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Workload and sex effects in comprehensive assessment of cutaneous microcirculation

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ABSTRACT

Introduction: Workload and sex-related differences have been proposed as factors of importance when evaluating the microcirculation. Simultaneous assessments with diffuse reflectance spectroscopy (DRS) and laser Doppler flowmetry (LDF) enable a comprehensive evaluation of the microcirculation. The aim of the study was to compare the response between sexes in the microcirculatory parameters red blood cell (RBC) tissue fraction, RBC oxygen saturation, average vessel diameter, and speed-resolved perfusion during baseline, cycling, and recovery, respectively.

Methods: In 24 healthy participants (aged 20 to 30 years, 12 females), cutaneous microcirculation was assessed by LDF and DRS at baseline, during a workload generated by cycling at 75 to 80 % of maximal age-predicted heart rate, and recovery, respectively.

Results: Females had significantly lower RBC tissue fraction and total perfusion in forearm skin microcirculation at all phases (baseline, workload, and recovery). All microvascular parameters increased significantly during cycling, most evident in RBC oxygen saturation (34 % increase on average) and perfusion (9-fold increase in total perfusion). For perfusion, the highest speeds (>10 mm/s) increased by a factor of 31, whereas the lowest speeds (<1 mm/s) increased by a factor of 2.

Conclusion: Compared to a resting state, all studied microcirculation measures increased during cycling. For perfusion, this was mainly due to increased speed, and only to a minor extent due to increased RBC tissue fraction. Skin microcirculatory differences between sexes were seen in RBC concentration and total perfusion.

1. Introduction

The primary challenge for the cardiovascular system during workload is to deliver adequate amount of oxygenated blood to active muscles. However, there are other demands as well, such as maintaining body temperature and blood pressure. After physical activity and during recovery, blood flow is redistributed to eliminate excess heat and to prevent syncope. Skin blood flow in non-glabrous areas is the primary site for heat elimination during physical activity, while the glabrous skin overtakes this role in recovery (Potočnik and Lenasi, 2016). The intrinsic myogenic activity in vascular smooth muscle cells (vasomotion) increases during and after exercise, which has been demonstrated using laser Doppler flowmetry and tissue reflectance oximetry (Dunaev et al., 2014; Kvernmo et al., 1998). The myogenic rhythms during exercise may increase oxygen consumption due to increased blood flow in the microcirculation (Dunaev et al., 2014). Regular exercise leads to functional and structural adaptations of the cardiovascular system. Individuals that train endurance have a higher core temperature and cutaneous blood flow at any level of workload (Lenasi and Strucl, 2010). Repeated exercise has a strong and independent beneficial effect on the cardiovascular system (Blair and Morris, 2009), that may be partly mediated through the direct impact of exercise on the vasculature (Green et al., 2008; Green et al., 2017; Joyner and Green, 2009). Repeated exercise during 8-weeks of cycling training increased forearm perfusion, possibly related to local adaptation of cutaneous microcirculation (Atkinson et al., 2018). In addition, repeated physical activity increased the responsiveness to various vasodilator stimuli in the cutaneous microcirculation (Wang et al., 2002; White, 2002). In patients

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with hypertension (Higashi et al., 1999), type 2 diabetes (Colberg et al., 2002), and coronary heart disease (Hambrecht et al., 2003), regular physical activity can improve endothelium-dependent vasodilation.

Besides aerobic capacity, age and sex are influencing factors on the cutaneous perfusion (Hodges et al., 2010; Tew et al., 2012). The incidence of cardiovascular disease is higher among males and postmenopausal females compared to premenopausal females, which might be explained by differences in estradiol levels (Arrick et al., 2016). Menstrual cycle and hormonal contraceptives have been suggested to impact thermoregulatory mechanisms due to variation in levels of estradiol and progesterone. Estradiol promotes vasodilation in the skin which results in heat dissipation and contribute to the lower blood pressure seen in young women compared to young men, while progesterone appears to promote heat conservation by stimulating less vasodilation (Charkoudian et al., 2017). Healthy young women have been suggested to have a higher cutaneous microvascular reactivity than men in response to vascular occlusion (Stupin et al., 2019). In 50 to 64-year-old individuals, sex and age differences in the microcirculation were investigated with brachial artery occlusion, using Periflux 6000 EPOS (Enhanced Perfusion and Oxygen Saturation) (Jonasson et al., 2020). The study found that females had lower red blood cell (RBC) tissue fraction in general, and higher peak oxygen saturation and lower perfusion at baseline.

Various imaging techniques have been used to study the microvasculature of skin, such as dermoscopy, capillaroscopy, Doppler sonography, laser Doppler flowmetry, perfusion imaging, laser speckle contrast imaging, and optical coherence tomography (Deegan and Wang, 2019). Although each imaging method has been effective in addressing a specific aspect, there is no single modality that can cover all aspects without any drawbacks. As a result, the advancement of cutaneous microvascular imaging may rely on combining multiple imaging methods to overcome the limitations of individual systems and enhance our imaging abilities in a synergistic manner (Deegan and Wang, 2019).

The skin blood flow, measured by laser Doppler flowmetry, increases during workload (Potočnik and Lenasi, 2016). However, further details of the distribution of blood flow regarding speed distribution, RBC tissue fraction, vessel diameter, and the changes in cutaneous oxygen saturation, have so far not been studied. The influence of sex during workload on these parameters is also unknown. The Periflux 6000 EPOS measures the abovementioned microcirculatory parameters simultaneously (Fredriksson et al., 2013; Fredriksson et al., 2020), providing new perspectives and a deeper understanding of the microcirculation.

The aim of the study was to compare the comprehensive cutaneous forearm microcirculation parameters between sexes during baseline, workload by cycling, and recovery. We hypothesized an overall increased cutaneous perfusion and RBC oxygen saturation related to workload. We expected the most prominent increase within parameters related to vessels with higher speeds in the microcirculation.

2. Materials and methods

2.1. Study population

Twelve women and 12 men, in the ages between 20 and 30 years old, mean 24 (standard deviation (SD) 2) years, participated in the study. They had no known cardiovascular, skin, or microvascular diseases. Their body mass index (BMI) ranged between 20.3 and 29.1 kg/m^2, with a mean of 24.7 (2.9) kg/m^2. They were non-smokers and did not take any vasoactive medications. Demographic details are presented in Table 1. All participants were informed to refrain from exercise and alcohol the day prior to and on the test day. They were asked to refrain from caffeine or nicotine intake the same day as the test. All participants gave their informed written consent prior to the onset of the experiment. The study was approved by the Swedish Ethical Review Authority, d.no. 201904713.

To determine how physically active the participants were, they were asked to answer two questions acquired from Olsson et al. (2016). The participants were asked how much time they spend exercising (e.g., running, ball games, or fitness class) every week, and how much time they spend being physically active, doing things such as gardening or walking.

Table 1
Demographics and physiological parameters for males and females. Data are presented as mean (SD), or number of participants in each group.

	Females, N = 12	Males, N = 12	p-value
Systolic blood pressure, mmHg	109 (8)	123 (7)	<0.001
Diastolic blood pressure, mmHg	67 (9)	76 (8)	0.031
Age, years	24 (2)	25 (3)	n.s.
BMI, kg/m^2	25 (3)	24 (2)	n.s.
Exercise			n.s.
30–60 min per week	3	2	
60–90 min per week	3	0	
90–120 min per week	2	5	
>120 min per week	4	5	
Skin temperature			
Baseline, °C	30.3 (0.6)	31.2 (0.4)	0.001
Cycling °C	28.3 (1.1)	29.2 (0.7)	0.032
Recovery, °C	30.1 (0.9)	30.8 (0.8)	n.s.
Revolutions per minute	72 (10)	75 (15)	n.s.
Workload, W	94 (24)	116 (26)	0.035
Workload/body weight (W/kg)	1.4 (0.4)	1.5 (0.4)	n.s.
Heart rate, baseline, beats per minute	68 (6)	66 (9)	n.s.
Heart rate cycling, beats per minute	151 (2)	151 (3)	n.s.
Heart rate, recovery, beats per minute	75 (5)	80 (14)	n.s.

p-values refer to Student's t-test between sexes, except for exercise where Mann-Whitney U-test was used.

2.2. Equipment

The data was collected using the Periflux 6000 EPOS system (Perimed AB, Järfälla-Stockholm, Sweden). The EPOS system has previously been described in detail (Fredriksson et al., 2013; Fredriksson et al., 2020; Jonasson et al., 2015; Fredriksson et al., 2017; Jonasson et al., 2019). Briefly, the system consists of one laser Doppler flowmetry (LDF) unit and one diffuse reflectance spectroscopy (DRS) unit. The units collect signals from the same point of skin tissue via a fiber-optic probe, where the length of the optical fibers was 2.8 m.

When the laser light (Class I laser, wavelength 785 nm) is scattered by moving red blood cells in the vessels, a tiny Doppler shift of the laser frequency occurs, and the LDF unit calculates a Doppler effect spectrum from the frequency content of all detected laser light.

The different chromophores in the tissue, including reduced and oxygenized hemoglobin, have characteristic light absorption as a function of wavelength. In addition, light scattering in tissue varies with wavelength. As a result of the absorption and scattering processes, white light that is backscattered from the tissue changes color depending on the tissue content, reflected in the wavelength spectra assessed with the DRS unit, in the wavelength interval 475–750 nm.

The EPOS system utilizes artificial neural networks to analyze the Doppler power spectra and the wavelength spectra from the LDF and DRS units to assess microvascular parameters, namely the RBC tissue fraction (%), the RBC oxygen saturation (%), the average vessel diameter (μm), and perfusion (% RBC × mm/s). The perfusion is separated into three speed regions, low speed (<1 mm/s), medium speed (1–10 mm/s), and high speed (>10 mm/s), as well as the total perfusion, i.e. the sum of the perfusion in the three speed regions. The sampling depth is about 0.5 mm from the skin surface. The system was set to collect and analyze 15 sets of spectra (DRS and LDF), per second.

The oxygen saturation and RBC tissue fraction parameters assessed with EPOS have been validated with physical phantoms giving an absolute root mean square (RMS) deviation of 5 %-units or better for

oxygen saturation and a relative RMS deviation of 11 % or better for the RBC tissue fraction (Fredriksson et al., 2017). Model-based validation showed similar results (Fredriksson et al., 2020). The speed-resolved perfusion has been validated with a flow phantom indicating a relative RMS deviation of 8 % or better (Jonasson et al., 2019).

The speed-resolved perfusion enables discrimination of low-speed nutritive flow from flow in larger vessels where the speed can be assumed to be higher (Fredriksson et al., 2013; Fredriksson et al., 2017). It should be noted that the oxygen saturation assessed with EPOS is the average oxygen saturation of all hemoglobin in the sampling volume, which is different from the oxygen saturation assessed with pulse oximetry that only considers the pulsatile part, i.e. the arterial oxygen saturation. Thus, the oxygen saturation values from EPOS are generally much lower than what would be expected from a pulse oximeter. The average vessel diameter is a quantity that can be assessed from the DRS wavelength spectra since the vessel diameter affects those spectra in a characteristic manner. Studying all these parameters simultaneously, including their interplay during changes in the microcirculation, enables new understanding of the microcirculation.

2.3. Study protocol

All experiments were done in the same room with a mean temperature of 21.9 (SD 0.8) °C. The participants lay down and rested for about 5 min before blood pressure was measured using an automated blood pressure cuff (Omron M7 intelli AFIB, Kyoto, Japan). The EPOS probe was placed, using double-adhesive tape (PF 105-1, Perimed AB and 3 M Tegaderm I.V. Transparent Dressing, 1633, 3 M, Maplewood, MN, USA), on the volar skin of the non-dominant forearm, around 15 cm above the wrist, avoiding hair, visible veins, tattoos, tendons, and scars. Data was continuously recorded at baseline (rest in supine position) for 20 min, during cycling for 25 to 35 min, and after the workload for another 35 min during recovery in supine position. The bicycle was placed close to the bed with the EPOS instrument in between, enabling continuous recording during the whole test from rest to cycling and back to rest, due to the long fiber optic cable.

Cycling was chosen as workload since the forearm could be kept relatively still, minimizing the risk of motion artefacts. The participants were also told to keep the measurement arm as still as possible throughout the test. They were offered a blanket to counteract cooling during the initial rest and the recovery period. Skin temperature was measured on the skin proximal to the probe, every five minutes at baseline and recovery, and every second minute during cycling, using a digital infrared thermometer (Fluke 572 CF, Fluke Corporation, Germany). Heart rate was measured continuously with a pulse oximeter, Nonin GO2 model 9570 (model 9570 Oximeter, Nonin Medical, Inc., USA).

The cycling was performed on a Monark Ergomedic 839 E (Grimaldi Industri AB, Stockholm, Sweden). Participants initially warmed up for about 5 to 10 minutes to allow them to reach their target heart rate. Once target heart rate was reached, participants were asked to cycle for another 20 min, at an effort level maintaining their target heart rate. The targeted heart rate was 75 % to 80 % of each participant's maximum heart rate that was estimated by subtracting age from 220 beats per minute (bpm) (Fox et al., 1971). The revolutions per minute and wattage on the exercise bike were adjusted to fit the target heart rate and were documented every other minute.

2.4. Statistical analysis

Data were analyzed using SPSS 28.0 (SPSS Inc., Chicago, Illinois, USA). The distribution of the interval and ratio scale variables were examined with Shapiro Wilk's test of normality. No demographic or physiological data violated the normality test. Therefore, Student's *t*-test was used to compare parameters between sexes, except for the ordinal data (the participants estimated amount of exercise and physical activity per week) where Mann-Whitney *U* test was used.

Microcirculatory data were considered depending on two factors in a general linear model ANOVA. The within-subject factor was workload with three levels: baseline (average of 20-min baseline period), during cycling (average of last 10 min of cycling) and end recovery (average of last 20 min of the 35-min recovery period). The between-subject factor was sex. Extreme outliers were identified as values deviating >3 times the interquartile range within each sex and activity phase (baseline, cycling, recovery). These values were excluded in the statistical analysis. Data were analyzed using a general linear model with repeated measures, if the Mauchly test of sphericity test was not violated. Pairwise comparisons were Bonferroni corrected. If Mauchly's test was violated, sex effects were compared using *t*-test at each activity phase. If data differed, they were statistically compared separated by sex, else as one group. For completeness, data were presented both separated by sex and as one group. The effect of workload was compared using the Friedman rank test for comparing repeated measures on either all data or separated by sex if significant sex effects were found in the former *t*-test.

3. Results

There were no significant differences between males and females for amount of exercise or level of physical activity. Females had a significantly lower systolic blood pressure (mean (SD) = 109 (8) vs. 123 (7) mmHg, $p = 0.000$) and diastolic blood pressure (67 (9) vs. 76 (8) mmHg, $p = 0.031$) compared to males. Females had a significantly lower skin temperature at baseline (30.3 (0.6) vs 31.2 (0.4) °C, $p = 0.001$) and at cycling (28.3 (1.1) vs 29.2 (0.7) °C, $p = 0.032$) compared to males. Females also had a lower workload compared to males (94 (24) vs 116 (26) W, $p = 0.035$), as shown in Table 1, but when normalized with body weight, there was no significant difference (workload/body weight).

3.1. Microcirculatory response

In Fig. 1, an example of the measured signals from one of the participants is shown. A 10 s moving average filter was applied on the signals for better visualization in the 90 min time scale. The shaded sections in the plots represent the signals that were averaged and analyzed; baseline, last 10 min of cycling, and last 20 min of recovery.

The microvascular response is summarized in Fig. 2. The oxygen saturation showed a significant workload effect ($p < 0.001$, ANOVA; Table 2). Cycling and end recovery values were higher than baseline ($p < 0.001$) and cycling values higher than end recovery ($p < 0.001$).

The RBC tissue fraction showed significant workload and sex effects ($p < 0.05$ and $p < 0.05$, respectively, ANOVA). Baseline and cycling values were significantly higher for males ($p < 0.05$ and $p < 0.05$, respectively).

The vessel diameter and perfusion parameters did not fulfil the Mauchly sphericity test and were, therefore, analyzed for sex effect using *t*-tests at each workload level and then for workload effect using Friedman rank ANOVA. The vessel diameter did not have a significant sex effect but a significant workload effect ($p < 0.001$).

Total perfusion was significantly higher for males at baseline ($p < 0.05$) and at end recovery ($p < 0.01$). There were significant workload effects for males and females ($p < 0.001$, respectively) with values during cycling being higher than at baseline. For speed-resolved perfusions (speed intervals <1 mm/s, 1–10 mm/s and > 10 mm/s, respectively), there were no sex effects. There were significant workload effects for all parameters ($p < 0.001$, respectively) with higher values during cycling than at baseline.

In Fig. 2, box plots are shown for RBC oxygen saturation, RBC tissue fraction, vessel diameter, and total perfusion, showing the differences between sexes and between baseline, cycling, and end recovery. The average speed-resolved perfusion grouped on sex is shown in Fig. 3 at baseline, cycling, and end recovery.

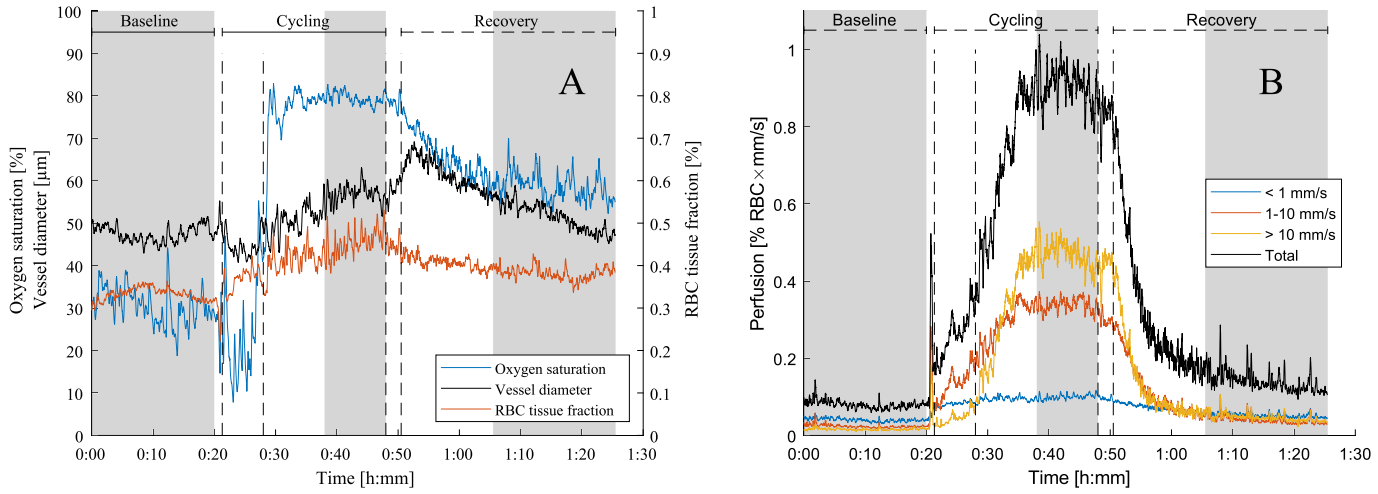

Fig. 1. Example of measured signals from one of the participants. Time periods where average values were calculated for baseline (0:00–0:20), last ten minutes of cycling (0:38–0:48), and last 20 min of recovery (1:06–1:26) are shaded. Time points for the start of cycling (0:21), reached target heart rate (0:28), end cycling (0:48), and start recovery, i.e., lying down (0:51) are marked with dashed black vertical lines. Movement artefacts can foremost be seen in the perfusion signals (B) just after 20 min when the participant moved to the ergometer.

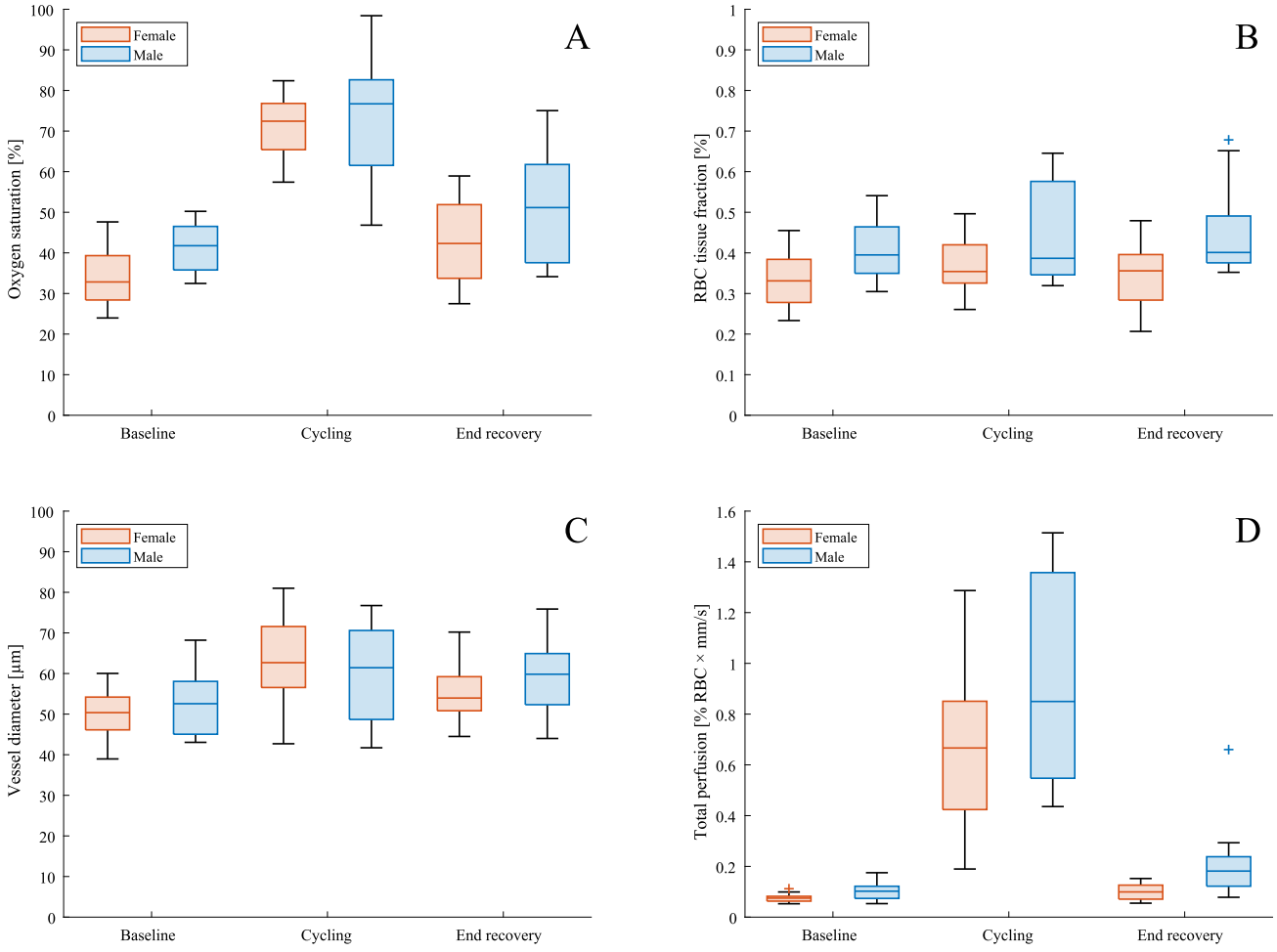

Fig. 2. Box plots of RBC oxygen saturation (A), RBC tissue fraction (B), vessel diameter (C) and total perfusion (D), during baseline, cycling and end recovery, grouped on sex. The plots show the median (horizontal line inside box), the lower and upper quartiles (lower and upper boundaries of the box), any outliers (+), and max and min that are not outliers (whiskers). Outliers are defined as values that differ >1.5 times the interquartile range from the lower or upper quartile, respectively. Mean and standard deviation values are provided in Table 2.

Table 2
Effect of workload and sex on microcirculation. Data are displayed as mean (SD) excluding extreme outliers and presented separated by sex and for all participants, respectively, for completeness. If sex effect was significant, data separated by sex are bold italic marked, otherwise data for both sexes (All) are bold italic marked (See Statistical analysis). Pairwise post-hoc comparisons are described in Microcirculatory response for clarity.

	Female	Male	All	Workload	Sex
RBC Oxygen saturation [%]	N = 12	N = 12	***N = 24***	***, [1]	n.s.[1]
Baseline	34 (7)	41 (6)	***38 (7)***		
Cycling	71 (8)	74 (14)	***72 (12)***		
End recovery	43 (10)	52 (14)	***47 (13)***		
RBC tissue fraction [%]	***N = 12***	***N = 11***	N = 23	*, [1]	*, [1]
Baseline	***0.33 (0.07)***	***0.40 (0.07)***	0.37 (0.08)		
Cycling	***0.37 (0.07)***	***0.44 (0.12)***	0.40 (0.10)		
End recovery	***0.34 (0.08)***	***0.43 (0.09)***	0.38 (0.09)		
Vessel diameter [μm]	N = 12	N = 12	***N = 24***	***, [2]	
Baseline	50 (7)	53 (8)	***52 (7)***		n.s.[3]
Cycling	64 (11)	61 (12)	***62 (11)***		n.s.[3]
End recovery	56 (8)	59 (9)	***57 (9)***		n.s.[3]
Total perfusion [% RBC × mm/s]	***N = 12***	***N = 11***	N = 23	***, [2]/***, [2]	
Baseline	***0.076 (0.017)***	***0.10 (0.028)***	0.086 (0.025)		*, [3]
Cycling	***0.67 (0.33)***	***0.89 (0.38)***	0.78 (0.37)		n.s.[3]
End recovery	***0.10 (0.03)***	***0.17 (0.07)***	0.14 (0.06)		**, [3]
Perfusion <1 mm/s [% RBC × mm/s]	N = 12	N = 12	***N = 24***	***, [2]	
Baseline	0.045 (0.013)	0.040 (0.008)	***0.042 (0.011)***		n.s.[3]
Cycling	0.089 (0.026)	0.088 (0.027)	***0.088 (0.026)***		n.s.[3]
End recovery	0.051 (0.017)	0.052 (0.014)	***0.051 (0.015)***		n.s.[3]
Perfusion 1–10 mm/s [% RBC × mm/s]	N = 11	N = 10	***N = 21***	***, [2]	
Baseline	0.030 (0.012)	0.042 (0.031)	***0.036 (0.023)***		n.s.[3]
Cycling	0.21 (0.08)	0.25 (0.16)	***0.23 (0.12)***		n.s.[3]
End recovery	0.044 (0.023)	0.054 (0.026)	***0.049 (0.024)***		n.s.[3]
Perfusion >10 mm/s [% RBC × mm/s]	N = 11	N = 9	***N = 20***	***, [2]	
Baseline	0.013 (0.009)	0.019 (0.016)	***0.015 (0.013)***		n.s.[3]
Cycling	0.42 (0.30)	0.50 (0.34)	***0.46 (0.31)***		n.s.[3]
End recovery	0.028 (0.041)	0.044 (0.035)	***0.035 (0.038)***		n.s.[3]

[1] Significance for the factor using a two-way general linear model ANOVA with sex and workload (repeated measure) as factors.
[2] Significant workload effect using Friedmans repeated measures rank test for each sex.
[3] Significance for sex difference using independent group t-test. (See Statistical analysis).
* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.

4. Discussion

The main findings in this study were that women had an approximately 25 % lower concentration of RBC and a 30 % lower total perfusion in forearm skin microcirculation at all three analyzed activity phases (baseline, cycling, and end recovery). At a workload by cycling at 75 % to 80 % of maximal age-predicted heart rate, total perfusion increased 9 times compared to baseline values for both sexes. The most pronounced change was seen in the speed-resolved signal for speeds >10 mm/s. The different speed regions increased by a factor of 2, 4 and 31 for <1 mm/s, 1–10 mm/s, and > 10 mm/s, respectively. This was associated with a 10 % increase in the RBC tissue fraction and a minor increase in average vessel diameter. Hence, the perfusion increase was mostly by increasing perfusion speeds and not by increasing the number of RBCs by vessel dilation or vascular recruitment.

An increase from baseline to cycling, then a decrease from cycling to end recovery, with higher levels at end recovery compared to baseline, were observed for all microcirculatory parameters. The oxygen saturation, vessel diameter, and all the speed-resolved perfusion parameters significantly increased from baseline to cycling and could be explained by physiological adaptations to workload, where stroke volume increases and cutaneous vasodilation occurs (Hellsten and Nyberg, 2015). The RBC oxygen saturation increased since there was an increased blood flow without having a proportionally increased oxygen demand. In the study by Fredriksson et al. (2022), vessel diameter was negatively correlated with the average values for RBC tissue fraction, RBC oxygen saturation, and total perfusion during measurements of cutaneous forearm flowmotion at rest. The authors hypothesized that the negative correlation could be explained by capillary recruitment, lowering the average vessel diameter and increasing RBC tissue fraction and RBC oxygen saturation. However, in this study the corresponding values were positively correlated during cycling, indicating vasodilation to be the major mechanism rather than capillary recruitment.

In the speed-resolved perfusion signal, the increase from baseline to cycling was largest for speeds >10 mm/s, a 31-fold increase, compared with speeds between 1 and 10 mm/s with a 6-fold increase and speeds

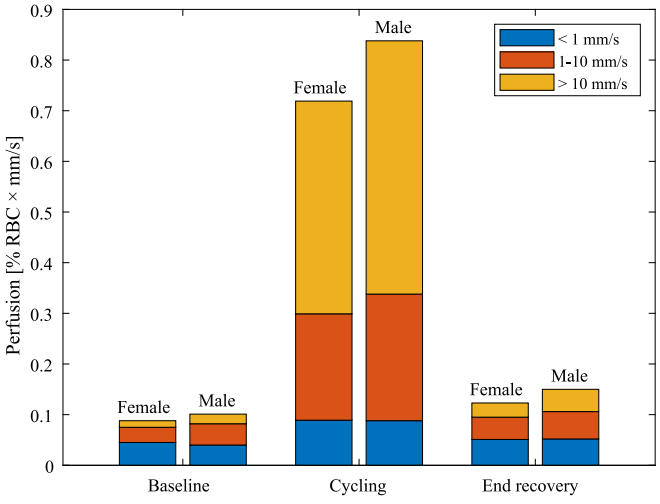

Fig. 3. Bar chart of the speed-resolved perfusion, divided into the three different speed regions; <1, 1–10, and >10 mm/s at baseline, cycling and end recovery for females and males. Note that the different speed regions do not exactly add up to the total perfusion seen in Table 2, due to different numbers of excluded values in total perfusion compared to the different speed regions.

<1 mm/s with a 2-fold increase. A possible explanation would be the different physiology of the blood vessels that each speed represent. The higher relative change in perfusion signal at speeds >10 mm/s can be explained by more smooth muscle cells in the larger vessels, resulting in greater vasodilation compared to smaller vessels (Moppett, 2012), and this has also been observed in response to local heating (Jonasson et al., 2015). Poole et al. reasoned about the lack of capillary recruitment in active skeletal muscle during exercise (Poole et al., 2008). In line with their reasoning, we speculate that during workload the skin is primarily increasing blood flow in larger vessels within the microcirculation. This is based on our findings of a limited increase in RBC tissue fraction and vessel diameter while blood flow with speeds above 10 mm/s had the most prominent response. If capillary recruitment, i.e. an increase in number of capillaries containing RBC, would have been the case, we had expected the most prominent perfusion increase in the lowest speed interval, assuming that blood flow speed in capillaries being low. This would then be accompanied by a decrease in average vessel diameter.

Our results are in line with Jonasson et al.'s work Jonasson et al. (2020), that demonstrated lower values for females in the perfusion signal and RBC tissue fraction at baseline and under provocation. It is known that females have higher levels of estradiol, which causes vasodilation by targeting vascular smooth muscle cells and endothelial cells (White, 2002). The fact that males had higher perfusion values was surprising since estrogen's potential to stimulate vasodilation would increase blood flow. Thus, there must be other explanations to our findings, such as higher levels of the vasoconstrictor endothelin (ET-1) reported in females (Evans et al., 1996), or the significantly lower blood pressure observed in females. It is known that females have lower blood pressure and skin temperature, possibly explained by estradiol's vasodilatory ability which results in lower blood pressure and increased heat dissipation (Charkoudian et al., 2017). The cutaneous microcirculation is directly connected to the skin temperature and can be one of the explanations to the observed sex differences. In addition, females generally have lower hemoglobin levels, hematocrit, and red blood cell count (Grau et al., 2018), which could explain their lower RBC tissue fraction. Another factor that could possibly explain our findings is the lower stroke volume during exercise reported in females, possibly explained by reduced sympathetic response and supported by lower levels of catecholamines (Wheatley et al., 2014). Additionally, males had significantly greater workload when cycling, indicating higher physical potential. Since we did not measure levels of estradiol, testosterone, hemoglobin, or catecholamine in the participants, we can only speculate that these factors had an impact on the sex related differences observed. Future studies should focus on the sex-related mechanisms in the microvascular function.

Age has been shown to be associated with cutaneous microcirculatory dysfunction. In aging, thermoregulatory responses are impaired and diseases such as hypertension and diabetes may worsen this further. This can be explained by mechanisms involving neural reflexes, sweat gland and vascular function. Increased physical activity can partly reverse the decreased heat dissipation responses in older. Also, it has been shown that older individuals have higher low-speed perfusion at baseline and lower peak oxygen saturation compared to young (Jonasson et al., 2020). Since the Periflux 6000 EPOS is a noninvasive way of measuring the microcirculation, it could possibly detect microvascular dysfunction before complications are presented. After menopause, levels of estradiol decrease, causing higher blood pressures in older females (Charkoudian et al., 2017). Therefore, it would be interesting to investigate sex related differences in older individuals.

As mentioned in the introduction, the vasomotion activity has previously been shown to change during and after exercise. In a newly published study by our group, we used frequency analysis of perfusion images to study the spatial heterogeneity of flowmotion, that is variations in blood perfusion induced by vasomotion (Hultman et al., 2023). That study showed a considerable spatial heterogeneity. This heterogeneity results in large uncertainty in flowmotion when assessed in a single point, as in this study. Thus, a study on changes in flowmotion in relation to exercise should preferably be done using perfusion imaging. However, a study on vasomotion utilizing perfusion imaging warrants effective algorithms minimizing the influence of motion artefacts, which is much less a problem with the current study.

The study is limited by the fact that physical performance levels were based solely on self-reported assessment by the participants, thus variations in individual fitness could possibly have had an impact on the results. However, all participants exercised for at least 30 min every week, which is likely above average. Endurance training improves cardiovascular adaptations, resulting in increased core temperature and cutaneous blood flow at any level of workload (Lenasi, 2014; Simmons et al., 2011). Therefore, our findings might not be representable for the 20 to 30-year-olds in Sweden. Another limitation was that the cycling time was not equal for all participants since the individuals reached their targeted heart rate at different times, resulting in various cycling times. The shortest cycling time was 24 min and the longest 35 min, an inconsistency that may affect the analysis. The study is further limited by the lack of measurement of estradiol levels or participants phase in menstrual cycle, which is only discussed as an explanatory factor.

5. Conclusion

Compared to a resting state, the local forearm cutaneous microcirculatory perfusion is increased during workload. This is mainly based on increased speed, although to a minor extent also RBC tissue fraction. Speed-resolved perfusion show the most prominent increase in larger vessel with speeds above 10 mm/s. Meanwhile the local oxygen saturation is also significantly increased. Skin microcirculatory differences between sexes were observed in signals related to RBC concentration and total perfusion.

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CRediT authorship contribution statement

Linda Samils: Formal analysis, Writing – original draft. **Joakim Henricson:** Formal analysis, Writing – review & editing. **Tomas Strömberg:** Formal analysis, Writing – review & editing. **Ingemar Fredriksson:** Conceptualization, Methodology, Formal analysis, Data curation, Writing – review & editing. **Fredrik Iredahl:** Conceptualization, Methodology, Resources, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

Dr. Fredriksson is part-time employed by Perimed AB, which is developing products related to research described in this publication.

Data availability

Data will be made available on request.

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