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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The role of physical fitness and body composition in detecting age-related differences in skeletal muscle oxidative function using near-infrared spectroscopy

Master's thesis in Human Movement Science

Supervisor: Mireille van Beekvelt

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Abstract

Introduction: Aging is associated with physiological declines of muscular strength and endurance, and evidence indicates that the oxidative capacity of the skeletal muscles is a key factor to these age-related changes. Near-infrared spectroscopy provides a non-invasive tool capable of assessing skeletal muscle oxygenation *in vivo*, and studies have shown that elderly have a reduced oxidative function and slower $\dot{V}O_2$ kinetics. However, physical exercise and a healthy lifestyle can strongly attenuate the decay in physical fitness, and therefore potentially modulate the response of the $\dot{V}O_2$ kinetics. **Aim**: The aim of this study was therefore to investigate whether differences in mitochondrial capacity can be detected between healthy young and elderly individuals, and to assess these findings in relation to parameters of physical fitness. **Methods**: 20 young (Y = 26.2 ± 3.0 years) and 20 elderly (E = 62.4 ± 4.3 years) men and women performed vascular occlusion test at rest, and submaximal cycling exercises at low and high intensity followed by repeated arterial occlusions. **Results**: Peak pulmonary oxygen uptake was significantly different between groups (Y = 47.4 + 7.6 vs E = 40.3 + 6.6 $mL \cdot min^{-1} \cdot kg^{-1}$). There were no group-differences in BMI, lean body mass or adipose tissue thickness. The $m\dot{V}O_2$ recovery kinetics at rest and after submaximal cycling exercise were not slower, and in some cases even faster, in E compared to Y. **Conclusion**: The mitochondrial capacity from recovery kinetics of $m\dot{V}O_2$ assessed by NIRS were not slower in E compared to Y. The data indicate that the $m\dot{V}O_2$ kinetics response is highly modulated by physical fitness, independent of age.

Sammendrag

Introduksjon: Aldring er assosiert med en reduksjon i muskulær styrke og utholdenhet, og nye funn indikerer at skjelettmusklenes oksidative kapasitet er en viktig faktor relatert til de aldersrelaterte endringene. Nær-infrarød spektroskopi (NIRS) er et ikke-invasiv verktøy som kan måle muskeloksygenering *in vivo*, og studier har vist at eldre personer har en redusert oksidativ funksjon og tregere muskeloksygenering. Likevel, fysisk trening og en sunn livsstil kan hemme forfallet i fysisk form, og derfor potensielt påvirke muskeloksygenerings-responsen. **Mål**: Målet med denne studien var derfor å undersøke hvorvidt man kan finne forskjeller i mitokondriell kapasitet mellom friske unge og eldre individer, og å vurdere funnene i sammenheng med bestemmende faktorer for fysisk form. **Metode**: 20 yngre (Y = 26.2 ± 3.0 år) og 20 eldre (E = 62.4 ± 4.3 år) menn og kvinner utførte en vaskulær okklusjonstest i hvile, og submaksimale sykkeltester på lav og høy intensitet etterfulgt av repeterte arterielle okklusjoner. **Resultater**: Maksimalt oksygenopptak var signifikant forskjellig mellom gruppene (Y = 47.4 + 7.6 vs E = 40.3 + 6.6 $mL \cdot min^{-1} \cdot kg^{-1}$). Det var ingen gruppeforskjeller i KMI, fettfri masse eller fettvev tykkelse. Muskeloksygenering i restitusjonsfasen etter hviletest og submaksimale sykkeltester var ikke tregere, og i enkelte tilfeller faktisk raskere i E sammenlignet med Y. **Konklusjon**: Mitokondriell kapasitet fra muskeloksygenering under restitusjonsfasen undersøkt med NIRS var ikke tregere i E sammenlignet med Y. Data fra studien indikerer at responsen i muskeloksygenering påvirkes i stor grad av fysisk form, uavhengig av alder.

Acknowledgements

I would first like to thank my supervisor Dr Mireille van Beekvelt for her honest, clear, patient and excellent guidance and cooperation throughout this project. She trusted us to make critical decisions for the final project protocol and supported us whenever we needed help. Even though the meetings and presentations in the final three months of the project were virtual, we received high quality feedback and support every single week. I would also like to thank Xianchun Tan and Arnt Erik Tjønna for their expert support with materials and technical equipment in the laboratory.

A project of this size would never be possible without the commitment and effort of the participating test subjects. Therefore, I would like to send a big thank you to everyone, especially friends and family, who have voluntarily performed experiments while allowing us to measure every possible variable of interest.

I must also express my gratitude to my family who have supported and encouraged me throughout my work with the master thesis, but also for their contributions during my entire education, from primary school through university.

Finally, I would like to thank my girlfriend, lab partner and project collaborator Martine Berg. Our great lab cooperation and continuous academic discussions contributed significantly to the execution of the experimental tests and the writing process. You enhance my ability to critically reflect by (rightly) challenging my work, arguments and opinions, making my writing and communication coherent and nuanced to the audience and other readers. We spent countless hours in the laboratory (and stuck at home) but have always remained colleagues and best friends for the duration of this master thesis.

…however, 2020 will also be remembered for the devastating effects of the coronavirus. We were lucky to finish our data collection in the middle of February, just three weeks before the national lockdown. The pandemic has highlighted the need for preparation and strong preventive measures to combat many of the current and emerging global health crises. No magic pill will ever resolve the public health issues of physical inactivity, obesity, racism and social inequality.

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Abbreviations

AO: Arterial occlusion

ATT: Adipose tissue thickness

BF%: Body fat percentage

BIA: Bioelectrical impedance analysis

Deoxy[Hb+Mb]: Deoxygenated hemoglobin and myoglobin

E: Elderly group

Hb: Hemoglobin

HIGH: High intensity, corresponding to 70% of MAP

HR: Heart rate

HR_{max}: Maximal heart rate

LBM: Lean body mass

LOW: Low intensity, corresponding to 50% of WR_{obla}

MAP: Maximal aerobic power

Mb: Myoglobin

$m\dot{V}O_2$: Skeletal muscle oxygenation

$m\dot{V}O_{2rest}$: Resting muscle oxygen consumption

NIRS: Near-infrared spectroscopy

Oxy[Hb+Mb]: Oxygenated hemoglobin and myoglobin

$p\dot{V}O_{2:}$ Pulmonary oxygen consumption

$p\dot{V}O_{2peak:}$ Peak pulmonary oxygen consumption

RAO: Repeated arterial occlusions

RPE: Rating of perceived exertion

SmO_2: Muscle tissue oxygen saturation

T_c: Time constant for the recovery of $m\dot{V}O_2$

TA: Tibialis anterior

VL: Vastus lateralis

VOT: Vascular occlusion test

WR_{obla}: Highest work rate before 4 mmol/L blood lactate

Y: Young group

1.0 Introduction

Advancing age is associated with physiological declines of muscular strength, balance and endurance. Age-related deteriorations of physical fitness seem to be inevitable and may eventually lead to poor muscle function and performance, increasing the risk of morbidity and mortality (Tieland et al., 2018). The magnitude of decline in physical fitness and function due to aging appears to be highly individual as the main determinants of muscular aging are multifactorial and complex, often attributable to biological, psychosocial and lifestyle factors across a lifespan (Tieland et al., 2018). On a population level, the individual variation includes a wide spectrum ranging from high functioning masters athletes (Mckendry et al., 2018), to active healthy elderly, pre-frail elderly and finally sarcopenic older adults in a poor state of health in need of care (Cruz-Jentoft et al., 2010).

The primary physiological mechanisms responsible for the age-related reductions in physical form and performance are loss of skeletal muscle mass and strength (Mitchell et al., 2012), in addition to a progressive reduction in maximal oxygen consumption ($\dot{V}O_{2max}$), the main determinant of aerobic capacity (Tanaka & Seals, 2008). $\dot{V}O_{2max}$ relies on the collective efficiency of the pulmonary function, the cardiovascular system, the oxygen carrying capacity of the blood and the intramuscular microvasculature and mitochondrial oxidative capacity to transport and utilize oxygen in working muscles (Valenzuela et al., 2020). However, it is unclear how and to what extent central and peripheral factors contributes to the decline in aerobic capacity. Even though a reduced cardiac output, mainly through a reduced maximal stroke volume and maximal heart rate, appears to be the primary factor affecting $\dot{V}O_{2max}$, there are several other factors (i.e. stiffening of the arteries or impaired mitochondrial biogenesis) that may negatively affect the aerobic system (Valenzuela et al., 2020). As the effects of aging on the peripheral mechanisms are less clear, this is of clinical relevance to investigate.

In the recent decades, there has been a growing interest to gain insight in the role of skeletal muscle oxidative capacity (i.e. microvascular and mitochondrial function) on muscle aging. (Andreux et al., 2018; Ferri et al., 2007; Layec et al., 2013; Picca et al., 2018). Several techniques and equipment that have been used to measure skeletal muscle oxygenation (i.e. muscle biopsies and magnetic resonance spectroscopy) are either invasive, expensive or technically challenging in exercise settings (Barstow, 2019). Conversely, near-infrared spectroscopy (NIRS) provides a functional tool well suited for clinical tests on humans, especially in exercise settings. Since the early pioneering work by Jöbsis (1977), the use of

NIRS as a measuring technique to investigate skeletal muscle oxygenation ($m\dot{V}O_2$) has been gradually increased (Ferrari et al., 2011). NIRS has been used in a wide population including healthy young adults (Okushima et al., 2016), clinical populations (Fuglestad et al., 2019) and individuals of different fitness levels (Brizendine et al., 2013), proving its versatility. NIRS has been shown to be a reliable, non-invasive and cost effective tool for assessing muscle blood flow and oxygenation, and may be used both at rest and during exercise (Lucero et al., 2018).

The vascular occlusion test (VOT) is the most common method developed for the clinical application using NIRS to assess oxygen utilization and microvascular function at rest (Gerovasili et al., 2010). By applying arterial occlusions (AO) using a pneumatic cuff on the upper and lower limbs, NIRS can measure concentration changes in oxygenated (oxy[Hb+Mb]) and deoxygenated (deoxy[Hb+Mb]) hemoglobin (Hb) and myoglobin (Mb) during periods of ischemia. The rate of appearance of deoxy[Hb+Mb] or disappearance of oxy[Hb+Mb] is then thought to reflect oxygen consumption, making it possible to indirectly assess oxygen utilization in the underlying muscle (Gerovasili et al., 2010; van Beekvelt et al., 2001a). Subsequently, Motobe et al (2004) showed how applying repeated arterial occlusions (RAO) immediately following exercise could be used to generate a time constant for the recovery kinetics of $m\dot{V}O_2$, which was thought to reflect oxidative capacity of the muscle. This method has since been validated as a marker for mitochondrial capacity (Ryan et al., 2012, 2013; Southern et al., 2014), and has been utilized in various muscles in the upper (Chung et al., 2018) and lower extremities (Zuccarelli et al., 2020). By using post-exercise recovery kinetics of oxygen metabolism, mitochondrial dysfunction can be evaluated by NIRS *in vivo* in clinical populations, proving its potential to explore the underlying mechanisms of aging (Ryan et al., 2014). Still, there are a few potential pitfalls using this method as it relies on the assumption that mitochondrial enzymes are maximally activated and that oxygen availability is not limited (Adami & Rossiter, 2018). Additionally, external and internal work rate and the extent of increase in $m\dot{V}O_2$ during exercise compared to resting levels should be addressed in order to make inferences on exercise intensity (Zuccarelli et al., 2020).

Despite the many advantages NIRS offers, very few studies have used NIRS to assess the effects of aging on $m\dot{V}O_2$. Three studies claim to have found an association between aging and reduced microvascular function after performing a VOT to evaluate vascular responsiveness (de Oliveira et al., 2019; Rosenberry et al., 2018, 2019), whereas only two

studies have used the RAO to evaluate mitochondrial capacity in young and older individuals (Chung et al., 2018; Lagerwaard et al., 2020). However, many of the studies have not addressed the potential confounding effects of physical fitness or body composition. It is well established that adipose tissue thickness attenuates the strength of the NIRS signals and may therefore confound the interpretation of the NIRS measurements (Craig et al., 2017; van Beekvelt, et al., 2001b). Additionally, it has been shown that individuals with high aerobic capacity have greater muscle oxidative capacity compared to inactive, sedentary or low fitness individuals (Brizendine et al., 2013; George et al., 2018). Moreover, inactivity and lack of regular exercise, which often coincide with age, is a major contributor to the age-related reduction in physical capacity (Tieland et al., 2018). Studies have shown that chronically trained elderly can possess aerobic fitness, muscular strength and physical function comparable to healthy young controls in their 20's (Mckendry et al., 2018). Thus, it appears to be a gap in the literature as the role of physical fitness and body composition on age-related $m\dot{V}O_2$ kinetics using NIRS remains undetermined.

The aim of this study was therefore to investigate whether differences in mitochondrial capacity from recovery kinetics of skeletal muscle oxygen consumption can be detected between healthy young and elderly individuals, and secondly to assess these findings in relation to parameters of physical fitness. Cycling exercises at different intensities were performed and NIRS data from two muscle sites were collected to provide additional insight on the $m\dot{V}O_2$ kinetics. It was hypothesized that the younger participants would show superior oxidative capacity compared to the older participants, but that the response would be modulated by physical fitness independent of age.

2.0 Methods

2.1 Participants

A total of 41 men and women between the ages of 20 to 73 were recruited to participate in the study. The participants were separated into groups of young (Y) and elderly (E) based on age, of which 21 were between ages 18-40 and 20 between ages 58-80. All subjects were healthy, recreationally active and had a BMI <30. None of the participants reported history of cancer, heart-related disease, pulmonary disease, metabolic diseases, muscle-, joint- or skeletal problems, current smoking or use of medications that would affect their hemodynamic responses to exercise prior to the study. Participants were recruited through friends and

family, social media, a local cycling club and gyms. One subject in the young group did not complete the study due to time restraints and was excluded from the results.

The study was approved by The Regional Ethical Committee for Medical and Health Research Ethics, Midt-Norge. Information about the protocols with possible risks and discomforts was given both in writing in advance (detailed information sheet) and verbally on the first test day, before written consents were obtained.

2.2 Experimental Protocol

Data collection was conducted at NTNU (Trondheim), between October 2019 – February 2020, and the experimental procedures were completed on three separate days. An overview of the project protocol is illustrated in Figure 1. Test day 1 and 2 were separated by a minimum of 48 hours and maximum 14 days. The participants were instructed to refrain from intensive exercise 24 hours preceding the tests, and to consume a large meal 2-3 hours before the test day 1 and 2. Body composition measurement using bioelectrical impedance analysis (BIA) was performed on a separate day following an overnight fast. Several tests and measurements not included in this study were performed as this study was part of a larger project. Only data and tests relevant for this thesis will be presented.

On the first day in the laboratory, the subject's height and weight was measured. Participants then performed the vascular occlusion test to examine muscle oxygenation at rest. This was followed by a lactate threshold test and a ramp incremental test to voluntary exhaustion on a cycle ergometer. The second test day consisted of a submaximal cycling exercise and anthropometric measurements.

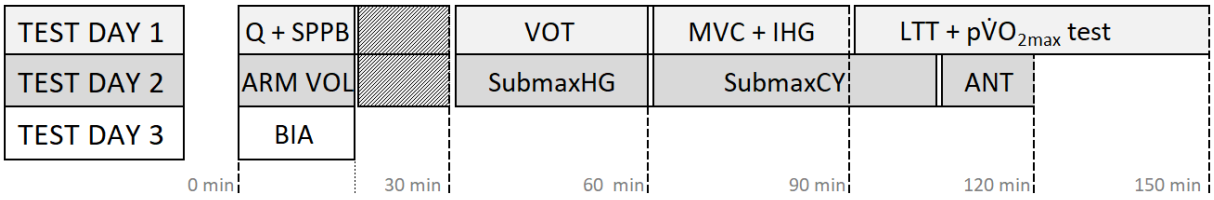

Figure 1. Overview of the project protocol. Abbreviations: Q = Questionnaire; SPPB = Short physical performance battery; VOT = Vascular occlusion test; MVC = maximal voluntary contraction test; IHG = Incremental handgrip test; LTT = Lactate threshold test; $pV\dot{O}_{2max}$ test = maximal oxygen uptake test; ARM VOL = arm volume test; SubmaxHG = Submaximal handgrip test; SubmaxCY = submaximal cycling exercise; ANT = Anthropometrics; BIA = Bioelectrical impedance analysis.

2.2.1 Vascular occlusion test (VOT)

The VOT was performed to measure muscle tissue oxygen saturation (SmO_2) at rest using NIRS. Subjects were placed in a semi-supine seated position on a bench with their legs extended and the right arm at the level of the heart. Inflatable occlusion cuffs were attached

proximally around the right leg. Following a brief baseline measurement period, a 1-minute occlusion was performed to familiarize with the protocol. After a resting period of 3-4 minutes, a 10-minute occlusion was performed, followed by 3-4 minutes of recovery measurements after cuff release. Participants were instructed to minimize movement during the entire test. The resting $m\dot{V}O_2$ values were also used as a baseline to assess relative changes after exercise.

2.2.2 Lactate threshold test (LTT)

The lactate threshold test was used to determine work rate (WR) and pulmonary oxygen consumption ($p\dot{V}O_2$) at the onset of blood lactate accumulation (OBLA), defined as blood lactate of > 4 mmol/L. After a warm-up of approximately 5 minutes at 50-100W, incremental cycling bouts of 4 minutes were performed. Depending on age and gender, three predetermined baseline protocols were used; for young men, starting WR was 100W with increments of 25W. For young women and elderly men, starting WR was 95W with increments of 20W. For elderly women starting WR was 75W with increments of 15W. Based on the participants experience with cycling and self-reported fitness level, the starting WR was individually adjusted with the aim to reach OBLA within 3-6 bouts. Participants were asked to maintain a self-selected cadence between 80-100 RPM.

$p\dot{V}O_2$ and heart rate (HR) was measured during the whole test. HR and rating of perceived exertion (RPE) were noted and blood samples from the fingertip were taken at the end of each cycling bout. The test was terminated when blood lactate of >4.0 mmol/L was measured. The highest WR achieved before surpassing OBLA was defined as WR_{obla}. Due to large inter-individual variations in blood lactate levels, some participants continued the test despite measuring blood lactate >4.0 mmol/L and some terminated the test at levels >4.0 mmol/L. The decision to continue or terminate the test was based on the examiners subjective observation of the participants HR and level of fatigue, and the participants RPE.

2.2.3 Ramp incremental cycling test ($p\dot{V}O_{2max}$ test)

Following the lactate threshold test, participants performed 5-10 minutes of recovery cycling at low intensity. Afterwards, a ramp incremental cycling test was completed to determine $p\dot{V}O_{2peak}$, maximal aerobic power (MAP) and maximal heart rate (HR_{max}), with the aim to reach exhaustion between 6-10 minutes. The $p\dot{V}O_{2max}$ test was initiated with a WR set at one level below WR_{obla} with increments of 25W/min for young men, 20W/min for young women and elderly men and 15W/min elderly women. Strong verbal encouragement was given throughout the test. The test was terminated when the subject was unable to continue pedaling

at a constant cadence or if cadence dropped below 60 rpm. $p\dot{V}O_2$ and HR was measured during the entire test. RPE was noted and blood lactate was measured immediately after exhaustion. The highest WR sustained for 60 seconds during the ramp test was set as MAP.

2.2.4 Submaximal cycling exercise (SubmaxCY)

Following a 5-minute warm up at 50-100W, two 5-minute cycling periods were performed, one at low intensity corresponding to 50% of WR_{obla} (LOW) and one at high intensity corresponding to 70% of MAP (HIGH). Immediately after LOW, a wooden block was placed underneath the right pedal with the foot locked in a fixed resting position almost perpendicular to the floor. Then, a series of RAO were applied in intervals of 5 seconds on/5 seconds off for 4 minutes followed by 10 seconds on/20 seconds of for another 4 minutes. The participants were instructed to minimize movement for the duration of the RAO. After 2 minutes of recovery cycling at 50-100W, the protocol was repeated at HIGH. RPE and HR was noted and blood lactate was measured immediately after each cycling bout. $p\dot{V}O_2$ was only measured during the cycling bouts while NIRS-measurements were recorded continuously.

2.2.5 Body composition measurements

Height and weight were measured using a mechanical telescopic measuring rod (Seca 222, GmbH & Co, Hamburg, Germany) and a flat scale (Seca 877, GmbH & Co, Hamburg, Germany). Limb circumference and skinfold thickness was measured on the muscle site of the two NIRS-probes (vastus lateralis and tibialis anterior) with a measuring tape and a skinfold caliper (Holtain; Crymych, UK). Skinfold thickness was calculated as the average of two skinfold measurements divided by 2. Lean body mass (LBM), fat mass (FM), body fat percentage (BF%) and body weight were measured using whole body bioelectrical impedance analysis (InBody770, Seoul, Korea). The participants performed the test between 06.30-08.00 in the morning following an overnight fast, wearing only light clothes. Women were asked to perform the test after the follicular phase of the menstrual cycle to reduce the effect of water retention.

2.3 Materials and data analyses

2.3.1 Cycling tests

All cycling tests were performed on a cycle ergometer (Lode Excalibur Sport, Lode B. V., Groningen, Netherlands) adjusted to the participants preferred seat height and handlebar position. Pulmonary gas exchange was measured (10 sec sampling time) continuously with a

stationary spiroergometric device in mixing chamber mode (Oxycon Pro, Jaeger GmbH, Hoechberg, Germany). The flow turbine was calibrated at the beginning of every test day using a 3-liter calibration syringe (Hans Rudolph Inc, Kansas City, MO, USA) for volume calibration. The gas analyzers were calibrated with an external gas source (Reissner-Gase, GmbH & Co; Lichtenfels, Germany) of known concentrations (15% O2, 5% CO2) before each test. A secondary spiroergometric device (Metalyzer 3B, Cortex Biophysik GmbH, Leipzig, Germany) was used in some test due to technical issues with the Jaeger system. Calibrations were carried out in accordance with the manufacturer's manual. HR was measured with a heart rate sensor belt (Wearlink, Polar Electro OY, Kempele, Finland) linked to a HR monitor watch (Polar RS800CX, Polar Electro OY, Kempele, Finland) with a sampling frequency of 5 seconds. Rating of perceived exertion was quantified using Borg's scale (Borg, 1982). Blood lactate was measured using a hand-held analyzer (Lactate Pro2 LT-1730, Arkray KDK, Kyoto, Japan).

The $p\dot{V}O_{2peak}$ was determined as the highest mean value achieved over a 30 second period during the ramp incremental test. The maximum HR measured during the incremental cycling test was set as HR_{max}. The $p\dot{V}O_2$ and HR at SubCY was determined as the mean value of the last 30 seconds for each cycling bout.

2.3.2 NIRS

As Hb and Mb has similar absorption spectra, it is difficult to distinguish their relative contribution to the NIRS signal (Davis & Barstow, 2013), and their signals are therefore combined in this study. The absorbance of NIR light in Hb and Mb is oxygen dependent, making it possible to differentiate between oxy[Hb+Mb] and deoxy[Hb+Mb], and subsequently make inferences on tissue oxygenation (Ferrari & Quaresima, 2012). The sum of oxy[Hb+Mb] and deoxy[Hb+Mb] represents total[Hb+Mb], whereas SmO_2 was calculated as oxy[Hb+Mb]*100/total[Hb+Mb] expressed in percent (%).

Changes in SmO_2 were recorded using four near-infrared spectrophotometers (Portamon, Artinis Medical Systems, Netherlands) placed longitudinally on the muscle belly of the vastus lateralis (VL) and tibialis anterior (TA) on the right leg, using wavelengths 845 nm and 762 nm and 10 Hz sampling frequency. The NIRS-probes were fastened with elastic bandage and tape to mitigate movement and covered in a piece of black cloth to minimize intrusion of external light. Excessive body hair at the optode sites was shaved with a razor. AO were performed with inflatable occlusion cuffs (Hokanson SC12L, Marcom Medical ApS, Denmark and Hokanson SC5, Marcom Medical ApS, Denmark) on the right leg. The cuffs

were rapidly inflated to 280 mmHg, and subsequently rapidly deflated, using an automated pressure regulator (Hokanson E20 Rapid Cuff Inflator, Marcom Medical ApS, Denmark).

Resting muscle oxygen consumption ($m\dot{V}O_{2rest}$) was calculated during the VOT as the linear slope of desaturation for a period of 3 minutes, 30 seconds after cuff inflation. The baseline measurement (baseline SmO_2%) prior to SubmaxCY protocol was calculated as the mean value of 30 seconds prior to test start, while end-exercise ΔSmO_2 (ΔSmO_{2end}) was calculated as the mean value from the last 30 seconds for each cycling bout. For the post-exercise measurements, the time constant for the recovery of $m\dot{V}O_2$ (T_c) was calculated from a linear regression of the SmO_2 signal in a 3 second period during the RAO, 0.5 seconds after cuff inflation. The values from all AO during the RAO period were then fit to a mono-exponential curve. Data from NIRS was processed in MATLAB R2019b.

2.4 Statistical analysis

Results are presented as means ± SD. To compare the group means of normally distributed values, independent samples T-tests were used. Independent samples Mann-Whitney U tests were used when the means of variables were not normally distributed. A two-way ANOVA was used to compare means between the groups during and after the SubCY tests. General linear models with repeated measures were used to test group differences in ΔSmO_2% during SubCY. As there were some cases with missing variables, linear mixed models with repeated measures was used to test group differences in T_c following SubCY. Whenever sphericity was violated according to Mauchly's test of sphericity, results from the mixed ANOVA were reported using the Greenhouse-Geisser correction. Pearson's correlation coefficient was used to assess the association between different continuous variables. Graphs and statistical analyses were made and processed in GraphPad Prism (GraphPad Software 8.4.2, CA, USA) and IBM SPSS Statistics version 26. Statistical significance was accepted at $p<0.05$.

3.0 Results

3.1 Participants

All of the 40 participants that participated in the study completed the entire test protocol, apart from one subject who discontinued the VOT due to discomfort. The tests were well tolerated, and no other adverse effects were reported. Physical characteristics and main cardiovascular and metabolic responses to peak exercise are presented in Table 1. As expected by design, age was significantly greater in E compared to Y (p <0.001). There were no differences in BMI, LBM or ATT between the groups. However, E had a higher BF% than Y (p<0.05). As

expected, the relative and absolute $p\dot{V}O_{2peak}$ and MAP were higher in Y compared with E (p<0.05). The incremental cycling test elicited HR_{max} corresponding to 100% and 102% of the age-predicted HR_{max} (calculated as 211 - 0.64·age), high RPE and blood lactate of 13.0 and 11.0 mmol/L, for Y and E respectively, indicating maximal effort (Nes et al., 2013).

Three data sets, two in Y and one in E, were excluded from all analysis using NIRS data due to poor NIRS signal quality or technical issues with the NIRS equipment. Additional exclusion of data sets is specified at each test.

Table 1. Physical characteristics and peak exercise responses.

	Young		Elderly	
	Mean (± *SD)*	Range	Mean (± *SD)*	Range
***n* (male/female)**	20 (14/6)		20 (13/7)	
Age (years)	26.2 (±3.0)	20-33	62.4 (±4.3) ***	58-73
Weight (kg)	77.7 (±12.4)	50.9-93.5	73.5 (±11.0)	45.9-88.9
Height (cm)	179.7 (±8.6)	163.0-195.5	175.5 (±9.2)	151.0-187.0
BMI (kg/m^2)	24.0 (±3.2)	19.2-29.3	23.7 (±2.3)	20.1-28.2
BF%	18.3 (±9.6)	5.9-42.7	21.9 (±4.8)*	12.5-30.2
LBM (kg)	63.2 (11.1)	44.0-81.4	57.5 (±9.8)	34.8-68.4
ATT VL (mm)	7.0 (±3.9)	2.8-15.0	6.5 (±3.0)	2.7-11.2
ATT TA (mm)	4.5 (±2.0)	2.3-9.7	3.5 (±1.6)	2.0-8.5
$p\dot{V}O_{2peak}$ (mL·min^{-1}·kg^{-1})	47.4 (±7.6)	30.6-56.4	40.3 (±6.6) **	28.3-54.5
$p\dot{V}O_{2peak}$ (mL·min^{-1})	3690 (±820)	2310-5070	3010 (±790) **	1610-4420
MAP (W)	299 (±73)	195-425	244 (±70) *	120-355
HR_{max} (beats·min^{-1})	195 (±5)	188-207	175 (±9) ***	157-195
RPE (Borg 6-20)	18.8 (±1.2)	17-20	18.5 ± (±0.8)	16-20
WR_{obla} (W)	171 (±55)	75-275	154 (±54)	75-275

BMI, body mass index; BF%, body fat percentage; LBM, lean body mass; ATT, adipose tissue thickness; VL, vastus lateralis; TA, tibialis anterior; $p\dot{V}O_{2peak}$, peak pulmonary oxygen consumption; MAP, maximal aerobic power; HR_{max}, maximal heart rate; RPE, rating of perceived exertion; $WR_{obla,}$ work rate at OBLA
* Significantly different between groups (p <0.05), ** (p <0.01), *** (p <0.001)

3.2 Muscle oxygen consumption during rest

Resting muscle oxygen consumption ($m\dot{V}O_{2rest}$), measured as the linear slope of deoxygenation during VOT, is presented in Table 2 for Y and E, including a separate sample for men and women. Five data sets in Y and two data sets in E were excluded due to poor NIRS signal quality or missing data. While $m\dot{V}O_{2rest}$ was not different between Y and E, there was a significant difference between men and women. As presented in Figure 2, a strong correlation between $m\dot{V}O_{2rest}$ and ATT in both VL (r^2 = 0.73; p<0.001) and TA (r^2 = 0.57;

p<0.001) was found, indicating a possible confounding effect of ATT. In addition, men had significantly less ATT than women at VL (5.3 ± 2.3 vs. 9.9 ± 3.2 mm; *p*<0.001) and TA (3.4 ± 1.1 vs. 5.3 ± 2.5 mm; *p*<0.05).

Table 2. Resting muscle oxygen consumption during VOT.

	Young (n=15)	Elderly (n=18)	Men (n=23)	Women (n=10)
m$\dot{V}O_{2rest}$ (%·s^{-1}) VL	-0.08 ± 0.03	-0.07 ± 0.03	-0.08 ± 0.03	-0.05 ± 0.02 $$
m$\dot{V}O_{2rest}$ (%·s^{-1}) TA	-0.14 ± 0.04	-0.15 ± 0.04	-0.16 ± 0.04	-0.12 ± 0.05 $

m$\dot{V}O_{2rest}$, resting muscle oxygen consumption; VL, Vastus lateralis; TA, Tibialis anterior. Values are mean ± SD.
$ Significant difference between men and women (*p*<0.05), $$ (*p*<0.01)

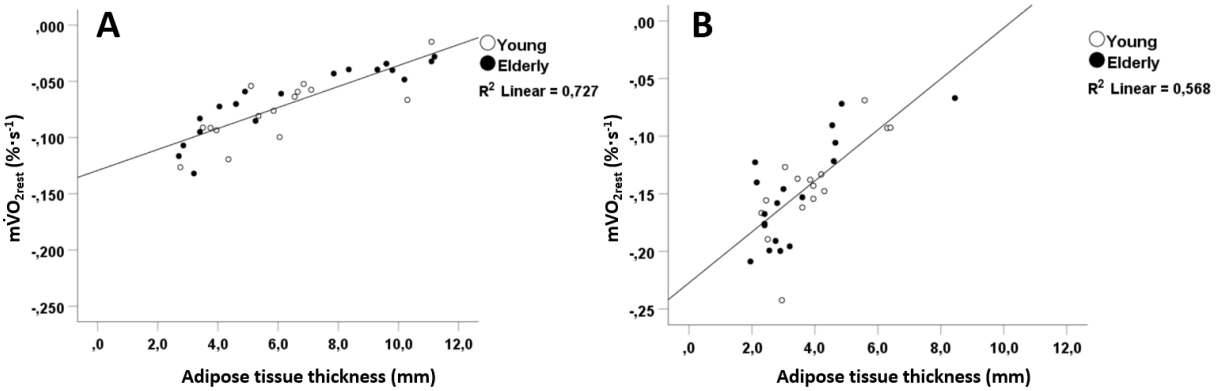

Figure 2. Correlation between mV̇O2rest and ATT in (A) vastus lateralis (VL) and (B) tibialis anterior (TA)

3.3 Muscle oxygenation kinetics in submaximal cycling exercise

Cardiovascular and metabolic responses to submaximal cycling exercise at LOW and HIGH intensity are presented in Table 3 and illustrated in Figure 3. Group mean values of work rate, p$\dot{V}O_2$, heart rate and blood lactate revealed similar internal and external work rates between the groups, at both submaximal intensities. In addition, no difference was observed in RPE after the HIGH intensity cycling bout (14.7 ±1.3 vs 14.5 ±1.6 for Y and E, respectively).

Table 3. Cardiovascular and metabolic responses to submaximal cycling exercises.

	LOW		**HIGH**	
	Young (n = 18)	Elderly (n = 18)	Young (n=18)	Elderly (n = 18)
Work rate (W)	89 (±25)	81 (±28)	217 (±49)	182 (±59)*
%p$\dot{V}O_{2peak}$	44.1 (±3.1)	47.4 (±4.6)*	82.4 (±4.1)	82.2 (±5.4)
%HR_{max}	58.4 (±3.2)	59.7 (±5.6)	84.7 (±3.4)	83.9 (±4.8)
[La^-] (mmol·L^{-1})	1.2 (±0.4)	1.4 (±0.5)*	7.0 (±1.3)	5.5 (±1.8)*

%p$\dot{V}O_{2peak}$, fraction of p$\dot{V}O_{2peak}$ 30 seconds before end of exercise; %HR_{max}, fraction of maximal heart rate 30 seconds before end of exercise; [La^-], blood lactate concentration 30 seconds after exercise; Values are mean±SD. * Significant difference between groups (*p*<0.05).

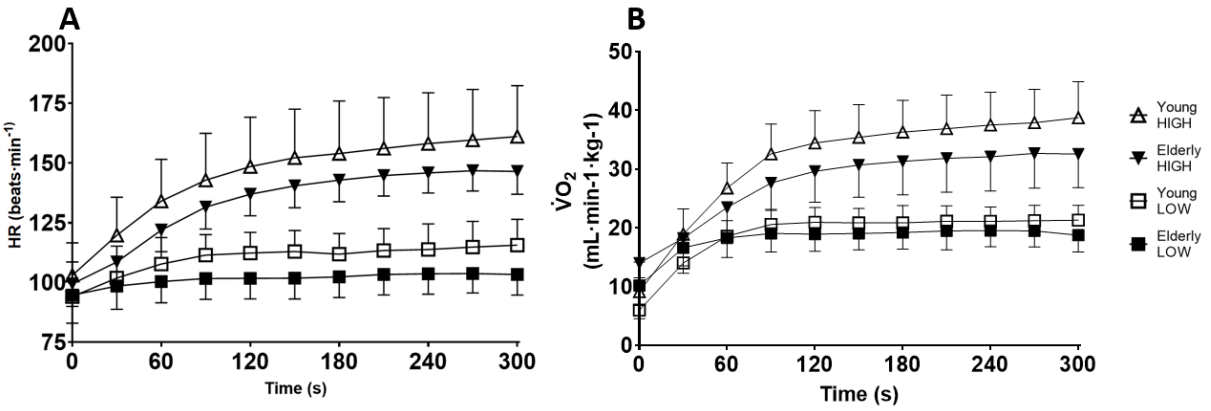

Figure 3. Cardiovascular (A) and p$\dot{V}O_2$ (B) responses to submaximal cycling exercises. Values are group means ± SD

3.3.1 m$\dot{V}O_2$ on-kinetics

Mean values of baseline SmO_2% and $\Delta SmO_2\%_{end}$ for VL and TA during HIGH and LOW intensity are presented in Table 4, whereas group responses are illustrated in Figure 4. For the pre-exercise measures, only baseline SmO_2% in TA was different between Y and E. Two data sets in each group were excluded due to poor NIRS signal quality or missing data.

For VL, there was a main effect of group on changes in $\Delta SmO_2\%_{end}$ ($F(1,34) = 23.0$, $p<0.001$). In addition, there was a main effect of intensity ($F(1,34) = 113.4$, $p<0.001$), and an interaction effect between intensity and group in VL ($F(1,34) = 5.4$, $p<0.05$). In TA, there was not a main effect of group on changes in $\Delta SmO_2\%_{end}$ ($F(1,34) = 0.4$, $p = 0.54$). However, there was a main effect of intensity ($F(1,34) = 131.7$, $p<0.001$), but no interaction effect between intensity and group was found ($F(1,34) = 5.4$, $p<0.05$).

Table 4. Pre- and end-exercise m$\dot{V}O_2$ during submaximal cycling.

	LOW		HIGH	
	Young (n=18)	Elderly (n=18)	Young (n=18)	Elderly (n=18)
Baseline SmO_2% VL	76.1 (±4.7)	72.1 (±8.5)	76.3 (±4.7)	73.2 (±8.1)
Δ $SmO_2\%_{end}$ VL	-3.8 (±2.6)	-0.5 (±1.8)***	-12.6 (±5.9)	-6.1 (±3.3)***
Baseline SmO_2% TA	71.8 (±6.3)	65.8 (±7.9)*	75.8 (±5.2)	68.5 (±9.3)**
Δ $SmO_2\%_{end}$ TA	-0.3 (±2.3)	0.2 (±3.8)	-12.6 (±6.3)	-11.2 (±7.6)

Baseline SmO_2%, 30 second average SmO_2% pre-exercise; $\Delta SmO_2\%_{end,}$ average ΔSmO_2% last 30 seconds of exercise; VL, vastus lateralis; TA, tibialis anterior. * Significantly different between groups (p <0.05), ** (p <0.01), *** (p <0.001).

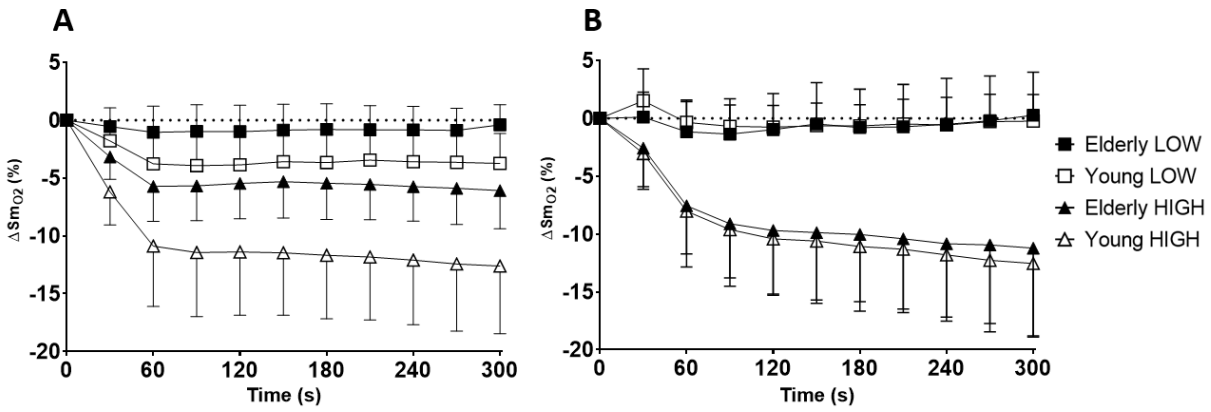

Figure 4. Group responses of $m\dot{V}O_2$ kinetics (ΔSmO2% from baseline SmO2%) in vastus lateralis (A) and tibialis anterior (B) during submaximal cycling at LOW and HIGH intensity. Values are group means ± SD.

3.3.2 $m\dot{V}O_2$ off-kinetics

The group responses of $m\dot{V}O_2$ recovery kinetics following LOW and HIGH intensity cycling bouts are presented in Figure 5 and 6. At the onset of recovery, $m\dot{V}O_2$ values in VL after LOW were 21 versus 22 times higher than at rest, for Y and E respectively. After HIGH, the equivalent measures were only 15 versus 19 times higher, for Y and E respectively. There were no significant differences between the groups. The monoexponential curve fits calculated from the recovery kinetics during the RAO were great ($0.87 \leq r^2 \leq 0.97$). Still, individual data sets with curve fitting $r^2 \leq 0.70$ were excluded. Therefore, five (Y) and four (E) data sets for VL, in addition to five (Y) and seven (E) in TA, were excluded due to poor data quality. There was a main effect of intensity on Tc in VL ($F(1,58) = 55.9$, $p<0.001$). In addition, there was a main effect of group in VL ($F(1,58) = 10.9$, $p<0.01$). Finally, there was an interaction effect between intensity and group in VL ($F(1,58) = 5.3$, $p<0.05$). In TA, there was not a main effect of intensity on Tc ($F(1,52) = 0.5$, $p = 0.50$). However, there was a main effect of group ($F(1,52) = 4.0$, $p = 0.05$), but no interaction effect between intensity and group was found ($F(1,52) = 3.4$, $p = 0.07$)

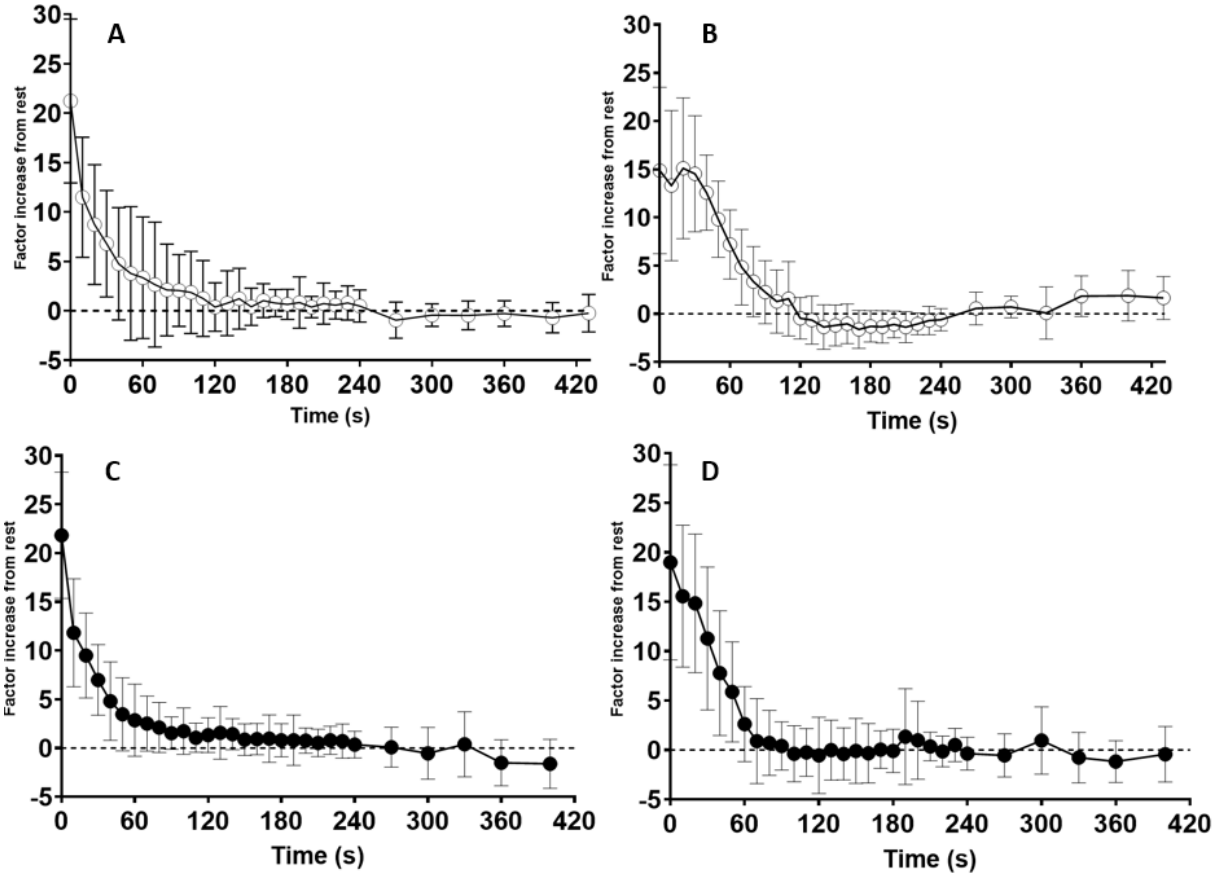

Figure 5. m$\dot{V}O_2$ as a factor increase from rest in vastus lateralis after LOW and HIGH for young (A and B) and elderly (C and D). Values are group means ± SD

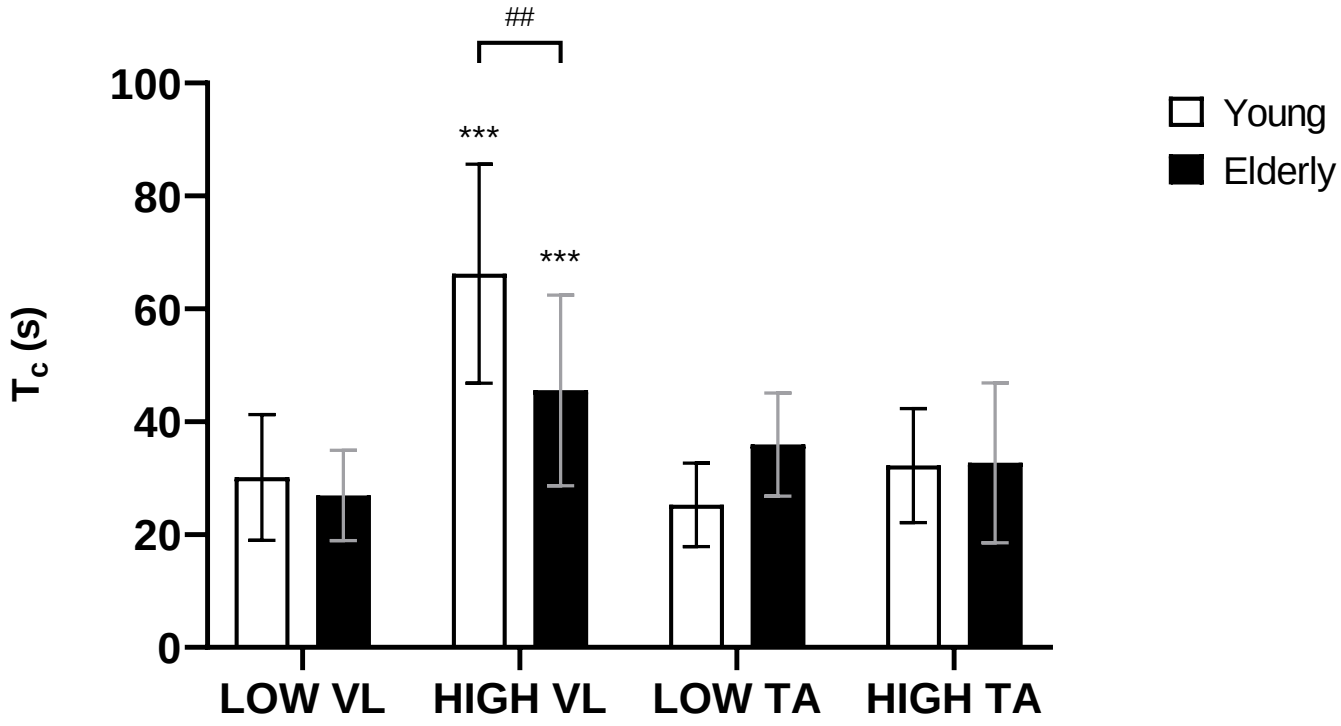

Figure 6. Time constant values (T_c) of recovery m$\dot{V}O_2$ in vastus lateralis (VL) for young (n=15) and elderly (n=16) and tibialis anterior (TA) for young (n=15) and elderly (n=13) after submaximal cycling at LOW and HIGH intensity. Values are means ± SD. *** Significant different from LOW $p<0.001$. ## Significant difference between groups ($p<0.01$)

3.4 Parameters of physical fitness

Additional correlation analyses were performed to investigate potential interaction effects of body composition and aerobic capacity on the $m\dot{V}O_2$ kinetics responses. ATT was positively correlated with T_c in VL after LOW ($r^2 = 0.33$, $p<0.01$) and $\Delta SmO_2\%$ in VL ($r^2 = 0.28$, $p<0.01$) and TA ($r^2 = 0.29$, $p<0.01$) after HIGH. In addition, LBM was negatively correlated with $\Delta SmO_2\%$ in VL ($r^2 = 0.29$, $p<0.01$) and TA ($r^2 = 0.19$, $p<0.01$) after HIGH. Furthermore, WR at SubCY was negatively correlated with $\Delta SmO_2\%$ after HIGH in VL ($r^2 = 0.32$, $p<0.001$) and LOW ($r^2 = 0.11$, $p<0.05$) and HIGH ($r^2 = 0.33$, $p<0.001$) in TA, indicating greater desaturation at higher WR. Finally, $p\dot{V}O_2$ at SubCY was negatively correlated with $\Delta SmO_2\%$ after LOW ($r^2 = 0.23$, $p<0.01$) and HIGH ($r^2 = 0.43$, $p<0.001$) in VL and HIGH ($r^2 = 0.19$, $p<0.01$) in TA.

4.0 Discussion

The primary objective of this study was to assess mitochondrial capacity from recovery kinetics of $m\dot{V}O_2$ between healthy young and elderly individuals. The main findings in this study was that $m\dot{V}O_2$ kinetics at rest and after submaximal cycling exercise were not slower, and in some cases even faster, in E compared to Y. Even though Y had a slightly higher $p\dot{V}O_{2peak}$ and MAP than E, the body composition of the two groups were similar as there was no difference in BMI, LBM or ATT. Additionally, the results shows that the $m\dot{V}O_2$ responses in both groups were highly affected by exercise intensity, and that the most distinct differences were seen at HIGH intensity. The present data indicates that assessment of physical fitness is of major importance when interpreting results using NIRS to examine the physiological effects of aging.

4.1 $m\dot{V}O_2$ kinetics

4.1.2 $m\dot{V}O_2$ rest

There were no group differences in $m\dot{V}O_{2rest}$ in VL or TA during the VOT. However, a strong correlation between $m\dot{V}O_{2rest}$ and ATT was found as presented in figure 2, consistent with previous findings in the literature (Craig et al., 2017; van Beekvelt, et al., 2001b). Although few studies have reported resting measurements during the VOT, the related evidence in the literature is inconsistent. As the studies vary in terms of measuring site, participant age, gender and health status, it is difficult to compare the results between the studies. One study found no difference in $m\dot{V}O_{2rest}$ in VL between young endurance trained athletes and inactive counterparts (Brizendine et al., 2013). Another study showed no difference in $m\dot{V}O_2$ rest in

the flexor carpi radialis between healthy young and elderly (de Oliveira et al., 2019). However, de Oliveria et al. (2019) did find a slower desaturation rate among a third group consisting of older adults with cardiovascular risk factors. Additionally, Rosenberry et al. (2018) found a markedly reduced desaturation rate in the flexor digitorum profondus during VOT between young and elderly.

Although it is beyond the scope of this thesis, the present study did find a significant difference in $m\dot{V}O_{2rest}$ between males and females, as presented in table 2. Still, it is unclear whether this might be due to differences in ATT or other possible factors (e. i. aerobic capacity or muscle quantity and quality of the underlying tissue).

4.1.2 $m\dot{V}O_2$ during exercise

The responses to submaximal cycling exercise showed both similarities and differences between the groups. The magnitude and pattern of tissue desaturation were almost identical in TA while the response in VL was blunted in E compared to Y, as seen in Figure 4 and Table 4. E had a significantly lower $\Delta SmO_2\%_{end}$ in VL at both LOW and HIGH intensity compared to Y, whereas no between group differences were observed in TA. Few studies have investigated the effect of whole-body exercise on skeletal muscle deoxygenation, but Okushima et al. (2016) showed that the magnitude of tissue desaturation in VL was associated with $p\dot{V}O_{2max}$ and absolute power during a ramp-incremental cycling exercise in young adults. Even though the relative work rate was similar between the groups in this study, the absolute work rate was 10% (8W) and 19% (35W) higher in Y, at LOW and HIGH respectively (Table 2). However, the differences in absolute work rate between the groups was only statistically significant in HIGH (p<0.05). On the other hand, an attenuated NIRS signal due to differences in muscle quality or quantity may also be an explanation. Although ATT did not differ between the groups at VL or TA, this study provided almost no information with regards to muscle quality (e. i. MRI) or muscular strength in the legs. Additionally, ATT was greater in VL (6.3 + 2.9 mm) compared to TA (3.7 + 1.4 mm) which may diminish the absolute quantification of the NIRS signals in VL.

4.1.3 $m\dot{V}O_2$ post-exercise

The T_c of $m\dot{V}O_2$ from the recovery phase following submaximal cycling showed that the response in E was not inferior to Y. A clear effect of exercise intensity was seen in VL, while the response in TA was more unclear (Figure 6). The results observed are to some degree unexpected as there was a considerable age difference and Y had a greater $\dot{V}O_{2peak}$ than E. Some studies have shown slower time constants for elderly versus young (Chung et al., 2018)

and low versus high fitness young individuals (Batterson et al., 2020; Brizendine et al., 2013; Lagerwaard et al., 2019). However, the magnitude of difference in $\dot{V}O_{2peak}$ between Y and E in this study (47.4 vs 40.3 $mL \cdot min^{-1} \cdot kg^{-1}$, respectively) was relatively low compared to other studies. In addition, $\dot{V}O_{2peak}$ values for E were ~11% higher than the national average for age group 60-69, whereas the equivalent for Y was ~7% lower compared to age group 20-29 (Loe et al., 2013). As $p\dot{V}O_{2peak}$ is not the sole determinant of aerobic capacity (Tanaka & Seals, 2008), it is possible that some of the difference in $p\dot{V}O_{2peak}$ is counteracted by other factors in E as a result of years of chronic exercise.

Another possible explanation may also be the discrepancy observed in $\Delta SmO_2\%_{end}$ in VL during SubCY. As previously discussed, the difference in $\Delta SmO_2\%_{end}$ in VL could be a result of several factors, and it is not clear whether this could also affect the $m\dot{V}O_2$-T_c. If Y truly had a greater tissue desaturation than E, it would be reasonable to assume that the following recovery period also would be longer. However, if the difference in tissue desaturation is a result of different signal quality, then that may affect the quantification of the NIRS signals for the recovery measurements. Clearly identifying the muscle belly of the VL was also more challenging on E than for Y, in contrast to the TA which is a more visual accessible muscle with less ATT.

4.1.4 Parameters of physical fitness

The correlation analyses showed mostly weak to moderate correlations between $m\dot{V}O_2$ kinetics and various factors of body composition and aerobic fitness. Apart from the strong correlation between ATT and $m\dot{V}O_{2rest}$, it is evident that no single factor alone affected the results. This further supports the notion that physical fitness should to be assessed broadly in order to better understand how different factors interact with or affect the NIRS data.

4.2 Assessing the physiological effects of aging using NIRS

The primary findings in this study are inconsistent with most studies in the literature using NIRS to examine the physiological effects of aging. Previous studies have found an age-related reduction in mitochondrial or microvascular function when comparing young versus elderly individuals (Chung et al., 2018; Lagerwaard et al., 2020; Rosenberry et al., 2018). However, these studies lack quantitative measures of physical fitness and have other questionable methodological considerations. Conversely, one study supports the findings in the present study by demonstrating that fitness level rather than aging determines $m\dot{V}O_2$ kinetics (George et al., 2018). Additionally, de Oliveria et al. (2019) showed no difference in

microvascular function between healthy young and elderly adults. Still, isolating the effect of age on mitochondrial and microvascular function is very challenging. Previous findings in the literature are conflicting, but it is hard to compare the results between the studies due to different methods, study populations and outcome variables.

Even though there has been very little research on NIRS and aging, it may be argued that the basis for comparison between groups have not been adequate in some of the prior studies. In the studies of Chung et al. (2018) and Rosenberry et al. (2018), the participants in the older groups had several cardiovascular risk factors and many were medicated. Additionally, there were no measures of aerobic capacity, body fat percentage or adipose tissue thickness. Furthermore, the participants in the older groups were predominantly female, whereas gender was more equally distributed in the younger groups. It is therefore questionable to conclude that aging *per se* was the primary factor attributable for their results.

In Lagerwaard et al. (2020), the aim was to compare mitochondrial capacity in young and elderly healthy with similar physical activity levels to negate the confounding effects of physical activity. They collected accelerometer data for seven days to establish physical activity levels and performed basic body composition measurements. However, as there were no measures of aerobic capacity or performance measures of endurance, it is unclear whether the groups had comparable fitness levels. The results of the study are therefore still potentially confounded by the effects of physical activity.

4.3 Methodological considerations

The primary limitations in the present study included missing or poor NIRS data quality and the lack of measures on muscle quality and strength. For the various tests, between 4 and 12 data sets were excluded, including three subjects who were excluded from all NIRS analyses. For one of the participants, all NIRS data were lost as a result of technical problems with the NIRS devices, while the other two instances were excluded due to poor NIRS data quality. However, common features for the latter included a BF%, BMI and ATT in the higher range which may interfere with the signals from the underlying muscle. A BMI >30 was one of the exclusion criteria, but it is evident from the physical characteristics (Table 1) that there is a large variation of BF% and ATT between the subjects of both groups. This shows including other measures of body composition may be of importance, especially when studying heterogeneous groups (e. i. different genders, ages and fitness levels).

As previously discussed, this study did not thoroughly quantify muscle quality (e. i. intramuscular fat) or strength (MVC or 1RM). However, limb circumferences at the NIRS muscle sites were measured, but revealed no differences between the groups at VL (Y = 48.2 + 4.5 vs E = 46.4 + 3.0 cm) or TA (Y = 38.1 + 2.8 vs E = 37.4 + 2.5 cm). It is therefore still unclear whether the muscle morphology of the groups differed, and if this could possibly aid to explain some of the observations.

5.0 Conclusion

In conclusion, this study demonstrated that the mitochondrial capacity from recovery kinetics of m$\dot{V}O_2$ assessed by NIRS were not slower in healthy elderly individuals compared to healthy young individuals. The data indicate that the m$\dot{V}O_2$ kinetics response is highly modulated by physical fitness, independent of age. Assessment of physical fitness is therefore of major importance when interpreting results using NIRS to examine the physiological effects of aging. Future research on the effects of aging using NIRS should implement measures of muscular capacity and muscle quality to negate the confounding effects of physical fitness.

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Appendix 1: Samtykkeskjema

INVITASJON TIL DELTAKELSE I FORSKNINGSPROSJEKT

Vi søker friske deltakere i alderen 18-40 år og 60-80 år som er moderat aktive på fritiden (mosjonister).

Bakgrunn og hensikt

Forskningsstudiet undersøker muskelfunksjon i aldring.

Måling av hele kroppens oksygenforbruk under aktivitet kan gi verdifull informasjon om kroppens fysiske form, og er i tillegg et indirekte mål for muskulært oksygenforbruk. Å måle oksygenforbruket til hele kroppen er forholdsvis enkelt, men krever fysisk aktivitet på høy intensitet. Å måle oksygenforbruket i en enkelt muskel er mer komplisert og har først i de siste tiår blitt tilgjengelig med nær-infrarød spektroskopi (NIRS). Ved å bruke denne metoden kan vi måle oksygenforbruket i de enkelte aktive muskler, og kan vi undersøke energiomsetningen i muskelen uten at det krever fysisk aktivitet på høy intensitet. I dette forskningsprosjektet vil oksygenforbruket til enkelte muskler bli studert både under helkroppsarbeid (sykling) og under isolert muskelarbeid (håndgrep-øvelse) for å undersøke om lokale målinger kan brukes for å studere muskelfunksjon i aldring.

Vi søker etter friske deltakere i alderen 18-40 år eller 60-80 år som er moderat aktiv på fritiden (mosjonister). For å delta i prosjektet kan du ikke ha en historikk med hjerte-, lunge- eller karsykdommer. Deltakere kan ikke ha bevegelsesproblemer eller metabolske sykdommer som fedme (BMI over 30), diabetes, ukontrollert høyt blodtrykk, og heller ikke være nåværende røyker. Studien gjøres i forbindelse med to mastergradsoppgaver ved institutt for nevromedisin og bevegelsesvitenskap, NTNU.

Hva innebærer studien?

Prosjektet er delt opp i **tre** testdager, som vil foregå med noen dagers mellomrom for å sikre fullstendig restitusjon. Hvis du ønsker å delta i studien må du avstå fra annen trening de dagene målingene gjennomføres, samt i minst 48 timer før testing. Under de forskjellige testene samles det

inn data om oksygenforbruk i hele kroppen, oksygenforbruk i muskulaturen, hjertefrekvens, hjertets minuttvolum og blodlaktat.

TESTDAG 1. På testdag 1 vil du bli stilt noen spørsmål om ditt aktivitetsnivå og nåværende helsestatus, før det gjennomføres noen enkle funksjonstester for balanse, ganghastighet og styrke. Etter dette vil vi feste elektroder på hals, bryst, under armhulen og på ryggen din, samt NIRS lysdioder på underarmen, låret og leggen. Dette utstyret må være på under resten av testene.
For å måle oksygenforbruket i lår- og underarmsmuskelen vil en mansjett rundt låret og armen pumpes opp en gang i henholdsvis 1 og 10 minutter som midlertidig klemmer av blodstrømmen (arteriell okklusjon). Denne målingen blir gjennomført i hvile i sittende posisjon, og det er viktig å sitte helt i ro for å kunne oppnå gode målinger.
Videre testes maksimal håndgrepsstyrke i en håndgrepdynamometer. Etter dette gjennomføres også en håndgripetest med gradvis økende belastning for å estimere armmuskelens maksimale evne til å ta opp oksygen under lokalt arbeid. Testdagen avsluttes med en sykkeltest med gradvis økende belastning for å estimere laktatterskel og kroppens maksimale evne til å ta opp oksygen. Sykkeltesten innebærer 5-6 målinger av blodlaktat som gjøres ved å hente en dråpe blod fra et stikk i fingeren. De sistnevnte testene krever maksimal innsats, og kan oppleves som krevende.
Det kan forventes å bruke opptil 2.5 timer på å gjennomføre testene på dag 1.

TESTDAG 2. På testdag 2 vil vi måle armvolum i en vannsylinder. Testdagen innebærer også to tester under fysisk aktivitet på lav, moderat og høy belastning. Test nr. 1 gjennomføres med et håndgrepergometer, test nr. 2 på sykkelergometer. Til slutt gjøres flere antropometriske målinger (omkrets og hudfoldtykkelse) på ulike punkter på kroppen som arm, lår, legg, rygg og hofte.
Det kan forventes å bruke 2 timer på å gjennomføre testene på dag 2.

TESTDAG 3. KROPPSANALYSE. Testen gjøres med en kroppsanalysemaskin, og vil foregå på morgenen (kl.06.30-09.00). For å få en gyldig test krever det at man faster fra kvelden før, og man må derfor møte opp på tom mage. En slik analyse gir et godt estimat av din kroppssammensetning som vekt, fettmasse og muskelmasse i de ulike kroppsdelene. Du vil få med deg dine resultater hjem når alle testdagene er gjennomført.
Det kan forventes å bruke 10 minutter på denne analysen.

Alle testdagene vil gjennomføres ved St.Olavs Hospital.

Mulig fordeler og ulemper

Denne studien samler inn normaldata som bidrar til nyttig kunnskap om forskjellene i oksygenforbruk under helkroppsarbeid og lokalt muskelarbeid, og kan gi oss kunnskap om mulige endringer i muskelfunksjon med aldring. Disse målingene kan ikke gi direkte informasjon om muskelfunksjonen og aldring hos den enkelte deltaker. Likevel kan vi tilby deg et oppsummerende skriv som inkluderer info om din kroppssammensetning (fett- og muskelmasse), kondisjon og styrkeresultater. Om ønskelig tilbyr vi også en oppsummerende samtale, med tolkning av dine resultater og enkel individuelt tilpasset treningsveiledning.

Maksimaltestene kan medføre noe ubehag, men de vil bli utført med kyndig personell til stede og etter etablerte prosedyrer. I tillegg kan mansjetten som brukes i studien oppleves som ubehagelig. Dette gjelder spesielt i starten av okklusjonen (avklemmingen), men ubehaget varer bare under selve testen og det medfører ingen skader. Utover dette vil ikke deltagelse i prosjektet medføre økt risiko eller ubehag utover det som vil oppleves i en normal treningssituasjon.

Hva skjer med testene og informasjonen om deg?

Testene du gjennomfører og informasjonen som registreres om deg skal kun brukes slik som beskrevet i hensikten med studien. For å sikre din anonymitet i studien vil det tildeles et deltakernummer som knytter deg til dine opplysninger og resultatene gjennom en navneliste. Vi registrerer ingen direkte personidentifiserbare opplysninger og det vil ikke være mulig å spore deg i dataene som lagres underveis i studien, eller i resultatene av studien når disse publiseres.

Frivillig deltakelse

Det er frivillig å delta i studien. Du kan når som helst og uten å oppgi noen grunn trekke deg fra prosjektet. Dersom du ønsker å delta, undertegner du samtykkeerklæringen på siste side. Om du nå sier ja til å delta, kan du senere trekke tilbake ditt samtykke. Dersom du senere ønsker å trekke deg eller har spørsmål til studien kan du kontakte masterstudentene ***Martine Berg*** (mb@stud.ntnu.no | tlf. 90914871) og ***Sigve Bakken Bolme*** (sigvebb@stud.ntnu.no | tlf. 93805784) eller prosjektleder Mireille van Beekvelt (mireille.van.beekvelt@ntnu.no | tlf. 73413283).

Prosjektet er godkjent av Regional komité for medisinsk og helsefaglig forskningsetikk, Midt-Norge.

Kapittel A- utdypende forklaring av hva studien innebærer

Bakgrunnsinformasjon om studien.

- Studien gjennomføres i forbindelse med et mastergradsprosjekt ved institutt for nevromedisin og bevegelsesvitenskap, NTNU.

Undersøkelser deltageren skal gjennomføre

- Som deltager møter du opp tre ganger på St. Olavs Hospital.
- Testdag 1: Måling av alder, vekt, høyde, balanse og ganghastighet, oksygenforbruk, hjertefrekvens, minuttvolum, blodlaktat og estimering av fettprosent. Samt en test med gradvis økende intensitet for hele kroppen (sykling) Måling av muskelmasse i bein og arm, maximalt kraft i arm og en test med gradvis økende intensitet for arm (håndgrep aktivitet). Måling av hjertefrekvens, minuttvolum, slagvolum og oksygenforbruk for hele kroppen og i armmuskulaturen.
- Testdag 2: Utføring av to submaksimale tester (1 sykling, 1 håndgrep aktivitet). Måling av hjertefrekvens, minuttvolum og oksygenforbruk for hele kroppen og i lår/arm-muskulatur.
- Testdag 3: Analyse av kroppssammensetning i en kroppsanalysemaskin.

Tidsskjema

- Testingen vil foregå i løpet av vinteren 2019 og våren 2020.

Mulige ubehag

- Mansjetten som blåses opp kan oppleves som ubehagelig og litt vond, men det er bare under avklemmingen.
- Det kan oppleves som ubehagelig når man presser mot utmattelse seg på de fysiske testene, på samme måte som under vanlig trening og konkurranse.

Kapittel B - Personvern, biobank, økonomi og forsikring

Personvern

Opplysninger som registreres om deg er kjønn, alder, høyde, vekt, omkrets på ulike steder, mål av hudfoldtykkelse på låret, oksygenforbruk i hele kroppen og i bein/arm-muskulatur, kroppssammensetning, minuttvolum, hjertefrekvens og slagvolum. All data samles inn og oppbevares avidentifisert. Bare prosjektmedarbeidere vil ha tilgang til data. NTNU ved dekanus på Det Medisinske Fakultetet (DMF) er databehandlingsansvarlig.

Rett til innsyn og sletting av opplysninger om deg og sletting av prøver

Hvis du sier ja til å delta i studien, har du rett til å få innsyn i hvilke opplysninger som er registrert om deg. Du har videre rett til å få korrigert eventuelle feil i de opplysningene vi har registrert. Dersom du trekker deg fra studien, kan du kreve å få slettet innsamlede prøver og opplysninger, med mindre opplysningene allerede er inngått i analyser eller brukt i vitenskapelige publikasjoner.

Forsikring

Forsøkspersonene i prosjektet er omfattet av pasientskadeloven.

Informasjon om utfallet av studien

Ved endt studie har alle deltakerne rett til å få informasjon på gruppenivå om utfallet/ resultatet av studien.

Samtykke til deltakelse i studien

Jeg bekrefter å ha mottatt muntlig eller skriftlig informasjon om studien.

Jeg samtykker til å delta i prosjektet og til at mine personopplysninger og mine date brukes slik det er beskrevet.

(Signert av prosjektdeltaker, dato)

Jeg bekrefter å ha gitt informasjon om studien

(Signert, rolle i studien, dato)

Apendix 2: Spørreskjema

Bakgrunn

Q1: Hvordan vil du beskrive helsa di nå?

Dårlig ☐ Ikke helt god ☐ God ☐ Svært god ☐

Q2: Røykevaner

(Sett ett kryss)

☐ Jeg har aldri røykt

☐ Jeg har røykt AV OG TIL tidligere

☐ Jeg røyker AV OG TIL nå (ikke daglig)

Q3: Omtrent hvor ofte har du i løpet av de siste 12 måneder drukket alkohol?

(Regn ikke med lettøl)

☐ Ikke drukket alkohol siste 12 måneder

☐ 1 gang i måneden eller sjeldnere

☐ 2-4 ganger per måned

☐ 2-3 ganger per uke

☐ 4 eller flere ganger per uke

☐ Jeg har aldri drukket alkohol

Q4: Har du drukket alkohol 24 timer før test? Ja/**Nei**

a. Hvis ja, hvor mange enheter? …….
b. Og type drikke (sprit, øl)? ……

Q5: Har du drukket koffein siste 6 t? Ja/Nei

a. Hvis ja, hvor mange enheter?
b. Hvis ja, hvilke type (eks. kaffe, energidrikk)?

Q6: Er du, eller har du vært i overgangsalderen?

a. Nei ☐ Ja ☐
b. Hvis ja, når var (omtrent) siste mensen (kvinner)? (årstall)

Din aktivitet

Q7: Hvor ofte driver du mosjon? (Ta et gjennomsnitt)

Med mosjon mener vi at du f.eks. går tur, går på ski, sykler, svømmer eller driver trening/idrett.

Aldri..... ☐

Sjeldnere enn en gang i uka..... ☐

En gang i uka..... ☐

2-3 ganger i uka ..... ☐

Omtrent hver dag ..... ☐

Q8: Dersom du driver slik mosjon, så ofte som en eller flere ganger i uka; hvor hardt mosjonerer du? (Ta et gjennomsnitt)

Tar det rolig uten å bli andpusten eller svett ..... ☐

Tar det så hardt at jeg blir andpusten eller svett..... ☐

Tar meg nesten helt ut ..... ☐

Q9: Hvor lenge holder du på hver gang? (Ta et gjennomsnitt)

Mindre enn 15 minutter..... ☐

15-29 minutter ..... ☐

30-60 minutter ..... ☐

Mer enn 60 minutter ..... ☐

Q10: Hvilken aktivitetsform gjør du mest av? (ranger 1-5 hvor 1 er mest)

Styrketrening med egen kroppsvekt..... ☐

Langrenn/rulleski..... ☐

Gåing..... ☐

Klatring..... ☐

Jogging..... ☐

Intervalltrening..... ☐

Sykling..... ☐

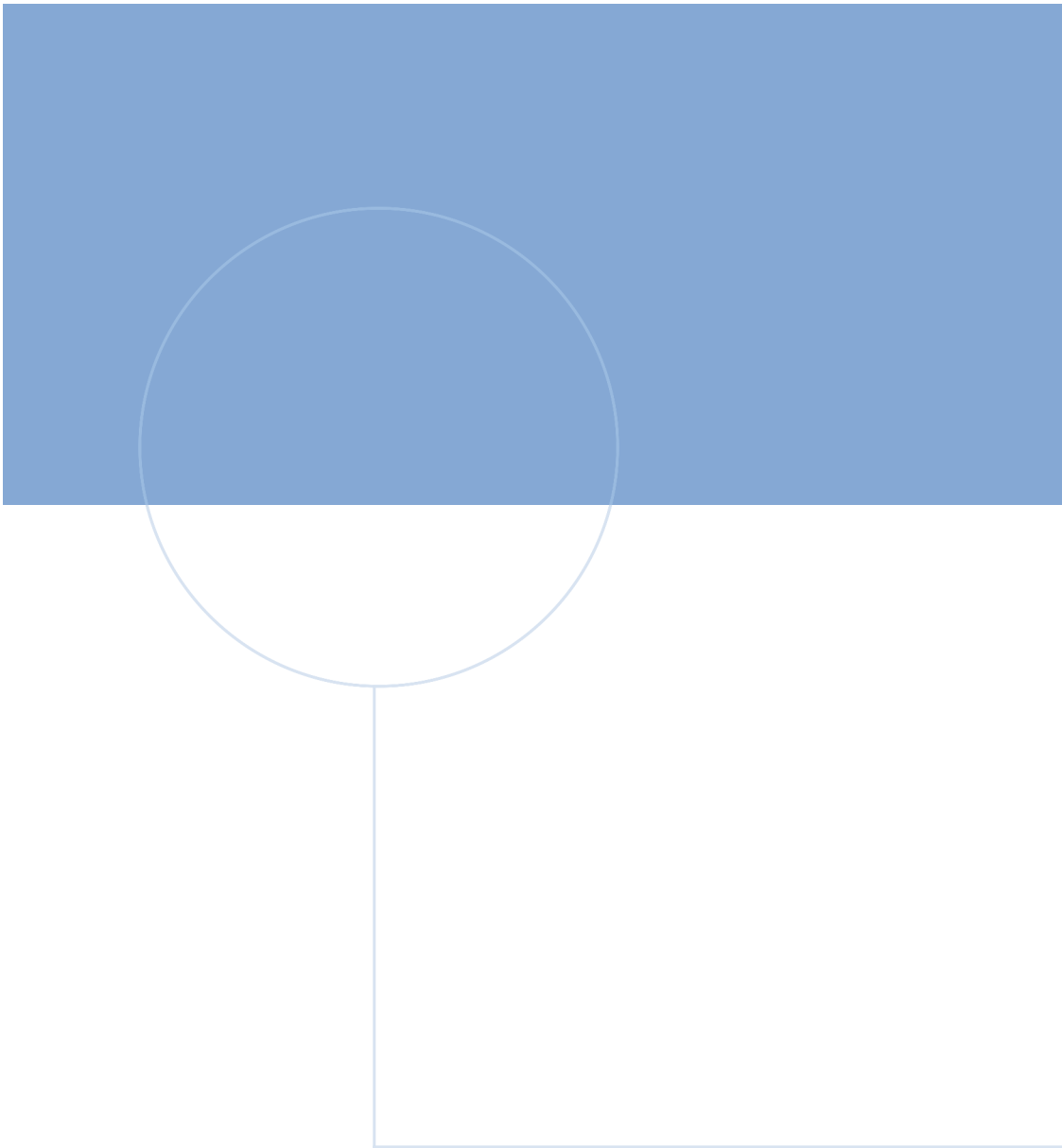
Ballidrett..... ☐

Yoga..... ☐

Styrketrening med vekter..... ☐

Annen Aerobic..... ☐

Q11: Har du unngått utmattende trening 24-48 timer før test?



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