Master's thesis

NTNU
Norwegian University of Science and Technology
Faculty of Medicine and Health Sciences
Department of Neuromedicine and Movement Science

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Age-related differences in muscle microvascular response and muscle oxygen uptake: a near infrared spectroscopy approach

Master's thesis in Physical Activity and Health - Exercise Physiology
Supervisor: Mireille van Beekvelt

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Investigating age-related differences in muscle microvascular response and muscle oxygen uptake: A Near infrared spectoscopy approach

Groups

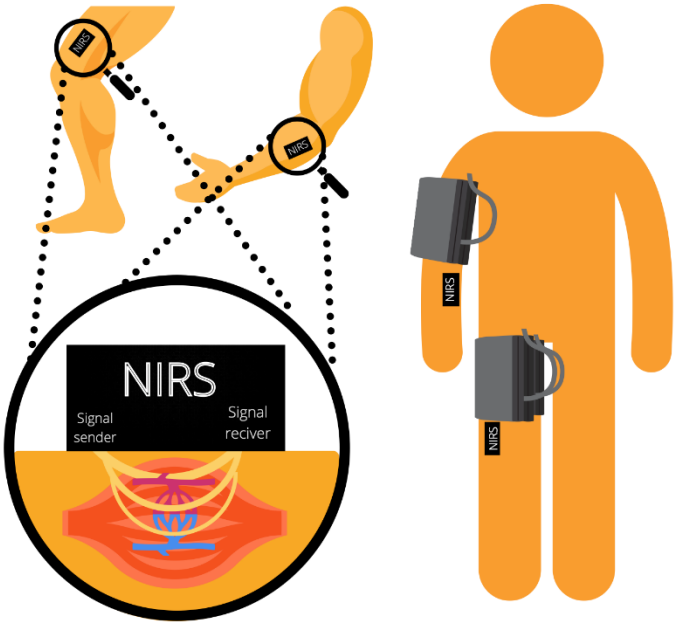
Young (n=19)
18-40 years old

Elderly (n=18)
60-80 years old

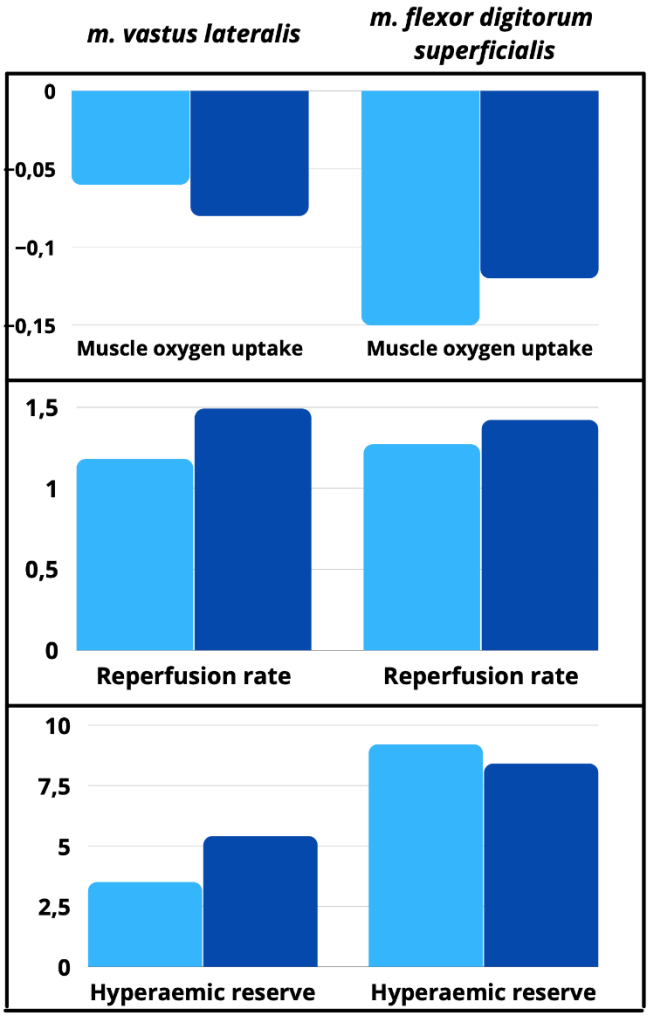
Methods

Near Infrared Spectroscopy (NIRS) together with an arterial occlusion:

Non-invasive method to measure skeletal muscle microvascular responsonse and muscle oxygen uptake.



Results



Reprefusion rate and hyperaemic reserve both reflect the muscles microvascular response.

Conclusion

We did not observe any significant difference in microvascular response and muscle oxygen uptake between elderly and young.

Abstract

Background: Blood flow to the limbs has shown to be significantly reduced in elderly when compared to their younger counterparts**.** The knowledge on the effect of age on microvascular function is still limited. Near infrared spectroscopy (NIRS) is a non-invasive method which can measure skeletal muscle microvascular responsiveness and muscle oxygen uptake. Although NIRS is thought to be a reliable method there are certain confounders like adipose tissue thickness over the muscle and physical fitness that should be considered. **Objective:** The main aim of this study was to further investigate if there is a difference in muscle microvascular response and muscle oxygen uptake (mVO_2) between elderly and young, taking into consideration physical performance parameters and adipose tissue thickness (ATT). The secondary aim of this study is to investigate the difference between an arterial occlusion fixed for time and an arterial occlusion fixed for the level of desaturation, between elderly and young. **Methodology:** A total of 37 participants completed the testing protocols, 19 young (25±0,5 years) and 18 elderly (69±1,4 years). To measure the participants microvascular response and mVO_2 two arterial occlusion tests (AOT) were performed, one with a fixed duration (AOT10) and one with a fixed desaturation (AOT20). The performance parameters measured were peak-incremental dynamic handgrip test (IHT-peak) and whole-body peak oxygen uptake (VO_2-peak). **Results:** No difference in microvascular response and mVO_2 between elderly and young, in the m. vastus lateralis (VL). In the m. flexor digitorum superficialis (FDS) there was observed a higher mVO_2 for the young group. We found a relationship between ATT for both microvascular response and mVO_2 in the VL. While in the FDS there was only observed a relationship between ATT and microvascular reperfusion rate (RR). We did also find a weak relationship between peak IHT-peak duration and the microvascular hyperaemic response (ΔSmO_2-max) in the FDS. Our study showed no difference in microvascular response and mVO_2 between elderly and young when using AOT20. While we did observe a difference in microvascular response and muscle oxygen uptake between AOT20 and AOT10. **Conclusion:** We found no difference in microvascular response and mVO_2 between elderly and young for neither AOT10 nor AOT20. We did observe that ATT did affect the NIRS-signals. We found a relationship between IHT-peak duration and microvascular hyperaemic response. We did observe a larger microvascular response for the AOT10 compared to AOT20.

Sammendrag

Bakgrunn: Blodstrømmen til arm og ben har vist seg å være betydelig redusert hos eldre sammenlignet med yngre. Kunnskap om effekten av alder på den mikrovaskulære funksjonen er fortsatt begrenset. Nær infrarød spektroskopi (NIRS) er en ikke-invasiv metode som kan måle muskelens mikrovaskulære respons og muskelens lokale oksygenopptak. Selv om NIRS antas å være en pålitelig metode, er det visse konfunderende faktorer som tykkelse på fettvev over muskelen og fysisk form som bør vurderes. **Mål:** Hovedmålet med denne studien var å undersøke om det er forskjell i muskelens mikrovaskulære respons og muskelens lokale oksygenopptak (mVO2) mellom eldre og unge, tatt i betraktning deltakernes fysiske ytelsesparametere og fettvevstykkelse (ATT). Det sekundære målet med denne studien var å undersøke forskjellen mellom en arteriell okklusjon som er fast for tid og en arteriell okklusjon med fast desatureringsnivået, mellom eldre og unge. **Metode:** Totalt 37 deltakere fullførte testprotokollen, 19 unge (25 ± 0,5 år) og 18 eldre (69 ± 1,4 år). For å måle deltakernes mikrovaskulære respons og mVO2 ble det utført to arterielle okklusjonstester, en med en fast varighet (AOT10) og en med en fast desaturering (AOT20). Ytelsesparametrene som ble målt var maksimal dynamisk gripetest (IHT-peak) og maksimalt oksygenopptak (VO2-peak). **Resultater:** Ingen forskjell i mikrovaskulær respons og mVO2 mellom eldre og unge, i m. vastus lateralis (VL). I m. flexor digitorum superficialis (FDS) ble det observert en høyere mVO2 for den unge gruppen. Vi fant en korrelasjon mellom ATT for både mikrovaskulær respons og mVO2 i VL. Mens det i FDS bare ble observert en korrelasjon mellom ATT og mikrovaskulær reperfusjonshastighet (RR). Vi fant også en svak korrelasjon mellom topp IHT-toppvarighet og mikrovaskulær hyperemisk respons (ΔSmO_2-max) i FDS. Studien vår viste ingen forskjell i mikrovaskulær respons og mVO2 mellom eldre og unge ved bruk av AOT20. Mens vi observerte en forskjell i mikrovaskulær respons og muskeloksygenopptak mellom AOT20 og AOT10. **Konklusjon:** Vi fant ingen forskjell i mikrovaskulær respons og mVO2 mellom eldre og unge for verken AOT10 eller AOT20. Vi observerte også at ATT påvirket NIRS-signalene. Vi fant en korrelasjon mellom IHT-peak varighet og mikrovaskulær hyperemisk respons. Vi fant også en større mikrovaskulær respons for AOT10 sammenlignet med.

Acknowledgements

I would like to thank my supervisor Mireille van Beekvelt for her help and guidance writing this thesis. I would also like to thank my co-workers in the laboratory. Lastly, I would also like to thank my fellow students for two wonderful years together.

List of abbreviations

SmO_2: Tissue saturation

mVO_2: Muscle oxygen uptake

RR: Reperfusion rate

ΔSmO_2-max: Hyperaemic reserve

ΔSmO_2-min: Relative tissue saturation decrease

SmO_2-baseline: Baseline tissue saturation

SmO_2-max: Absolute tissue saturation max

SmO_2-min: Absolute tissue saturation min

VO_2-peak: Whole-body peak oxygen uptake

IHT-peak: Peak incremental handgrip test

MVC: Maximal voluntary contraction

ICT: Incremental cycling test

ATT: Adipose tissue thickness

FDS: m. flexor digitorum superficialis

VL: m. vastus lateralis

EG: Elderly group

YG: Young group

CVD: Cardiovascular disease

BMI: Body mass index

MM_RA: Muscle mass right arm

BP: Blood pressure

RPE: Rate of perceived exertion (6-20)

BF%: Body fat %

HR-resting: Resting heart rate

HR-peak: Peak heart rate

La-peak: Peak blood lactate

Introduction

The number of people over 60 years of age is estimated to increase from 900 million to 2 billion between the years 2015 to 2050 (World Health Organization, 2017). Ageing is associated with increased risk of disease and functional decline (Burton & Sumukadas, 2010). It has been reported that after the age of 50 the loss of skeletal leg muscle mass is between 1-2% every year. Over time this loss may develop into more severe skeletal muscle weakness and wasting resulting in functional decline, also known as Sarcopenia (A. J. Cruz-Jentoft et al., 2010; Alfonso J Cruz-Jentoft et al., 2018).

The risk of disease, especially cardiovascular disease (CVD), is known to increase considerably with age. CVD is estimated as the leading cause of death in the world, with over 17 million registered deaths in 2008. This number is expected to increase to 23,6 million by the year 2030 (Mendis, Puska, Norrving, & Organization, 2011; Smith et al., 2012). It has been suggested that there is a relationship between low skeletal muscle mass and vascular dysfunction, but whether it is vascular dysfunction causing low skeletal muscle mass or vice versa has not been established (Dvoretskiy et al., 2020).

Research has shown that physical activity and exercise participation decreases with age, negatively affects the cardiovascular- and skeletal muscle system which causes a decline in peak aerobic exercise capacity. It is well known that peak aerobic exercise capacity, also measured as whole-body peak oxygen uptake (VO_2-peak), correlates well with quality of life and functionality in elderly (Fleg, 2012; McPhee et al., 2016).

With age vascular stiffening and endothelial dysfunction are thought to arise, resulting in a diminished capacity to cope with the fluctuating pressure that occurs during the cardiac cycle. As the elasticity of the vessels are diminished the pressure needed to perform the cardiac cycle has to be higher to sufficiently push the blood through the vascular system (Taylor & Johnson, 2008, pp. 15-16, 17-18). This compensation mechanism causes a heightened systolic blood pressure which is associated with an increased risk for developing CVD (Stevens et al., 2016). Endothelium, made up of endothelial cells, covers the inner layer of all cardiovascular structures, including the heart. In response to shear stress, caused by the rush of blood in the blood vessels, the endothelial cells release nitric oxide which works as a signalling molecule for vasodilating the blood vessels (McArdle, Katch, & Katch, 2015, p. 336). In dysfunctional endothelium the endothelial cells do not release enough nitric oxide causing the blood vessels

to not vasodilate sufficiently. Endothelial dysfunction is believed to be one of the first signs of developing CVD (Taylor & Johnson, 2008, pp. 15-16).

Vascular stiffness and endothelial dysfunction have a negative impact on blood flow to the muscles, causing a diminished delivery of oxygen rich blood to the muscles tissue which in turn may results in a decreased peak aerobic exercise capacity (Fleg, 2012; Konukoglu & Uzun, 2017; Widmer & Lerman, 2014). Endothelial dysfunction has also shown to be negatively affected by a sedentary lifestyle, independent of age (Taylor & Johnson, 2008, pp. 15-16; Widmer & Lerman, 2014). Blood flow to the limbs appear to be significantly reduced in elderly when compared to their younger counterparts. (Taylor & Johnson, 2008, p. 12). It is believed that microcirculation in the skeletal muscles has a large impact on the diminished exercise capacity seen with age (Beere, Russell, Morey, Kitzman, & Higginbotham, 1999; Proctor & Joyner, 1997; Ridout, Parker, & Proctor, 2005). Microvascular vessels, specifically arterioles, are known to be the main regulator of blood flow to the limbs. Arterioles are highly dynamic vessels due to its vasodilating-/vasoconstricting properties, accounting for more than 60% of the resistance in the cardiac cycle (Silverthorn, 2013, p. 519). It has been suggested that the age-related alterations happening in the macrovascular vessels expands to the microvascular vessels (Muller-Delp, 2006). The knowledge on the effect of age on microvascular function is still limited when compared to the knowledge we have about age-related effects on macrovascular vessels.

Near infrared spectroscopy (NIRS) is a non-invasive method which has shown to be reliable when measuring skeletal muscle microvascular responsiveness, muscle oxygen uptake and muscle saturation (Jones, Chiesa, Chaturvedi, & Hughes, 2016). Performing an arterial occlusion together with NIRS has shown to be a reliable method with a good day to day reliability for measuring the muscle oxygen uptake and muscle microvascular response in a resting state (Iannetta et al., 2019; Zhang, Hodges, & McCully, 2020). The arterial occlusion causes an ischemic state in the muscle by not letting any blood flow in or out of the limb (Jones et al., 2016). The NIRS-devices continuously measures the concentration changes in oxygenated (O_2Hb)- and deoxygenated haemoglobin and myoglobin (HHb), from which muscle oxygen saturation is calculated.

Prior studies using NIRS has shown an inferior muscle microvascular response and muscle oxygen uptake in elderly individuals when compared to their younger counterparts. (Horiuchi & Okita, 2020; Rosenberry et al., 2018). Although using NIRS is thought to be a reliable method

there are certain confounders like adipose tissue thickness over the muscle and physical fitness that should be considered. The adipose tissue thickness between the NIRS-device and the muscle of interest is a methodological confounding factor, as it affects how much of the NIRS-signal reaches the muscle. As for the physical fitness this is a physiological confounding factor, as an elderly individual with superior physical fitness most likely has a different muscle physiology compared to an age matched individual with lower physical fitness (Harridge & Lazarus, 2017). There has also been reported that NIRS-measurements are sensitive to arterial occlusion durations, as a longer occlusion has shown produces greater microvascular response (Iannetta et al., 2019; McLay, Gilbertson, Pogliaghi, Paterson, & Murias, 2016). It appears that the tissue desaturation level during an arterial occlusion affects the level of microvascular response. A study by Rosenberry et al. (2018) observed that elderly and young, with different microvascular responses during an arterial occlusion with fixed time, showed no difference in microvascular response when matching the level of tissue desaturation.

The main aim of this study is therefor to further investigate if there is a difference in muscle microvascular response and muscle oxygen uptake between elderly and young, taking into consideration physical performance parameters (i.e., peak aerobic exercise capacity) and adipose tissue thickness. The hypothesis is that the difference, or lack thereof, in microvascular response and/or muscle oxygen uptake is not solely due to age but may differ because of differences in the physical performance parameters and adipose tissue thickness between the groups.

The secondary aim of this study is to investigate the difference between an arterial occlusion fixed for time and an arterial occlusion fixed for the level of desaturation. We will also investigate if there is a difference between elderly and young when using an arterial occlusion fixed for the level of desaturation. With this approach we aim to test if the muscle microvascular response, muscle oxygen uptake and/or desaturation time will differ between the groups (elderly and young) and the occlusion tests (time-fixed vs desaturation-fixed). The hypothesis is that we will see a similar microvascular response between the groups regardless of other differences between the groups (i.e., fitness/age). We do hypothesize that there will be a difference in microvascular response between the arterial occlusion tests (time-fixed vs desaturation-fixed), as the occlusion durations most likely will differ. We do not expect that the mVO_2 will affected by different occlusion durations.

Methodology

This study is a part of a larger project collecting normative data with the aim to investigate non-invasive muscle markers in aging skeletal muscles. The methods that were not related nor interfered with our results are not presented in the methodology section.

Ethical approval

This study was approved by the Norwegian Regional Ethical Committee for Medical and Health Research Ethics (REC). All participants got verbal- and written information about the possible risks/discomforts of the testing protocols. Before being included in the study all participants had to sign an informed consent.

Participants

The data collection was conducted at the Norwegian university of science and technology in Trondheim, between November 2020 and January 2021. A total of 39 people participated in our study and were split into two groups, a young group (YG) and an elderly group (EG). Participants included in the YG had to be between the ages of 18-40 years old, participants included in the EG had to be between the ages of 60-80 years old. Two of the elderly participants did not finish, one due to fainting during the occlusion test and one due to abnormal heart rate measurements during the incremental cycling test. A total of 37 participants completed the testing protocols, 19 young (25±0,5 years) and 18 elderly (69±1,4 years. All included participants were recreationally active, had no present- or former history of pulmonary or- cardiovascular disease, had a body mass index (BMI) < 30 and were non-smokers. Two of the participants reported their left hand as their dominant one.

Study design

All the participants visited the lab on three separate occasions. The testing days were divided into two days with one to three days in between, while body composition analysis (BIA) was performed on a separate day.

On the first test day the participants went through an arterial occlusion test with a fixed duration of 10 minutes, a maximal voluntary contraction (MVC) test, an incremental handgrip test (IHT) and an incremental cycling test (ICT). On the second test day a vascular occlusion test with a fixed desaturation level of 20% was performed. The participants were instructed to avoid vigorous exercise and alcohol intake 24-48 hours before the test days and to avoid

caffeine intake at least 6-hours before the test. The NIRS-devices were attached to the right forearm and right thigh of the participants before starting the protocols.

Experimental protocol and equipment

Arterial occlusion tests

To measure the participants microvascular response and muscle oxygen uptake (mVO_2) two arterial occlusion tests were performed on two separate days, one with a fixed duration (AOT10) and one with a fixed desaturation (AOT20). In preparation for the arterial occlusion tests on both days the participants were instructed to sit down on a bench in a comfortable position, with the lower body in a supine position and the torso slightly upright. Following that two pneumatic tourniquet cuffs (Hokanson SC12L; Marcom Medical ApS, Denmark) were attached, one to the right upper arm and one to the right upper thigh. To obtain baseline NIRS-measurements the participants were asked to sit as still as possible, with an emphasis on the limbs where the NIRS-devices were attached. When the cuffs were inflated, they applied a pressure of 300 mmHg, stopping all inflow-/outflow of blood to the limbs.

On the first testing day, before the AOT10 was performed all participants started with a familiarization occlusion period of 1-minute, which was not included in the analysis. When the cuff pressure was released, participants had a 3-minute resting period. The second period of AOT lasted 10-minutes (AOT10), with a 7-minute resting period after cuff pressure release. The participants were instructed to sit as still as possible throughout the whole procedure, both before-, during- and after the occlusion.

On the second testing day the AOT20 was performed for the duration it took for the SmO_2 in the FDS to decrease by 20% from baseline levels. After cuff release there was a 3–5-minute resting period. All participants were instructed to sit as still as possible throughout the whole procedure, both before-, during- and after the occlusion.

Maximal voluntary contraction (MVC) and incremental handgrip test (IHT)

MVC test was performed after the AOT10. The participants handgrip strength was assessed with a handgrip dynamometer (Lafayette Instruments, Model No. 5030L1, Indiana, USA). The test was performed with a 2-second maximal contraction with a total of 3-trails, with 1-minute rest between each trail. The trail with the highest measurement counted as the participants MVC-score, measured in kilograms (kg). All participants used their right arm

with an approximately 90-degree angle at the elbow, in the same sitting position as during the arterial occlusion test.

The IHT was performed after the MVC test. The goal of the IHT was to assess the participants max dynamic handgrip load as a measure for forearm muscle endurance. The test was performed on a custom-made handgrip dynamometer, with the arm being supported at the elbow and wrist and the forearm in an upward angel and upper arm at the level of the heart. All participants had to use their right arm due to the design of the equipment. A rhythm of 1-second on and 1- second off was set to a metronome, for guiding the contraction and relaxation phases of the hand during the test. All participants started the IHT at a weight of 2,5 kg for 1-minute, then the weight was increased by 250 grams every 15-seconds until exhaustion. Exhaustion was determined by the participant not being able to follow the rhythm, using the upper arm muscles and/or stopping.

Lactate threshold test and Incremental cycling test

The ICT test was performed after the IHT. This test was performed on a cycle ergometer (Lode Excalibur Sport, Lode B. V., Groningen, Netherlands). Before the warmup, a blood lactate sample was taken from the participant using a lactate analyser (Lactate Pro2 LT-1730, Arkray KDK, Kyoto, Japan), after that the participants were instructed to do 10 minutes of warmup at a cadence >60. The work rate (WR) during the warmup was below 95-100w for elderly and young men, and under 75-95w for elderly and young women. After the warmup, the participant did a lactate threshold test (LTT) perfoming 4-minute stages increasing the WR until blood lactate reached >4mmol/l, known as lactate threshold. Next the participant got a 5–10-minute active rest before the ICT, which started on the second last WR of the LTT. The ICT was performed by increasing the WR every minute (15-25W/min) until the participant reached VO_2-peak, characterized by a respiratory exchange ratio (RER) ≥1, blood lactate (BL) >8 and/or not being able to obtain an RPM >60. During both the LTT and ICT pulmonary gas exchange was measured using a circuit indirect calorimetry (Oxycon Pro, Jaeger GmbH, Hoechberg, Germany). Calibration of the turbine was done every day using a 3-liter calibration syringe (Hans Rudolph Inc, Kansas City, MO, USA) and calibration of the gas analyser was done every day with a gas bottle (Reissner-Gase, GmbH & Co; Lichtenfels, Germany). The participants VO_2-peak was measured as the highest 30-second average oxygen uptake. The WR-peak was measured as the highest WR sustained for 1-minute and the heart rate peak (HR-peak) was measured as the highest recorded heart rate during the test.

Body composition analysis

On the third day the participants body composition was measured with a bioimpedance analysis (Inbody 720, BIOSPACE, Seoul, Korea). According to the producers of the product the participant had to fast from 11:00pm the night before the analysis. The test itself was performed with the participant standing barefoot on marked electrodes and holding one electrode in each hand. The test itself took approximately 1-minute.

Near-infrared spectroscopy

NIRS uses near infrared light which is absorbed by oxygenated- and deoxygenated haemoglobin (Hb)/myoglobin (Mb) in the tissue of interest. The light absorption of oxy(Hb+Mb) and deoxy(Hb+Mb) differs from each other, making it possible to distinguish them with NIRS (Jones et al., 2016). However, Hb and Mb have similar optical characteristics making it impossible to distinguish them from each other using NIRS (M. L. Davis & Barstow, 2013). We used a continuous wave near infrared spectrophotometer (Portamon, Artinis Medical Systems, Netherlands) to measure the tissue saturation (SmO_2). The two NIRS-devices were attached longitudinally on the muscle belly of the m. flexor digitorum superficialis (FDS) on the right arm and m. vastus lateralis (VL) on the right leg. Excessive hair was removed with a shaver before placing the NIRS-devices. The NIRS devices were taped on the muscle and then fastened with an elastic bandage (Elastoquick Sport, Snogg, Norway) to mitigate movement. To minimize external light pollution the NIRS-devices were covered with a black cloth under the elastic bandage. The NIRS devices used a wavelength of 845nm and 761nm sampled at 10 Hz with a source-detector distance at 31, 35 and 39mm.

Calculations of mVO_2 were done for both occlusions (AOT10 and AOT20) using SmO_2. mVO_2 was calculated as the initial linear slope decrease in SmO_2, after the arterial occlusion was set. For the AOT10 this was done over a 3-minute regression period with a 30-second delay, but for the AOT20 this was done by using a 45-second regression period with a 10-sec delay. Reperfusion rate (RR) was calculated as the initial linear slope increase in SmO_2, after the arterial occlusion was released. For both AOT10 and AOT20 this was measured with a 3-second regression period with a 0,5-second delay. The reason for the delay when calculating mVO_2 and RR was to avoid possible artifacts of the cuff inflation/deflation. The relative SmO_2 decrease (ΔSmO_2-min) was calculated from baseline tissue saturation (SmO_2-baseline) to minimum tissue saturation (SmO_2-min). The relative SmO_2 increase (ΔSmO_2-max) was calculated form SmO_2-baseline to maximum tissue saturation (SmO_2-max). The RR reflects

the speed of the reperfusion of the muscle tissue and ΔSmO_2-max reflects the muscles hyperaemic reserve, both giving an indication of the microvascular response (Barstow, 2019; Gerovasili, Dimopoulos, Tzanis, Anastasiou-Nana, & Nanas, 2010).

After the whole protocol was finished the skinfold where the NIRS-devices had been placed was measured with a skinfold calliper (Holitain, Crymmych, UK), to estimate the amount of subcutaneous fat there was between the skin and the muscle. Skinfold measurements were then converted to adipose tissue thickness (ATT) by dividing the skinfold value by 2 (skinfold/2=ATT).

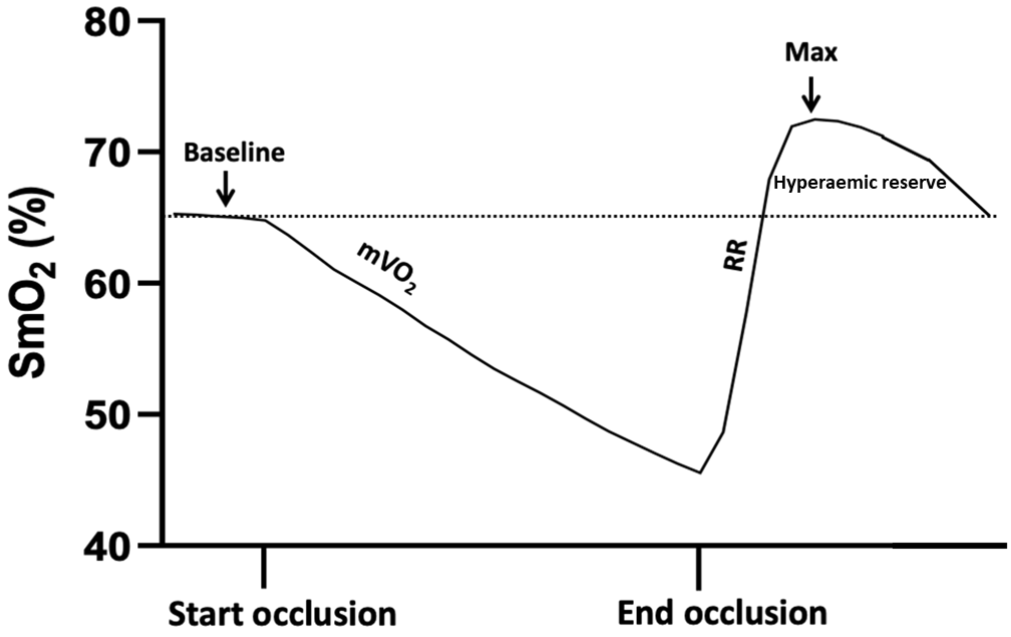


Figure 1. Representative NIRS-measurement profile of tissue saturation (SmO_2) during an arterial occlusion test. Baseline SmO_2 (SmO_2-baseline) measured 2-min before the occlusion. As the arterial occlusion is set the SmO_2 decreases creating a downwards slope from which muscle oxygen uptake (mVO_2) is calculated. The lowest point of SmO_2 is the minimum saturation (SmO_2-min) during the occlusion. At the arterial occlusion release the SmO_2 increases creating an upwards slope from which reperfusion rate (RR) is calculated. The maximum saturation (SmO_2-max) is the highest SmO_2 after the occlusion. The relative increase for SmO_2-baseline to SmO_2-max reflecting the hyperaemic reserve (ΔSmO_2-max).

Statistical analysis

All values are presented as mean ± standard deviation (SD). An independent sample t-test was performed on all normally distributed data and a non-parametric Man Whitney U-test was performed on all not-normally distributed data to compare the physical characteristics, performance variables and AOT10 derived NIRS data for the VL and FDS between the

groups. A repeated ANOVA was used to compare the NIRS data from the AOT10 vs AOT20. The data that was not normally distributed was transformed before including it in the repeated ANOVA test. To compare differences between groups for AOT20 an independent sample t-test and a non-parametric Man-Whitney U test was performed, depending on what data was normally distributed. A paired sampled t-test was performed to compare the AOT10 occlusion duration vs AOT20 occlusion duration. A person correlation was used to investigate the relationship between the physical performance variables and ATT and the NIRS-data. A spearman correlation was used for the data that was not normally distributed. The significance level for all statistical analysis was set at $p<0,05$, and all analysis were done in SPSS (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp).

Results

Out of the 37 participants some of the data had to be excluded due to poor quality of the NIRS-signals. Table 1 presents the participants physical characteristics and performance variables. There was no significant difference for height, weight,
Body mass index (BMI), resting heart rate (HR-resting), body fat (BF), and muscle mass right arm (MM_RA) between the groups. The EG had significantly higher blood pressure (BP) and significantly lower ATT, for both FDS and VL, compared with the YG. The ATT range for the FDS was 1,7-5,8mm in the YG and 1,2-4,1mm in the EG. The ATT range for the VL was 3,2-17,2mm in the YG and 1,5-9,1mm in the EG. MVC and IHT-peak load in kilograms was not significantly different between the groups, but IHT-peak duration was significantly higher in the EG. VO_2-peak, RER, peak blood lactate (La-peak), HR-peak and rate of perceived exertion (RPE) were significantly higher in the YG.

Table 1. Physical characteristics and physical performance variables.

	Young (n=19) Mean ±SD	**Elderly (n=18) Mean ±SD**	**p-value**
Sex (M/F)	7/12	12/6	
Age (Years)	25,47±2,3	69,28±6,0	<0,001
Height (cm)	174,1±10,3	171,6±8,8	0,435
Weight (kg)	68,2±10,2	70,9±11,3	0,445
BMI ($kg \cdot m^{-2}$)	22,2±2	23,3±3	0,198
BP (mmHg)	122/74	140/83	0,001
HR-resting	64,2±8	62±9	0,46
ATT_FDS (mm)	3,3±1,1	2,5±0,9	0,018
ATT_VL (mm)	8,5±3,9	4,9±1,9	0,001
MM_RA (kg)	2,8±0,7	3,0±0,7	0,371
BF (%)	20,6±6,6	22,6±6,7	0,35
MVC (kg)	42,4±9,3	42,1±12,1	0,93
IHT-peak (kg)	11,9±2,1	12,6±1,7	0,291
IHT-peak (sec)	579,5±125,1	665,0±104,4	0,03
VO_2-peak ($ml \cdot kg \cdot^{-1} min \cdot^{-1}$)	48,2±8,2	39,2±8,9	0,003
WR-peak (W)	266,8±76,0	213,6±60,1	0,024
RER	1,0±0,1	0,9±0,1	0,001
La-peak ($mmol \cdot L^{-1}$)	14,1±3,2	8,8±2,3	<0,001
RPE-peak	18,2±0,8	17,1±1,2	0,002
HR-peak (bpm)	184,6±9,6	154,2±24,2	<0,001

Body mass index (BMI); Blood pressure (BP); Resting hear rate (HR-resting); Peak heart rate (HR-peak); Adipose tissue thickness (ATT); m. flexor digitorum superficialis (FDS); m. vastus lateralis (VL); Muscle mass right arm (MM_RA);Body fat (BF); Maximal voluntary contraction (MVC); Peak incremental handgrip test (peak-IHT); Whole-body peak oxygen uptake (VO_2-peak); Work rate peak (WR-peak); Peak blood lactate peak (La-peak); Millimoles per litre ($mmol \cdot L^{-1}$); Rate of perceived exertion (RPE); Significant difference ($p<0,05$) between the groups.

Table 2 shows the NIRS-measurements for the EG and YG during the AOT10. No significant difference for microvascular response (RR and ΔSmO_2-max) or mVO_2 was observed between the EG and YG, for both muscles (FDS and VL). This was also the case for the ΔSmO_2-min showing no significant difference between EG and YG, for both muscles (FDS and VL). The SmO_2-baseline was significantly higher in the YG compared to the EG, for both muscles (FDS and VL). Figure 2 shows a visual representation of the average SmO_2 response for both groups (YG and EG) during AOT10 for the VL and FDS.

We also observed that ATT positively correlated with mVO_2 ($r= 0,8$; $p<0,001$), negatively correlated with RR ($r=-0,36$; $p=0,03$), and ΔSmO_2-max ($r=-0,76$; $p<0,001$) in the VL. In the

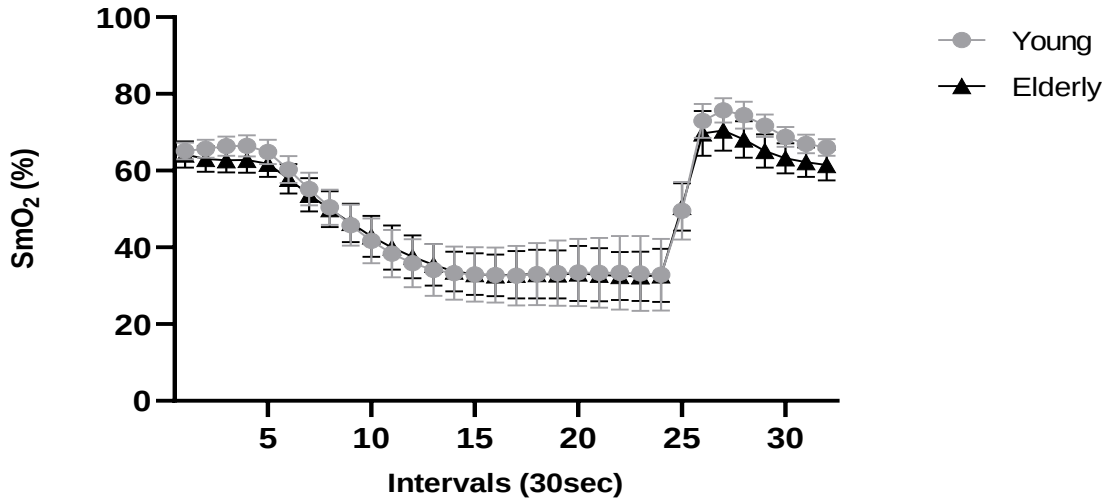
FDS ATT positively correlated with RR (r=0,53; p=0,022) but did not correlate with mVO_2 (r=0,11; p=0,53) or ΔSmO_2-max (r=-0,09; p=0,58). There were no significant correlations between the physical performance parameter VO_2-peak and mVO_2 (r=-0,21; p=0,22), RR (r=0,16; p=0,36), or ΔSmO_2-max (r=0,12; p=0,47) in the VL. No significant correlations were found between the performance parameter IHT-peak duration and mVO_2 (r=0,18; P=0,29) or RR (r=-0,057; p=0,75), but ΔSmO_2-max (r=-0,37; p=0,003) showed a negative correlation for the FDS.

There was a significant difference between the groups for the SmO_2-max for both FDS (YG: 76,2±3,1% EG: 71,5±5,2%; p=0,001) and VL (YG: 83±2,6% EG: 79,3±1,8%; p<0,001). This was also the case for the SmO_2-min as a significant difference was observed between the groups for VL (YG: 60,9±9,3% EG: 51,6±11%; p=0,024), but no difference was observed for FDS (YG: 32,9±9,4% EG: 32,7±6,9%; p=0,80).

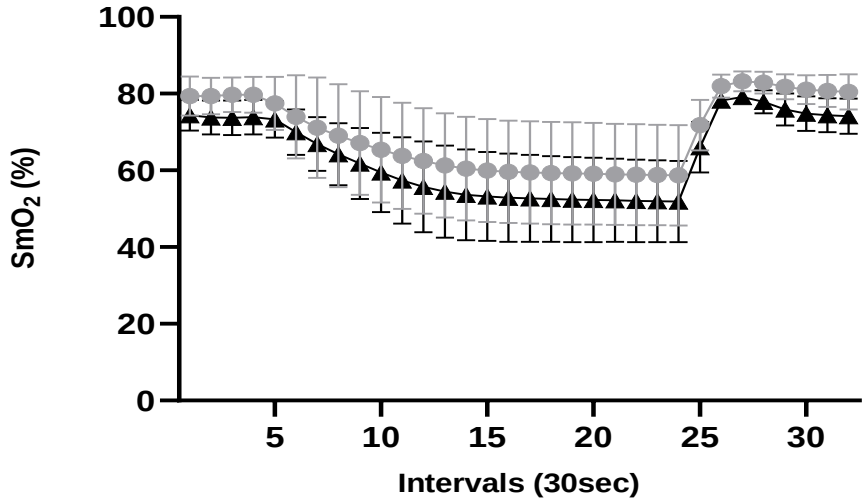
Table 2. NIRS-measurements during the fixed time occlusion for the FDS and VL.

	AOT10		
	Young Mean±SD	**Elderly Mean±SD**	**p-value**
SmO_2-baseline_VL (%)	79,8±4,6	74,6±4,0	0,001
mVO_2_VL (%·s^{-1})	-0,06±0,03	-0,08±0,04	0,14
ΔSmO_2-min_VL (%)	18,6±5,4	22±6,8	0,11
RR_VL (%·s^{-1})	1,18±0,55	1,49±0,54	0,10
ΔSmO_2-max VL (%)	3,5±2,9	5,4±3,5	0,10
SmO_2-baseline_FDS (%)	66,5±2,7	62,8±3,4	0,007
mVO_2_FDS (%·s^{-1})	-0,15±0,03	-0,12±0,03	0,012
ΔSmO_2-min_FDS (%)	34±9,7	30,1±8,5	0,12
RR_FDS (%·s^{-1})	1,27±0,7	1,42±0,7	0,56
ΔSmO_2-max _FDS (%)	9,2±4,5	8,4±3,5	0,50

Muscle oxygen uptake (mVO_2); Reperfusion rate (RR); Tissue saturation baseline (SmO_2-baseline); Relative tissue saturation decrease (ΔSmO_2-min); Hyperaemic reserve (ΔSmO_2-max) m. vastus lateralis (VL); m. flexor digitorum superficialis (FDS); Arterial occlusion 10-minutes (AOT10); m. vastus lateralis (VL); m. flexor digitorum superficialis (FDS); Significant difference (p<0,05) between the groups.



A



B

Figure 2. A) A visual representation of the tissue saturation (SmO_2) group responses mean±SD before-, during- and after the time-fixed arterial occlusion test (AOT10) in the m. flexor digitorum superficialis (FDS). B) A visual representation of the tissue saturation (SmO_2) group responses mean±SD before-, during- and after the time-fixed arterial occlusion test (AOT10) in the m. vastus lateralis (VL).

Figure 3 shows the mean group measurements during AOT20 for RR, ΔSmO_2-max, mVO_2, ΔSmO_2-min and SmO_2-baseline in the FDS. No difference was observed between the EG and YG in microvascular response (RR and ΔSmO_2-max) or mVO_2. The YG used an average of 156±34sec to reach 20% desaturation compared to 148±35sec in the EG, but this difference was not statistically significant (p=0,82). During the AOT20 a significant difference in the FDS was observed between the groups for the SmO_2-min (YG: 46,6±3,8% EG: 43,8±4%;

p=0,047) and SmO_2-max (EG: 69,9±2,5% YG: 72,5±3,9%; p=0,028). Figure 3 depicts all relevant p-values not reported in the text.

Figure 3 also shows that RR and ΔSmO_2-min had a significant main effect for test, showing superior values for the AOT10 compared to AOT20 independent of the groups. The mVO_2 also showed a main effect of test showing a superior value for the AOT20 compared to AOT10, independent of the groups. The occlusion duration for AOT10 (600±0,4sec) and AOT20 (153±34,5sec) was significantly different from each other (p<0,001). Figure 3 includes all relevant p-values not reported in the text.

There was a main effect for group in SmO_2-baseline (P=0,001) and SmO_2-max (P=0,001), showing a higher tissue saturation for the YG independent of the tests. No main effect for group was found for RR (P=0,58), mVO_2 (P=0,53), SmO_2-min (P=0,23), ΔSmO_2-min (P=0,31) and ΔSmO_2-max (P=0,67). No interaction effect for test and group was found in RR (P=0,34), mVO_2 (P=0,34), SmO_2-min (P=0,56), SmO_2-baseline (P=0,58), SmO_2-max (P=0,06), ΔSmO_2-min (P=0,40) and ΔSmO_2-max (P=0,36).

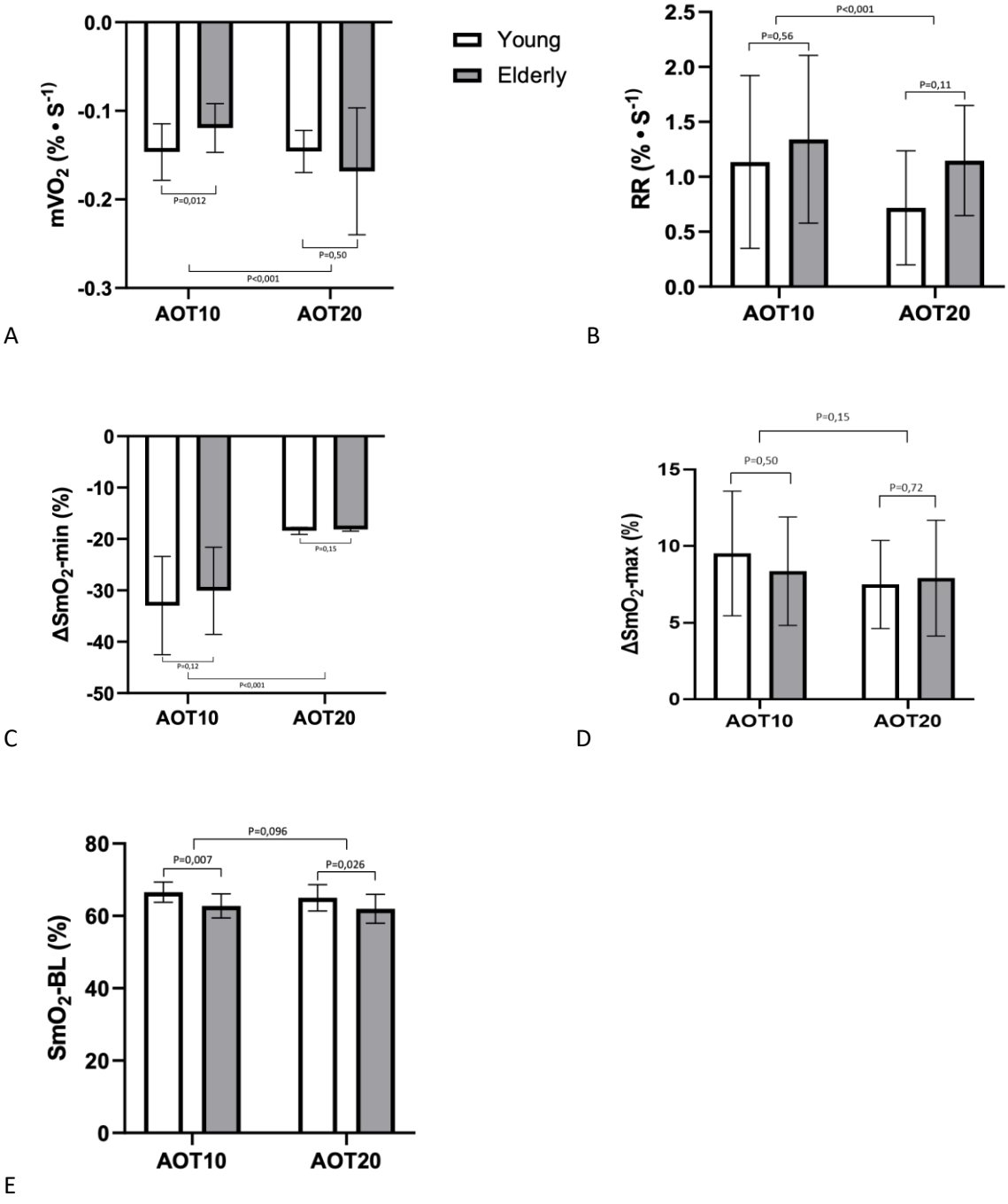


Figure 3. Showing differences between AOT10 vs AOT20, and young (white) vs elderly (grey) in the m. flexor digitorum superficialis (FDS). A) Muscle oxygen uptake (mVO_2), B) Reperfusion rate (RR), C) Relative tissue saturation decrease from baseline (ΔSmO_2-min), D) Hyperaemic reserve (ΔSmO_2-max), and E) Tissue saturation baseline (SmO_2-BL). Significant value between groups ($p<0,05$).

Discussion

The main purpose of this study was to investigate the difference in muscle microvascular response (RR and ΔSmO_2-max) and mVO_2 between elderly and young, taking into consideration physical performance parameters and ATT. The secondary aim of this study was to investigate the difference between elderly and young when the desaturation levels were fixed, and to investigated if there was any difference between AOT20 and AOT10.

The main findings of this study were twofold: 1) We did not observe any difference in microvascular response (RR and ΔSmO_2-max) and mVO_2 between elderly and young, in the VL. However, in the FDS there was observed a higher mVO_2 for the young group, but no difference in microvascular response (RR and ΔSmO_2-max) between the groups. 2) We also observed a relationship between the ATT for both microvascular response (RR and ΔSmO_2-max) and mVO_2 in the VL. While in the FDS there was only observed a relationship between ATT and microvascular response, measured as RR. We did also find a weak inverted relationship between the performance parameter, IHT-peak duration, and the microvascular response, measured as ΔSmO_2-max in the FDS.

The secondary findings of this study showed no difference in microvascular response (RR and ΔSmO_2-max) or mVO_2 between elderly and young, when using AOT20. While we did observe a difference in microvascular response (RR and ΔSmO_2-max) and mVO_2 between the AOT20 and AOT10.

Previous research has suggested that microvascular response is negatively affected by advancing age (Muller-Delp, 2006). A study done by Rosenberry et al. (2018), using NIRS, showed that elderly had an diminished microvascular response and lower mVO_2 in FDS when compared with their younger counterparts. This partly coincides with what we found in the FDS, showing a higher mVO_2 for the YG but no significant differences in the microvascular response (RR and ΔSmO_2-max) between the groups. Another study by Horiuchi and Okita (2020) measuring the forearm muscles found a significant difference in microvascular response when comparing young, middle aged and older women. They concluded that the microvascular response decreases with age, although not finding any significant difference for the speed of the microvascular RR between the groups. These findings partly coincide with our results as we found no statistical difference in RR between the groups either. Even though

no statistical differences were found in Horiuchi and Okita (2020) or our study both showed a tendency towards a slower RR in the elderly.

In contrast to our study, neither Rosenberry et al. (2018) nor Horiuchi and Okita (2020) measured the participants physical fitness, measured as VO_2-peak, even though that is believed to affect the endothelial vasodilatory function (Montero, 2015; Muller-Delp, 2006). A study by McLay et al. (2016) showed that when comparing two groups with different fitness levels, the group with the higher VO_2-peak also had a faster microvascular RR.

This association between VO_2-peak and RR does not match our findings as we observed no significant difference in the microvascular response between the groups, even though the YG had a better VO_2-peak compared to the EG. Some methodological differences from our study were that McLay et al. (2016) investigated the tibialis anterior muscle, which is active differently than the VL during cycling (Ericson, 1986). Also McLay et al. (2016) used age matched male groups in their twenties, whilst our groups were not age matched nor the same sex. It has been suggested that the VO_2-peak testing protocols is not as fitted for elderly populations (Poole & Jones, 2017; Sidney & Shephard, 1977). We could speculate that this was the case for our elderly population when looking at the RER values, which did not reach our set VO_2-peak criteria, which could have underestimated the VO_2-peak in our elderly group.

Our results did not show any correlations between the VO_2-peak and the microvascular response (RR and ΔSmO_2-max) for the VL. This agrees with our previous findings as our YG, which had a higher VO_2-peak, showed no better microvascular response than the EG. A study by Buscemi et al. (2013) showed that VO_2-peak correlated with flow-mediated dilatation (FMD) for the brachial artery, which has shown to correlate well with NIRS derived RR in the forearm (R. N. Soares, Somani, Proctor, & Murias, 2019). The only relationship that was found between performance parameters and NIRS-measurements was the negative correlation between duration of the IHT and ΔSmO_2-max in the FDS, suggesting that the better performance on the longer the participants lasted during the IHT the lower microvascular hyperaemic reserve, measured as ΔSmO_2-max. The FDS is known to be the main muscle used during dynamic grip movements, however, other forearm muscles are also known to be recruited (Johanson, James, & Skinner, 1998). It has been suggested that individuals have different recruitment patterns during dynamic handgrip exercise, at a submaximal intensity (Johanson et al., 1998). One could therefor speculate if the recruitment pattern would change

even more during a dynamic handgrip exercise with increasing intensity, which could perhaps partly explain the reason for our observation that a better incremental hand grip test duration the lower the ΔSmO_2-max. As the correlation we found was weak it might not have as large of a clinical factor, but further research would be needed to determine this.

It is well known that the amount of adipose tissue between the NIRS-device and the muscle do confound the NIRS-measurements. A study by van Beekvelt, Borghuis, van Engelen, Wevers, and Colier (2001) showed that mVO_2 NIRS-measurements from the FDS were negatively affected by a higher ATT. The finding of their study is in line with with our findings for the VL outcome measures, but not for the findings in FDS. One differences between van Beekvelt et al. (2001) and our study is that our participants had a smaller mean ATT (EG: 3,3±1,1 and YG: 2,5±0,9 mm) and a smaller ATT range (EG: 1,2-4,1mm and YG: 1,7-5,8mm) for the FDS compared to the participants in van Beekvelt et al. (2001), which had a mean ATT of 3,7±1,9 with a range of 1,4-8,9mm. van Beekvelt et al. (2001) also hypothesized, with a simplified model which did not take into account the different scattering properties of fat and muscle tissue, that only 49% of the signal reached the muscle for the participant with 8,9mm ATT, but 92% for the participant with 1,4mm ATT. The ATT over the VL had a much larger mean (EG: 4,9±1,9mm and YG: 8,5±3,9mm) and range (EG: 1,5-9,1 and YG: 3,2-17,2mm) much larger than for the FDS. This might explain why we found a correlation between ATT and NIRS-measurements in the VL and not in the FDS, as the ATT for FDS was not large enough overall to negatively affect the NIRS-measurements.

As for the relationship between ATT and the microvascular response (RR and ΔSmO_2-max) we found a strong relationship between ΔSmO_2-max and a weak relationship between RR for the VL, showing that a higher ATT correlated with a lower ΔSmO_2-max and RR measurements. The FDS showed an opposite relationship, which seems illogical as a larger ATT is known to scatter more of the light signals before reaching the muscle tissue (Barstow, 2019; van Beekvelt et al., 2001). One of the explanations for this might be that at the release of the arterial occlusion the blood flow increases rapidly to the limb, also causing an increase in skin perfusion. A study by S. L. Davis, Fadel, Cui, Thomas, and Crandall (2006) suggest that small changes in skin blood flow might affect the NIRS-measurements for SmO_2. S. L. Davis et al. (2006) is methodologically different from our study, as they used heat to increase the skin blood flow. However, from a physiological standpoint the principle of the rush of blood to the skin tissue after an arterial occlusion or when actively heating up the skin tissue would be somewhat the same, as both methods increase the skin perfusion.

A review by Barstow (2019) suggested that using SmO_2 (measured as the ratio oxy-(Hb+Mb)/total-(Hb+Mb)) could be largely affected by the increase of blood flow to the tissue. Barstow (2019) suggests that oxy-(Hb+Mb) might be higher in fat tissue which might overestimate the oxy-(Hb+Mb) with increased blood flow, as it is known that fat tissue does not extract as much oxygen as muscle tissue. This suggestion might explain why we see a strong relationship between ΔSmO_2-max in the VL but not the FDS, as the VL has a much larger ATT that might have overestimated the relationship ΔSmO_2-max and ATT. Although, as we did not measure blood flow this remains a speculation and further investigation would be needed.

Our study showed no difference in body fat percentage but a difference in ATT between the groups, with the YG having a larger ATT for both FDS and VL. One reason for this might be that there were more females in the YG compared to the EG. It has been suggested that females usually have more adipose tissue on the thighs compared to males, which might be one of the explanations for the larger ATT seen over the VL for the YG (Karastergiou, Smith, Greenberg, & Fried, 2012).

Previous research has shown that the arterial occlusion duration might affect the NIRS-measurements for tissue saturation. McLay et al. (2016) and Rosenberry et al. (2018) found a dose-response relationship between the occlusion duration and muscle microvascular response, as a longer occlusion duration yielded a larger microvascular response. This partly agree with our findings as the AOT10, which had a longer occlusion duration and desaturation level than AOT20, also had a faster RR. But for the ΔSmO_2-max we found no significant difference between the occlusion tests, however there was a tendency towards a higher ΔSmO_2-max for the AOT10 compared to the AOT20.

We also observed a difference in mVO_2 between AOT10 and AOT20, showing a higher mVO_2 in AOT20. This is not in agreement with neither Rosenberry et al. (2018) or Rogerio N. Soares, Somani, Al-Qahtani, Proctor, and Murias (2019), which both showed no statistical difference between mVO_2 when using different occlusion durations. A methodological difference from our study is that we did the AOT10 and AOT20 on separate days, compared to both Rosenberry et al. (2018) and Rogerio N. Soares et al. (2019) which did both occlusion tests on the same day. It has been shown that the day-to-day variability in measuring mVO_2 is small (Zhang et al., 2020), although there is a risk of inconsistent placement of the NIRS-

device between the two test days. If the possible misplacement of the NIRS-device between the two test days was the reason for this finding cannot be fully determined.

For AOT20 the microvascular response (RR and ΔSmO_2-max) between elderly and young in our study showed no statistical difference. Rosenberry et al. (2018) had a similar approach showing that when performing a 3-minute occlusion for the young group and a 5-minute occlusion for the elderly group the desaturation levels were the same between the groups, causing no difference in microvascular response between the groups. Rosenberry et al. (2018) findings for microvascular response are in line with our findings, showing that when matching the tissue desaturation levels the microvascular response does not differ. However, Rosenberry et al. (2018) showed that the elderly group used 2-minutes longer to reach the same desaturation level as the young group, while our study showed that the time duration to reach 20% desaturation was similar for the elderly and young. One possible reason for this difference in Rosenberry et al. (2018) might be because of differences in ATT and physical fitness between the groups. However, Rosenberry et al. (2018) did not measure the ATT nor the physical fitness making it impossible to determine if this had an impact on the NIRS-measurements. Another reason might be that our study population were more homogenous for the FDS than Rosenberry et al. (2018) study population. We can speculate that this was the case as the mVO_2 between elderly and young for our study were not different when matching the desaturation levels with 20%, but for Rosenberry et al. (2018) there was a difference in mVO_2 between elderly and young when matching desaturation levels. This was also the case for the grip strength, as our study showed no difference in MVC between the groups, but for Rosenberry et al. (2018) the MVC score was significantly higher in the young group.

Methodological considerations

One of the limitations for our study was that we had to exclude some data due to poor NIRS-signal quality, especially for the RR- and mVO_2 measurements. Two of the participants in the young group had to be excluded for all FDS NIRS-measurements because of poor signal quality. Another limitation was the skewed distribution of sexes between the groups which made it somewhat harder to determine if there was an age-related difference, because of the difference in adipose tissue distribution we found between the sexes. A methodological difference when comparing our findings to the research we cited is that we occluded two limbs simultaneously as compared to one limb. When occluding two limbs simultaneously the blood redistribution might be affected which in turn might affect the microvascular response

measurements, which must be taken into consideration when comparing our results too other studies.

Conclusion

In conclusion we found no difference in microvascular response between elderly and young for our study population, although we did find a higher mVO_2 in the young for the FDS. We observed a relationship between a large ATT and low mVO_2-values. We also found a weak relationship between the IHT-peak duration and microvascular hyperaemic reserve, measured as ΔSmO_2-max. We did not find any difference in microvascular response or mVO_2 between elderly and young when using AOT20. But we did see a tendency towards a larger microvascular response for the AOT10 compared to AOT20.

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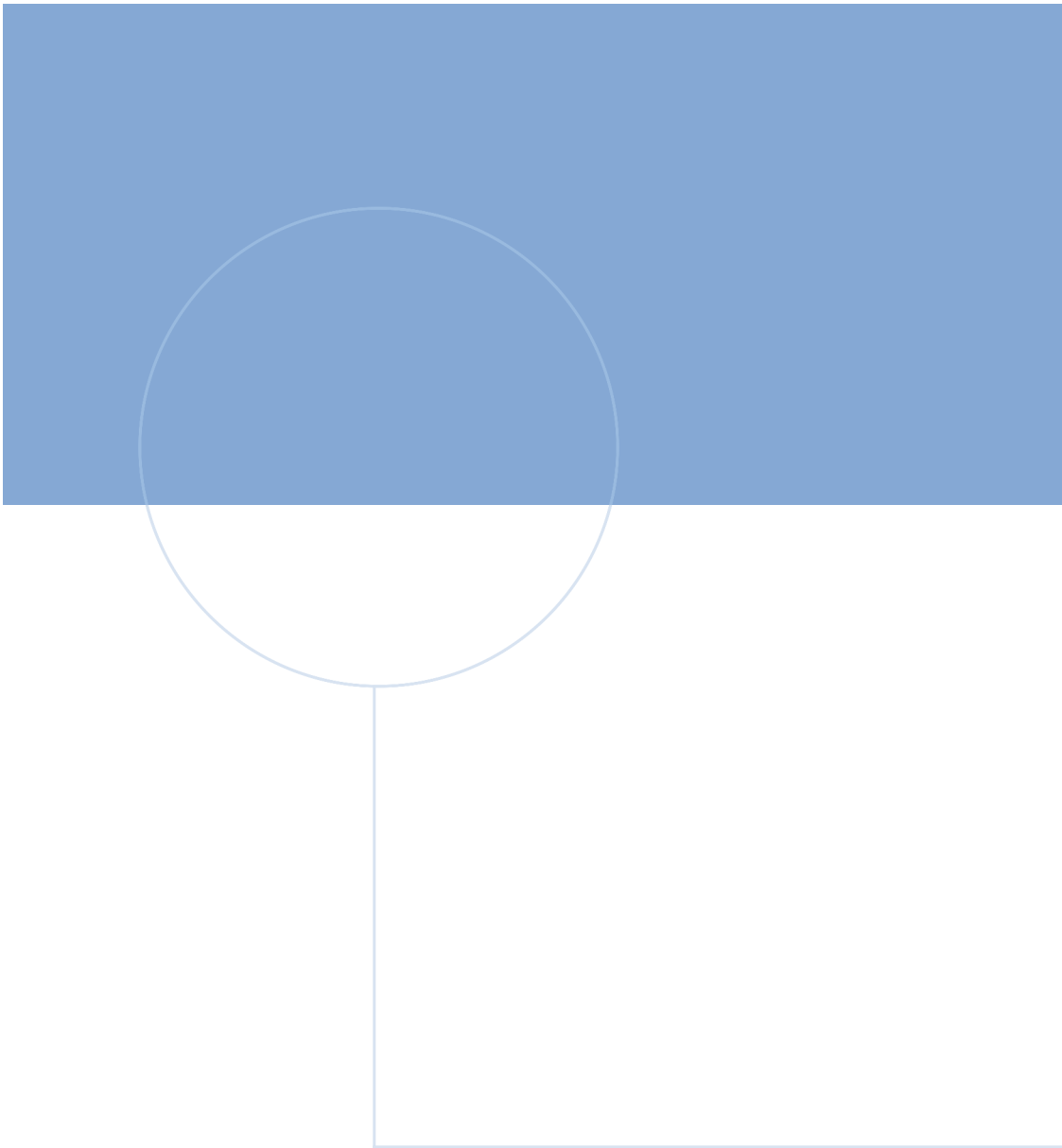

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