The effect of two exercise modalities on skeletal muscle capillary ultrastructure in individuals with type 2 diabetes

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Running title: High-intensity training, capillary ultrastructure and type 2 diabetes

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Abstract

Type 2 diabetes is associated with microvascular dysfunction, but little is known about how capillary ultrastructure is affected by exercise training. To investigate the effect of two types of exercise training on skeletal muscle capillary ultrastructure and capillarization in individuals with type 2 diabetes, 21 individuals with type 2 diabetes were allocated (randomized controlled trial) to 11 weeks of aerobic exercise training consisting of either moderate-intensity endurance training (END; n=10), or low-volume high-intensity interval training (HIIT; n=11). Skeletal muscle biopsies (m vastus lateralis) were obtained before and after the training intervention. At baseline, there was no difference in capillarization, capillary structure and exercise hyperaemia between the two groups. After the training intervention, capillary-to-fiber ratio increased by 8±3% in the END group (P<0.05) and was unchanged in the HIIT group with no difference between groups. Endothelium thickness increased (P<0.05), basement membrane thickness decreased (P<0.05) and the capillary lumen tended (P=0.07) to increase in the END group, whereas these structural indicators were unchanged after HIIT. In contrast, skeletal muscle endothelial nitric oxide synthase (eNOS) increased after HIIT (P<0.05), but not END, whereas there was no change in vascular endothelial growth factor (VEGF), superoxide dismutase (SOD)-2 or NADPH oxidase after both training protocols. In contrast to END training, HIIT did not alter capillarization or capillary structure in individuals with type 2 diabetes. In conclusion, HIIT appears to be a less effective strategy to treat capillary rarefaction and reduce basement thickening in type 2 diabetes.
**Key words:** Endothelium, capillary, microcirculation

ClinicalTrials.gov ID no: NCT02001766

**INTRODUCTION**

The prevalence of type 2 diabetes (T2D) is rapidly growing, which has led to an estimate that the number of T2D patients will increase to 439 million worldwide by 2030\(^1\). T2D is associated with an increased risk of cardiovascular disease, which is mainly caused by structural defects of larger vessels, but they can also be due to changes in the integrity of the microcirculation. Pathological processes caused by microcirculatory degeneration are designated diabetic microangiopathies which may affect the capillary structure and function in many tissues and organs throughout the body. Changes in microvasculature can alter tissue perfusion and substrate delivery (such as \(O_2\) and glucose)\(^2\), particularly affecting organs heavily dependent on their microvasculature supply. The most prominent examples of such diabetic microangiopathies are nephropathy in the kidney, retinopathy in the eye and neuropathy of the nervous system. In the lower limbs, diabetic microangiopathies may be involved in the etiology of peripheral vascular disease, with degeneration of capillaries and an associated poor wound healing and potentially leading to even amputation\(^3\).

The organ- and tissue specific diabetic microangiopathies may be influenced by a diversity of pathogenic mechanisms that appear to depend upon the architecture of the tissues and the topology of the capillary system. For the kidney, pericapillary basement membrane (CBM) thickening is elicited mainly by the interplay between metabolic factors in the glomerulum acting on the epithelial podocytes and changes in the hemodynamic forces acting on the capillary endothelial cells\(^4\). In the retina, on the other hand, hypoxia appears to be important for the initiation of the angiogenesis\(^5\). The pathophysiology accounting for the onset of the diabetic microangiopathy in skeletal muscles in T2D patients has been less intensively characterized, but an increase in CBM thickness is a central issue.
that appears to be influenced by a combination of various factors including glucose-dependent hyperosmosis, increased hydrostatic pressure and an inflammatory state.

Exercise training represents a well-described intervention to improve glycemic control in individuals with T2D. However, little is known about the effects of exercise training in general, and especially HIIT, on capillaries in T2D. In insulin-dependent diabetes, a combination of training and long-term insulin control and diet has been found to reduce the CBM thickening in individuals with type I diabetes, and in elderly individuals with T2D, a nine month exercise intervention lead to thinning of the CBM. In recent years, high-intensity interval training (HIIT) has been found to be a preferable time-efficient exercise modality to improve glycemic control in individuals with T2D. However, the effect of HIIT on the vasculature in T2D remains largely unexplored.

The aim of the present study was to evaluate the effect of 11 weeks of low-volume HIIT compared with moderate-intensity endurance training (END) on skeletal muscle capillary ultrastructure, capillarization and leg blood flow during exercise in T2D patients. We hypothesized that both exercise modalities would reduce CBM thickness. Skeletal muscle CBM thickness was the primary outcome, while capillarization, protein content and leg blood flow were secondary outcomes.

Methods

Participants

Twenty-one individuals with type 2 diabetes were studied. Before inclusion, all participants underwent a standardized medical check including blood chemistry analysis, a resting 12-lead electrocardiogram and an oral glucose tolerance test (OGTT). Exclusion criteria were exogenous insulin treatment, smoking, unstable weight (change >5 kg/6 months), any illness that contraindicated...
physical activity, evidence of renal, liver or cardiovascular disease. All participants were in stable treatment for their T2D and none of the participants changed their medication during the study. The study was approved by the Ethics Committee of the Capital Region of Denmark (H-2-2011-070), and signed informed consent was obtained from all participants before enrolment into the study.

**Experimental design**

After baseline tests, the included participants were given opaque sealed envelopes randomly allocating them to either END (n=10) or low-volume HIIT (n=11). The participants were studied on two experimental days (A and B), separated by one day before and after an 11 week training period. Data presented in the current manuscript are from day B, whereas data on glycemic control and body composition obtained from day A and B and have been reported previously. Before and after the exercise intervention, VO\textsubscript{2}peak (Cosmed Quark b, Rome, Italy) was determined by an incremental cycling test on a bicycle ergometer (839E; Monark, Varberg, Sweden). Body mass was calculated from whole-body dual-energy x-ray absorptiometry scanning (Prodigy, GE Healthcare).

**Exercise interventions**

Participants entered an 11-week bicycle intervention consisting of either 40 min/session (END) or 20 min/session (HIIT), 3 days/week. Each training session was initiated with a brief 5 minute standardized warm-up (40% of W\textsubscript{peak}), where after participants in the END group performed 40 minutes of cycling at 50% of W\textsubscript{peak} and participants in HIIT performed 20 minutes of cycling consisting of 10 times 1 min at 95% W\textsubscript{peak} and 1 minute of active recovery (20% W\textsubscript{peak}). Heart rate was measured continuously during each training session (Team\textsuperscript{2} system, Polar, Kempele, Finland). When including the warm-up, the total duration of the exercise protocol was 75 min per week in HIIT group and 135 min per week in the END group.
Experimental day

Participants refrained from caffeine and alcohol intake for 24 hour and exercise for 48 hour before the experiment. On the day of the experiment the participants refrained from taking their anti-diabetic medication and arrived in a fasting state (≥10 h) at 08.00 am. A muscle biopsy was obtained from m. vastus lateralis of the experimental leg using the Bergström-technique under local anesthesia (lidocaine 20%). The biopsies were dived in two: One part was immediately frozen in liquid nitrogen and stored at -80°C until further analysis. The second part was chemically fixed in a 6.25% (v/v) glutaraldehyde solution buffered with 0.1 M sodium cacodylate-HCl (pH 7.4) and stored at 4°C until analysis. After 2 h of supine rest, the subjects performed 1 h of one-legged knee extensions (6W).

Measurements and analysis

Capillarization assessed by light microscopy

The chemically fixed biopsies were divided into 2-3 pieces, each with a volume of approximately 0.5 mm³, after which they were post-fixed in 1% (w/v) OsO₄, stained en bloc and embedded in Epon 812 (Fluka, Buchs, Switzerland), as previously described. One-micrometer thick semi-thin sections were cut using diamond knives and stained with toluidine blue. Transverse or slightly oblique sections through the muscle (size of approximately 1mm²) were cut from two randomly selected Epon blocks for the morphometric analysis. Obliquely sectioned blocks were re-sectioned after rotation if the smallest and widest diameters of the majority of the muscle fibers differed more than 10% (which corresponds to a slope in the cutting direction of about 6°). Using systematic sampling strategy 10 photographs of each section at a magnification of x63 in a Leica DMR light microscope (Leica Microsystems, Heerbrugg, Switzerland) were acquired. Using these light micrographs, the number of muscle fibers and capillaries were quantified taking into account the forbidden line rule. The mean cross-sectional fiber area was estimated by point counting, and capillary profile density and capillary-to-fiber ratio were calculated, as previously described.
Capillary ultrastructure assessed by transmission electron microscopy

Ultra-thin sections (80-90 nm in thickness) of the Epon blocks were prepared with an Ultracut ultramicrotome (Reichert-Jung, Bensheim, Germany), floated on 200-mesh molybdenum grids (Plano, Wetzlar, Germany) and contrasted with uranyl acetate and lead citrate, as previously described15. The inspection was carried out using a transmission electron microscope (Philips EM 400)14.

Morphometry analysis

Ultra-thin sections from two randomly selected Epon-embedded blocks of each muscle biopsy were used for the morphometric analysis. For the quantification of the capillary ultrastructure, 17-20 randomly selected capillaries were photographed on each of the ultrathin sections in the TEM at a final magnification of x8.900 using a MORADA digital camera (OSIS, Münster, Germany).

Photo-images of the capillaries were opened in ImageJ using a touch-sensitive computer system. Lines were drawn with a digital pen around the lumen (lumen/endothelial cell-transition), along the abluminal endothelial cell (EC) surface (EC/BM-transition), at the CBM/endomysium transition and around the pericyte (PC) surface of the capillaries on the micrographs, as previously described 17. By processing with ImageJ, the values for the areas (Alumen, AEC/BM, ABM/Endo, APC) and perimeters=circumferences (Clumen, CEC/CBM, CBM/Endo, CPC) of the structures of interest were obtained and then computed to gain structural indicators that describe quantitatively the compartmental capillary ultrastructure: the mean cross-sectional area (A) of the capillary and each of its compartment, the mean area density (AA) of each compartment relative to the capillary area ABM/Endo and the mean arithmetic circumference (C). The mean arithmetic thickness (T) was calculated as A / C and computed as T (lumen) = 2 * Alumen / Clumen, T (EC) = 2 * [(AEC/BM - Alumen) / (CEC/BM + Clumen)], T (PC) = 2 * Apc / Cps and T (BM) = 2 * [(ABM/Endo - AEC/BM - APC) / (CBM/Endo + CEC/BM + CPC)]17.

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**Quantification of protein content in skeletal muscle**

The muscle biopsies were freeze-dried and dissected free of non-muscle tissue. The samples were homogenized in a fresh batch of lysis buffer (Qiagen Tissuelyser II; Retsch, Haan, Germany) and then centrifuged. The supernatant (lysate) was used for analysis. The concentration of total protein in the lysate was determined in triplicate by BCA protein assay (Pierce Biotechnology Inc, Rockford, IL, USA).

**VEGF protein analysis**

VEGF protein in the muscle lysates were determined by electrochemiluminescence assay kit for VEGF_{165} (Meso Scale Diagnostics, Maryland, USA) according to the manufacturer’s guidelines. The values were normalized to the total protein content in each sample.

**eNOS, SOD-2 and NADPH oxidase analysis**

Lysate proteins were separated using SDS gels (Bio-Rad Laboratories) and transferred (semidry) to PVDF membranes (Immobilon Transfer Membrane, Millipore). The membranes were incubated with primary antibodies to eNOS (610297 BD Transduction Laboratories), SOD-2 (#06-984, Millipore, Billerica, US), NADPH oxidase (#610912, BD transduction, San Jose, Ca, USA) or GAPDH (ab9484, Abcam, Cambridge, UK). Secondary antibody horseradish-peroxidase-conjugated goat anti-rabbit (P-0448, Dako, Glostrup, Denmark) or horseradish-peroxidase-conjugated goat anti mouse (Jackson ImmunoResearch, USA) was used for detection of the proteins. Subsequent to exposure (Kodak Image Station, 2000MM) and quantification (Kodak Molecular Imaging software), the protein content was expressed in arbitrary units related to human standards.
Leg blood flow

Leg blood flow was measured at rest and during exercise (15, 30, 45 and 60 min) with an ultrasound Doppler technique (Logic E9, GE Healthcare, Pittsburgh, PA, USA) equipped with a probe operating an imaging frequency of 9MHz and Doppler frequency of 4.2–5.0MHz. The site of blood flow velocity was measured in a common femoral artery distal to the inguinal ligament but above the bifurcation into the superficial and profound femoral branch to avoid turbulence from the bifurcation. All recordings were assessed using an insonation angle ≤60°. The sample volume was maximized according to the width of the vessel, and kept clear of the vessel walls. A low-velocity filter (velocities <1.8ms⁻¹) rejected noise caused by turbulence at the vascular wall. Doppler tracings and B-mode images were recorded continuously, and Doppler tracings were averaged over ~8 heart cycles at the time of blood sampling. The femoral artery diameter during the systole was determined under a perpendicular insonation angle at supine and seated rest, assuming negligible changes in vessel-diameter during exercise.

Statistical analyses

Baseline differences were tested using a one-way ANOVA. To test for the effect of training within the two groups, a two-way repeated measure ANOVA was conducted (SigmaPlot v12.5, Systat Software Inc., USA). To test for the effect of training between the two groups, a two-way ANOVA was conducted. After a significant time, group or time by group interaction, pairwise differences were identified using the Holm-Sidak post hoc procedure. Data are presented as means with standard deviation (SD). Due to insufficient sampling material, capillary ultrastructure was only analyzed in 18 out of the 21 participants.
Results

Participant characteristics

There was no difference in baseline characteristics before the intervention (Table 1). After the training interventions, VO₂peak was improved in both groups, whereas HbA1c, resting heart rate and body mass were only reduced in the HIIT group (P<0.05).

Capillaryization and leg blood flow

Skeletal muscle capillary area and capillary density did not change with exercise training in either group (Table 2). Capillary-to-fiber ratio increased with END training (P<0.05), whereas it did not change with HIIT. No difference was observed between groups. There was no difference in resting and exercising leg blood flow within or between groups.

Capillary ultrastructure

After END training, mean arithmetic thickness of endothelial cells was increased by 30±27% (P<0.05), whereas the thickness of the basement membrane was reduced by 21±7% (P<0.05) and capillary lumen tended (P=0.07) to be increased (Figure 1 + 2). After HIIT, no change in mean arithmetic thicknesses of endothelial cells, CBM, pericytes or capillary lumen were observed.

Quantitative expression patterns

There were no differences in VEGF, SOD-2, NADPH oxidase within or between groups (Figure 3 + 4). eNOS protein content increased with exercise training in the HIIT group, whereas no difference was observed in the END group (figure 5).
Discussion

The aim of this investigation was to compare the effect of 11 weeks of END training and HIIT on capillarization and capillary ultrastructure in vastus lateralis (VL) muscle biopsies of T2D patients. We furthermore studied whether the expression levels of proteins with known impact on pericapillary basement membrane (CBM) growth in skeletal muscles were affected by the training regimes. The major observations were that: 1. END training, but not HIIT, resulted in an increased capillary endothelial cell thickness, a reduced CBM thickness and tendency toward a larger capillary lumen, 2. END training, but not HIIT, resulted in an increased capillary-to-fiber ratio, and 3. HIIT, but not END, training led to a higher eNOS protein expression. There were no changes in the skeletal muscle expression of VEGF-A, NADPH oxidase or SOD-2 protein with either HIIT or END training.

Type 2 diabetes is associated with a thickening of the CBM, that is thought to impair the exchange of substrates across the vessel. An important observation was that END training lowered the thickness of the CBM by 21%. The thinning of the basement membrane with END training is in agreement with previous studies showing an ~35% reduction in CBM thickness after 9 month training in individuals with T2D, a ~17% reduction in hypertensive patients and a ~22% reduction in non-diseased persons after eight weeks of ergometer training. The outcome of these investigations indicates that END training generally reduces CBM thickness.

We have used a recently devolved morphometric approach based on tablet-based image analysis to quantify the capillary phenotype in skeletal muscles of diabetic patients before and after END and HIIT. In contrast to previously established morphometric methods, our technique delivers not only data on the thickness of the CBM but facilitates also the simultaneous assessment of several other structural indicators specific for the capillary phenotype. Thus, the findings of our study go beyond the data previously published on capillary ultrastructure in skeletal muscle of diabetic patients. An interesting observation was the tendency towards a larger capillary lumen after END training.
training, but not HIIT. The effect of exercise training of T2D patients on the capillary lumen in skeletal muscle has not previously been reported. However, findings in rats suggest that exercise training prevents the decrease in luminal diameter usually observed in T2D\textsuperscript{21}. We suggest that the continuous high levels of blood flow through the skeletal muscle capillaries during endurance exercise-induced a passive expansion of the capillary lumen\textsuperscript{22}.

Another finding was that the endothelial cell (EC) profiles of the capillaries were more expanded after training in VL biopsies of T2D patients after END but not after HIIT. Substantial EC swelling of capillaries in skeletal muscle was repetitively shown to be a systemic response triggered by a local ischemic event in humans\textsuperscript{23,24} and rodents\textsuperscript{15,25,26}. Furthermore, amiloride (a Na\textsuperscript{+}/H\textsuperscript{+} exchange blocker) prevented this reaction after ligation-induced ischemia and mild electrical stimulation suggesting that EC swelling is related to acidosis\textsuperscript{27}.

We found that END training, but not HIIT, increased the capillary-to-fiber ratio in the VL of the participants. This is in agreement with previous reports showing that endurance training triggers capillary growth in skeletal muscle of healthy individuals\textsuperscript{28,29}, individuals with essential hypertension\textsuperscript{14} as well as T2D patients\textsuperscript{30}. The effect of HIIT on capillarization in skeletal muscle has been studied less intensively. In accordance with our findings, a study with healthy individuals indicated that HIIT only represents a weak stimulus for angiogenesis\textsuperscript{31}. In contrast, HIIT interval-style soccer training showed a 7%-increase of the capillary-to-fiber ratio in T2D patients\textsuperscript{32}. It is likely that the 45% lower training volume and ~36% lower training-derived metabolic demand with HIIT in the present study resulted in an insufficient stimulus for the induction of angiogenesis compared to END training. Conclusively, we suggest that longer END training periods with moderate elevated blood flow rather than HIIT with short periods with peak leg blood flow are required to induce angiogenesis in skeletal muscles of individuals with T2D.
Investigations with animal models and studies analyzing diabetic patients with retinopathy or nephropathy have led to the identification of several molecular systems underlying BM-thickening. Higher levels of reactive oxygen species (ROS) and nitric oxide (NO) may directly contribute as redox-sensitive transcription factors to the thickening of the CBM. In addition, ROS may activate five major biochemical processes thereby acting as indirect modulator of BM thickening. The most important of these ROS-regulated processes is glycation, which refers to the non-catalytic appendage of monosaccharide moieties to proteins. For example, glycation augments the cross-linkage in type-IV collagen which renders it more resistant to degradation and turnover. The levels of ROS and NO in skeletal muscle are essentially controlled by eNOS and SODs and NADPH-oxidase, which thus may be important for the establishment of an enlarged CBM in T2D patients. VEGF-A is another factor that has an impact on the CBM accumulation, since treatment with anti-VEGF antibodies attenuated glomerular CBM thickening and mesangial matrix expansion. Because these proteins are involved in CBM thickening in skeletal muscle, we hypothesized their expression levels are down-regulated in diabetic patients if the training is accompanied with thinning of CBM. To validate this hypothesis, we determined the levels of eNOS, SOD-2, NADPH-oxidase and VEGF in the pre- and post-exercise muscle biopsies of the T2D patients that underwent either END training or HIIT. The immunoblot analysis revealed that with exception of eNOS in the extracts of the patients undergoing HIIT the quantitative expression patterns of the four proteins were not significantly different suggesting that these proteins are not regulated in skeletal muscle by the training regimes. It cannot be excluded, however, that the time between the last training bout and the obtainment of the muscle biopsies (3-5 days) was too long to detect an effect of the exercise training and that the VO2peak test 48 hours before the biopsy masked any differences between the groups. Alternatively, other molecules than those being established players in the CBM growth could be responsible for the reduction of the CBM in skeletal muscle of T2DB patients after END training. One candidate for such a crucial role in CBM remodeling is MMP-2, which has been shown to be up-regulated in VL muscles of T2D patients after 8 weeks of endurance exercise. Increased eNOS levels after HIIT are in
agreement with the observation that eNOS expression is rapidly and sustainably susceptible to alterations in blood flow which may vary more during HIIT with short periods of peak leg blood flow than during END training periods with moderate elevated blood flow. However, since HIIT was not accompanied by thinner CBM, this up-regulation of eNOS is presumably not related to the growth of this entity.

In conclusion, HIIT did not alter capillarization and capillary ultrastructure in individuals with T2D. In contrast, END training induced angiogenesis, increased endothelial thickness and reduced basement membrane thickness.

**Perspective**

Low-volume HIIT appears to be effective in improving glycemic control in individuals with T2D\textsuperscript{11,12}, but compared to endurance training, HIIT appears to be a less effective strategy to treat the capillary rarefaction and thickening of the CBM observed in T2D. A possible explanation is the lower training volume during HIIT compared to endurance training, and combination endurance training and HIIT may therefore be needed to optimally treat the impaired glycemic control and the microvascular complications associated with type 2 diabetes.

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Not applicable

**Declaration of interests**

The authors declare that they have no competing interests

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Authors’ contributions

Conception or design of the work: SPM, KMW and BKP.

Acquisition, analysis or interpretation of data for the work: SPM, KMW, UWI, GMW, NM, YH and OB

Drafting the work or revising it critically for important intellectual content: SPM, KMW, UWI, GMW, NM, YH, BKP and OB

All authors approved the final version of the manuscript.

Reference list


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Table 1 - Participant characteristics before and after 11 weeks of endurance training (END) or high-intensity interval training (HIIT).

<table>
<thead>
<tr>
<th></th>
<th>END Before</th>
<th>END After</th>
<th>HIIT Before</th>
<th>HIIT After</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>n (male/female)</strong></td>
<td>10 (7/3)</td>
<td>11 (6/5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Age (years)</strong></td>
<td>57±9</td>
<td>53±7</td>
<td></td>
<td></td>
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<tr>
<td><strong>Time since diagnosis (years)</strong></td>
<td>5±4</td>
<td>7±4</td>
<td></td>
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<tr>
<td><strong>Medication</strong></td>
<td></td>
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<tr>
<td>Metformin</td>
<td>8</td>
<td>9</td>
<td></td>
<td></td>
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<tr>
<td>DPP-4 inhibitor</td>
<td>0</td>
<td>2</td>
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<td>Sulfonylureas</td>
<td>1</td>
<td>3</td>
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<tr>
<td>GLP-1 analogues</td>
<td>0</td>
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<tr>
<td>No medication</td>
<td>1</td>
<td>0</td>
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</tr>
<tr>
<td><strong>HbA1c (%)</strong></td>
<td>6.9±0.9</td>
<td>6.8±0.8</td>
<td>6.8±0.9</td>
<td>6.6±0.9*</td>
</tr>
<tr>
<td><strong>VO2peak</strong></td>
<td></td>
<td></td>
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<tr>
<td>Absolute (L/min)</td>
<td>2.4±0.5</td>
<td>2.6±0.5*</td>
<td>2.5±0.5</td>
<td>2.8±0.7**</td>
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<tr>
<td>Relative (mL/kg/min)</td>
<td>28±6</td>
<td>31±8*</td>
<td>29±6</td>
<td>35±7**</td>
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<tr>
<td><strong>Blood pressure</strong></td>
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<tr>
<td>Systolic (mmHg)</td>
<td>133±18</td>
<td>137±26</td>
<td>142±14</td>
<td>140±17</td>
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<tr>
<td>Diastolic (mmHg)</td>
<td>83±7</td>
<td>80±10</td>
<td>85±5</td>
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<tr>
<td><strong>Resting heart rate</strong></td>
<td>67±13</td>
<td>61±9</td>
<td>67±12</td>
<td>62±10*</td>
</tr>
<tr>
<td><strong>Body composition</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body mass (kg)</td>
<td>86±11</td>
<td>85±11</td>
<td>85±12</td>
<td>84±12**</td>
</tr>
<tr>
<td>Body Fat (%)</td>
<td>28±6</td>
<td>27±6</td>
<td>29±8</td>
<td>28±8</td>
</tr>
<tr>
<td>Experimental leg mass (kg)</td>
<td>12.6±1.9</td>
<td>12.6±2.1</td>
<td>12.1±1.7</td>
<td>12.1±1.7</td>
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<td><strong>Lipids</strong></td>
<td></td>
<td></td>
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<tr>
<td>Total cholesterol (mmol/L)</td>
<td>4.8±1.1</td>
<td>4.7±1.1</td>
<td>4.9±1.0</td>
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<td>HDL cholesterol (mmol/L)</td>
<td>1.3±0.4</td>
<td>1.2±0.3</td>
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<td>LDL cholesterol (mmol/L)</td>
<td>3.0±0.9</td>
<td>2.8±1.0</td>
<td>2.8±1.0</td>
<td>2.7±0.9</td>
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<td>Triglycerides (mmol/L)</td>
<td>1.3±0.5</td>
<td>1.8±1.0</td>
<td>2.3±1.7</td>
<td>1.7±0.8</td>
</tr>
</tbody>
</table>

Data presented as mean±SD. Statistical differences are indicated by *P<0.05 or ** P<0.01, within group before vs. after.
Table 2 - Capillarization in skeletal muscle and leg blood flow before and after 11 weeks of endurance training (END) or high-intensity interval training (HIIT)

<table>
<thead>
<tr>
<th></th>
<th>END</th>
<th>HIIT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
</tr>
<tr>
<td>Fiber area (μm²)</td>
<td>2927±589</td>
<td>3011±630</td>
</tr>
<tr>
<td>Capillary density (cap/mm²)</td>
<td>471±46</td>
<td>485±32</td>
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<tr>
<td>Capillary-to-fiber ratio</td>
<td>1.45±0.30</td>
<td>1.55±0.28*</td>
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<tr>
<td>Resting leg blood flow (L/min)</td>
<td>0.25±0.04</td>
<td>0.24±0.03</td>
</tr>
<tr>
<td>Leg blood flow (L/min) during knee-extensions (6W)</td>
<td>1.42±0.13</td>
<td>1.26±0.18</td>
</tr>
</tbody>
</table>

Data presented as mean±SD. Statistical differences is indicated by *P<0.05, within group before vs. after.
**Figure legends:**

Fig. 1. Transmission electron microscopy to demonstrate the ultrastructure of capillaries in skeletal muscle biopsies. Shown are representative micrographs of capillary profiles from muscle biopsies obtained before and after 11 weeks of endurance exercise training (END; upper panel) or low-volume high-intensity interval training (HIIT; lower panel). Note the thinner basement membrane (BM) around the endothelium (EC) in the participants that performed END (white arrowheads). Capillaries in individuals with type 2 diabetes are often surrounded by degenerating pericytes (PC) (white asterisk) within the pericapillary basement membrane layer. Occasionally, the BM revealed to be built up of several concentric sheets of undefined lamellae (black arrows).

Fig. 2. Morphometry of the capillary ultrastructure in muscle biopsies obtained before and after 11 weeks of endurance training (End) or low-volume high-intensity interval training (HIIT). Data presented as mean±SD. Significant differences is indicated by *P<0.05, different from before training biopsies.

Fig. 3. VEGF content in muscle biopsies obtained before and after 11 weeks of endurance training (END) or low-volume high-intensity interval training (HIIT). Data presented as mean±SD.

Fig. 4. NADPHox (upper panel) and SOD-2 (lower panel) content in muscle biopsies obtained before and after 11 weeks of endurance training (END) or low-volume high-intensity interval training (HIIT). Data presented as mean±SD.
Fig. 5. eNOS content in muscle biopsies obtained before and after 11 weeks of endurance training (END) or low-volume high-intensity interval training (HIIT). Data presented as mean±SD. Significant differences is indicated by *P<0.05, different from before training biopsies.