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Alpha adrenergic receptor blockade increases capillarisation and O_2 extraction and lowers blood flow in contracting human skeletal muscle

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Abstract

Chronic α1-adrenergic receptor blockade is known to increase capillarization through elevated shear stress in rodents, but its effect on angiogenesis in humans is unknown. Moreover, a higher capillary density is thought to be an important mechanism underlying the increase in skeletal muscle oxygen extraction following training, but this physiological effect has never been directly demonstrated.

Purpose: To investigate the effect of increased basal shear stress on angiogenesis, and the role of enhanced skeletal muscle capillarisation on blood flow and oxygen extraction.

Methods: Limb haemodynamics and oxygen extraction were measured at rest and during one-leg knee-extensor exercise (12 and 24W) in 10 healthy untrained young men before and after 4 weeks treatment with an α_1 receptor-antagonist (Terazosin, 1-2 mg day^{-1}). Biopsies were taken from the m. vastus lateralis.

Results: Resting leg blood flow was > 6 hours following Terazosin treatment ($P<0.05$). Basal capillary-to-fibre ratio was 1.68±0.07 and increased to 1.89±0.08 after treatment ($P<0.05$). Leg oxygen extraction during knee-extensor exercise was higher (4-5% ; $P<0.05$), leg blood flow and venous lactate levels lower (6-7%; $P<0.05$) and leg VO_2 similar after Terazosin treatment.

Conclusion: These results demonstrate that daily treatment with an α-adrenergic receptor blocker and a consequent increase in resting blood flow induces capillary growth in human skeletal muscle, likely due to increased shear stress. The increase in capillarisation led to enhanced O_2 extraction in the exercising leg, concomitant with a lower blood flow and venous lactate levels.

Introduction

Angiogenesis is a complex process controlled by a number of pro- and anti-angiogenic factors, where the balance between these factors determines whether capillary growth or regression will occur (Egginton, 2009). Several physiological factors, of both mechanical and chemical origin, influence capillary growth in skeletal muscle by promoting the formation or release of a number angiogenic growth factors (Egginton, 2011). Studies in rodents have shown that a period of chronically elevated blood flow induced by addition of the α-adrenergic receptor blocker, prazosin, to drinking water leads to increased muscle capillarisation (Dawson & Hudlická, 1989; Ziada et al., 1989; Zhou et al., 1998; Baum et al., 2004). The elevated basal blood flow in skeletal muscle leads to an increased frictional force by the blood on the capillary endothelium, which is registered by mechanosensors and transduced into endothelial cell activation and upregulation of angiogenic proteins (Milkiewicz et al., 2001; Egginton, 2011). In a previous study on human subjects, we showed that a period of increased blood flow and muscle stretch by passive movement of the lower leg induced angiogenesis in sedentary individuals (Hoier et al., 2010), however, the isolated effect of a chronic increase in resting blood flow has not been previously examined in humans.

One of the most responsive angiogenic factors is endothelial nitric oxide synthase (eNOS), which can both be activated and increase in expression in response to elevated shear stress (Lamontagne et al., 1992; Baum et al., 2004; Williams et al., 2006a). eNOS has been shown to be important for shear stress-induced angiogenesis (Baum et al., 2004), an effect likely mediated in part by NO stimulation of vascular endothelial growth factor (VEGF) production (Tsurumi et al., 1997; Da Silva-Azevedo et al., 2002). VEGF is thought to be one of the most important growth factors regulating capillary supply in muscle (Wagner, 2011). Genetically modifie rodens without muscle-specific VEGF have low levels of basal capillarisation in skeletal muscle, indicating a role for

VEGF in regulation of developmental angiogenesis, but the animals also lack the ability to increase muscle capillarization in response to exercise training (Olfert, 2010). The angiogenic effect of VEGF as well as its expression can be countered by the anti-angiogenic factor thrombospondin-1 (TSP-1)(Olfert et al., 2006; Malek & Olfert, 2009). One of the underlying mechanisms may be the interference of eNOS signalling by TSP1 (Isenberg et al., 2009), and this anti-angiogenic factor may therefore also have important implications for shear stress-induced angiogenesis.

Aerobic exercise training has marked effects on the cardiovascular system and skeletal muscle, enhancing systemic oxygen transport capacity as well as local oxidative capacity. One important adaptation is an increase in peripheral O_2 extraction, and a consequent lowering of blood flow to the exercising limb when exercise is performed submaximally at the same workload (Kiens et al., 1993; Mortensen et al., 2012; Mortensen et al., 2014). This effect of training is thought to be, at least in part, attributed to a parallel increase in skeletal muscle capillarisation (Andersen & Henriksson, 1977) which reduces O_2 diffusion distance and mean erythrocyte transit time. The microvascular transport of oxygen to tissue is also influenced by the distribution and tortuosity of capillaries (Al-Shammari et al., 2014). In addition, exercise training results in many other local adaptations, such as mitochondrial proliferation and optimised blood flow distribution pattern, which may also improve oxygen extraction efficiency (Hellsten & Nyberg, 2015). Due to the complex feedback regulation associated with metabolic demand during exercise, the isolated effect of an increase in capillarisation on O_2 extraction and blood flow has not previously been examined.

The aim of the present study was to investigate the effect of a chronic increase in resting blood flow and a consequent elevated vascular shear stress on skeletal muscle capillarisation, O_2 extraction and blood flow in humans. The hypothesis was that an increase in vascular shear stress would induce an

increase in skeletal muscle capillarisation through upregulation of pro-angiogenic factors. Moreover, we hypothesised that an increase in capillarisation would increase skeletal muscle O_2 extraction and consequently lower blood flow during exercise. To this end, we obtained muscle biopsies and measured resting and exercising haemodynamics and blood gasses before and after a four week period of treatment with an α-receptor antagonist to increase skeletal muscle blood flow in untrained individuals.

Methods

Ten young, untrained healthy males were studied (24±2 years, 71±3 kg and 180±2 cm). The study was approved by the Ethics Committee of the capitol region of Denmark, and conducted in accordance with the guidelines of the Declaration of Helsinki. Written informed consent was obtained from all individuals before enrollment into the study. Before the first experimental day the individuals visited the laboratory on two occasions to become accustomed to the one-leg knee-extensor model and to perform an incremental bicycle ergometer exercise test in which pulmonary maximal oxygen uptake (VO_{2max}) was determined with a metabolic cart (CPET system, Cosmed, Rome, Italy).

Terazosin intervention

Subjects received oral Terazosin (α_1 antagonist; Sinalfa, Amdipharm, Dublin, Ireland), with an estimated half-life of 12 hours, for 4 weeks. During the first week, subjects ingested 1 mg every night before bed, and for the three subsequent weeks they ingested 2 mg each night. The subjects completed an experimental day before the intervention period and 3 days after the last Terazosin ingestion. In four subjects, resting leg blood flow was measured during the first six hours after

Terazosin administration and in all subjects resting blood flow was assessed each week throughout the intervention period.

Experimental days

After local anaesthesia (lidocaine 20%), catheters were placed into the femoral artery and vein of the experimental leg, and a muscle biopsy was obtained from m. vastus lateralis using the Bergström-technique with suction. Following 30 min of supine rest, the subjects completed 5 min of passive leg movement (lower leg was strapped to the ergometer and was moved passively at a frequency of 60 r.p.m) followed by active one-legged knee-extensor exercise (5 min at 12 and 12 W). Blood samples were drawn at rest (supine and seated in the knee extensor ergometer), during passive leg movement (at 0.5, 1.5 and 4 min) and during active exercise (at 2.5 and 4.5 min).

Measurements

Leg blood flow (LBF) was measured with ultrasound Doppler (Logic E9, GE Healthcare) equipped with a linear probe operating an imaging frequency of 9 MHz and Doppler frequency of 4.2-5.0 MHz. The site of blood velocity measurements in the common femoral artery was distal to the inguinal ligament, but above the bifurcation into the superficial and profound femoral branch to avoid turbulence. All recordings were obtained at the lowest possible insonation angle, and always below 60°. The sample volume was maximized according to width of the vessel, and kept clear of the vessel walls. A low-velocity filter (velocities <1.8 $m.s^{-1}$) 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. Vessel diameter was determined after each Doppler recording. Arterial diameter was recorded during systole from arterial B-mode images with the vessel parallel to the transducer. Intra-arterial pressure was

monitored with transducers (Pressure Monitoring Kit, Baxter) positioned at the level of the heart. Leg mass was calculated from whole-body dual-energy x-ray absorptiometry scanning (Prodigy, GE Healthcare).

Analysis

Blood samples

Blood gases, haemoglobin, and lactate were measured using an ABL725 analyzer (Radiometer, Glostrup, Denmark).

Immunohistochemistry

Liquid N_2-frozen biopsy samples were cut using a cryostat into 8 μm thick transverse sections, attached to coated slides, and fixed by immersion in acetone (-20˚C for 30 sec) followed by 2% formaldehyde (2 min at room temperature). The sections were rinsed in 10 mM phosphate-buffered saline containing 1% bovine serum albumin (PBS–BSA) and blocked for 1 h with PBS–BSA. The lectin Ulex Europaeus agglutinin-1 (UEA-1) was used as endothelial cell marker (DAKO A/S, Glostrup, Denmark). The distribution of muscle fibers containing MHC I or MHC IIa was determined with the use of the antibodies MHCI (M8421, Sigma, St Louis, Missouri, USA) and anti-human MHC II (N2.261, Developmental Studies Hybridoma Bank, University of Iowa). The sections were incubated with biotinylated secondary antibody (DAKO A/S), with binding visualized using an ABComplex kit with alkaline phosphatase (ABComplex/AP, DAKO A/S).

Capillaries and muscle fibers were visualised at a total magnification of x 400. On average 300 fibers were counted per biopsy. Morphometric data were calculated using digitised images and the image analysis software ImageJ (NIH). The number of capillaries per fiber (C:F), the fiber cross-

sectional area (FCSA, μm^2), and capillary density (CD, mm^{-2}) were computed from all biopsies with adequate muscle preservation.

Using samples from a subset of 5 individuals, binary images were then imported into an analysis package to estimate capillary distribution (Al-Shammari et al., 2014). A region of interest (ROI) was placed over the images to allow unbiased sampling to calculate C:F and CD (Egginton, 1990), and used to quantify the area of tissue supplied by a single capillary (capillary domain) based on the tessellation of Voronoi polygons representing that area of tissue closer to one capillary than another. The domain areas were normalised by logarithmic transformation to form a parametric data series and the SD calculated. This area-based approach permits calculation of the local heterogeneity in capillary spacing (logSD).

Western blot

Biopsies were freeze-dried and dissected free from fat, blood and connective tissue. The biopsy was homogenized in homogenization buffer (10% glycerol, 20 mM sodium pyrophosphate, 150 mM NaCl, 50 mM HEPES, 1% NP-40, 20 mM β-glycerophosphate, 2 mM Na_3VO_4, 10 mM NaF, 2 mM PMSF, 1 mM EDTA and EGTA, aprotinine, leupeptine and benzamidine) while kept on ice at all times. The protein concentration of lysate samples was determined by a BSA protein assay (Pierce, Rockford, IL, USA). Lysate proteins were separated using SDS gels (Bio-Rad Laboratories) and transferred (semidry) to PVDF membranes (Immobilion Transfer Membrane, Millipore). The membranes were incubated with primary antibodies to VEGF (A-20, Santa Cruz Biotechnology, Santa Cruz), FLK-1 (sc19530, Santa Cruz Biotechnology), eNOS (610297 BD Transduction Laboratories), TSP-1 (ab85762, Abcam, USA). Secondary antibody horseradish-peroxidase-conjugated goat anti-rabbit (P-0448, Dako, Glostrup, Denmark) 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.

Statistical analysis

A two-way repeated measures ANOVA was performed to detect treatment-induced changes. After a significant F-test, pairwise differences were identified using Tukey posthoc procedure. The significance level was set at $P<0.05$ and data expressed as means ±SEM. Steady state conditions were reached after the first 0.5 min of passive leg movement and means were obtained at 0.5, 1.5 and 4 min. No statistical difference was seen between measurements obtained at 2.5 and 4.5 min during active exercise, and data presented are therefore the mean of these measurements.

Results

Blood flow

Acute terazosin administration increased femoral arterial resting blood flow by 23±22, 39±7 and 40±5 % after 2, 4 and 6 hours, respectively. During chronic Terazosin treatment, resting femoral arterial blood flow was increased from 195±23 $ml.min^{-1}$ to 301±41 $ml.min^{-1}$ (2 weeks) and 271±33 $ml.min^{-1}$ (4 weeks) (both $P<0.05$ vs. baseline).

Skeletal muscle morphology

Global analysis of capillary to fiber ratio (C:F) (1.69±0.08 vs. 1.90±0.08 with Terazosin) and capillary density (335±17 vs. 416±60 capillaries $mm^{2-1}$with Terazosin) were higher after Terazosin treatment ($P<0.05$; Figure 1). Similar values were obtained from the samples used for higher resolution analysis (C:F: 1.52±0.06 vs. 1.78±0.08 with Terazosin (n=5; $P<0.05$)), confirming a representative subset. Here, capillary domain area was similar in both samples (1740±63 vs.

1883±69 μm^2) as was ICD (46.1±0.9 vs. 47.6±0.9 µm). Importantly, the quantitative measure of capillary homogeneity, logSD, was unchanged (0.155 vs. 0.145 µm) (Figure 2).

Performance

There were no differences in VO_2max (3.5±0.1 vs. 3.3±0.1 l min^{-1} with Terazosin) or peak workload (297±11 vs. 300±11 W with Terazosin) obtained during incremental cycling and knee-extensions (57±3 vs. 60±3 W with Terazosin) before and after Terazosin treatment (Figure 3).

Haemodynamics

Seated resting leg blood flow and vascular conductance were lower after Terazosin treatment ($P<0.05$), whereas arterial blood pressure was unchanged (Figure 4). Terazosin treatment did not alter hemodynamics during passive leg movement. After Terazosin treatment, leg O_2 extraction during exercise at 12 and 24 watts was higher ($P<0.05$), leg blood flow and vascular conductance were lower ($P<0.05$), leg VO_2 similar, and femoral venous lactate levels lower (Figure 5; $P<0.05$).

Protein content of angiogenic and angiostatic factors in skeletal muscle

There were no differences in VEGF, FLK, TSP-1 or eNOS protein content before and after Terazosin treatment (Figure 6).

Discussion

The aim of the present study was to determine the effect of an increase in vascular shear stress on angiogenesis, O_2 extraction and blood flow in skeletal muscle. The results show that an elevated resting blood flow accomplished by a four week period of α1-adrenergic blockade increases skeletal muscle capillarisation in humans. Moreover, for the first time we demonstrate that the

functional consequences of an increase in skeletal muscle capillarisation without other concomitant aerobic adaptations are an increase in O_2 extraction, a lower blood flow to the exercising limb, and lower venous lactate levels.

We show that four week treatment with an α_1-adrenoreceptor antagonist results in a chronic elevation in resting limb blood flow and an increase in skeletal muscle capillarisation. The increase in femoral artery perfusion is of similar magnitude to that previously reported during acute adrenergic antagonism in hypertensive men (Scarpelli et al., 1981). Importantly, capillary growth during α_1-adrenoreceptor blockade only appears to occur in tissues with elevated blood flow, suggesting that capillary shear stress is indeed the underlying mechanism of the increase in skeletal muscle capillarization (Ziada et al., 1989). This angiogenic response appears to be independent of the vasodilator used to elevate shear stress (Egginton et al., 2016). In a previous study, we examined the effect of four weeks regular passive leg movement on muscle capillarisation and found a small, but significant increase in capillary supply (Hoier et al., 2010). Passive leg movement induces an approximate 2-3 fold increase in blood flow and a 20% passive stretch of the muscle (Hellsten et al., 2008), thus the effect of shear stress versus stretch on angiogenesis could not be distinguished in this model. The current findings of increased capillarisation with increased resting blood flow suggest that chronic elevation of shear stress is a sufficiently potent stimulus to promote capillary growth. A comparison between these studies would also indicate that low level more long term (>8 hours) as opposed to a higher level, but temporary (90 min) increase in shear stress may be more effective in inducing angiogenesis, which may have important implications for translation of these findings into clinical angiotherapy.

The quantitative measure of homogeneity in capillary distribution, logSD, represents the degree of variability amongst capillary domains e.g. a decrease would show a more regular pattern of capillary distribution. Canonical sprouting angiogenesis is likely responsive to the local metabolic environment and as such is expected to produce a more homogeneous distribution of capillaries, whereas the lack of a myocyte response to hyperaemia would suggest a more stochastic angiogenic response, expected to result in an increased capillary supply of similar spatial distribution. The logSD values are consistent with this expectation, adding further support to the concept of direct endothelial cell mechanotransduction of elevated shear stress (Olfert et al., 2016).

It is known that a period of exercise training can increase oxygen extraction and lower blood flow in the muscle during submaximal exercise (Kiens et al., 1993; Mortensen et al., 2012; Mortensen et al., 2014). As increased skeletal muscle capillarisation and increased muscle oxidative capacity are common adaptations to aerobic exercise training, it has been assumed that the increased oxygen extraction has been a result of these adaptations. However, no in vivo study in either animals or humans has directly demonstrated the effect of increased capillarisation on muscle oxygen extraction and blood flow. In the present study, O_2 extraction in the exercising leg was increased in parallel with an increase in capillarisation. Since the Terazosin treatment was followed by a 3 day wash out period before the experimental day, this effect was not related to the acute effects of the vasodilator. Increased capillary density improves local O_2 delivery by lowering the diffusion distance from capillary to skeletal muscle fibre, and increasing the mean capillary transit time of erythrocytes (Krogh & Lindhard, 1913). Although leg VO_2 was not significantly different before and after the Terazosin treatment, improved O_2 diffusivity and consequently improved support for aerobic metabolism likely explain the lower femoral venous lactate levels following exercise after treatment. The increased O_2 extraction and lower blood flow during exercise is similar to the

changes observed after a period of aerobic exercise training, supporting the concept that increased capillarisation and subsequent improved O_2 diffusion is a major driver for increased O_2 extraction in the trained state. Although this has commonly been assumed, to date no studies have directly demonstrated this effect of capillarisation in muscle.

Despite the improved conditions for O_2 diffusion, we did not observe an increase in maximal O_2 uptake or peak workload during cycling or peak workload during knee-extensions. The lack of effect of an increased capillary supply on maximal O_2 uptake is consistent with the concept that capacity of the systemic circulation to deliver O_2 to exercising muscles is the major factor limiting maximal O_2 uptake (Andersen & Saltin, 1985; Mortensen et al., 2005). The lack of effect during knee-extensions may suggest that mitochondrial oxidative capacity rather than diffusion capacity is the rate limiting step during small-muscle mass exercise (Blomstrand et al., 1997), although we did not determine leg VO_2 during maximal knee-extensions to verify this.

The mechanism(s) by which an increase in capillary shear stress induces angiogenesis has been suggested to be linked to an increase in eNOS and NO formation (Baum et al., 2004), and NO is known to influence VEGF expression (Tsurumi et al., 1997; Da Silva-Azevedo et al., 2002). Despite the increase in resting blood flow and capillarisation, there were no changes in protein levels of eNOS, VEGF, VEGF receptor 2 or TSP-1 following the intervention period. With the exception of eNOS these observations are in agreement with most reports in humans that these proteins are not altered with training or passive movement (Høier et al., 2012; Høier & Walker, 2013). In contrast, levels of eNOS are generally enhanced with aerobic training (Hellsten & Nyberg, 2015), and both acute and repeated passive movement increases expression of eNOS mRNA (Hellsten et al., 2008; Hoier et al., 2010). The response of angiogenic factors in rodent

skeletal muscle differ somewhat from what is generally found in humans in that levels of VEGF, VEGF receptor 2, TSP-1 in mice and rats appear to be more readily altered by a period of stimuli such as muscle contraction, shear stress or passive stretch (Williams et al., 2006b; Olenich et al., 2013). This may be linked to the greater stimulus intensity leading to a more rapid angiogenic response in rodents. A possible explanation for the lack of effect on eNOS and VEGF expression following Terazosin treatment is that the muscle biopsies were obtained 3 days after treatment ended.

In conclusion, increased resting blood flow leading to enhanced capillary shear stress induces angiogenesis in human skeletal muscle. Moreover, an increase in skeletal muscle capillarisation increases O_2 extraction and lowers blood flow, and appears to lower anaerobic metabolism at the same exercise workload. Increasing capillarisation could therefore be an important treatment for patients with microvascular dysfunction in skeletal muscle to improve metabolic efficiency.

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Table 1

	Before					After			
	Rest		Exercise			Rest		Exercise	
	Supine	Seated	Passive	12 W	24W	Supine	Seated	Passive	12 W
Hemoglobin (g l^{-1})									
FA	13.9±0.4	14.1±0.4	14.2±0.3	14.5±0.3	14.4±0.3	13.5±0.2	14.0±0.3	14.0±0.3	14.4±0.3
FV	13.7±0.5	13.7±0.5	14.1±0.4	14.5±0.5	14.5±0.5	13.2±0.2	13.8±0.3	13.8±0.3	14.2±0.2
PO_2 (mmHg)									
FA	97±5	97±2	106±5	102±3	105±2	101±1	99±1	100±3	103±3
FV	37±2	29±2	35±2	26±1	26±1	33±2	25±1*	31±2	24±1*
O_2 Saturation (%)									
FA	97.6±0.4	97.4±0.4	97.6±0.3	97.7±0.4	98.0±0.2	97.6±0.4	97.5±0.3	97.6±0.3	97.8±0.3
FV	66.4±4.1	50.3±4.5	62.2±4.5	41.3±1.8	37.7±2.5	61.6±3.5	43.2±2.1*	57.3±3.4	36.4±1.5*
O_2 content (ml l^{-1})									
FA	184±6	187±5	189±4	193±5	192±4	180±3	185±4	186±4	192±3
FV	123±10	94±10	116±14	81±5	69±8	111±8	81±4*	108±8	70±3*

Figure legends

Figure 1. Capillaries per muscle fiber (A) and capillary density (B) in skeletal muscle (m. vastus lateralis) before and after four weeks of treatment with an α_1-receptor blocker to increase vessel wall shear stress. * different compared to before treatment, $P<0.05$

Figure 2. Frequency distribution of capillary domain areas following five weeks Terazosin supplementation showing similar, log-normal distribution patterns, despite an increase in capillary supply.

Figure 3. Maximal oxygen uptake (A), maximal workoad during maximal cycling (B) and maximal workoad during maximal knee-extension before and after four weeks of treatment with adrenoreceptor blockage to increase vascular shear stress.

Figure 4. Leg blood flow, mean arterial pressure and leg vascular conductance during resting conditions, passive leg movement and knee-extension before and after four weeks of Terazosin treatment. * different compared to baseline, $P<0.05$

Figure 5. Leg a-vO_2 difference, leg VO_2 and femoral venous lactate under resting conditions, passive leg movement and knee-extension before and after four weeks treatment. *$P<0.05$ vs. baseline.

Figure 6. Protein levels for pro-angiogenic and angiostatic factors in skeletal muscle before and after four weeks Terazosin treatment. a) Vascular endothelial growth factor (VEGF), b) VEGF receptor 2 (Flk-1), c), thrombospondin-1 (TSP-1), d) endothelial nitric oxide synthase.

Figure 1.

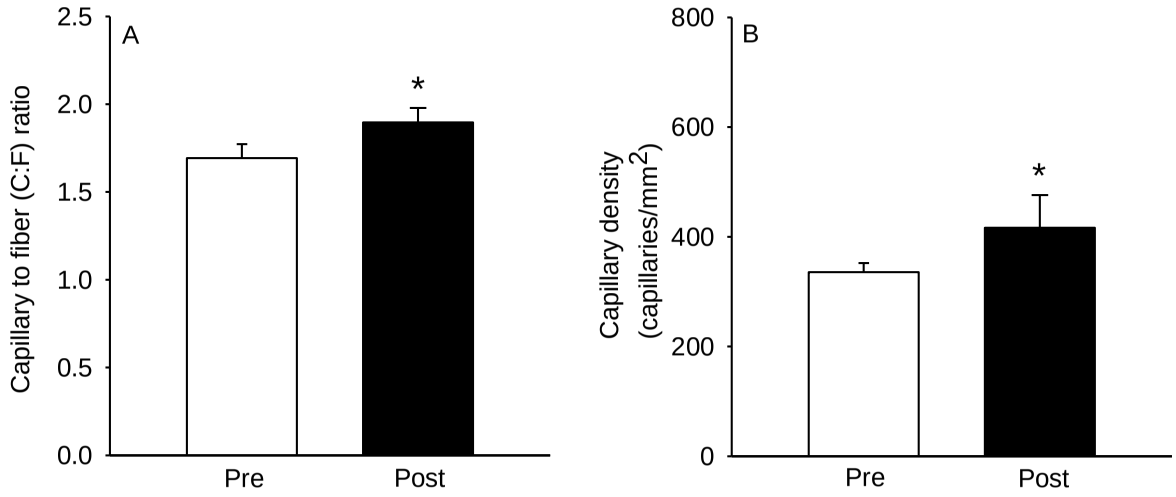

A
Capillary to fiber (C:F) ratio
2.5
2.0
1.5
1.0
0.5
0.0
*
Pre
Post
B
Capillary density (capillaries/mm2)
800
600
400
200
0
*
Pre
Post

Figure 2.

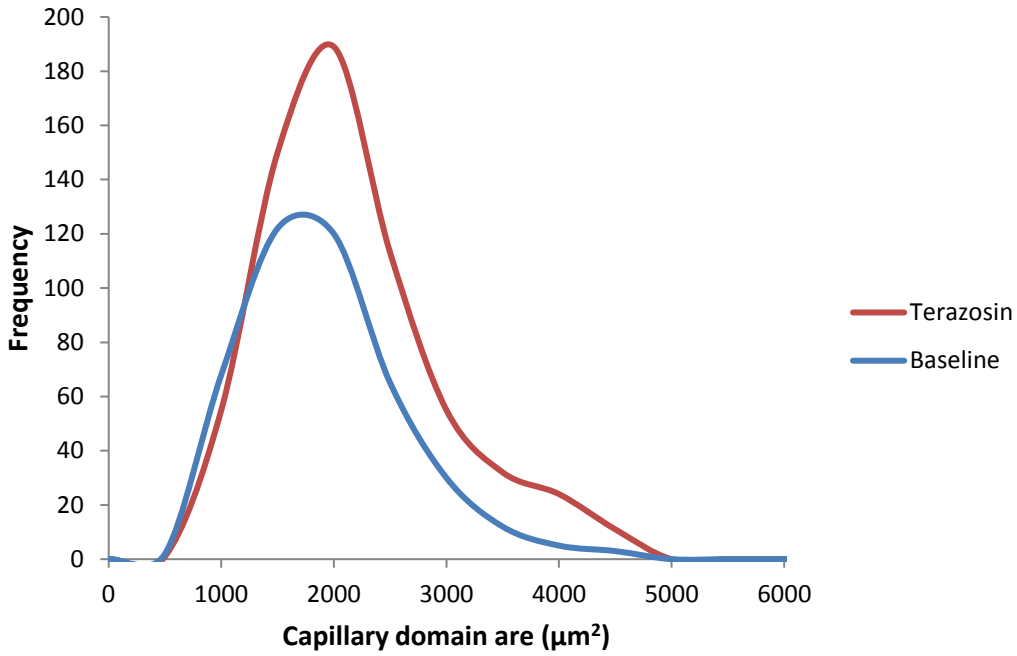

200
180
160
140
120
100
80
60
40
20
0
Frequency
0
1000
2000
3000
4000
5000
6000
Capillary domain are (µm²)
Terazosin
Baseline

Figure 3.

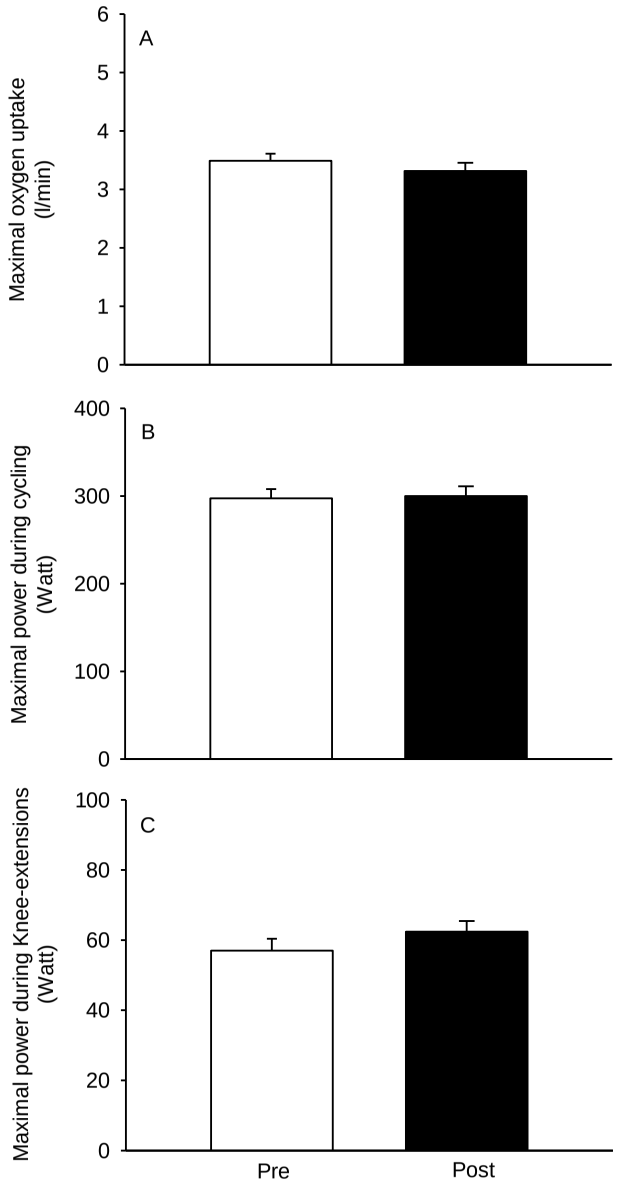

A
Maximal oxygen uptake (l/min)
0
1
2
3
4
5
6
B
Maximal power during cycling (Watt)
0
100
200
300
400
C
Maximal power during Knee-extensions (Watt)
0
20
40
60
80
100
Pre
Post

Figure 4.

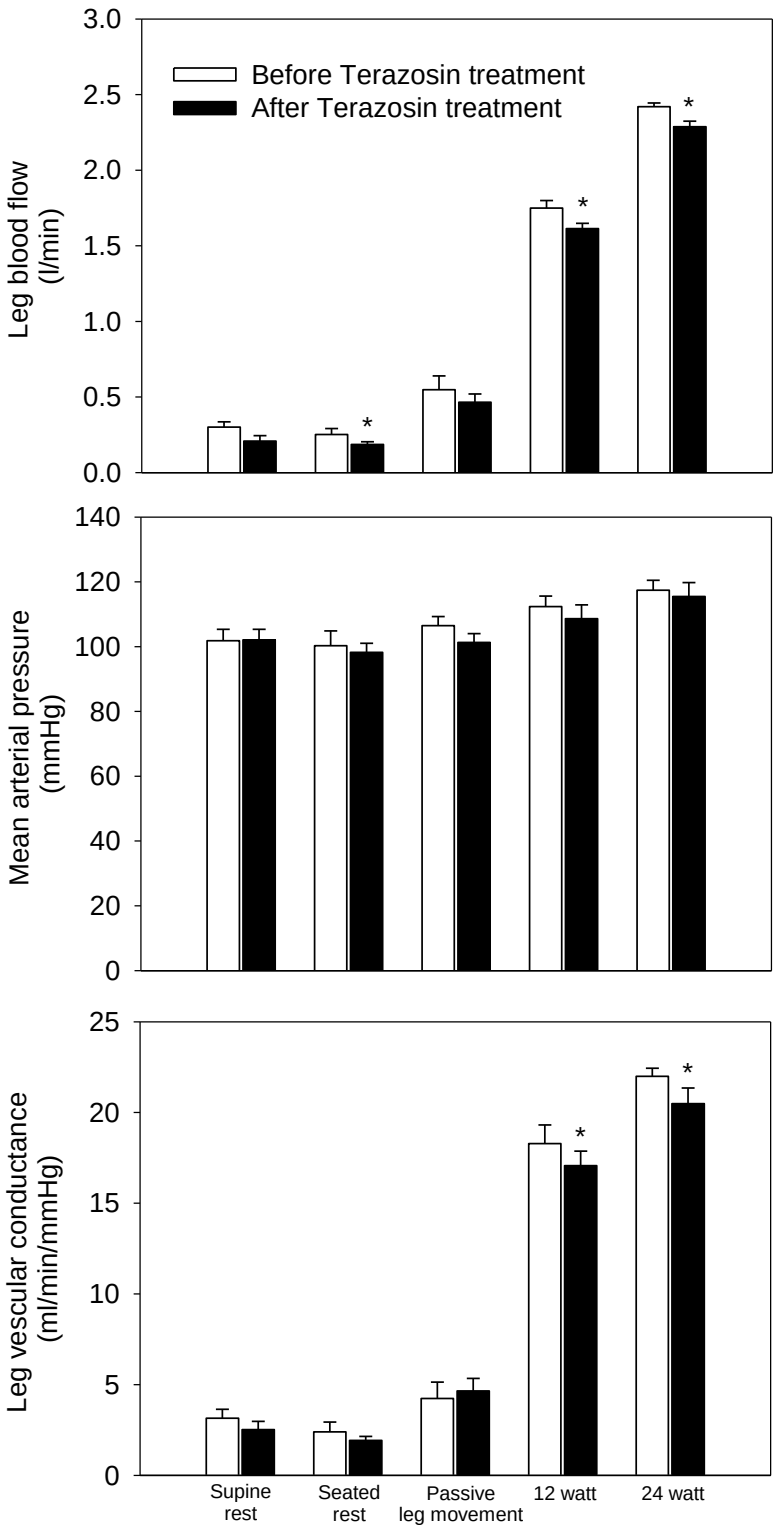

Before Terazosin treatment
After Terazosin treatment
Leg blood flow (l/min)
Mean arterial pressure (mmHg)
Leg vescular conductance (ml/min/mmHg)
Supine rest
Seated rest
Passive leg movement
12 watt
24 watt

Figure 5.

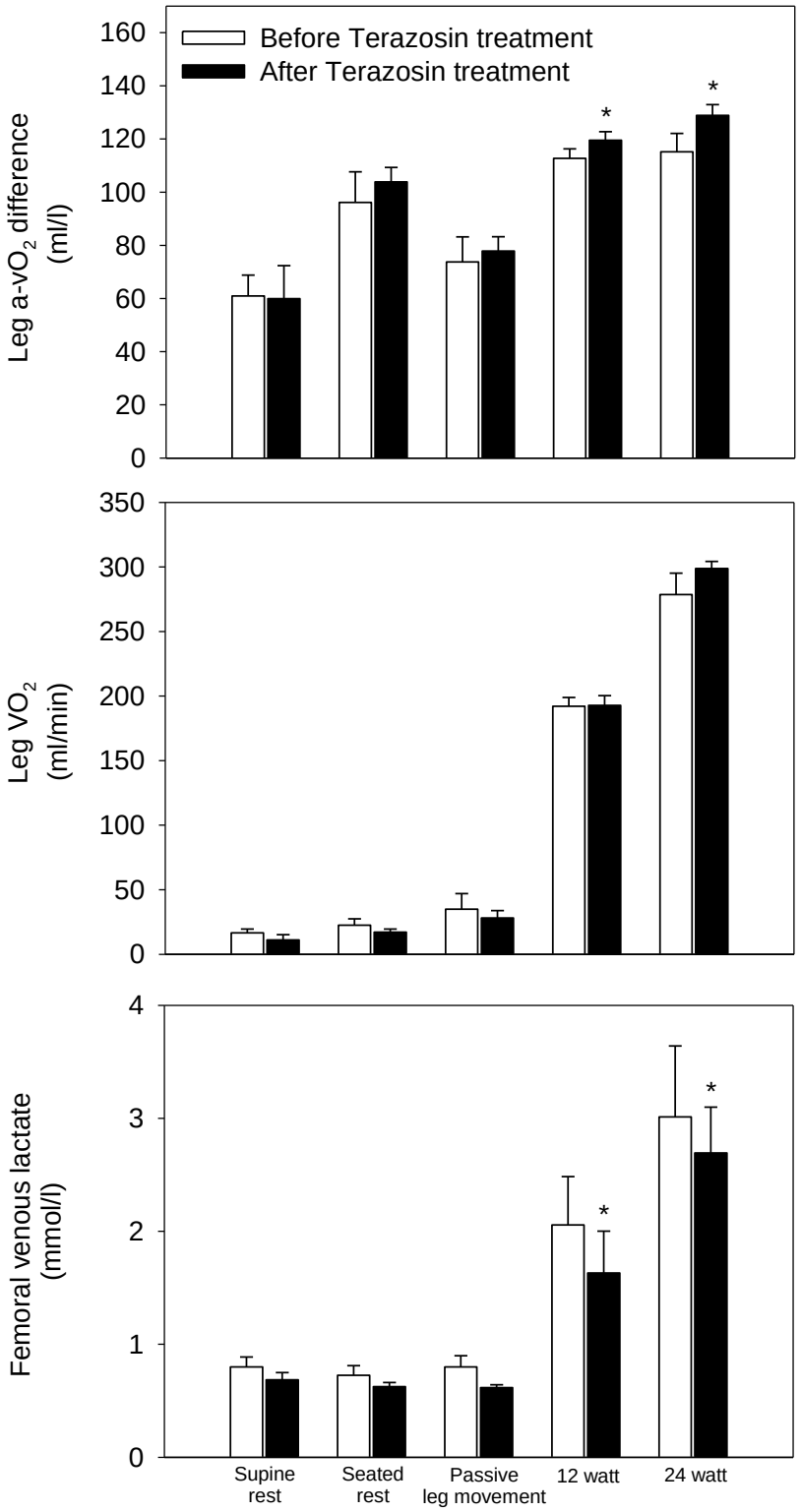
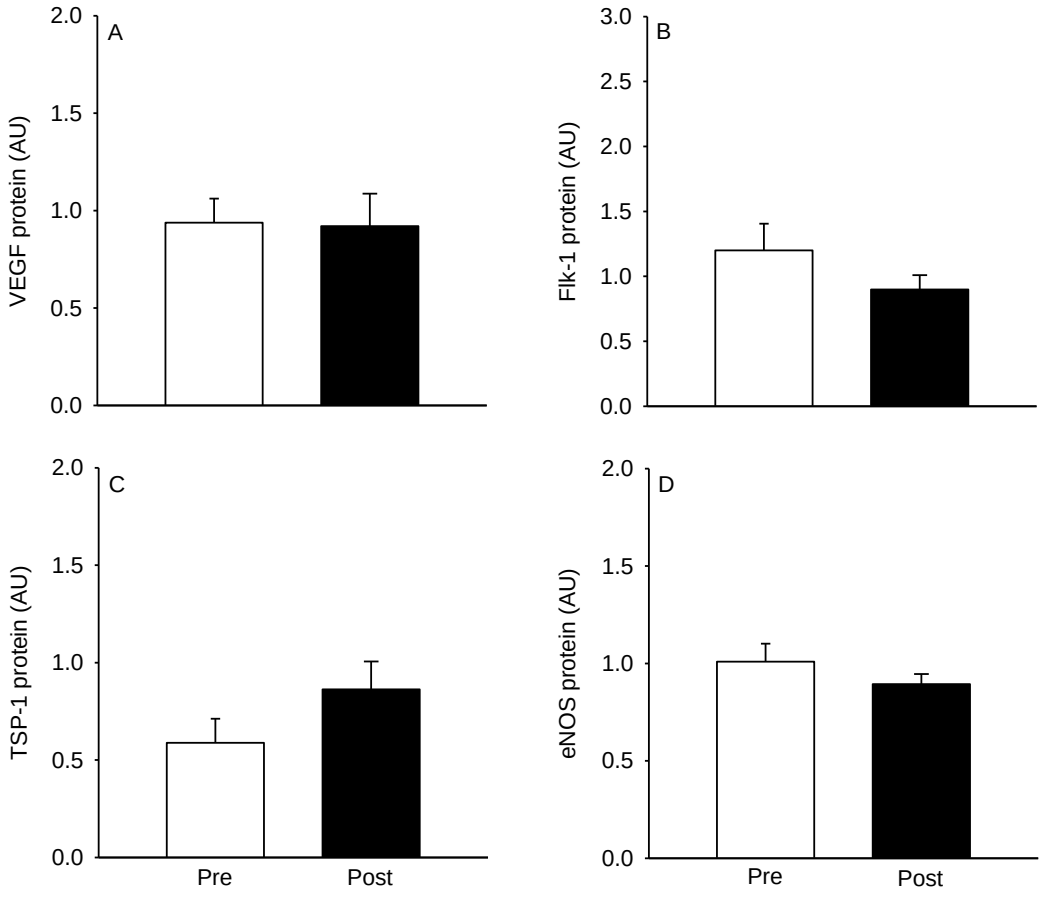

Before Terazosin treatment
After Terazosin treatment
Leg a-vO2 difference (ml/l)
Leg VO2 (ml/min)
Femoral venous lactate (mmol/l)
Supine rest
Seated rest
Passive leg movement
12 watt
24 watt
*

Figure 6.


A
VEGF protein (AU)
2.0
1.5
1.0
0.5
0.0
B
Flk-1 protein (AU)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
C
TSP-1 protein (AU)
2.0
1.5
1.0
0.5
0.0
Pre
Post
D
eNOS protein (AU)
2.0
1.5
1.0
0.5
0.0
Pre
Post