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 skeletal muscle oxidative function during whole-body exercise and isolated muscle work

Master's thesis in Human Movement Science

Supervisor: Mireille van Beekvelt

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Abstract

Introduction: Ageing is associated with reduced function in many systems of the body, where the magnitude of deterioration characterizes 'successful and pathological ageing'.
Skeletal muscle oxidative capacity is identified as a key factor for healthy ageing. Near infrared spectroscopy is a non-invasive method that can continuously measure tissue oxygenation and hemodynamics *in vivo*. Few studies have investigated local muscle oxidative function in healthy elderly during exercise. **Aim:** The aim of this study was to investigate if differences in muscle oxidative capacity could be detected between a group of healthy young and healthy elderly, during two different exercise modes. A whole-body model and an isolated muscle exercise model were used to differentiate between central and peripheral effects. **Methods:** 20 young (Y: 26.2 ± 3.0 years) and 20 elderly (E: 62.4 ± 4.3 years) men and women participated. A vascular occlusion test was performed at rest. Submaximal exercise bouts of dynamic handgrip exercise (SHG) and cycling (SCY) were performed at various intensities, followed by application of arterial occlusions. HR, SV, CO was continuously measured during all tests. **Results:** Y had a higher handgrip MVC (Y = 54.7±14.5 vs E= 41.8±9.5, kg). There were no differences in local muscular endurance (Y= 13.2±2.3 vs E= 12.6±2.4, kg). Peripheral measurements by NIRS showed a main effect of group in SCY, but not in SHG. Systemic measurements showed no main effect of group in either exercise mode. **Conclusion:** The results of this study demonstrate that muscle oxidative function was not impaired in healthy elderly individuals. Measurements of systemic variables indicate that muscle oxidative capacity during SHG and SCY was not affected by different group responses in central factors.

Keywords: NIRS, muscle oxidative capacity, ageing

Sammendrag

Introduksjon: Aldring er assosiert med en redusert funksjon a flere systemer i kroppen, hvor omfanget av funksjonstapet karakteriserer 'sunn eller patologisk aldring'. Skjelettmuskulaturens oksidative kapasitet har blitt identifisert som en nøkkelfaktor for sunn aldring. Nær-infrarød spektroskopi er en ikke-invasiv metode som kontinuerlig kan måle muskel oksygenering og hemodynamikk *in vivo*. Få studier har undersøkt lokal muskel oksygenering hos friske eldre personer under aktivitet. Mål: Målet med denne studien var å undersøke om forskjeller i muskelokygenering kan oppdages mellom en gruppe friske eldre og yngre personer under to ulike aktivitetsmoduser. En helkropps modell og en isolert muskel model ble brukt for å differensiere mellom sentrale og perifere effekter. Metode: 20 yngre (Y: 26.2 ± 3.0 år) og 20 eldre (E: 62.4 ± 4.3 år) menn og kvinner deltok i studien. En vaskulær okklusjonstest ble gjort i hvile. Submaksimale arbeidsperioder i en dynamisk håndgripeøvelse (SHG) og helkropps sykkeltest (SCY) ble gjennomført, etterfulgt av arterielle okklusjoner. HR, SV og MV ble målt kontinuerlig under alle testene. **Resultater:** Y hadde høyere håndgripestyrke (MVC) **(**Y = 54.7±14.5 vs E= 41.8±9.5, kg). Det var ingen forskjeller i lokal muskulær utholdenhet mellom gruppene (Y= 13.2±2.3 vs E= 12.6±2.4, kg). Perifere målinger gjort med NIRS viste en gruppeforskjell under SCY, men ikke under SHG. Systemiske målinger viste ikke forskjeller mellom gruppene i noen av aktivitetsmodusene. Konklusjon: Resultatene fra denne studien viste at muskel oksidativ funksjon ikke var svekket i friske eldre individer. Målinger av de systemiske variablene indikerte at muskel oksidativ kapasitet ikke ble påvirket av ulik grupperespons i sentrale faktorer, under SHG og SCY.

Nøkkelord: NIRS, muskeloksygenering, aldring

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Foremost, I would like to express my sincere gratitude to my supervisor Dr. Mireille van Beekvelt for the help and guidance you have provided throughout the whole process of research and writing of this master thesis. I especially thank you for sharing your knowledge and invaluable insight into the research field, and for the trust and patience you have shown in letting us influence this project. I appreciate the great conversations that extend further from the scientific life and 'MATLAB scripts'.

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Abbreviations

AO	arterial occlusion
ATT	adipose tissue thickness
BIA	bioelectrical impedance analysis
CO	cardiac output
FDS	m. flexor digitorum superficialis
FFM	fat free mass
Hb	hemoglobin
HR	heart rate
LBM	lean body mass
Mb	myoglobin
MRI	magnetic resonance spectroscopy
MVC	maximal voluntary contraction
$m\dot{V}O_2$	muscle oxygen consumption
NIRS	near-infrared spectroscopy
O_2	oxygen
O_2Hb	oxyhaemoglobin
O_2Mb	oxymyoglobin
$p\dot{V}O_2$	pulmonary oxygen consumption
RPE	rating of perceived exertion
SCY	submaximal cycling exercise test
SD	standard deviation
SHG	submaximal handgrip exercise test
SmO_2	muscle oxygen saturation
SmO_{2min}	maximal muscle oxygen desaturation
SV	stroke volume
tHb	total haemoglobin
TPR	total peripheral resistance
VL	m. vastus lateralis
$\dot{V}O_2$	central oxygen consumption
$\dot{V}O_{2peak}$	peak central oxygen consumption during maximal incremental cycling test
VOT	vascular occlusion test
WR_{OBLA}	Highest work rate before 4 mmol/L blood lactate
WR_{PEAK}	Maximum work rate (watt) derived from maximal incremental cycling test

1 Introduction

Ageing is associated with reduced function in many systems of the body (Plowman & Smith, 2017); such as a progressive loss of skeletal muscle mass and reduced aerobic capacity. The "rate of muscle ageing" however seems to vary greatly between individuals (Degens & Korhonen, 2012), as physiological processes are influenced at a wide range of levels; from the molecular to the populational level (Kent & Fitzgerald, 2016). Given the importance of skeletal muscle for health, it is crucial to identify the underlying mechanisms of successful and pathological ageing. As the first world countries now are facing a demographic shift where the proportion of elderly in the population is rising, the need to understand the age-related changes in skeletal muscle is intensified.

When the loss of muscle strength and muscle mass severe, and is accompanied by low physical performance, a condition of sarcopenia may manifest. Sarcopenia, i.e. loss of muscle mass or muscle failure rooted in adverse muscle changes that accrue over a life course; although it is primarily considered as a geriatric syndrome, it is now recognized that the development of sarcopenia begins earlier in life, and may be seen as early as in the fourth decade of life (Sayer et al., 2008). Ultimately, sarcopenia may dramatically impact the individual's quality of life as it increases the likelihood of adverse outcomes like fall injuries, mobility disorders, problems to perform activities of daily living, loss of independence and increased risk of hospitalization (Cruz-Jentoft et al., 2019), causing major personal, social and economic burdens for individuals and society (Mitchell et al., 2012).

Sarcopenia has been strongly linked with poor aerobic fitness (Cruz-Jentoft et al., 2019), which again is a good predictor for morbidity and mortality. Maximal aerobic capacity reflects the cardiovascular system's ability to deliver and use oxygen (Valenzuela et al., 2020), a capacity that progressively declines in normal ageing as well as pathological ageing, independently of training status. In healthy sedentary men, peak oxygen uptake (VO_{2peak}) declines at a rate of as much as 10-15% per decade after the age of 30 (Valenzuela et al., 2020). In general, reduced VO_{2peak} in ageing is in part caused by a reduction in peak cardiac output, primarily attributable to a reduced peak heart rate and lower maximal stroke volume (SV) (Ferri et al., 2007). However, there is also evidence that reduced oxidative capacity, decreased mitochondrial density and reduced citrate synthase activity, at the skeletal muscle level, contributes to the reduction in peak

oxygen uptake and the decline in exercise tolerance and aerobic power with ageing (Ferri et al., 2007).

The role of mitochondria in skeletal muscle involves the process of oxidative phosphorylation, or aerobic production of energy (ATP) (Kent & Fitzgerald, 2016). During activities of daily living, as well as during exercise of low to moderate intensity, the skeletal muscles relies primarily on energy from oxidative metabolism (Takafumi Hamaoka & McCully, 2019). It is well known that exercise training can stimulate an increase in mitochondrial content in the skeletal muscle, and that inactivity has the opposite effect. Physical activity and training maintained through life slows the physiological deterioration seen in inactive individuals, whilst disease or poor nutrition can speed up the "rate of ageing". Levels of physical activity typically go down as we get older, and disease or health issues are more prevalent; hence it is difficult to distinguish if changes in oxidative function are related to a 'pure age' effect, or what is related to disuse and disease.

Moreover, aerobic fitness as measured by means of pulmonary gas exchange analysis, provides information of whole-body oxygen uptake during exercise but cannot directly asses if the age-related decline oxygen transportation (central component) is accompanied by a proportional decline in peripheral components, like mitochondrial ATP synthesis (Layec et al., 2016). Important information to understand the complex mechanisms of the biochemical processes of ATP synthesis can be obtained from biopsy-based methods and *in situ* animal models, but these methods are often invasive and measured in non-physiological conditions. (Kent & Fitzgerald, 2016). *In vivo* studies that provide information on skeletal muscle function in real-life condition, where circulatory and regulatory systems are intact, are therefore needed (Takafumi Hamaoka & McCully, 2019) (Ryan et al., 2014).

Near infrared spectroscopy (NIRS) is a non-invasive method that can continuously measure tissue oxygenation and hemodynamics during rest or exercise. NIRS is a relatively inexpensive and simple method, making it very applicable for studies on different population cohorts and under various conditions (Chung et al., 2018) (McCully et al., 1994). The technique utilizes the oxygen-dependent absorption of NIR-light by hemoglobin and myoglobin to monitor tissue oxygenation (Van Beekvelt et al., 2001). By applying local ischemia during or after exercise, NIRS-determined changes in muscle tissue saturation can provide a quantitative evaluation of local muscle oxygenation kinetics (T. Hamaoka et al., 1996).

Application of NIRS to study aging

NIRS has been used to assess microvascular and mitochondrial function in healthy subjects as well as in different patient groups, and holds great potential for quantitative assessment of skeletal muscle oxidative function in geriatric populations (Rosenberry et al., 2018). At present there is very little research done using NIRS to study the effects of *age*. In total, 9 studies were found that used NIRS in older 'healthy' populations. These studies investigated either forearm muscles (Chung et al., 2018; de Oliveira et al., 2019; Rosenberry et al., 2018), vastus lateralis (Brizendine et al., 2013; George et al., 2018; Iannetta et al., 2019), or calf-muscles; i.e. gastrocnemius or tibialis anterior (Baker et al., 2017; George et al., 2018; Lagerwaard et al., 2020; Layec et al., 2016), using a "whole-body" exercise model or "isolated muscle" model. The leg-modus is usually used as a whole-body model where systemic changes are significant and can potentially influence exercise performance. During isolated muscle work using the arm-modus, relatively small muscle mass is involved therefore little to no systemic changes are expected; thus peripheral changes can be separated from systemic changes, allowing one to fully test the "oxidative potential" of the investigated muscles (Ferri et al., 2007). For a geriatric population of various health-status, the arm-model is more widely applicable in a clinical setting. Although the results of previous studies are promising, a common limitation among existing studies of age-effect are the potential confounding and interacting effects of physical fitness status, body composition and disease. More studies are needed to evaluate age-related differences in muscle metabolism in various muscle groups and during various exercise models using NIRS.

Therefore, the aim of this study was to investigate whether age-related differences in local muscle oxygenation ($m\dot{V}O_2$) can be detected between a group of healthy young and healthy elderly. The primary objective of this thesis is to compare upper- and lower body $m\dot{V}O_2$ during exercise at various intensities. In addition, two exercise modes were used in order to distinguish between central and peripheral effects for both groups.

It is hypothesized that muscle oxidative function will not be limited in a group of healthy elderly at rest. However, it is also hypothesized that young have a superior oxidative function during exercise, and that elderly may compensate with a higher contribution from systemic factors.

2 Methods and materials

2.1 Participants

Twenty healthy elderly and twenty-one healthy young individuals were recruited from advertisements through friends and family, social media, a local cycling club, and organized training groups. Individuals were recruited if they did not report a history of cancer, heart-related disease, pulmonary disease or muscle-, joint- or skeletal problems. None of the participants were obese (BMI >30), current smokers, had metabolic disease or used medications that could affect hemodynamic responses to exercise. All participants were recreationally active. All but one of the subjects were right hand dominant.

All volunteers received a detailed information sheet in advance of the first test day. Each participant was verbally informed of the purposes, testing procedures and possible risks and discomforts of participation, before giving written informed consents prior to the tests. The study was approved by The Regional Ethical Committee for Medical and Health Research Ethics, Midt-Norge.

2.2 Exercise protocols

The data collection was conducted at NTNU in Trondheim, between October 2019 to February 2020, in a well-ventilated laboratory at 18-20°C, under the supervision of two master students. All participants completed test day 1 and test day 2 in the mentioned order and separated by a minimum of 48 hours, within the timeframe of 2 weeks. In addition, body composition was measured by bioelectric impedance (BIA) on a separate day, either between or after the other test days. Participants were asked to refrain from intensive exercise 48 hours preceding tests days.

The full test battery is presented in Fig. 1. On test day 1 the participants general health and activity habits was screened through a structured interview questionnaire. The resting blood pressure was measured (OSZ 5 easy, Welch Allyn, Jungingen, Germany) before commencing to other tests. A vascular occlusion test (VOT) was performed to determine muscle oxygen consumption at rest. After that participants carried out two incremental exercise tests to voluntary exhaustion: first as an isolated muscle exercise test using a handgrip dynamometer, and the second as a whole-body exercise using a cycle ergometer. Test day 2 consisted of a submaximal

handgrip test and a submaximal cycling test, where work intensities were set based on the results from maximal tests on day 1.

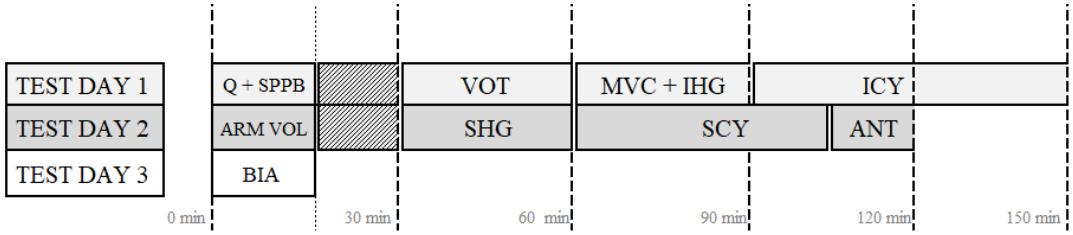


Figure 1. Day-by-day test battery with timeline.
Q= Questionnaire, SPPB= Short Physical Performance Battery, VOT= Vascular occlusion technique, MVC= maximal voluntary contraction force of handgrip, IHG= Incremental handgrip test, ICY= Incremental cycling test, ARM VO = measurement of hand and underarm volume by water-displacement, SHG= Submaximal handgrip exercise, SCY= Submaximal cycling exercise, ANT= anthropometric measurements, BIA= Bioelectric Impedance Analysis

The order of the tests was fixed to prevent crossover effects, however in this thesis they will be presented in a different order. All handgrip exercises were performed on a custom-built handgrip dynamometer and all cycling tests were performed on a cycle ergometer (Lode Excalibur Sport, Lode B. V., Groningen, Netherlands).

2.2.1 Vascular occlusion test (VOT)

The subjects were placed in comfortable semi-supine seated position on a medical examination bench and instructed to rest and minimize movement for the rest of the test. The right arm was placed in a horizontal position with the elbow and forearm at the level of the heart. Pillows were placed under the arm and knees for support and comfort. Fig. 2 shows the timeline and events for the VOT. The test started with 5 minutes of baseline resting measurements (NIRS) before two arterial occlusions (AO) were applied on the right arm and leg simultaneously (as described in 2.3.1). First a 1 min occlusion (VOT_{1min}) to familiarize with the technique, and then a 10 min occlusion (VOT_{10min}), with 3-4 min rest between the two. After the cuff release, 5 minutes of resting NIRS measurements were recorded.

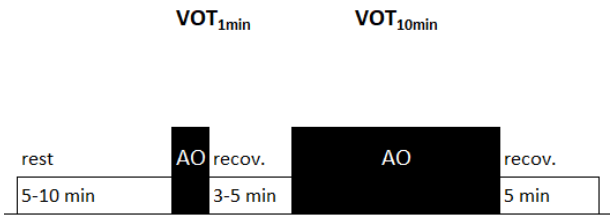


Figure 2. Event of the vascular occlusion test.
***rest**= participant resting in seated position; **AO**= arterial occlusion; **recov.** = recovery*

2.2.2 Incremental Cycling Test (ICY)

The incremental cycling protocol (ICY) included a lactate threshold test (LTT) and an incremental cycling test ($\dot{V}O_{2max}$). The LTT started after a 5-10 min warm up (<100W). Following the warm-up, the participants cycled 4-minute consecutive bouts at increasing work rates (WR). The predetermined start load and increments were based on subjects age and gender, and chosen with the aim to reach lactate threshold within 4-6 bouts. The predetermined protocols were as following (*start load, W + increments, W*): elderly women (*75W+15W*), elderly men and young women (*95W+20W*), young men (*100W+25W*). Blood lactate was measured during the last 30 seconds of each cycling bout, and the LTT was terminated when a blood lactate measurement of ≥4.0 mmol/L occurred. The purpose of the LTT was to determine the WR at the onset of blood lactate accumulation (OBLA), and WR_{OLBA} defined as the highest WR achieved before reaching OBLA defined as 4.0 mmol/L. Due to inter-individual variability in blood lactate levels, for 5 of the subjects the LTT was not terminated at 4.0 mmol/L. For 4 subjects a decision to continue the LTT after measuring ≥ 4.0 mmol/L was made based on examiners overall assessment of subject's exhaustion, heart rate and subjects own perceived rate of exertion (RPE). The subjects then cycled one or two additional stages. For 1 subject LTT was terminated *before* reaching 4.0 mmol/L due to same considerations.

After termination of the lactate threshold test participants cycled 5-10 min at a low intensity (50-100W) before the $\dot{V}O_{2max}$ test commenced. The starting work rate for the $\dot{V}O_{2max}$ test was set at one stage below $WR_{OBLA.}$ The workload increased every minute by 15-25 W (E) or 20-25W (Y), until voluntary exhaustion. Exhaustion was defined as being uncapable of maintaining a cadence over 60 rpm. Strong verbal encouragement was given throughout the test to promote maximal effort. Pedaling frequency was digitally displayed to the subjects as an external focus. Immediately after exhaustion RPE using the BORG-scale was noted and blood lactate was measured. The purpose of the $\dot{V}O_{2max}$ test was determine peak pulmonary oxygen consumption ($p\dot{V}O_{2peak}$) and peak power output (MAP). $p\dot{V}O_{2peak}$ was defined as the highest 30sec average of $p\dot{V}O_2$ during the ramp test. MAP was defined as the highest work rate sustained for a full 60 seconds during the ramp test.

2.2.3 Submaximal cycling exercise (SCY)

A cycling exercise mode was used as a whole-body model. Pulmonary and skeletal muscle oxygen consumption were measured during and after continuous submaximal exercise bout at work rates corresponding to 50% WR_{OBLA} (LOW) and 70% of WR_{OBLA} (HIGH). The cycling protocol is shown in Fig. 5. The test started with 5 minutes warm up at >100W, followed by a 5 min work bout at LOW intensity. Immediately following the exercise bout, a wooden block was placed underneath the right pedal, fixating the foot in a resting position perpendicular to the floor. Then a series of repeated arterial occlusions (RAO) were applied for '5 seconds on/5 seconds off' and '10 seconds on/20 seconds off', for 4 and 4 minutes, respectively. After 2-3 minutes of recovery cycling the protocol was repeated at HIGH intensity. RPE was noted and blood lactate was measured immediately after each cycling bout. Only the first occlusion following exercise was used for the purpose of this study.

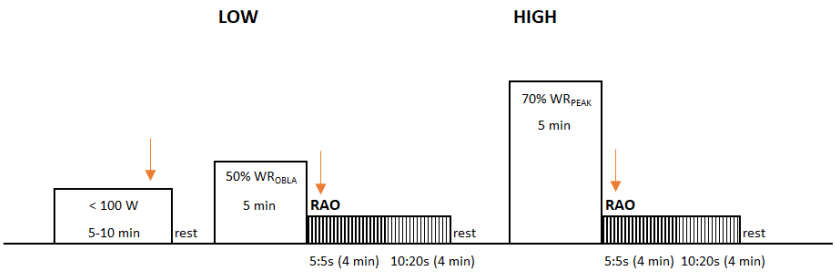


Figure 5. Submaximal cycling test (SHG) with events.
RAO= repeated arterial occlusion,→ = blood lactate measurement.

2.2.4 Incremental Handgrip Test (IHG)

The maximal workload for handgrip exercise was tested by means of an incremental handgrip test. Participants were placed in the same position as during the VOT. One of the examiners explained the test procedure before letting the participant perform a short practice of the handgrip exercise. Adjustments and feedback were given before starting the test. The position of the handgrip during the test are shown in Fig. 3 (A+B). The opened handgrip position (A) was determined as an approximately 90-degree angle in the index finger and marked with blue tape as a visual guidance marker. The closed handgrip position (B) was determined as the handle touching the handlebar. The contraction rate was standardized using a metronome signaling 2 beats per 60 bpm; the first beep signaling to open the grip, the second beep signaling to close the grip, resulting in 30 contractions per minute.

A B

Figure 3. Handgrip positions.

↑ = blue tape marking the open handgrip position; ↑ = handlebar, marking the closed handgrip position.

All participants followed the same protocol starting the handgrip test at a 2.5 kg load, with increments of 250 gram/15 seconds. The test was terminated when subjects reached voluntary exhaustion or if the examiner concluded that the subject was not following the rhythm, failing to close grip or changing the technique, i.e. changing body positioning or clearly relaying on other muscle groups. The final load completed for a full 15 seconds was determined as the maximum handgrip load (HG_{max}).

Additionally, maximal voluntary isometric grip force (MVC) was tested using a handheld handgrip dynamometer (Lafayette Instruments Model 5030L1, Indiana, USA). In a sitting position, with a 90 degree angle in the elbow, each subject had three attempts to squeeze the handle with full force for approx. 3 seconds, with a minimum om 60 s rest between each attempt. The highest score (kg) obtained was determined as MVC.

2.2.5 Submaximal handgrip test (SHG)

A dynamic handgrip exercise test was used to perform isolated muscle work at submaximal intensities with constant load, as shown in Fig. 4. Four submaximal workloads of 30% (HG_{30}), 50% (HG_{50}), 70% (HG_{70}) and 90% (HG_{90}) were calculated from HG_{max}. The SHG test was performed using the same positioning and movements as previously described in IHG test (see 2.2.2). Baseline resting measurements were recorded for 5 minutes, before an AO was applied on the right arm for 45 seconds. All participants performed 4 x 60 sec bouts of dynamic handgrip exercise, with 5 minutes of rest between each set. Immediately following each 60 s work-bouts

an AO was applied at the upper arm. At the onset of the AO, the participant was instructed to "freeze" and relax their hand in the position currently held.

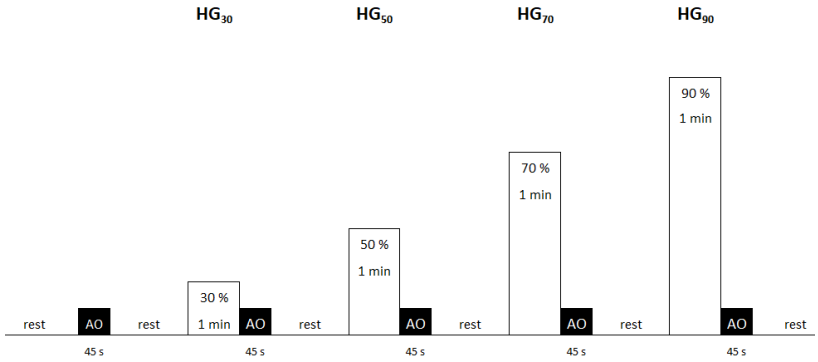


Figure 4. Events of submaximal handgrip exercise test. Workload is expressed as % of HG_{max}.
AO = arterial occlusion

2.3 Measurements and equipment

2.3.1 Measurements of peripheral variables by NIRS

This study used NIRS to obtain continuous measurements of relative skeletal muscle oxygen saturation (SmO_2), representing the dynamic balance between O_2-supply and O_2-consumption. In muscle tissue NIR light is absorbed by hemoglobin (Hb) and myoglobin (Mb).

NIRS is based on the oxygen-dependent absorption of near-infrared by Hb and Mb (Willingham & McCully, 2017). In this paper Hb and Mb are combined as it is difficult to distinguish their relative contribution to the NIRS signal due to their similar absorption spectra.

The NIRS device simultaneously uses the modified Beer-Lambert Law and SRS methods to calculate changes in SmO_2, as the ratio of oxy[Hb+Mb] to total hemoglobin (tHb) x 100, expressed in percent (%) (Takafumi Hamaoka & McCully, 2019). tHb equals is the sum of oxy[Hb+Mb] + deoxy[Hb+Mb].

Muscle oxygen saturation of the flexor digitorum superficialis (FDS) and vastus lateralis (VL) was continuously measured throughout all tests using two near-infrared spectrophotometers (Portamon, Artinis Medical Systems, Netherlands) submitting light at the wavelengths 845 nm and 762 nm, and measuring with a 10 Hz sampling frequency. The NIRS devices were placed on the muscle belly of the FDS and VL of the right arm and right leg. When necessary, body hair was shaved at the optode sites before securing the NIRS device with elastotape and Velcro straps. Black cloth was used to cover the device to prevent contamination of ambient light.

From the VOT test NIRS signals were used to calculate a baseline tissue saturation (SmO_{2REST}), maximum desaturation ($\Delta SmO2_{min}$), percentage change in tissue saturation (ΔSmO2), and rate of muscle resting oxygen consumption ($m\dot{V}O_{2REST}$). $SmO2_{REST}$, was calculated by a 30s average SmO_2 before onset of arterial cuff occlusion. SmO_{2min} was defined as the lowest measured SmO_2 value, calculated from a 30 s average at the end of the VOT_{10min}. ΔSmO2 was calculated as the percentage change in tissue saturation from baseline to maximum desaturation. $m\dot{V}O_{2REST,}$, is measured as the linear slope in muscle oxygen desaturation, calculated for 180 s period starting 30 seconds after cuff inflation.

The NIRS-signals from the cycling and handgrip exercise were used to calculate delta (Δ) values of SmO2 (ΔSmO2) as the percentage change from SmO_{2REST}. ΔSmO_2 was calculated as the average of the final 30 seconds of exercise.

NIRS was also used to calculate $m\dot{V}O_2$ from the arterial occlusion after SCY and SHG. All arterial occlusions were performed with inflatable cuffs (Hokanson SC5L and Hokanson SC10L, Marcom Medical ApS, Denmark) attached proximally around the upper arm and thigh. The cuffs were rapidly inflated using a pressure regulator (Hokanson E20 Rapid Cuff Inflator, Marcom Medical ApS, Denmark) preset to a pressure of 280 mmHG. During arterial cuff occlusion the blood flow is stopped, preventing oxygen-rich blood to flow into the forearm and leg, any reduction in forearm tissue oxygenation is directly related to oxygen consumption, the slope of SmO_2 represents an indirect measure of $m\dot{V}O_2$ (%/s).

For cycling exercise, $m\dot{V}O_2$ was determined by the linear regression of tissue saturation from the first AO during the RAO, calculated for 3 s, starting 0.5 s after cuff inflation. For handgrip exercise, $m\dot{V}O_2$ was calculated for each AO following the four exercise bouts, calculated for 30 s, 5 s after cuff-inflation.

At the end of tests days, skinfold thickness was measured with a skinfold caliper (Holtain, Crymych, UK), at both sites of the optode placement. Adipose tissue thickness (ATT) was calculated as an average of two skinfold measurements at each site divided by 2.

2.3.2 Measurements of systemic variables

Heart rate (HR) was measured continuously during all tests. Maximal heart rate (HR_{max}) was determined as the highest 5s average HR measured during the incremental cycling test by 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 5Hz. Pulmonary gas exchange and ventilation were measured during the cycling tests using open circuit spirometry (Oxycon Pro, Jaeger GmbH, Hoechberg, Germany or Metalyzer 3B, Cortex Biophysik GmbH, Leipzig, Germany). The ergospirometry 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, and an external gas source with known concentrations (15.0% O_2 and 5.0% CO_2), (Reissner-Gase, GmbH & Co; Lichtenfels, Germany) for gas calibration.

For the cycling exercise blood lactate was measured from small drops of blood from the fingertip by using a hand-held analyzer (Lactate Pro2 LT-1730, Arkray KDK, Kyoto, Japan) . Rating of perceived exertion (RPE) after cycling exercise was noted using BORG's scale (scores of 6-20) (Borg, 1982).

The systemic hemodynamic variables cardiac output (CO) and stroke volume (SV) were monitored continuously throughout all exercise tests on both days using bioimpedance cardiography (PhysioFlow Lab1, Manatec, Biomedical, Macheren, France). Thorough skin preparation (i.e., shaving, alcohol wiping and skin abrasion) was done to ensure optimal conductivity between electrode and the skin. A total of 6 electrodes (BlueSensor R., Ambu, Copenhagen, Denmark) were placed on each subject according to manufacturer's descriptions for use in exercise mode (*PhysioFlow Software User Manual*, 2017); one set of electrodes were placed on the left base of the neck (at the supraclavicular fossa) and one set on left side of the spine on the back (at the level the xiphiod process) of each subject. The final two were placed at the mid sternum and under the arm(on the rib closest left ventricle), to monitor single electrographic signals (ECG) (Richard et al., 2001).

The device was calibrated during a 30 sec period in which the autocalibration was performed in a comfortable sitting position during rest. Data were sampled in exercise mode and averaged over 10 s during the measurement. All data was exported after the measurement with the sample frequency of 0.1 Hz and subsequently imported in MATLAB for data analysis.

All NIRS, Physioflow and Jaeger/Cortex data were processed in MATLAB R2019b. For the statistical analysis of peripheral and systemic variables, only the end-exercise average values from SCY and SHG were used.

2.4 Statistical analysis

A Shapiro-Wilkinson test of normality was used to test variables for normal distribution. For normally distributed variables group means were compared by independent samples t-tests. To compare mean values of variables that were not normally distributed, like age and body fat percentage (BF%), an Independent Samples Mann-Whitney U test were used.

For peripheral variables during handgrip exercise, differences in ΔSmO_2 and $m\dot{V}O_2$ between the two groups and between intensities were compared using a two-way ANOVA (GLM with repeated measures) with a LSD- comparison test as a post-hoc analysis. When assumption of sphericity was violated according to a Mauchly's test of sphericity a Greenhouse-Geisser correction was used. For the cycling exercise, a mixed model analysis was used due to some missing values. For systemic variables, end-exercise values of CO, SV, HR and pVO2 were compared using a two-way mixed ANOVA. Interaction effects were tested using post-hoc analysis using LSD. Statistical analyses and graphics were done using commercially available software IBM SPSS Statistics 26 and GraphPad Prism v.8.4.2 (GraphPad Software, CA, USA). Results were presented as mean±SD unless other is specified. Significance was accepted at $p<0.05$.

3 Results

3.1 Participants

A total of 40 subjects completed all tests, with one exception where the VOT_{10min} was terminated before completion due to discomfort. In parts of the statistical analysis some data sets were excluded due to missing data points or poor signal quality. The number of included datasets is presented in each section. Table 1 presents the physical characteristics of the participants and the main parameters of physical fitness. Average height, weight and BMI were similar between the two groups, but BF% was significantly lower in Y compared to E. There were no significant group differences in adipose tissue thickness (ATT) of FDS or VL.

Table 1.

Physical characteristics by group.

	Young		Elderly		
	Mean (±*SD)*	Range	Mean ± *SD*	Range	Sig.
n (M:F)	20 (14:6)		20 (13:7)		
Age, yrs	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	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	*
ATT FDS, mm	3.1 (1.9)	1.6 - 7.5	3.0 (1.2)	1.4 -5.3	
ATT VL, mm	7.0 (3.7)	2.8 – 15	6.5 (3.1)	2.7 – 11.2	
MVC, kg	54.7 (±14.5)	30-72	41.8 (±9.5)	24-56	*
HG_{max}, kg	13.2 (±2.3)	9.5-16.8	12.6 (±2.4)	8.8-17.3	
pVO_{2peak}, mL/kg/min	47.4 (±7.6)	30.6-56.4	40.3 (±6.6)	28.3-54.5	*
HR_{max}, beats·min^{-1}	195 (±5)	188-207	175 (±9)	157-195	***
MAP, W	299 (±73)	195-425	244 (±70)	120-355	*
WR_{OBLA}, W	171 (±55)	75-275	154 (±54)	75-275	
[La^-] at WR_{OBLA}, mmol/L	3.6±1.1		3.3±0.8		

BMI = body mass index, **BF%** = body fat percentage as measured by BIA; **ATT** = adipose tissue thickness; **FDS** = m. flexor digitorum superficialis; **VL** = m. vastus lateralis; **MVC** = maximal voluntary contraction handgrip; **HG_{max}** = maximal load during incremental handgrip test; **MAP** = maximal aerobic power; **WR_{OBLA}** = Work rate at lactate threshold; [**La] at WR_{OBLA}** = blood lactate measurement at WR_{OBLA}, Sig. *($p < .05$) ** ($p < 0.01$) *** ($p < 0.001$)

3.2 Muscle oxygenation during rest

The main results from the vascular occlusion test for both muscles are presented in Table 2. Baseline tissue saturation, $SmO_{2baseline}$, was similar between the groups in both FDS and VL. ΔSmO2 was similar between groups in FDS, however in the VL, there was larger mean change from baseline measurements to maximum desaturation in Y compared to E. Resting muscle oxygen consumption, $m\dot{V}O_{2REST}$, measured as the linear slope of desaturation during the arterial occlusion showed no significant differences between groups in either of the muscles. Maximum desaturation during AO, SmO_{2min}, was also similar between groups in both muscles.

Table 2.
Main results from VOT

	Young (*n* = *19)*	Elderly (*n* = 20)	Sig.
FDS			
$SmO_{2baseline}$ (%)	64.1 ±5.4	60.4 ±7.5	
ΔSmO_2 (%)	-26.9 ±8.6	-21.7 ±20.1	
$m\dot{V}O_{2REST}$ (%·s^{-1})	-0.10 ±0.05	-0.12 ±0.06	
SmO_{2min} (%)	37.2 ±9.6	35.8 ±9.4	
VL			
$SmO_{2baseline}$ (%)	78.0 ±4.7	76.4 ±5.7	
ΔSmO_2 (%)	-26.0 ±7.2	-18.3 ±6.5	**
$m\dot{V}O_{2REST}$ (%·s^{-1})	-0.11 ±0.07	-0,08 ±0.03	
SmO_{2min} (%)	51.9 ±8.7	58.1 ±11.7	

$SmO_{2baseline}$ = resting muscle tissue saturation before vascular occlusion; **ΔSmO_2** = percentage change in muscle oxygen saturation from $SmO_{2baseline}$ to maximal desaturation; **mVO_{2REST}** = rate of muscle oxygen consumption during vascular occlusion test (%·s^{-1}); **SmO_{2min}** = muscle oxygen saturation during final 30 seconds of arterial occlusion; Sig. * (p<0.05)** (p<0.01) ***(p<0.001)

3.3 Cycling exercise

Two submaximal cycling bouts were performed, first at a LOW intensity and then a HIGH intensity. Table 4 presents data and physiological parameters measured from submaximal cycling protocol. As shown, at LOW intensity there were no significant group differences in internal workload (%HRmax, [La$^-$] and RPE) or external workload (WR). At LOW intensity, the %p$\dot{V}O_2$ was on average a little higher in E compared to Y (p<0.05).

During HIGH intensity, internal workload (%p$\dot{V}O_{2PEAK,}$ $\%HR_{MAX}$) was similar between the groups. Y measured higher blood lactate levels after the HIGH intensity bout. During HIGH the average WR was significantly higher for Y compared to E by 35W or ~19%.

Table 3.

Cardiovascular and metabolic responses to submaximal cycling exercises.

	LOW		HIGH	
	Young	**Elderly**	**Young**	**Elderly**
WR, 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) *
RPE, 6-20	-	-	14.7 (±1.3)	14.5 (±1.6)

Note. Young, n=18, Elderly, n=18. **WR**= work rate in watts, **%VO2peak**= average of O2 measurements of last 30 s of exercise in percentage of $\dot{V}O_{2PEAK}$, **$\%HR_{MAX}$**= percent heart rate of maximal heart rate 30 seconds before end of exercise, **[La$^-$]** = blood lactate concentration 30 seconds after exercise,* Sig. difference between groups (p<0.05).

Peripheral changes during submaximal cycling exercise

Fig. 6 (A) shows a typical example of the NIRS signal of tissue saturation for an individual, during the two submaximal cycling intensities. There were no significant differences in absolute baseline levels for SmO_2 before LOW (76.1±4.7 vs 72.3±8.4%) and HIGH (75.7±5.8 vs. 73.3±7.8%), for Y and E respectively. Group responses for both intensities are shown in Fig. 6 (B+C). As seen in the figure, SmO_2 declined as cycling commenced, before stabilizing after approx. 1 min.

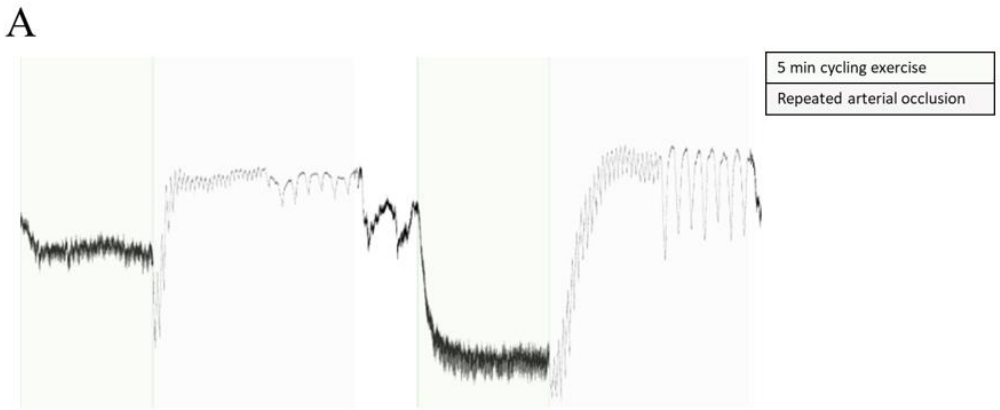
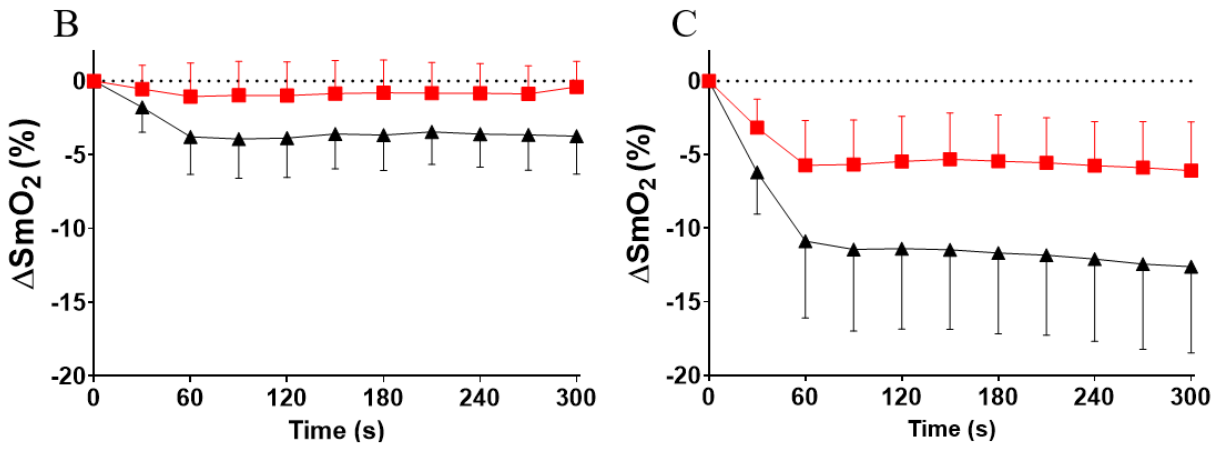


Figure 6. **(A)** Representative raw signal (NIRS) for one individual during SCY protocol.

ΔSmO_2 was more pronounced in Y compared to E, at both LOW and HIGH intensity. This was confirmed as mixed models analysis revealed a significant main effect of group, $F(1,38.76)=24.99$, $p< 0.001$, ES= 0.40, as well as a main effect of intensity, $F(1.337,45.45)=117.09$, $p<0.001$, ES=0.78. For end-exercise ΔSmO_2 values there was also a significant interaction effect of the two factors, $F(2,72.94)=13.99$, $p<0.001$, ES=0.28).

Post-hoc analysis showed a significant difference in ΔSmO_2 between LOW intensity ($p<0.05$) and HIGH intensity ($p<0.01$), for both groups.



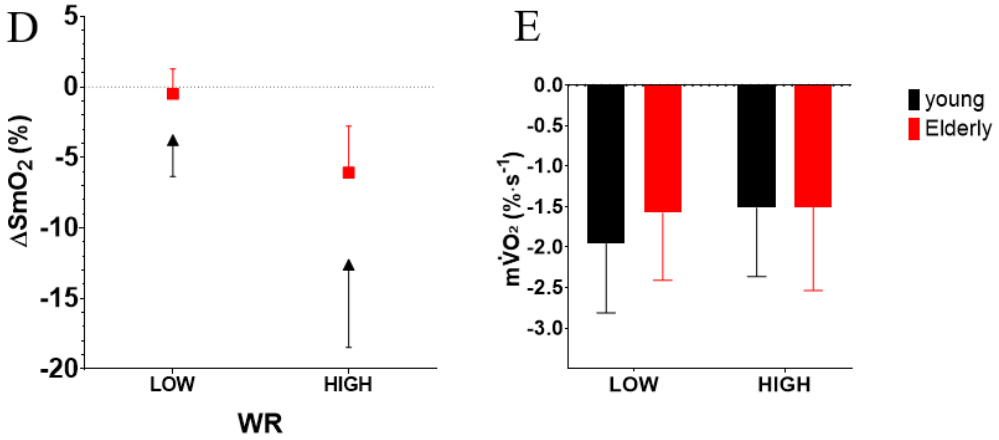


Figure 6. **(B)** Group response (ΔSmO_2) during cycling bout at LOW intensity **(C)** group response during cycling bout at HIGH intensity. **(D)** End-exercise group mean for LOW and HIGH intensity, **(E)** desaturation rate under AO after exercise. *Young n=18, Elderly n=18.*

In addition, $m\dot{V}O_2$ was measured immediately following CY exercise. $m\dot{V}O_2$ after LOW intensity was higher than $m\dot{V}O_2$ after HIGH intensity. Statistical analysis revealed a significant main effect of intensity, $F(2, 66.45)= 98.22$, $p< 0.001$, but no significant main effects of group, $F(1,34.47)= 0.46$, $p = 0.5$, or interaction effect between the two, $F(2, 66.45) = 1.35$, $p = 0.26$. $m\dot{V}O_2$ was significantly higher after exercise compared to $m\dot{V}O_{2REST}$ for both intensities ($p<0.001$). The calculated factor increase from $m\dot{V}O_{2REST}$ to end of exercise $m\dot{V}O_2$ after LOW intensity was equal to values of ≈20 (Y) ≈21 (E), and end of exercise $m\dot{V}O_2$ after HIGH intensity equal to values of ≈13 (Y), ≈18 (E).

Central changes during submaximal cycling exercise

Fig. 7 shows the mean group response in all systemic variables at both intensities, throughout the 5-min cycling bouts. No group differences were found for baseline measurements of the systemic variables HR, CO, SV and $p\dot{V}O_2$. For end-exercise values, HR was the only variable that revealed a main effect of group, $F(1,36)=5.1$, $p<0.05$, ES=0.12). However, HR average in %of HRmax was the same between groups (shown in table 4).

Mixed models analysis of the other systemic variables revealed following results; for stroke volume (SV) there was a significant main effect of intensity $F(1,36)=8.07$, $p<0.01$, no main effect of group $F(1,35)=0.11$, $p=0.73$, no interaction effect $F(1,35)= 0.99$, $p = 0.32$. Post-hoc LSD analysis showed that there was a significant difference between LOW-HIGH for Y. For

cardiac output (CO) there was also a significant main effect of intensity, $F(1,35) = 121.5$, $p<0.001$, and again no main effect of group, $F(1,36)=0.235$, $p=0.63$, and no interaction effect $F(1,35) = 0.236$, $p=0.629$. For $p\dot{V}O_2$ there was again a main effect of Intensity $F(1,27) = 486.4$, $p<0.001$, however also a main effect of group, $F(1,27)=6.93$, $p<0.05$, and interaction effect $F(1,27)=6.33$, $p<0.05$.

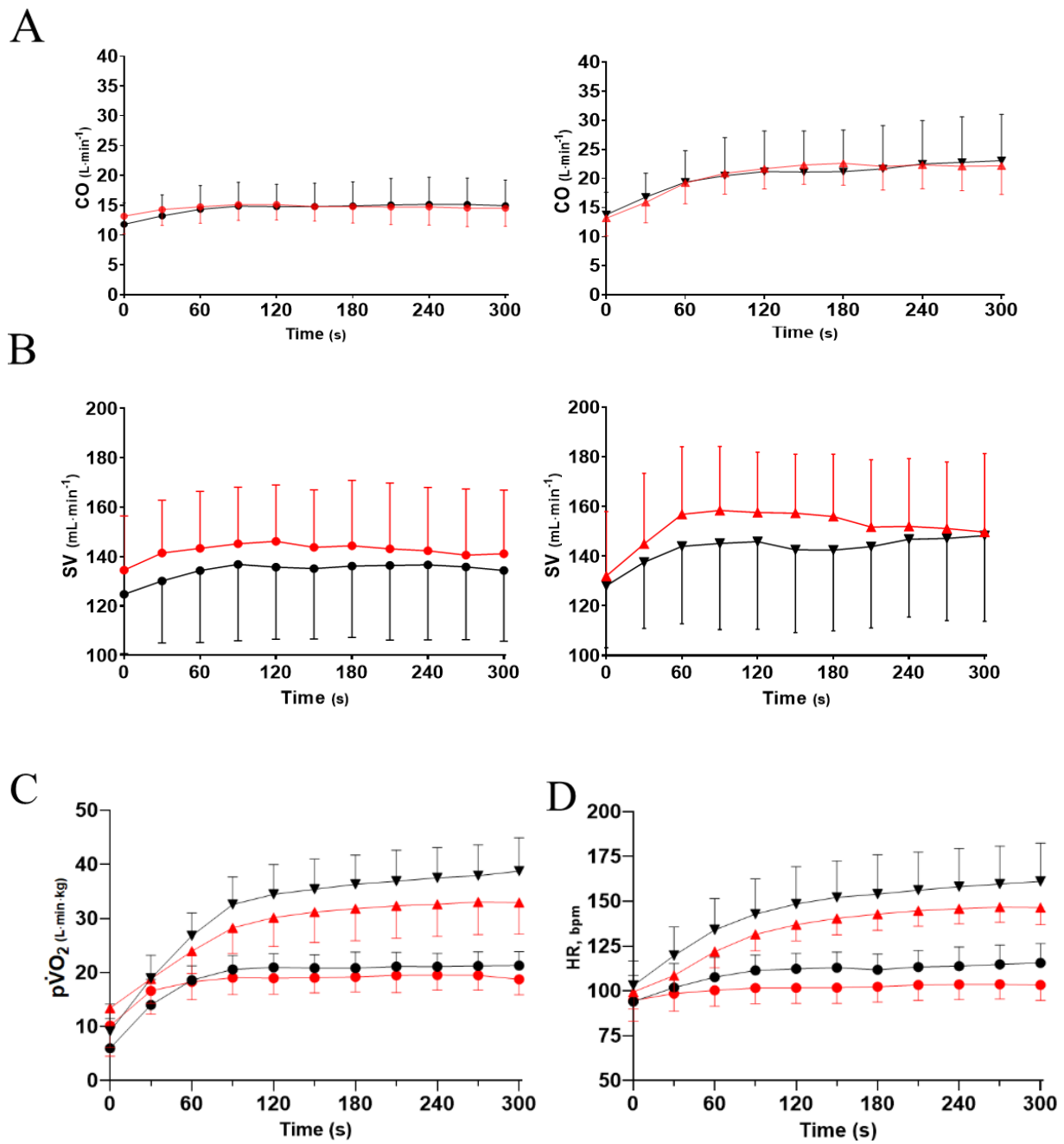


Figure 7. Group responses in systemic variables during SCY for **(A)** Cardiac ouput **(B)** Stroke volume **(C)** pulmonary oxygen uptake (**D)** heart rate

3.4 Handgrip exercise

Maximal handgrip strength was measured in both groups. It was found that on average the elderly had a 30% lower MVC than young (Table 1.). On the contrary there were no significant differences between groups regarding time to exhaustion and hence maximal load, HG_{max}, on the incremental handgrip test (as shown in table 3). The average external workload (kg) during the four submaximal bouts of dynamic handgrip exercise was similar between groups, as submaximal workload was calculated as $\%HG_{max.}$

Table 4.
Workloads at submaximal handgrip exercise bouts

		%HGmax			
	n	**30%**	**50%**	**70%**	**90%**
Young	20	4.0 (±0.7)	6.7 (±1.2)	9.4 (±1.6)	12.0 (±2.1)
Elderly	19	3.7 (±0.6)	6.2 (±1.1)	8.7 (±1.6)	11.1 (±2.0)

Note. Values are presented as mean±SD in kg. * Significant difference between groups ($p<0.05$)

Peripheral changes during handgrip exercise

A representative example of the NIRS signal in a typical individual subject during submaximal handgrip exercise protocol is shown in Fig. 6 (A). Fig 6. *(B+C)* shows the group response from start to finish, of the lowest (HG_{30}) and highest (HG_{90}) work intensity. As seen in Fig. 2, the same trend was seen in all individuals during the 60 s of rhythmic handgrip exercise; tissue saturation typically decreases as the handgrip exercise begins before reaching a plateau during the first 30 s of exercise.

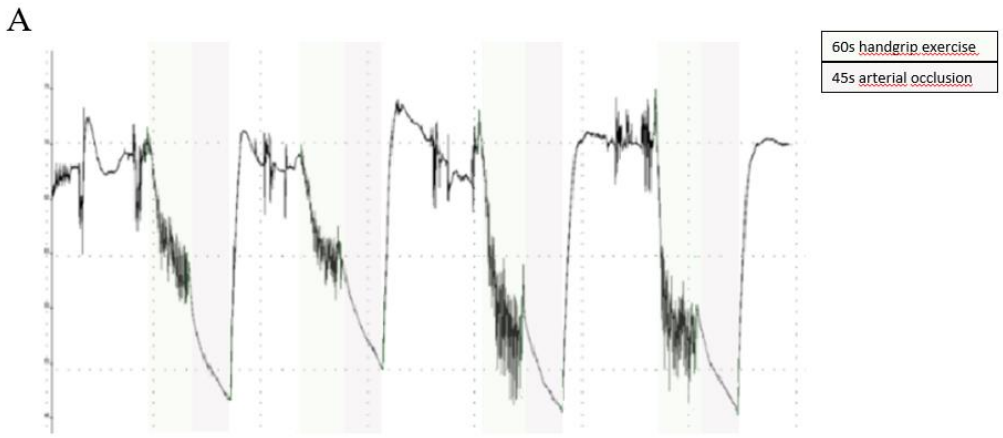


Figure 8. **(A)** Representative raw signal (NIRS) for an individual during SHG.

End-exercise delta values are shown in Fig. 6 (D). Statistical analysis showed that there was only a main effect of intensity $F(1.64,59.17)= 86.18$, $p< 0.001$, ES=0.71, and no main effect of group $F(1,36)= 2.34$, $p>0.05$, ES=0.06, and no interaction effect $F(4,144)= 1.68$, $p>0.05$, ES=0.05. Post-hoc analysis showed that ΔSmO_2 decreased significantly with each work rate (all $p<0.001$).

In addition, $m\dot{V}O_2$ was measured immediately following each HG exercise bout and once during the rest prior to the first work bout. A paired samples t-test showed no significant difference in $m\dot{V}O_{2REST}$ from VOT (-0.10±0.06) and resting $m\dot{V}O_2$ on before the HG test (-0.11±0.07) in FDS ($p= 0.53$). During the post exercise arterial occlusion there were no significant group differences in the rate of desaturation at any of the intensities (Fig. 7). Similar to the results in ΔSmO2, analysis showed a significant main effect of intensity, $F(2.34, 81.74)= 26.36$, $p<0.001$, ES=0.58, however no main effect of group, $F(1,36)=0.039$, $p>0.05$, or in the interaction between the two $F(4, 140) = 0.1554$, $p>0.05$. Post-hoc test showed differences in $m\dot{V}O_2$ between all levels of intensity.

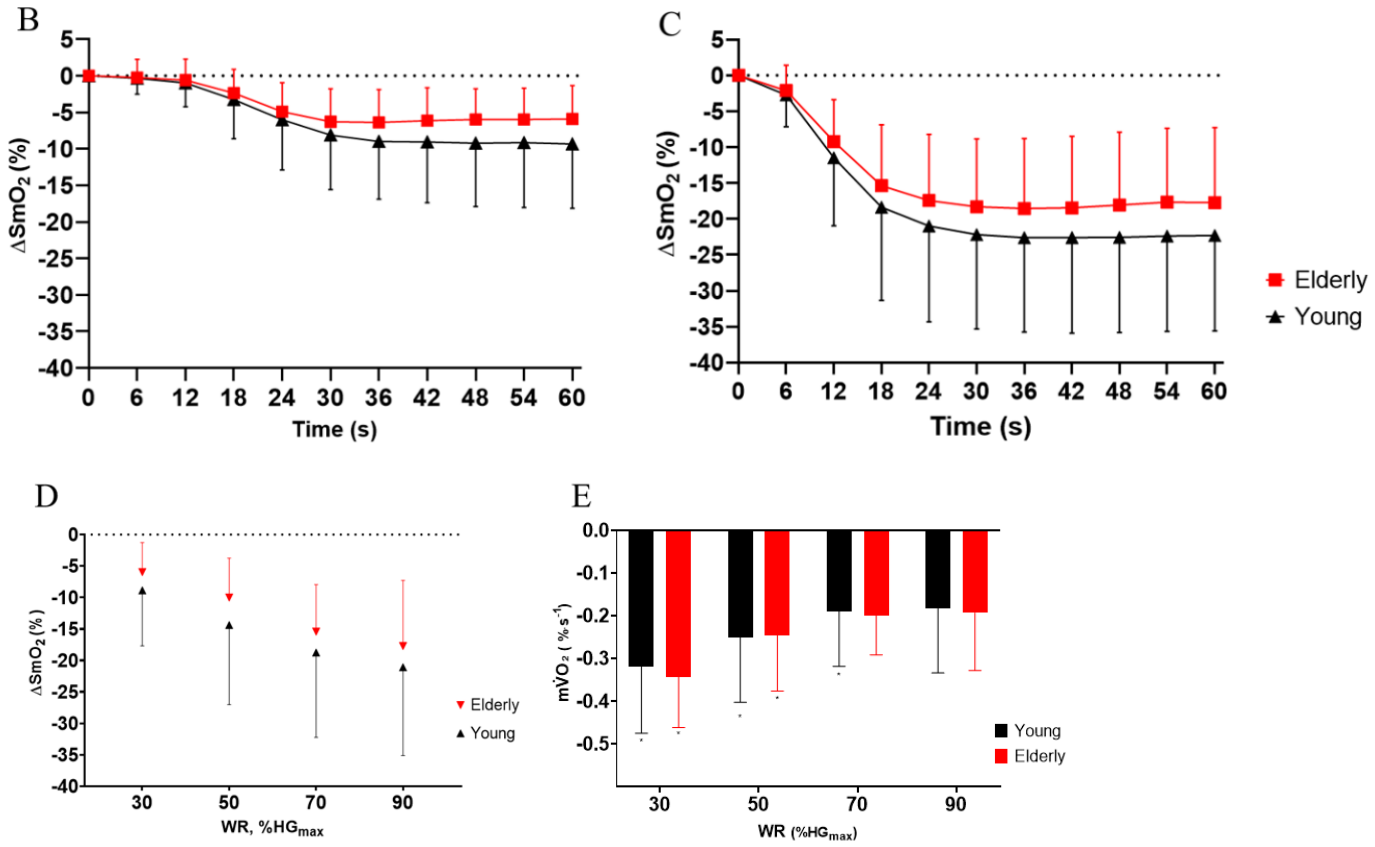


Figure 8. Group responses during **(B)** 30% of HGmax (C) 90% of HGmax. **(D)** End-exercise mean for all intensities of SHG, **(E)** desaturation rate during post-exercise AO. Young, *n*=19, Elderly, *n*=19

Systemic changes during handgrip exercise

As expected, little to no changes in the systemic variables occurred during HG exercise. For HR, a two-way ANOVA showed a main effect of group, $F(1,36)= 5.1$, $p<0.05$, ES=0.12, but no main effect of intensity or interaction effect. The average HR at baseline was significantly different between groups, where elderly group had a lower HR equivalent of 6-8 bpm compared to the young. A significant main effect of group was also found for both stroke volume (SV), $F(1,35)=11.43$, $p<0.01$, ES =0.25, and cardiac output (CO), $F(1,36)=17.10$, $p<0.001$, ES= 0.32. As with HR, there was no main effect of intensity or an interaction effect in SV and CO.

Table 5.
Heart rate responses during submaximal handgrip exercise test

		%HGmax							
		30%		**50%**		**70%**		**90%**	
	n	Baseline HR	End-exercise HR	Baseline HR	End-exercise HR	Baseline HR	End-exercise HR	Baseline HR	End-exercise HR
Young	20	71 ±9	70 ±10	70 ±9	71 ±11	70 ±9	71 ±8	69 ±10	73 ±9
Elderly	19	64 ±7	65 ±7	64 ±7	66 ±6	64 ±7	66 ±7	63 ±6	66 ±7

Note. Values are presented as group means±SD in bpm.

4 Discussion

The primary objective of this study was to determine the effects of age on local muscle oxidative capacity. The main findings showed that there were no group differences in $SmO_{2baseline}$, ΔSmO_2, SmO_{2min} and $m\dot{V}O_{2REST}$ in both muscles during rest, with the exception of a slightly greater ΔSmO_2 in VL for Y vs E.

From the cycling exercise there were significant group differences in ΔSmO_2, where Y had a greater desaturation during cycling bouts. There were no group differences in $m\dot{V}O_2$. Cycling intensity had an effect on both variables, as desaturation is more pronounces at HIGH compared to LOW, and rate of muscle tissue desaturation was higher after LOW compared to HIGH.

From the handgrip exercise there were no differences between the groups in ΔSmO2 or $m\dot{V}O_2$, but there was a clear effect of intensity. Measures of systemic variables showed that elderly and young had a similar response during the handgrip exercise in all the variables. This was also the case during cycling exercise.

Although it is not clear what the results indicate, the strengths of this study include the simultaneous measurements of local muscle oxidative function by NIRS, and systemic responses by pulmonary gas-exchange, polar and physioflow; allowing us to differentiate between central and peripheral responses to exercise. Also, by using two exercise modes and various intensities, we have information from different conditions as limitations in muscle oxidative function or performance may vary dependent on work mode, intensity or muscle groups involved.

4.1 Muscle oxygenation during rest

The results from VOT were in agreement with the hypothesis; namely that in a resting condition the muscle oxidative response would not differ between the two age groups. Since all participants were healthy and physically active it was unlikely that oxidative function would be significantly impaired during rest. This is in agreement with de Oliveria et al (2019) who found no significant difference in magnitude of desaturation (ΔSmO_2) or rate of desaturation ($m\dot{V}O_2$) under a 5 min cuff-occlusion in young healthy and older healthy. However, they did find a significantly lower ΔSmO2 and $m\dot{V}O_2$ for a third group consisting of older adults with CVD risk factors. However, these results contradict previous findings of Rosenberry et. al. (2018) who studied the forearm muscles of healthy young and elderly using a 5-min cuff occlusion protocol during rest. They found a higher rate of desaturation during AO, as well as a larger maximum desaturation during ischemia, in young compared an older group.

The inconsistency between results could possibly be explained by the higher average age of their elderly group (Our study: 62±4.3 years vs. Rosenberry et. al: 73 ± 5 years), where additional muscle deterioration is plausible factor. Another explanation for the inconsistencies could be that findings are confounded by a detraining effect accompanied with ageing. In our study, self-reported physical activity levels were collected by a questionnaire, where 16 out of 20 elderly reported being physically active '*almost every day'* (~5-7 days per week). Further, 14 out of 20 stated the duration of each exercise session was typically 30-60 min, and 18 out of 20 E reported that most of their weekly exercise training was of moderate intensity. In comparison, none of the elderly in Rosenberry et. al (2018) reported doing moderate-vigorous activity, however 9 out 14 young did. Based on these self-reported activity habits from both studies, one can argue that the elderly group of our study were in better trained, although there are no objective measures of aerobic fitness to compare. Reported handgrip MVC strengthens this

argument, as E of our study had a considerable higher average MVC (our study: 41.8±9.5kg versus Rosenberry: 20.9±5.3kg). Even when stratifying our elderly group by gender, referring to the fact that in the elderly group of Rosenberry et al. (2018) consisted of 8 women and 2 men; the 7 females of our study still scored ~10kg higher (our study: elderly female = 31.1±3.8 kg) on average, compared to elderly group of Rosenberry et. al (2018). Keeping in mind that the cut-off value for handgrip MVC in sarcopenia is 16kg for women and 27 kg for men (Cruz-Jentoft et al., 2019), some of the individuals in Rosenberry et al (2018) scored very close to this.

A general argument, in all studies investigating the effects of age, is that it is necessary to consider which parameters of physical fitness are accounted for, as they may interact and affect the outcome. Interpretations of results should be done with caution, as classification and categorizations like “healthy”, “physical active” or “elderly” are diverse.

4.2 Muscle oxygenation during exercise

In this study, a cycling exercise was used as a whole-body exercise model, and a handgrip exercise as an isolated exercise model. As expected, an immediate desaturation of the working muscles was observed in both exercise modes. Intensity had a clear effect on desaturation in both exercise modes as a response to increased muscle metabolism to match the metabolic demand of each intensity. The isolated muscle exercise showed very similar responses in the two groups, however during cycling the desaturation was more pronounced in the younger group. Post-exercise there were no group differences in rate of oxygen desaturation.

Very few studies have investigated age-effect on m$\dot{V}O_2$ during or after exercise using NIRS. Ferri et. al (2007), used a similar study design of two exercise models, an incremental cycling exercise and an incremental dynamic knee-extension exercise, with elderly and young. In agreement with this study, they found a greater peak Δdeoxy[Hb+Mb] in Y vs. E in the cycling test. Still, this was likely a result of the higher VO_{2peak} in Y, and therefore a higher workload at peak exercise. When comparing Y and E on same workloads, Y had a lower Δdeoxy[Hb+Mb], which may be attributable to the fact that for Y this was a lower workload relative to maximal capacity. On matching absolute workloads, the results indicated that elderly possibly had to recruit more motor units to maintain performance.

In this study, Y and E had a similar WR_{OBLA}, and therefore a similar external load during LOW. Yet, Y had a greater desaturation during LOW than E, which contradicts the findings of Ferri et al. (2007). It is not clear whether the findings reflect a physiological difference or if the signals are confounded by measurement errors or poor NIRS signal quality. During HIGH intensity the external workload was significantly higher for Y compared to E, even though the internal workload ($\%p\dot{V}O_2$, $\%HR_{max}$) demonstrated that the relative workload was similar between the groups. A study by Okushima et. al (2016) found a correlation between magnitude of desaturation and VO_{2peak} during maximal cycling exercise (Okushima et al., 2016). In this study Y had a greater desaturation than E during HIGH, which might be explained by the difference in absolute workload and pVO2, as shown in Table 3 and Fig. 7(C).

The greater desaturation in VL for Y during cycling bout could be a possible explanation for the post-exercise $m\dot{V}O_2$, where no differences were found between E and Y.

The findings from studies using an isolated muscle model are also inconsistent. Our results are in agreement with Kutzuwaza et. al (2001) who found no difference in oxygenation in an exercising forearm between young and elderly, measured simultaneously by NIRS and P-MRS (Kutsuzawa et al., 2001). However, these results differ from those of Ferri et. al (2007). They found a greater desaturation in E vs Y from the knee extension exercise, whereas this study found no differences in desaturation between E and Y during handgrip exercise.

Ferri et al. (2007) also measured central and peripheral effects during exercise and found that HR was not significantly elevated at peak knee extension exercise. HR measured as $\%HR_{max}$ was not different between E and Y, indicating that systemic factors were not a limitation during isolated muscle exercise. In the handgrip exercise, relatively small muscle mass is involved, therefore we did not expect systemic variables to change considerably. The results from the present study showed minimal changes in all systemic factors, at all of the studied intensities, confirming the notion that central factors were not limiting in E during isolated muscle work.

Interestingly, handgrip MVC was significantly different between E and Y, but there was no difference in HGmax from the the incremental handgrip test (Table 1). The results of the MVC test are not surprising as studies have shown that muscle strength decreases with age (Dodds et al., 2014). As the incremental handgrip exercise is not an established method, it was unclear if

lower handgrip MVC was accompanied by lower local muscular endurance. This may be an indication that muscle oxidative function is maintained despite loss of strength.

Muscle group heterogeneity in muscle ageing

We cannot directly compare the results found in FDS with those of VL of this study. Conflicting evidence in research on age-related differences could be a result of which muscles are being investigated. Research supports that "rate of muscle ageing" not only varies greatly between individuals, but also across muscle groups (Mitchell et al., 2012). Large MRI-based studies found a greater loss in muscle mass of the lower limbs compared to upper limbs (Janssen et al., 2000). Muscle atrophy, or muscle loss, seems to be greater in the type-2 muscle fibers, presumably because type-2 fibers are recruited at intensities >60% of MVC (Mckendry et al., 2018; Mitchell et al., 2012; Tieland et al., 2018)

A study by Kent and Fitzgerald (2016) using muscle PCr recovery kinetics, found a muscle-by-age interaction when they investigate the vastus lateralis and tibialis anterior of older and younger of different physical activity levels. They found a stronger correlation between the oxidative capacity and minutes of moderate-vigorous physical activity for the VL than for the TA. The finding suggests that activity intensity may influence some muscles more than other muscles with regard to muscle mitochondrial capacity in age (Kent & Fitzgerald, 2016).

NIRS has been used to study microvascular responsiveness and recovery kinetics of young and elderly. Lagerwaard et at. (2020) also studied elderly and young that were matched for physical activity level. They investigated the $m\dot{V}O_2$ recovery kinetics of locomotor muscles (VL, gastrocnemius and tibialis anterior), where they argue that there was a age-related decline in mitochondrial capacity in the VL and GA, but not TA (Lagerwaard et al., 2020). Another study by Soares et al. (2018) assessed microvascular responsiveness between upper and lower limbs, in a group of trained and untrained young. Their results showed a main effect of both training status and muscle group, and an interaction of the two, but only in leg muscle and not in the FDS, suggesting that adaptations to exercise training are more prominent in the trained lower limbs compared to upper limbs in young. Although these two studies had different outcome variables than this study, their findings indicate that microvascular adaptations may differ across muscle groups and training level. The present study found a group differences between E and Y in VL, but not in FDS. If this reflects a greater decline in oxidative function across muscle group is

difficult to say. The vastus lateralis typically consists of mixed fiber, whereas the FDS is more homogeneous dominated by type-1 fibers, this may support the muscle-intensity correlation, and possible larger muscle atrophy in VL (Kent & Fitzgerald, 2016).

Physical fitness

There is still very little research on age-related declines in mitochondrial function capacity using NIRS, and amongst the existing research there is an inconsistency in the results, where some report age-related declines and other not. As already discussed, some of the discrepancies may be explained by which muscle group was investigated (i.e often vastus lateralis, calf-musles or FDS), or in what circumstance (resting, whole-body exercise or isolated muscle work). However, in many cases differences could be explained by a lack of objective measures of physical activity and fitness level.

Authors have claimed that muscle mass and aerobic fitness can be reasonably maintained up to about age of 60 (Narici et al., 2003) with physical training and activity. In this study multiple parameters of physical fitness (pVO_{2peak}, lactate threshold, MVC, maximal handgrip 'endurance') and body composition (impedance analysis, anthropometric measures like arm length, circumference, volume, skinfold thickness), were collected in order to have a more accurate focus on the *age*-effects on muscle oxidative function. From these measures we can demonstrate that the elderly of this study were both healthy and physically active. Even though Y had a significantly higher VO_{2peak}, as was expected, the results revealed that E had a slightly higher $p\dot{V}O_{2peak}$ than the average healthy population of that age-category, whereas Y were slightly below average of their age-category (Loe et al., 2013).

The lack of significant group differences in handgrip exercise could partly be due to the fitness level of the elderly group, also it is possible that the differences observed in cycling exercise would have been of greater magnitude if the elderly had been of a relatively lower fitness level.

Back to the example of Ferri et al (2007), the mean age of the older group was higher than E of this study population, also the relative difference in VO_{2peak} between their groups were considerably greater compared to this study.

Although E had a lower aerobic fitness, it is possible that they were better cyclist. Among E cycling was commonly used in daily activity, but also a preferred exercise form, compared to Y that reported a larger variety in preferred training activity as well as more individuals combining

endurance and resistance training. It is possible that the similarities observed in WR_{OBLA} could be related to the specificity of cycling training in elderly.

4.3 Methodological considerations

There were some instances where data collection was not successful. Measurement and signal complications were mostly related to nonbiological factors like subject compliance to protocol and instrument errors.

There were some group differences in Physioflow signal quality, where more often in E than Y the signal was lost. It was of our experience that the preparations and attachment of equipment required a little more time and precision in E, due to factors like looser skin and less pronounced muscles. For PhysioFlow, loose skin, especially on the neck may also have contributed to some poor signal quality on high intensity, as the sensors move more and loose contact more easily, therefore there may be some measurement bias due to the fact that this was more problematic in one group compared to the other.

Furthermore, in some instances there may have been some issues in signal quality related to adipose tissue thickness. For the cycling test 4 datasets were excluded based on poor signal quality, where of 3 of these may be related to high ATT.

There were no differences in ATT between E and Y. In total 8 subjects measured ATT_{VL} >10mm, 5 (Y) vs 3 (E). Of these 8 subjects, 6 were women (Y=3, E=3), which is not unexpected as women naturally had a higher body fat percentage, confirmed by the body composition measurements by BIA. There was no correlation between ATT and $m\dot{V}O_{2rest}$ in FDS ($r^2 = 0.007$), in contrast to VL ($r^2 = 0.73$). A possible explanation for this inconsistency may be that ATT was significantly lower in FDS compared to VL. If SmO_2 is the primary NIRS outcome variable, correction for ATT may not be necessary, as both narrator and numerator will be affected to similar degree (Barstow, 2019), however ATT at VL still have affected the signal quality, and potentially the magnitude of ΔSmO_2 or $m\dot{V}O_2$ seen in cycling exercise, but not in FDS.

As NIRS device is sensitive to underlying fat mass, studies in the future should consider if high ATT could be an exclusion criterion. There is no cut-off value for skinfold thickness and signal quality, however we saw some issues in signal quality of subjects with ATT>10mm.

In this study end-exercise values were used to compare mean differences between groups in peripheral factors; magnitude and rate of tissue desaturation; as well as systemic variables HR, SV, CO, $p\dot{V}O_2$. We are aware that a reduction of data to end-point values for statistical analysis may not be the most suited approach to compare systemic variables, as the responses can fluctuate more at onset and in the course of exercise. However, as the main aim of this study was to compare the two groups, and not the response over the whole exercise bouts, the use of standardized end-exercise point is justified. The mean group response was presented in Fig. 7. And a visual inspection of the 10-point timeframe, the mean group responses are not markedly different.

Conclusion

In conclusion, the results of this study demonstrate that muscle oxidative function was not impaired in healthy elderly individuals. Measurements of systemic variables indicate that the results were not affected by different responses in central factor between the groups.

As the absolute workload during the handgrip exercise was similar between E and Y, and no differences were seen in muscle oxidative function as measured by NIRS, this holds promise that this exercise-model hold potential for future studies of elderly. Considering the high levels of physical activity and fitness of the elderly group the results also support that physical activity attenuates age-related declines in muscle oxidative function.

More studies are needed to understand the complexity of muscle ageing as the rate of ageing may vary over the course of a lifetime and between individuals. An accelerated decline in muscle function may lead to pathological ageing or sarcopenia, with severe outcomes for the individual. NIRS is a valuable method to gain insight into local muscle oxygen consumption across a span of age groups and muscle groups. With a future aim to identify biomarkers of pathological ageing in early stages.

Future studies should include also individuals of higher age than in the current study; whilst also addressing fitness levels, activity habits, of their study cohorts.

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Appendix 1

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)

Appendix 2

Spørreskjema 2

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..... ☐

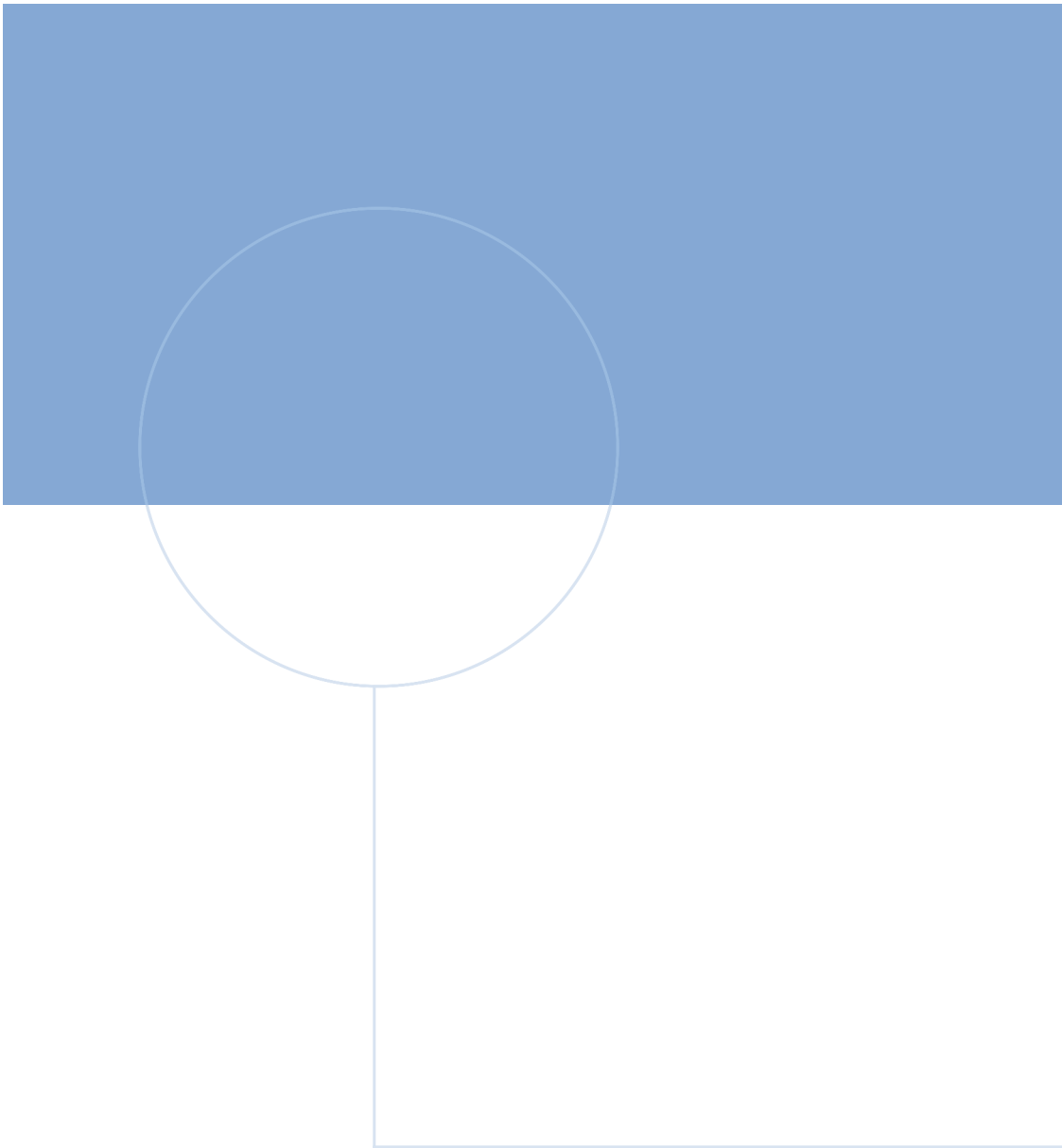
Ballidrett..... ☐

Yoga..... ☐

Styrketrening med vekter..... ☐

Annen Aerobic..... ☐

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



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