved in CONT, in the TRAIN group hypertension rate fell from 79% (59-91%) to 41% (24-61%) resulting in lower systolic and diastolic pressures than CONT (-12±3 and -6±2 mmHg; $P < 0.008$). Arterial stiffness declined (-17% augmentation index; $P = 0.048$) and reactive hyperemia increased (20%; $P = 0.028$) post –treatment in TRAIN vs. CONT. Blood constituents associated with arterial stiffness and with prothrombotic state (high-sensitive C-reactive protein, fibrinogen, platelets and erythrocytes) remained unchanged in TRAIN and CONT groups. In summary, six-month of intense aerobic exercise program reduces both arterial stiffness and microvascular dysfunction in MetS patients despite unchanged blood borne cardiovascular risk factors. Training lowers blood flow resistance in central and peripheral vascular beds in a coordinated fashion resulting in clinically relevant reductions in hypertension.

ClinicalTrials.gov Identifier: NCT03019796.

Key words: physical fitness; metabolic syndrome X; vascular stiffness; pulse wave velocity; reactive hyperemia.

INTRODUCTION

Metabolic syndrome (MetS) is a cluster of disorders that increase the risk of developing cardiovascular disease (CVD) and diabetes [1]. MetS is associated with poor blood pressure control [2] which increases their risk of developing CVD and thus, there is a growing interest in therapies that reduce blood pressure in this population. Chronic hypertension seems to induce changes in the tunica media of arteries resulting in arterial stiffness [3]. Current evidence suggests that arterial stiffness precedes hypertension in older adults [4]. Arterial stiffness can be assessed using applanation tonometry by measuring carotid-femoral pulse wave velocity (PWV) and by measuring the shape of the radial pulse waveform and calculating the augmentation index. In recent years, the use of these pulsatile measurements of pressure have gained popularity because they better predict coronary artery diseases risk than traditional measures of brachial blood pressure using sphygmomanometry [5].

MetS patients have increased arterial stiffness even when adjusting for associated factors such as age and gender[6]. In fact, MetS accelerates the age-associated increase in aortic stiffness in men and women [7]. In MetS patients, eight weeks of intense aerobic exercise lowers arterial stiffness (PWV) but not brachial artery pressures [8]. This suggests that pulsatile blood pressure measures may be more sensitive than traditional brachial artery pressure measurements to readily identify the effects of exercise training on hemodynamics. Nevertheless, even when studies are based only on pulsatile measurements, there is no unanimous agreement on the effect of exercise training on blood pressure. For instance, a recent meta-analysis suggests that continuous moderate-intensity aerobic exercise training, without weight loss, does not improve arterial stiffness in obese adults [9]. High-intensity aerobic interval training elicits superior peripheral adaptations in comparison with continuous moderate-intensity training [10] although its effect on arterial stiffness has not been explored.

Studies suggest that obesity and MetS are characterized by endothelial dysfunction in peripheral vascular beds, such as the subcutaneous microcirculation, that prevents adequate

tissue perfusion resulting in oxidative stress and inflammation [11,12]. Others argue that MetS has mainly a central effect that accelerates arterial stiffness [13] while a third group support that MetS affects both macro and microcirculation [14]. To our knowledge, no study has systematically evaluated the central and peripheral cardiovascular effects of aerobic interval training in the same MetS individuals using an intervention study rather than a cross-sectional design. That data may help to reveal which vascular bed is more responsive and conversely which is more resistant to the effects of aerobic high-intensity interval training on lowering blood pressure.

The aim of this study was to determine the hemodynamic effects of prolonged (6 months) high-intensity aerobic interval training (AIT) in MetS patients with high prevalence of hypertension and thus increased risk of developing CVD. Importantly, we examined both, central and peripheral vessel adaptations, with the hypothesis that exercise training would improve both vascular beds in a coordinated fashion. Our primary outcome was arterial stiffness measured using pulsatile indexes (i.e., PWV and augmentation index) [15].

METHODS

Study design and population. A randomized, pretest-posttest control group design was used. Subjects were recruited, clinically screened and completed the treatments and testing in the order presented in Figure 1 while the study complied with the CONSORT statement [16]. Fifty subjects were randomly assigned to a TRAIN or CONT group, balancing the number of women allocated in each group. All participants were physically inactive (exercise < 1 day per week) and weight stable (i.e. ± 2 kg) for at least 6 months prior to the study. Participants were enrolled based on fulfillment of ≥3 MetS criteria as per harmonized definition using Europid waist circumference cut-points (80 cm for women and 94 cm for men) [1]. Elevated blood pressure for the MetS is defined as systolic pressure ≥130 mmHg and/or diastolic ≥85 mmHg measured at the brachial artery or taking antihypertensive drug treatment [1]. Exclusion criteria

included use of medications known to affect weight or appetite and/or any disease associated with exercise intolerance. Women were not under hormone replacement therapy. Screening included physical examination to measure BMI, resting blood pressure, waist circumference (2 cm above the iliac crest), 12-lead ECG at rest and during an exercise stress test from which we obtained the maximal heart rate for each individual and blood biochemistry. All subjects provided written, witnessed, informed consent and the study was approved by the local Hospital's Ethics Committee in accordance with the Declaration of Helsinki.

Intervention program. Subjects in the CONT group were instructed to remain sedentary during the 6 months of study. Subjects were instructed to maintain a steady dietary pattern which was analyzed monthly using a 3-day meal diary (CESNID®, Barcelona, Spain). The supervised training program consisted of 45 min sessions of pedaling exercise, 3 days per week for a total of 6 months. Attendance to at least 85% of the sessions was required. In every exercise session, participants wore a heart rate monitor and workloads were self-adjusted to reach the target heart rate. Each exercise session consisted of a 10-min warm-up, followed by 4 bouts of 4 min of pedaling at an intensity that elicited 90% of maximal heart rate (i.e., HR_{MAX}) interspersed with 3 min active recovery periods at 70% HR_{MAX}. The average oxygen consumption rate during each exercise session in the TRAIN group was 26.5±4.7 $mLO_2/kg/min$ which corresponded to 7.6±1.3 METs. HR_{MAX} was re-evaluated monthly during a maximal cycling bout to exhaustion and workloads adjusted accordingly to maintain training stimulus constant. This intermittent exercise protocol was previously shown to improve cardiorespiratory fitness and be tolerable in the MetS population [10,17,18].

Clinical investigation. Before and after the 6 months of intervention (exercise or no-exercise) blood pressure and pulse-wave contour measurements were assessed at rest in the morning after an overnight fast. For the TRAIN group, post-training measurements were scheduled at least 48 h after the last exercise training session to examine the chronic effects of exercise training rather than the acute most recent exercise session. On a different day, post

occlusion reactive hyperemia was assessed in the cutaneous circulation. In addition, percent body fat was determined by dual energy X-ray absorptiometry scan (DXA Hologic Serie Discovery Wi QDR, Bedford, USA). Finally, subjects were referred to a clinic where blood was drawn in the morning after a 10 h overnight fast pre-and-post intervention. Subjects were instructed to complete the 3 visits (two to the lab and one to the clinic) within the same week.

Central arterial stiffness measurements. After 20 min of undisturbed supine rest on a gurney, brachial blood pressures (systolic and diastolic blood pressures; SBP and DBP) were measured in triplicate on the left arm with a calibrated ECG gated electro-sphygmomanometer (Tango; Sun Tech Medical, NC, USA). The first reading was discarded and the mean of the two following readings with a coefficient of variation < 10% was used, with additional readings if required [19]. Following, aortic systolic and diastolic pressures were calculated by applanation tonometry in the radial artery using high fidelity pressure contours (SphygmoCor; AtCor Medical, Sydney, Australia). The SphygmoCor system synthetizes a central (ascending aortic) pressure waveform from the radial pressure waveform that has been validated against the intra-arterial recorded wave [20]. Augmentation index (AI) was calculated as the maximal systolic pulse wave peak minus the pressure at the inflection point expressed as percentage of pulse pressure. Because AI is influenced by heart rate, [21] AI data were normalized to a heart rate of 75 beats·min^{-1} (AI@75HR) before analysis. Only measurements within the default specifications which were, average pulse height >80 units, pulse height and diastolic variation <5% and quality index >80%, were averaged.

Carotid-to-femoral pulse wave velocity (PWV) was measured by applanation tonometry (AtCor Medical, Sydney, Australia) as an index of central arterial stiffness. ECG-gated waveforms were recorded and time delay calculated from the foot-of-the-wave. Aortic distance was calculated from the carotid to the suprasternal notch and from the suprasternal notch to the femoral artery at the groin. All measures (AI and PWV) were taken in duplicate and the average of those two readings reported. However, when differences between the two

readings were present, additional readings were performed averaging two consecutives readings with coefficient of variation <12%. The same trained researcher took all pulsatile measurements. The intra-subject day-to-day reliability of the pressure wave contour measurement in our lab was established on 5 subjects at the same time of the day resulting in a mean coefficient of variation (CV) of 8.1% for PWV and 10.1% for AI.

Peripheral post-occlusion reactive hyperemia. On a different day, subjects arrived to the laboratory after an overnight fast having abstained from drinking tea or coffee in the 12 hours prior to attendance. Subjects were instrumented with a deflated sphygmomanometer cuff around their mid arm and a laser Doppler fluximeter probe (DRT4; Moor Instruments, Axminster, UK) was affixed with a flat holder and adhesive tape to the ventral right forearm (12 cm proximal from the wrist crease). After 20 min of lying supine with the arm rested on the gurney, the sphygmomanometer cuff was inflated 30 mmHg above resting systolic blood pressure for 3 min [22,23]. A satisfactory blood flow occlusion was evidenced by the loss of cutaneous pulsatile flow and a steadily low blood flow display [22]. After 3 min, the cuff was quickly deflated inducing reactive hyperemia. Participants were instructed to remain as still as possible during cuff deflation. The peak flux value above baseline and the time to reach it were recorded as indexes of microvascular reactivity [24]. Intra-subject reproducibility for this technique in our lab results in a CV <15%.

Blood analyses. Before and after the intervention and following an overnight fast a 6-mL blood sample was drawn into a blood collection tube containing EDTA (Vacutainer®; Becton-Dickinson, USA) and analyzed for erythrocytes, leukocytes, fibrinogen and platelets (BC 5800 Mindray, Bio-Medical Electronics Ltd, China), high-sensitive C-reactive protein (hsCRP) using immune-turbidimetry, HDL-c using accelerator selective detergent method, blood triglycerides (TG) with glycerol-3-phosphate oxidize method, and total cholesterol (T Chol) by an enzymatic method with a single aqueous reagent. Low-density lipoprotein-cholesterol (LDL-c) was calculated as proposed by Friedewald [25]. Plasma glucose was analyzed using the

glucose oxidase-peroxidase method. All the above analyses were run in an automated Mindray BS 400 Chemistry Analyzer (Mindray Medical Instrumentation, USA). Insulin concentration was measured in duplicate using chemiluminescent micro-particle immunoassay (Architect ci4100, Abbott Laboratories, USA).

Statistical analysis. Normality was evaluated by the Shapiro-Wilk test. Sample size calculation revealed that 18 subjects per group were sufficient to detect a moderate (Cohen's effect size; ES) Group x Time interaction effect for PWV, assuming a power of 0.8 and an α–error probability of 0.05. Differences between groups at baseline were analyzed using a *t* test for independent samples. The effects of the intervention, were tested using a two-way (Time x Group), mixed-model ANOVA. If an interaction existed, test for simple effects were explored using Bonferroni post-hoc test. Effect size (ES [26]) of time-group interaction effect were calculated using partial Eta square, based on the following criteria; >0.14 large effect, moderate 0.14-0.06 moderate effect; <0.06 small effect. All analyses were performed with SPSS version 21 (Chicago, IL). Data presented as mean ± SD. Statistical significance level was set at $P \leq 0.05$.

RESULTS

Participant characteristics. Subjects reported unchanged dietary pattern (amount and composition; CESNID, Barcelona, Spain) during the study. Attendance to the exercise sessions averaged 95% (85-100%). Age of the subjects was 53.5±8.9 years and there were 8 women (17%) among the 46 individuals that composed all the sample (Figure 1). Sex and age did not have significant interactions with time in any of the main outcome measures (i.e., AI@75HR, PWV and reactive hyperemia). While anthropometric did not vary in CONT group after 6 months, in the TRAIN group there were modest, yet significant reductions in body weight (91.0±1.6 vs. 89.7±12.4 kg, P = 0.019), BMI (32.8±3.3 vs. 32.3±3.1 kg·m^{-2}, P=0.014), and percent body fat (36.0±6.6 vs. 35.4±6.6 %, P = 0.001) following 6 months of AIT. Six months of AIT in the TRAIN group also resulted in significant reductions in two MetS parameters, waist circumference and blood pressure (Table 1). Other parameters associated with arterial stiffness did not significantly change in either group (i.e., total cholesterol, LDL cholesterol, hsCRP, insulin concentrations; Table 2). Furthermore, indexes of blood cellular content and prothrombotic state did not vary with training from CONT (Table 2).

Hemodynamic measurements. While no changes were detected in CONT, after 6 months of AIT, participants in TRAIN reduced brachial artery systolic and diastolic blood pressures (Table 1) lowering the prevalence of hypertension from 79% (95%CI; 59-91%) to 41% (95%CI; 24-61%) according to MetS elevated blood pressure thresholds [1]. A significant group-time interaction was found for systolic and diastolic blood pressure (F = 6.23, P= 0.016, ES = 0.124 and F = 4.31, P = 0.044, ES = 0.089, respectively). Thus, after 6 months, arterial pressures declined by 6-9% with exercise training in comparison to CONT resulting in post-treatment lower systolic (-12±3 mmHg; P = 0.001) and diastolic (-6±2 mmHg; P = 0.007) pressures (Table 1). Aortic pressures were also reduced after TRAIN by 7.8% in systolic (127±17 to 117±12 mmHg; P = 0.002) and 8% in diastolic (85±10 to 78±6 mmHg; P = 0.001) resulting in post-treatment lower systolic (-13±4 mmHg; P = 0.030) and diastolic (-6±2 mmHg;

P = 0.020) pressures than CONT. A significant group-time interaction was found for pulse wave velocity (PWV) and augmentation index (AI) (*F* = 4.02, *P* = 0.048, *ES* = 0.082 and *F* = 3.81, *P* = 0.023, *ES* = 0.096, respectively). PWV decreased in TRAIN from 8.5 ± 2.1 to 7.8 ± 2.3 $m \cdot s^{-1}$ (*P* = 0.05, Figure 2A) while it remained at baseline level in the CONT group. However, post-treatment, PWV was not significantly lower in TRAIN compared to CONT. AI@75 HR was reduced after training (TRAIN) from 24.7 ± 11.6 % to 21.9 ± 9.2 % (*P* = 0.038; Figure 2B) while remained unchanged in CONT. Therefore, after 6 months (i.e., follow-up) AI@75 HR was lower in TRAIN than CONT (17% lower; *P* = 0.048; Figure 2B). A significant group-time interaction was found for post occlusion reactive hyperemia flux (*F* = 4.71, *P* = 0.035; *ES* = 0.074). PORH after 3 min of occlusion of the cutaneous forearm vessels was unchanged in CONT but increased after AIT in TRAIN (303 ± 129 vs. 377 ± 147 %, *P* = 0.015; Figure 3A). Therefore, after 6 months (i.e., follow-up) PORH was higher in TRAIN than CONT (20% higher, *P* = 0.028, Figure 3A). However, the time to peak flow, was not affected by treatment in any group (11.6 ± 2.3 vs.11.9 ± 2.2 seconds; *P* = 0.59; Figure 3B).

DISCUSSION

The aim of the present study was to determine the blood pressure response to six months of an intense aerobic interval training (AIT) program in a sample of metabolic syndrome (MetS) participants with high prevalence of hypertension (77-79%; Table 1) and thus elevated risk for suffering cardiovascular diseases [13]. After AIT, we observed a marked reduction in brachial artery systolic and diastolic blood pressures (Table 1) reducing by half the prevalence of hypertension in the TRAIN group. Of novelty, our data reveals that the effect of AIT on lowering blood pressure was mirrored by reductions in central artery stiffness (i.e., PWV and AI; Figure 2) and improvements in peripheral vessel vasodilation capacity (i.e., reactive hyperemia; Figure 3). Previous studies support that both macro and microcirculation

resistances to flow are contributing to hypertension in the MetS [14,27] and we currently report that both are blunted by AIT training (Figure 2B and 3A) likely allowing to half of our sample to regain blood pressure control.

Epidemiological studies reveal that from early to late adulthood (i.e., 20-90 years) systolic blood pressure increases approximately 14% while AI increases fivefold and PWV twofold [28]. PWV has been shown to be an independent determinant of the longitudinal increase in systolic blood pressure [29] and thus arterial pulse-wave contour measurements are regarded as more sensitive than traditional brachial blood pressure measures to detect the alterations in vascular structure and function that occur with aging or disease status such as MetS. However, we observe similar central vasculature effects of exercise training with both measurements. Namely, brachial systolic pressure (Table 1) and pulsatile PWV and AI@75HR (Figure 2), both were reduced by approximately 8% after six months of training. Thus, although central artery stiffness is better assessed by PWV and AI, the reduction in brachial systolic blood pressure after exercise training seems to reflect the reductions in arterial stiffness facilitating the evaluation of the effects of exercise training.

Increased central arterial stiffness results in that most of the pulsatile energy of the forward pressure wave is transferred to the vasculature, potentially damaging vessel structures [30]. We measured central arterial stiffness and peripheral vessel reactive hyperemia and detected that both improved with intense aerobic interval training. Epidemiological studies have shown an association between increased arterial stiffness and reduced microvascular response to ischemia with aging and cardiovascular disease risk factors [31]. Exercise training of the duration and intensity used in this study simultaneously lowers the resistances to flow in central and peripheral vessels in MetS patients. MetS is an important population to study since they have not yet transition to more advance stages of cardiovascular disease (i.e., heart failure, coronary artery diseases, stroke, and peripheral

vascular disease) and seem to be still responsive to life-style therapies that include intense aerobic exercise.

It is tempting to speculate that the simultaneous improvements in peripheral vascular reactivity and arterial stiffness are driven by an external dominant factor that improves along with exercise training. We observed modest, yet significant reduction in body mass and percent fat mass following 6 months of AIT (i.e., -1.7%). Indeed, we [32] and others [33] have shown that weight and fat loss are associated with reductions in blood pressure in this population. However, it is unlikely that the reductions in blood pressure (6 to 13 mmHg comparing TRAIN and CONT at the end of intervention; Table 1) are due to the modest weight loss in the 6 months of TRAIN (1.3 kg). Furthermore, other factors associated with hypertension like elevated blood lipids and hyperglycemia were not affected by the 6 months of TRAIN (Table 1 and 2). Therefore, the improvement in arterial stiffness and microvascular response to ischemia, seem to pertain to the direct effects in the vasculature of the repeated bouts of interval exercise and not by lowering of blood glucose, lipids, or body fat.

Skin circulation is of prime interest because its dysfunction is associated with the pathogenesis of many diseases such as diabetes mellitus, hypertension and obesity [34] and because its vasodilation capacity has been shown to diminish with age. A decreased ability of the endothelium to induce vasodilation in response to occlusion (i.e., reactive hyperemia) is linked to classic risk factors for MetS and cardiovascular diseases risk factors including dyslipidemia, hypertension, inactivity and obesity [35]. While there is no clear consensus on the acute endothelial response to a single bout of exercise, aerobic training seems to consistently improve endothelial function. Furthermore, more profound improvements in vasodilation are observed with high-intensity rather than continuous, lower-intensity exercise training [10]. In agreement with the cited literature, we observed a significant increase (i.e., 20% Figure 3B) in cutaneous microcirculation reactive flow after 6 months of intense training when compared with MetS that remained sedentary.

Blunted microvascular reactivity, assessed by hyperemic flow, has been related to cardiovascular disease risk factors [36] like increased total cholesterol [37], reduced HDL cholesterol [31], high blood triglycerides [38], hyperinsulinemia and low insulin sensitivity [39]. We presently report that after six months of exercise training, the increase in hyperemic peripheral flow (Figure 3B) occurred despite unchanged blood cholesterol (HDL and total), triglycerides (Table 1) or insulin (Table 2). On the other hand, high-sensitive C-reactive protein (hs CRP) in MetS patients has been regarded as a predictor of coronary heart diseases and thus a contributor of the elevated aortic stiffness in this population [40]. Increases in hs CRP could provoke platelet hyperreactivity, promote fibrinogen biosynthesis and increase erythrocyte aggregability resulting in a prothrombotic state [41]. Conversely, all these events seem to be reversed by aerobic exercise training. We did not find reductions in hs CRP with 6 months of exercise training (Table 2) and neither subject's fibrinogen, platelets, leukocytes or erythrocyte count. Our results suggest a minor role for this unspecific inflammation marker (i.e., hs CRP) and other markers of prothrombotic state in the reductions in aortic stiffness observed with exercise training.

Our study is not free of limitations. Our subjects were all diagnosed with MetS and most of them were taking medications that were neither withdrawn nor were the doses lowered during the study. We deemed inappropriate to withhold antihypertensive medication during the study in patients with increased cardiovascular disease risk. It is currently unclear if exercise training lowers blood pressure through mechanisms different to medication, so a possible interaction between medication and exercise training could have existed in our data resulting in an overestimation of the effects of exercise in individual cases. Other limitations of this study were the lack of subject familiarization prior to obtaining measurements, and no control over tobacco usage. Furthermore, there is no gold standard measurement for microvascular function and several other indices besides post occlusion reactive hyperemia flux exist that were not assessed in the present study.

In summary, 6 months of AIT at a frequency of three times per week reduced the prevalence of hypertension from 79% (59-91%) to 41% (24-61%) in MetS subjects upon a 6-13 mmHg reduction in diastolic and systolic blood pressure. The mechanism of the normalization of blood pressure, involved a simultaneous reduction in central arterial stiffness and an augmented capacity of the peripheral microvasculature to vasodilate. These responses seemed to be mediated directly by the training stimulus since we observed minimal changes in other risks factors associated with arterial stiffness (dyslipidemia, hyperglycemia or body fat) or with blood prothrombotic state.

Acknowledgements. The authors report no conflicts of interest. This work was partly funded by a grant from the Spanish Ministry of Economy and Competivity (DEP-2014-52930-R).

REFERENCES

1. Alberti KG, Eckel RH, Grundy SM, et al. Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. *Circulation.* 2009;120(16):1640-1645.

2. Kjeldsen SE, Naditch-Brule L, Perlini S, Zidek W, Farsang C. Increased prevalence of metabolic syndrome in uncontrolled hypertension across Europe: the Global Cardiometabolic Risk Profile in Patients with hypertension disease survey. *J Hypertens.* 2008;26(10):2064-2070.

3. Safar ME, O'Rourke MF. *Arterial stiffness in hypertension.* Vol 23. Edinburgh (UK): Elsevier; 2006.

4. Mitchell GF. Arterial stiffness and hypertension: chicken or egg? *Hypertension.* 2014;64(2):210-214.

5. Weber T, Auer J, Lamm G, O'Rourke MF, Eber B. Arterial stiffness, central blood pressures, and wave reflections in cardiomyopathy-implications for risk stratification. *J Card Fail.* 2007;13(5):353-359.

6. Safar ME, Thomas F, Blacher J, et al. Metabolic syndrome and age-related progression of aortic stiffness. *J Am Coll Cardiol.* 2006;47(1):72-75.

7. Scuteri A, Cunha PG, Rosei EA, et al. Arterial stiffness and influences of the metabolic syndrome: a cross-countries study. *Atherosclerosis.* 2014;233(2):654-660.

8. Donley DA, Fournier SB, Reger BL, et al. Aerobic exercise training reduces arterial stiffness in metabolic syndrome. *J Appl Physiol.* 2014;116(11):1396-1404.

9. Montero D, Roberts CK, Vinet A. Effect of aerobic exercise training on arterial stiffness in obese populations : a systematic review and meta-analysis. *Sports Med.* 2014;44(6):833-843.

10. Tjonna AE, Lee SJ, Rognmo O, et al. Aerobic interval training versus continuous moderate exercise as a treatment for the metabolic syndrome: a pilot study. *Circulation.* 2008;118(4):346-354.

11. Grassi G, Seravalle G, Brambilla G, et al. Impact of the metabolic syndrome on subcutaneous microcirculation in obese patients. *J Hypertens.* 2010;28(8):1708-1714.

12. Rizzoni D, De Ciuceis C, Porteri E, Semeraro F, Rosei EA. Structural alterations in small resistance arteries in obesity. *Basic Clin Pharmacol Toxicol.* 2012;110(1):56-62.

13. Ingelsson E, Sullivan LM, Murabito JM, et al. Prevalence and prognostic impact of subclinical cardiovascular disease in individuals with the metabolic syndrome and diabetes. *Diabetes.* 2007;56(6):1718-1726.

14. Edgell H, Petrella RJ, Hodges GJ, Shoemaker JK. Central versus peripheral cardiovascular risk in metabolic syndrome. *Front Physiol.* 2012;3:38.

15. Safar ME, Balkau B, Lange C, et al. Hypertension and vascular dynamics in men and women with metabolic syndrome. *J Am Coll Cardiol.* 2013;61(1):12-19.

16. Begg C, Cho M, Eastwood S, et al. Improving the quality of reporting of randomized controlled trials. The CONSORT statement. *JAMA.* 1996;276(8):637-639.

17. Mora-Rodriguez R, Ortega JF, Hamouti N, et al. Time-course effects of aerobic interval training and detraining in patients with metabolic syndrome. *Nutr Metab Cardiovasc Dis.* 2014;24(7):792-798.

18. Stensvold D, Tjonna AE, Skaug EA, et al. Strength training versus aerobic interval training to modify risk factors of metabolic syndrome. *J Appl Physiol.* 2010;108(4):804-810.

19. Schjerve IE, Tyldum GA, Tjonna AE, et al. Both aerobic endurance and strength training programmes improve cardiovascular health in obese adults. *Clin Sci (Lond).* 2008;115(9):283-293.

20. Chen CH, Ting CT, Nussbacher A, et al. Validation of carotid artery tonometry as a means of estimating augmentation index of ascending aortic pressure. *Hypertension.* 1996;27(2):168-175.

21. Wilkinson IB, MacCallum H, Flint L, Cockcroft JR, Newby DE, Webb DJ. The influence of heart rate on augmentation index and central arterial pressure in humans. *J Physiol.* 2000;525 Pt 1:263-270.

22. Keymel S, Sichwardt J, Balzer J, et al. Characterization of the non-invasive assessment of the cutaneous microcirculation by laser Doppler perfusion scanner. *Microcirculation.* 2010;17(5):358-366.

23. Yvonne-Tee GB, Rasool AH, Halim AS, Rahman AR. Reproducibility of different laser Doppler fluximetry parameters of postocclusive reactive hyperemia in human forearm skin. *J Pharmacol Toxicol Methods.* 2005;52(2):286-292.

24. Roustit M, Cracowski JL. Non-invasive assessment of skin microvascular function in humans: an insight into methods. *Microcirculation.* 2012;19(1):47-64.

25. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem.* 1972;18(6):499-502.

26. Cohen J. Statistical Power Analysis for the Behavioural Sciences. In: 2nd ed. New Jersey: Lawrence Erlbaum Associates; 1988:569.

27. Czernichow S, Greenfield JR, Galan P, et al. Macrovascular and microvascular dysfunction in the metabolic syndrome. *Hypertens Res.* 2010;33(4):293-297.

28. Vaitkevicius PV, Fleg JL, Engel JH, et al. Effects of age and aerobic capacity on arterial stiffness in healthy adults. *Circulation.* 1993;88(4 Pt 1):1456-1462.

29. Najjar SS, Scuteri A, Shetty V, et al. Pulse wave velocity is an independent predictor of the longitudinal increase in systolic blood pressure and of incident hypertension in the Baltimore Longitudinal Study of Aging. *J Am Coll Cardiol.* 2008;51(14):1377-1383.

30. Mitchell GF. Effects of central arterial aging on the structure and function of the peripheral vasculature: implications for end-organ damage. *J Appl Physiol.* 2008;105(5):1652-1660.

31. Mitchell GF, Vita JA, Larson MG, et al. Cross-sectional relations of peripheral microvascular function, cardiovascular disease risk factors, and aortic stiffness: the Framingham Heart Study. *Circulation.* 2005;112(24):3722-3728.

32. Mora-Rodriguez R, Ortega JF, Guio de Prada V, et al. Effects of Simultaneous or Sequential Weight Loss Diet and Aerobic Interval Training on Metabolic Syndrome. *Int J Sports Med.* 2015.

33. Stevens VJ, Obarzanek E, Cook NR, et al. Long-term weight loss and changes in blood pressure: results of the Trials of Hypertension Prevention, phase II. *Ann Intern Med.* 2001;134(1):1-11.

34. Jonk AM, Houben AJ, de Jongh RT, Serne EH, Schaper NC, Stehouwer CD. Microvascular dysfunction in obesity: a potential mechanism in the pathogenesis of obesity-associated insulin resistance and hypertension. *Physiology (Bethesda).* 2007;22:252-260.

35. Currie KD, McKelvie RS, Macdonald MJ. Flow-mediated dilation is acutely improved after high-intensity interval exercise. *Med Sci Sports Exerc.* 2012;44(11):2057-2064.

36. Mitchell GF, Parise H, Vita JA, et al. Local shear stress and brachial artery flow-mediated dilation: the Framingham Heart Study. *Hypertension.* 2004;44(2):134-139.

37. Binggeli C, Spieker LE, Corti R, et al. Statins enhance postischemic hyperemia in the skin circulation of hypercholesterolemic patients: a monitoring test of endothelial dysfunction for clinical practice? *J Am Coll Cardiol.* 2003;42(1):71-77.

38. Gokce N, Duffy SJ, Hunter LM, Keaney JF, Vita JA. Acute hypertriglyceridemia is associated with peripheral vasodilation and increased basal flow in healthy young adults. *Am J Cardiol.* 2001;88(2):153-159.

39. Serne EH, Stehouwer CD, ter Maaten JC, et al. Microvascular function relates to insulin sensitivity and blood pressure in normal subjects. *Circulation.* 1999;99(7):896-902.

40. Sattar N, Gaw A, Scherbakova O, et al. Metabolic syndrome with and without C-reactive protein as a predictor of coronary heart disease and diabetes in the West of Scotland Coronary Prevention Study. *Circulation.* 2003;108(4):414-419.

41. Chen YW, Apostolakis S, Lip GY. Exercise-induced changes in inflammatory processes: Implications for thrombogenesis in cardiovascular disease. *Ann Med.* 2014;46(7):439-455.

FIGURE CAPTIONS

Figure 1. CONSORT schematic representation of the study procedures.

Figure 2. Changes in A) pulse wave velocity (PWV) from the carotid to the femoral artery and B) augmentation index at 75 heart rate (AI@75HR) in the TRAIN (*N*=23) and CONT (*N*=23) groups before and after 6 months of treatment. Data are means ± SD. * Significant difference from baseline within that group. † Significantly different from CONT at that time point (all; *P*<0.05).

Figure 3. Changes in A) the time to peak flow and B) peak forearm reactive hyperemia flow (% of baseline) in the TRAIN (*N*=23) and CONT (*N*=23) groups before and after 6 months of treatment. Data are means ± SD. * Significant difference from baseline within that group. † Significantly different from CONT at that time point (all; *P*<0.05).

Table 1. Evolution of metabolic syndrome factors and prevalence of hypertension in the TRAIN and CON groups.

	TRAIN (*N*=23)		CONT (*N*=23)		TRAIN vs CONT at Baseline
	Baseline	**Follow-up**	**Baseline**	**Follow-up**	***P* value**
Waist circumference (cm)	106±7	104±6 *†	108±6	109±5	0.191
HDL-cholesterol (mmol·L^{-1})	0.93±0.18	0.98±0.24	0.89±0.15	0.90±0.21	0.205
Glucose (mmol·L^{-1})	6.42±1.36	6.35±1.60	6.11±0.75	6.18±0.69	0.332
Triglycerides (mmol·L^{-1})	1.42±0.86	1.44±0.98	1.44±0.98	1.34±0.63	0.938
Systolic Blood Pressure (mmHg)	136±17	127±12 *†	138±16	140±12	0.709
Diastolic Blood Pressure (mmHg)	84±10	77±6 *†	84±11	83±8	0.777
Prevalence of hypertension in % (95% CI)	79.3 (59.2-91.0)	41.4 (23.9-61.3)	76.5 (56.2-89.2)	82.4 (62.6-92.9)	

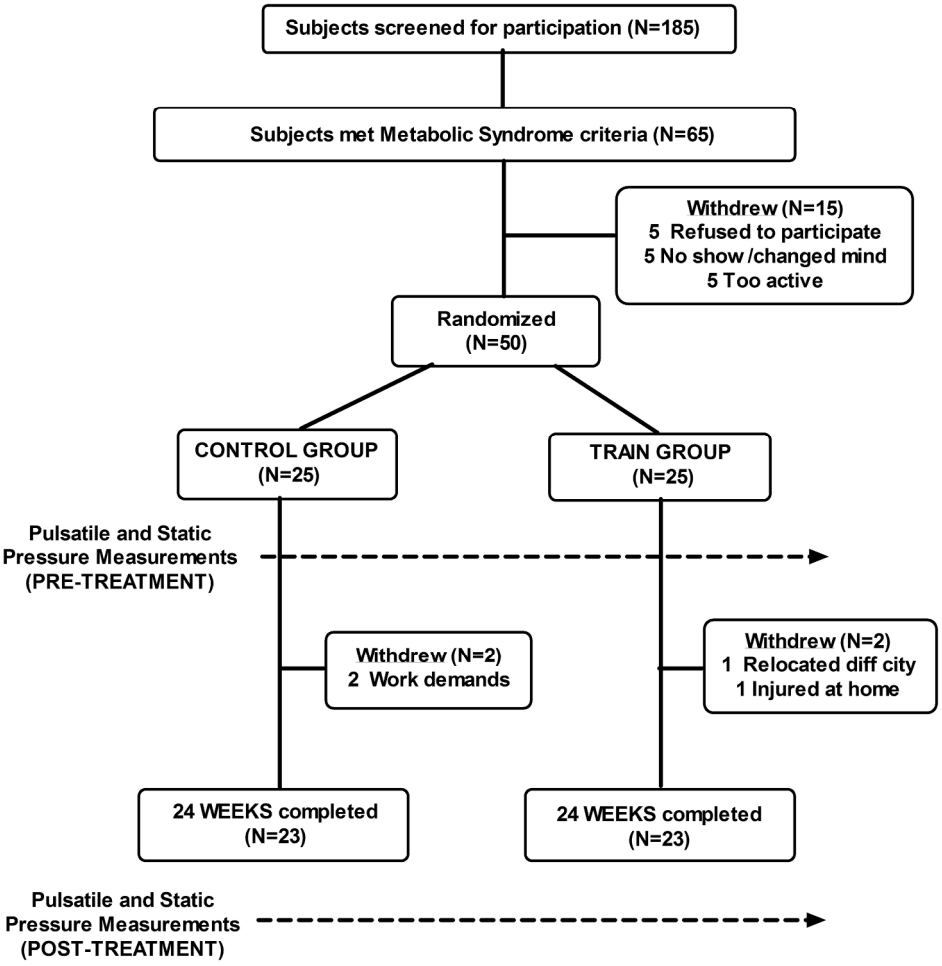
Data are presented as mean ± SD for 46 metabolic syndrome patients, divided into the TRAIN and CONT groups. Systolic and diastolic blood pressures measured by ECG gated sphygmomanometer at the brachial artery. * Significantly different from Baseline within that group. † Significantly different from CONT at that time point.

Table 2. Blood parameters associated with viscosity, prothrombotic state and arterial stiffness.

	TRAIN (*N*=23)		CONT (*N*=23)		TRAIN vs. CONT at Baseline
	Baseline	Follow-up	Baseline	Follow-up	P value
Erythrocytes ($10E^6 \cdot \mu l^{-1}$)	4.88±0.4	4.84±0.4	5.03±0.3	4.94±0.3	0.191
Platelets ($10E^3 \cdot \mu l^{-1}$)	242±52	238±44	228±39	223±28	0.228
Leukocytes ($10E^3 \cdot \mu l^{-1}$)	5.9±1.3	6.5±1.7	5.5±1.1	5.9±2.3	0.312
Fibrinogen ($\mu mol \cdot L^{-1}$)	8.4±2.0	8.2±1.3	8.3±2.3	8.1±1.1	0.906
hs CRP ($\eta mol \cdot L^{-1}$)	28±21	28±21	22±18	25±22	0.278
Insulin ($\rho mol \cdot L^{-1}$)	87±32	82±36	91±36	93±38	0.725
Total-cholesterol ($mmol \cdot L^{-1}$)	4.8±0.8	4.7±1.0	4.7±0.8	4.9±0.7	0.758
LDL-cholesterol ($mmol \cdot L^{-1}$)	3.2±0.8	3.0±0.9	3.1±0.8	3.2±0.5	0.831

Data are presented as mean ± SD for 46 metabolic syndrome patients divided into the TRAIN and CONTROL groups. hs CRP stands for high-sensitive C reactive protein.

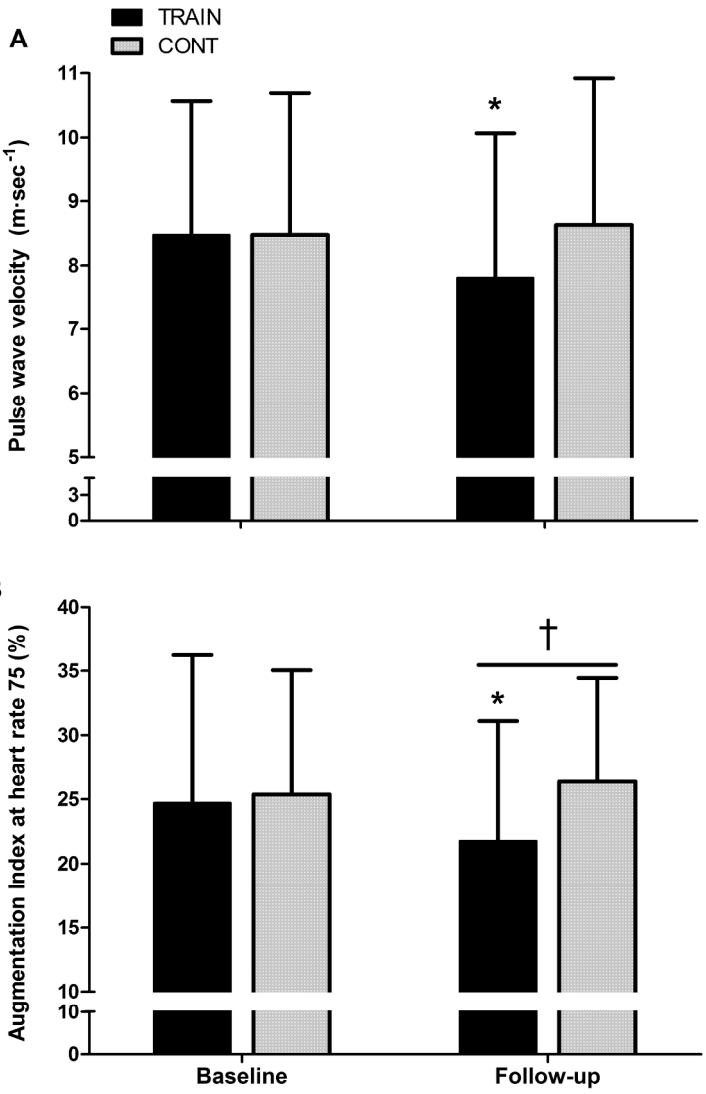
Figure 1



CONSORT schematic representation of the study procedures.

224x277mm (300 x 300 DPI)

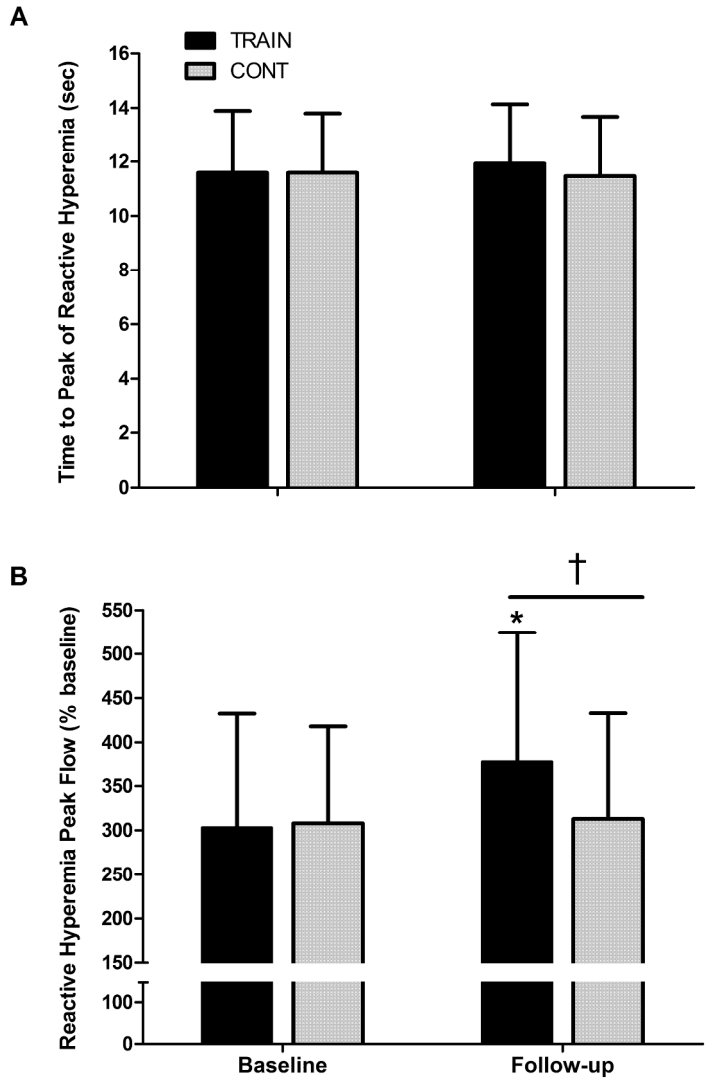
Figure 2



Changes in A) pulse wave velocity (PWV) from the carotid to the femoral artery and B) augmentation index at 75 heart rate (AI@75HR) in the TRAIN (N=23) and CONT (N=23) groups before and after 6 months of treatment. Data are means ± SD. * Significant difference from baseline within that group. † Significantly different from CONT at that time point (all; P<0.05).

254x350mm (300 x 300 DPI)

Figure 3



Changes in A) the time to peak flow and B) peak forearm reactive hyperemia flow (% of baseline) in the TRAIN (N=23) and CONT (N=23) groups before and after 6 months of treatment. Data are means ± SD. * Significant difference from baseline within that group. † Significantly different from CONT at that time point (all; $P<0.05$).

258x366mm (300 x 300 DPI)