Gender differences in microvascular function across muscles in the lower leg using blood-oxygen level dependent MR Imaging

Lars Molbo

Department of Health Science and Technology

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Lars Molbo

Supervisors:
Ryan Godsk Larsen
Lasse Riis Østergaard

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Abstract

Purpose Gender differences in the macrovasculature are well documented, however, little is known about gender differences in the microvasculature. Blood-oxygen-level-dependent (BOLD) MRI represents the ratio of oxygenated blood to deoxygenated blood and BOLD-MRI signal intensity (SI) time courses during reactive hyperemia provides a tool to investigate gender- and intermuscular differences in microvascular function. This study tested the hypotheses that, 1) females would have a decreased time-to-peak (TTP) compared to males across all muscles, 2) females would have an increased hyperemic peak value (HPV) compared to males across all muscles, 3) TTP and time-to-half-peak (TTHP) would provide similar conclusions.
Methods Thirty healthy, young men (n = 18) and women (n=12) went through reactive hyperemia caused by five minutes of cuff occlusion at the thigh followed by two minutes of reperfusion and hyperemia. BOLD-MRI SI time courses were acquired to monitor hyperemic responses while positioned supine in a 3T MR scanner. Tibialis anterior (TA), peroneal group (PG), soleus (SO) and gastrocnemius medial (GM) were manually segmented on T2-weighted MRI and transferred to BOLD-MRI using multimodal image registration. Relative changes in SI were calculated for each muscle, and TTP, TTHP and HPV were calculated and compared using three mixed design two-way (muscle, gender) repeated measures ANOVAs.
Results Females had significantly shorter TTP ($P< 0.002$) and TTHP ($P<0.002$) compared to males. Specifically, in females, TTP was shorter TA (28.4%), PG (33.9%), SO (19.7%) and GM (15.4%) compared to males. Similarly, in females, TTHP was shorter in TA (27.5%), PG (41.6%), SO (14.7%) and GM (18.8%) compared to males. Additionally, TTP in TA was shorter compared to PG (25.1%; $P<0.002$), SO (14.3%; $P<0.023$) and GM (15.6%; $P<0.011$). Similarly, TTHP in TA was shorter compared to PG (31.1%; $P<0.007$), SO (16%; $P<0.028$) and GM (26%; $P<0.000$). HPV was larger in SO compared to TA (2.2%; $P<0.027$) and GM (3.6%; $P<0.001$).
Conclusions A gender effect was found for TTP and TTHP with females having shorter TTP and TTHP compared to males across TA, PG, SO and GM. While this study did not cover the underlying mechanisms, a possible cause, could be a decreased sympathetic nerve activity in females compared to males. No gender effect was found for HPV, suggesting similar vasodilatory capacity in healthy young men and women. Findings of reduced TTP and TTHP in TA compared to PG, SO and GM, and higher HPV in SO compared to TA, PG and GM are consistent with the literature. Finally, TTP and TTHP showed similar results, and TTHP is suggested as a more robust and operator independent parameter. The results from this study contribute with new knowledge about gender effects on microvascular function and the underlying mechanisms should be investigated in future studies.

Introduction

The cardiovascular system is responsible for the transportation of blood throughout the body. A healthy cardiovascular system is essential for the transportation of nutrients, oxygen, hormones and cellular waste products. The cardiovascular system can be separated into the micro- and macrovasculature. The macrovasculature consists of the arteries and veins which are the largest blood vessels in the cardiovascular system. These are primarily concerned with the overall transportation of blood throughout the body. The microvasculature consists of the arterioles, capillaries and venules which are responsible for the exchange of oxygen, hormones, nutrients and waste products between the blood and the tissue. Thus, a healthy microvasculature is essential to all human function (1).

Traditionally vascular function has been investigated in the macrovasculature, commonly investigating flow-mediated dilation (FMD), using doppler ultrasound to measure the expansion of artery diameter in conduit arteries, during increased blood flow (2–4). More recently blood oxygen-level dependent (BOLD) MRI has been used to investigate function of the microvasculature (5–8). BOLD-MRI represents the ratio of oxyhemoglobin to deoxyhemoglobin in the smaller blood vessels due to the different magnetic properties of oxyhemoglobin and deoxyhemoglobin. Practically this means that BOLD signal intensity can express levels of oxygenated blood in specific regions of interest (ROI) (5,8,9).This is very useful as it allows for the investigation of regional differences in microvascular function, such as between different muscles.

The relationship between BOLD-MRI SI and oxygen levels in the microvasculature was demonstrated in a study by Toussaint et al., where a thigh cuff was inflated for five minutes to cause ischemia, followed by deflation of the cuff causing rapid reperfusion and hyperemia. This study showed that BOLD-MRI SI dropped steadily during ischemia and increased rapidly during reperfusion and hyperemia (8). Building on the work by Toussaint et al., Ledermann used a similar protocol of ischemia-reperfusion to examine microvascular function in patients with peripheral artery occlusive disease (PAOD). The study showed that TTP from cuff release until peak BOLD SI was significantly longer in participants with PAOD compared to healthy participants, likely due to PAOD patients having an impaired inflow of oxygenated blood. Furthermore, the peak BOLD signal intensity, which expresses the HPV, was significantly lower in participants with PAOD compared to healthy participants (10–12). The decreased peak BOLD-MRI SI in PAOD participants could be due to decreased vasodilatory capacity as blood volume has been shown to be closely correlated to BOLD-MRI SI (13–16). TTP, HPV and TTHP have since been used in studies using cuff occlusion to induce reactive hyperemia as indicators for microvascular function in multiple research areas (5,7,12,17,18). The methods for obtaining parameters such as TTP and HPV are not well documented in published studies (17), and comparing results of TTP and TTHP could be relevant, to reach more standardized parameters for microvascular function when using BOLD-MRI SI time courses.

Gender differences in vascular function have been researched thoroughly, showing that females tend to have better vascular function than males and that this could be explained by increased levels of estrogen

(2,6,19–22). This knowledge however comes from studies using doppler ultrasound on large arteries. While a lot is known about the transportation of blood in the cardiovascular system, little research has been done concerning microvascular function. Considering the importance of the microvasculature, where several vital exchanges between blood and tissue take place (1), it seems essential to uncover whether gender has an influence on microvascular function. With BOLD-MRI the tool is available to investigate whether a gender effect is evident in the microvasculature.

BOLD-MRI has some limitations and challenges that require attention (17) and to extract parameters of the BOLD response (e.g. TTP) for separate muscles, accurate segmentation of distinct muscle groups in the BOLD-MRI is required. Standard practice is to manually draw ROIs on BOLD-MRI (5,7–10,17,23), however the signal to noise ratio, as well as low resolutions in BOLD-MRI, makes it hard to do accurate manual segmentation of muscle groups. A possible solution to this could be to conduct segmentation on T2 weighted MRI where the resolution and signal to noise ratio is better and thus provide more defined muscle fascia. The more accurate T2 MRI segmentations could then possibly be transferred to BOLD-MRI using image registration. However, image registration could be challenging due to differences in resolution as well as the lack of clear landmarks present in both image types. With accurate segmentation done on T2 weighted MRI transferred to BOLD-MRI it would be possible to investigate differences in microvascular function across individual muscles.

With BOLD-MRI giving the opportunity to examine microvascular function across muscles, the aim of this study was to investigate whether gender influences microvascular function in the gastrocnemius, SO, TA and the PG using BOLD-MRI SI time courses of reactive hyperemia following five minutes of cuff occlusion. Additionally, to compare results of TTP and TTHP to help standardize parameters for microvascular function in the field. It was hypothesized that females would have greater overall microvascular function compared to males. Specifically, 1) females would have lower TTP compared to males across all muscles, 2) females would have higher HPV compared to males across all muscles, 3) TTP and TTHP would provide identical conclusions.

Methods

All data were collected as part of a larger research study by Associate Professor Ryan Godsk Larsen from the Department of Health Science and Technology at Aalborg University. The development of the MATLAB script to segment muscles, extract SI values and determine relevant parameters as well as all writing, data treatment, data analysis and statistics were done by the author of this manuscript.

Participants

Thirty healthy, young men (n = 18) and women (n=12) volunteered to participate in the study. All participants were non-smokers, not taking any medications or supplements known to affect metabolism or blood flow and eligible for MR procedures. All participants practiced at most 3 hours of structured exercise per week. All participants provided informed consent after experimental procedures and potential risks of the study were explained to them. The study was approved by the Ethics Committee of North Denmark (N-20130029) and in accordance with the Declaration of Helsinki.

Experimental Protocol

Participants were instructed to avoid strenuous physical activity, anti-inflammatory medication, and antioxidant supplementation for 24 hours before the experimental session. MR scans were conducted at the MR Research Center at Aalborg University Hospital. Conditions were standardized by having all sessions performed between 8 and 11 in the morning following an overnight fast.

A separate habituation session was done before each MRI session with participants being familiarized with the cuff occlusion procedure. A pressure cuff (VBM Medizintechnik GmbH, Sulz, Germany) was placed around the distal part of the thigh and manually inflated to 240 mmHg within 3-4 seconds to inhibit blood flow to the lower leg. Following 5 minutes of occlusion the cuff was deflated within 1-2 seconds.

Reactive hyperemia BOLD-MRI session

Participants were positioned in a supine position within the MR scanner approximately 15 minutes before the first MR scan. To limit motion artifact and participant discomfort during the MR session, padding was placed around the leg and knee. Before each BOLD-MRI session T2 weighted MRI were acquired. Each BOLD session consisted of 30 seconds of rest to provide baseline measurements of BOLD-MRI SI, 5 minutes of cuff occlusion to cause ischemia and 2 minutes following cuff release to monitor reperfusion and hyperemia.

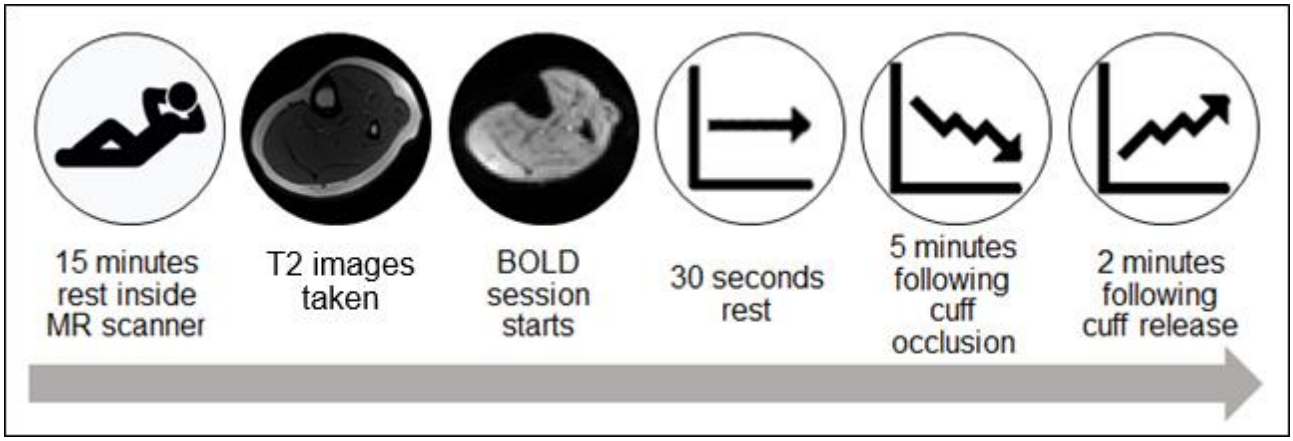

Figure 1. Experimental protocol for obtaining T2 weighted MRI and BOLD-MRI during reactive hyperemia.

Magnetic resonance imaging

The MR imaging acquisitions were performed on a 3T MR scanner (Signa HDxt, General Electrics, Milwaukee, WI, USA) using an8-channel extremity coil. T2 weighted MRI [TR = 1500 milliseconds, TE = 24 milliseconds, echo train length = 4, FOV = 18 cm, slice thickness = 10 mm, number of slices = 3, slice gap = 1 mm, acquisition matrix 320 x 224, NEX = 1] were acquired, as images of higher resolution and quality, compared to BOLD-MRI, were needed for the manual segmentation. To monitor reactive hyperemia during the BOLD session, one-shot gradient echo images [TR = 1000 milliseconds, TE = 40 milliseconds, FOV = 18 cm, slice thickness = 10 mm, acquisition matrix 64 x 64, NEX = 1, flip angle = 90º] were acquired continuously for the 7.5-minute duration of the BOLD session.

Data analysis

A custom-written MATLAB script was created to extract TTP, TTHP and HPV values from BOLD-MRI SI time courses during reactive hyperemia. First an average BOLD image was created based on the entire BOLD session. This image was resized to the same size as the T2 weighted MRI. Multimodal image registration was used to align the T2 weighted MRI to the enlarged average BOLD-MRI, allowing for segmentation to be done on the transformed T2 weighted MRI and then transferred to BOLD-MRI. Specifically, each ROI was manually drawn on the transformed T2 weighted MRI, and an image of all ROIs superimposed to the transformed T2 weighted MRI was shown to the operator to visually inspect the accuracy of the segmentation. Next, all ROIs were scaled down to the resolution of BOLD-MRI, and an image of all resized segmentations superimposed to BOLD-MRI was shown to the operator, again to inspect the accuracy of the transformation (Figure 2). The mean SI of each ROI was extracted for each of the 450 images and an SI

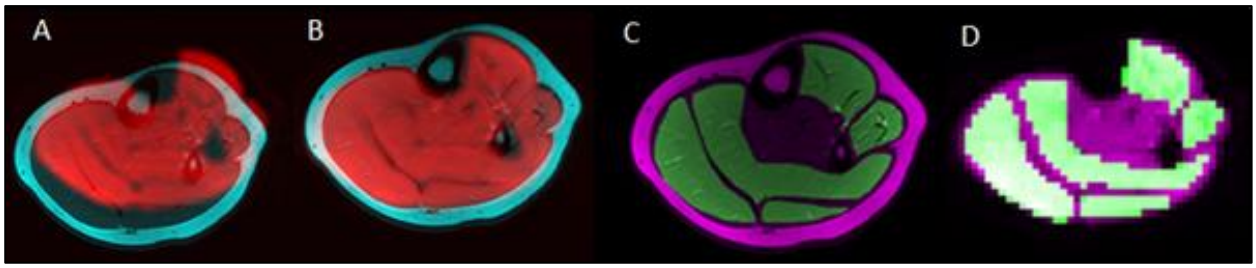

Figure 2. A: BOLD-MRI superimposed to T2 weighted MRI. B: BOLD-MRI superimposed to T2 weighted MRI following multimodal image registration. C: Manual segmentation done on transformed T2 weighted MRI. D: Resized manual segmentation masks superimposed to BOLD-MRI.

curve was plotted. A moving average filter was used to reduce noise in the signal as this was sometimes problematic due to the fact that the SI following the HPV was often very close to the HPV. This meant that noise would sometimes move the time of the HPV to later than expected. Each ROI was normalized to its baseline values, the baseline was calculated as the average SI of the 10-25th image during the BOLD session. TTP was extracted as the time from cuff release until the HPV. TTHP was extracted as the time from cuff release until half of the HPV. HPV was extracted as peak SI divided by baseline SI to represent the HPV as a

relative increase compared to rest (Figure 3).

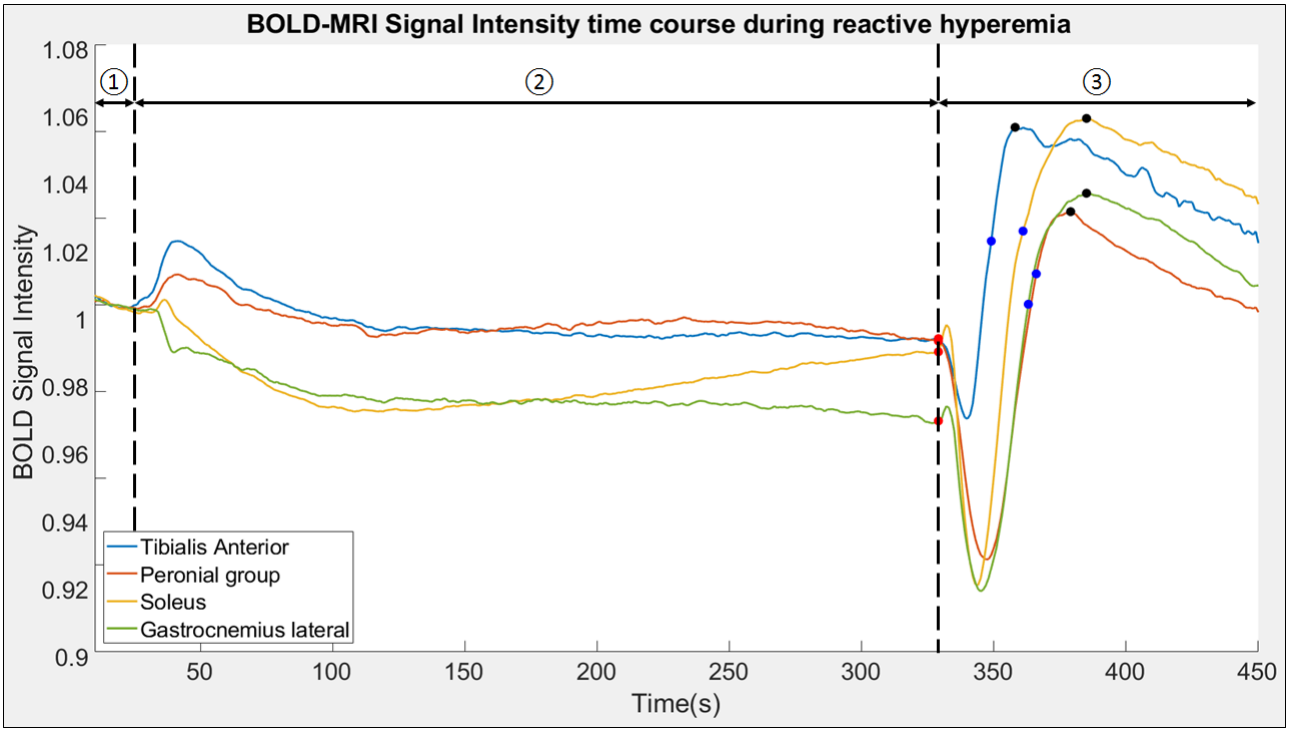

Figure 3. An entire BOLD-MRI signal intensity time course during reactive hyperemia with red marks representing the SI at cuff release, blue marks representing the point of half peak SI and black marks representing the hyperemic peak value. 1: Resting period, 2: Cuff occlusion period, 3: Reperfusion and hyperemia period.

Statistical analysis

Statistical analysis was carried out using SPSS version 25 (IBM Corp., Armonk, New York). Seven unpaired t-tests were used to find significant differences in the descriptive data from both genders. Three mixed design two-way (gender, muscle) repeated measures ANOVAs were used to test gender-based differences in TTP, TTHP and HPV across muscles (TA, PG, SO and GM). Main effects for both gender and muscles were found, but no interaction effects. In case of main effect of muscle, Bonferroni post hoc tests were done to identify differences between muscles.

Results

	Male			Female			
Characteristics	**Means**	**±**	**SD**	**Means**	**±**	**SD**	**P<0.05**
Age(years)	23.1	±	2.4	23.1	±	2.9	
Height(m)	180.8	±	8.1	169.3	±	6.8	*
Body mass(kg)	77.0	±	13.7	62.8	±	10.7	*
Body mass index(kg/m²)	23.4	±	2.7	21.8	±	2.8	
Systolic blood pressure(mmHg)	124.2	±	9.6	112.5	±	7.3	*
Diastolic blood pressure(mmHg)	71.6	±	9.7	74.5	±	8.1	
Physical activity (MET-min/week)	2786.6	±	1905.5	2781.8	±	1437.1	

Table 1. Male and female participant characteristics with significant differences marked between genders.

Significant differences between genders were found in height (P<0.000), body mass (P<0.005) and systolic blood pressure (P<0.002). With males being taller, heavier and having a higher systolic blood pressure. No significant differences were found in age(P<0.977), body mass index (P<0.131), physical activity (P<0.994) and diastolic blood pressure (P<0.434) (Table 1).

Significant main effects were found by gender(P<0.002) and muscle group(P<0.001) but no interaction effect(P<0.098) for TTP. Females had a shorter TTP in all muscle groups compared to males. The Bonferroni post hoc test found significant differences between TA and PG(P<0.002), SO(P<0.023) and GM(P<0.011) with TA having a significantly shorter TTP compared to the other muscle groups. Similarly, a significant main effect by gender(P<0.002) and muscle group(P<0.002) but no interaction effect(P<0.051) was found for TTHP. Females had shorter TTHP in all muscle groups compared to males. The post hoc test revealed significant differences between TA and PG(P<0.007), SO(P<0.028), and GM(P<0.000) with TA having significantly shorter TTHP compared to the other muscle groups. For HPV a significant main effect by muscle group(P<0.000), no significant main effects by gender(P<0.657) and no interaction effect(P<0.897) were found. The post hoc test revealed significant differences between SO and TA(P<0.027), PG(P<0.001) and GM(P<0.000), with SO having significantly higher HPV values compared to the other muscles (Figure 4).

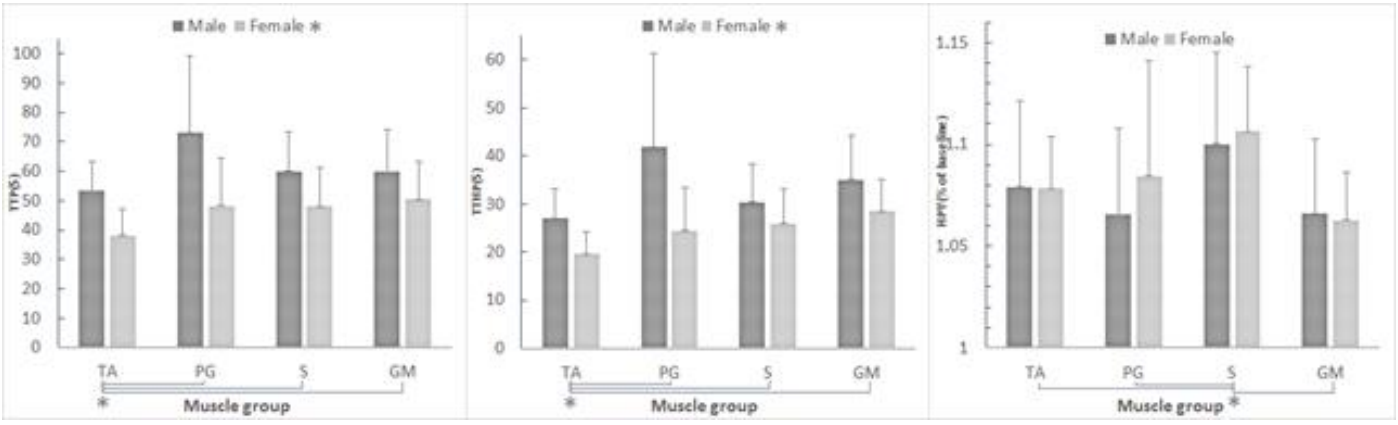

*Figure 4. Mean values and standard deviations for TTP, TTHP and HPV from left to right. Significant differences are marked with *.*

Discussion

This is the first study to investigate gender differences in parameters related to microvascular function using BOLD-MRI. The primary findings of this study are the significantly shorter TTP and TTHP across all muscles in females compared to males, as well as the finding that no significant gender effect exists in HPV. Additionally, this study found intermuscular differences in measures of microvascular function showing TA having significantly shorter TTP and TTHP compared to other muscles, while SO has significantly higher HPV compared to other muscles.

Gender differences

The decreased TTP and TTHP values found in females compared to males in this study suggests an improved ability to supply oxygenated blood to the microvasculature for females. Specifically, this study found that females had 28.4%, 33.9%, 19.7% and 15.4% shorter TTP in TA, PG, SO and GM, respectively, compared to males and 27.5%, 41.6%, 14.7% and 18.8% shorter TTHP in TA, PG, SO and GM, respectively, compared to males. These findings compare well to findings in the macrovasculature where females have shown improved vascular function compared to males as well. One study investigated differences in FMD between genders by inflation of an occlusion cuff for five minutes and measurement of the peak brachial artery diameter following cuff deflation. The peak brachial diameter relative to baseline brachial diameter was significantly larger in females across multiple populations grouped based on Framington risk scores (22). This was supported by another study using a similar occlusion protocol that found increased FMD for females compared to males as well (2). Interestingly, this study investigated the wall shear stress of the brachial artery simultaneously to the brachial artery diameter and found that females experience less wall shear stress while having a larger increase in brachial artery diameter. Finally, they compared the change in brachial artery diameter and change in wall shear stress each minute during reactive hyperemia. They saw female brachial artery diameter dilated sooner relative to the wall shear stress compared to males, suggesting an improved sensitivity in the regulation of artery diameter (2). The improved sensitivity in regulation of the artery diameter is consistent with the present results of shorter TTP and TTHP for females found in this study. An improved sensitivity in females compared to males could be corresponding to the decreased sympathetic nerve activity found in females compared to males, possibly improving vasodilation in females (24,25). Similarly, estrogen has shown a positive effect on vasodilation and could be related to the differences found as well (3). The HPV has been used as an indicator for vasodilatory capacity and based on the results of this study, nothing suggests that any differences in capacity between genders exist in the microvasculature (13–16). However, the decreased TTP and TTHP values found in females compared to males adds to the current knowledge of the microvasculature. These findings are relevant to understanding the key processes in the microvasculature, such as the exchange of oxygen, that are essential in all physical activity. The findings of this study warrant for future studies into the underlying mechanisms that cause the differences we see in the microvasculature.

Intermuscular differences

Some research on intermuscular differences in the microvasculature exists, however it has rarely been the primary focus. One study on young healthy participants performed a similar ischemia-reperfusion protocol to the one used in this study. BOLD-MRI was acquired during five minutes of cuff occlusion followed by partial deflation of the cuff to 30 mmHg below systolic blood pressure. They found 30.1% shorter TTP in TA compared to SO and 32.5% shorter TTP for gastrocnemius (17). The fact that TTP is shorter for TA is similar to what we found in this study, but differences are seen in the magnitude of the differences. Specifically, this study found that TA had 14.3% and 15.6% shorter TTP compared to SO and GM and 16% and 26% shorter TTHP compared to SO and GM, respectively. The differences in magnitude could possibly be explained by partial deflation of the cuff. The partial deflation might cause relatively more occlusion on SO and gastrocnemius compared to TA, due to the tibia mitigating some of the pressure applied to TA, which would result in relatively shorter times for TA. Another study investigated participants with PAOD and performed cuff induced ischemia for five minutes, followed by complete deflation for five minutes allowing reperfusion and hyperemia. The study used healthy control group in which they found 10.3% shorter TTP in TA compared to SO and 8.5% shorter TTP compared to gastrocnemius. Again, the findings are very similar to the ones found in this study, but for this one the decrease in TA is slightly smaller. The two participant groups were vastly different with an average age of participants in the mentioned study of 64 ± 6.7 compared to 23.1 ± 2.7 years for participants in this study. Additionally, the participants in this study had lower systolic, diastolic blood pressure and BMI compared to the control group in the mentioned study. The broad differences between the two groups of participants tested strengthens the findings, as they appear to apply on multiple different populations. Both the PAOD study (10) and the study on healthy young participants (17) investigated the HPV as well. In young healthy participants SO had a non-significantly larger HPV of 1.9% compared to TA and a significantly larger HPV of 2.9% compared to gastrocnemius. These values are very similar to the ones found in this study where SO had a 2.2% increase compared to TA and 3.6% compared to GM. The increased HPV in SO could possibly be explained by it having dual blood supply from both the posterior tibial and peroneal vessels (26) as well as SO having a higher capillary density and myoglobin content (7).

TTP vs TTHP

This study used both TTP and TTHP as parameters for vasodilatory function. Unfortunately, the methods of obtaining these parameters are not transparent in the literature (17) and they are not equally easy to standardize. Finding the peak SI is simple, but because the SI following peak SI remains very close to maximum, noise is prone to move the TTP to a later point than expected. However, finding the time frame which is closest to half of the HPV is simple and not prone to any operator bias. In this study, TTP and TTHP conclude the same significant differences and considering this, TTHP could be used more reliably in future studies as it is much easier to standardize compared to TTP.

Strengths of image registration

Accurate segmentation of BOLD-MRI can be difficult and prone to operator bias due to the resolution and signal to noise ratio is poor compared to other types of MRI. The use of multimodal image registration allowed for the segmentation to be done on T2-weighted MRI and then transferred to BOLD-MRI. This is very useful for accurate segmentations of muscles in the lower leg, as muscle fascia are often hard to see, if not impossible. Especially for SO this is problematic, as it borders to all the other muscles and has little of its outline defined by its border to the outer layer of fat in the calf. The tool developed for this study to segment and analyze BOLD SI in separate muscles could be mimicked in future studies, especially considering the use of image registration to reduce operator bias. Verifying the accuracy of the segmentations is however hard to quantify and must be done by visually inspecting the similarity of the segments made on the T2-weighted image and comparing them to the transferred BOLD segmentations. This is a common issue in image segmentation as in the case of this study, we would need a ground truth image of calf muscle segmentation.

Effects of menstrual cycle

This study did not control for the menstrual cycle of female participants and studies on the macrovasculature have reported variations in vasodilatory function during the menstrual cycle, partly due to increased levels of estrogen (4,27,28). Ideally, female participants would have been tested during the menstrual phase of their cycle, as estrogen levels in females have shown to be similar to males during that phase (3). Assuming the increase in vasodilatory function found in the macrovasculature transfers to the microvasculature, the increased vasodilatory function during the follicular and luteal phase are small in magnitude (7% increased FMD) (20) compared to the previously mentioned gender effects found in this study.

Conclusions

In conclusion, the results of this study show that gender influence the hyperemic response of the microvasculature during reactive hyperemia. Specifically, TTP and TTHP were shorter across all muscles in females compared to males while no differences were found for HPV. The present study does not elucidate the underlying mechanisms responsible for gender differences in the microvasculature, but possible mechanisms could include lower sympathetic nerve activity in females. Intermuscular differences were comparable with the existing literature, showing decreased TTP and TTHP in TA compared to PG, SO and GM and SO having increased HPV compared to TA, PG and GM. Further studies could benefit from the use of image registration as this is an efficient tool to minimize operator bias in segmentations of muscles. Lastly, no differences in the results of TTP and TTHP were found, and TTHP is suggested as a more robust parameter for investigation microvascular function compared with TTP. The gender effects found in this study contributes with new knowledge in the field of microvascular function and the underlying mechanisms should be investigated in future studies.

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