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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Investigating if an arm lift procedure is capable of highlighting aging-related differences in microvascular function, using Near-infrared Spectroscopy

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

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Kunnskap for ei betre verd

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Abstract

Sarcopenia pose serious detrimental consequences for the individuals affected. Current methods of diagnosis are cumbersome and require advanced equipment and personnel, and currently no method of early detection is available. Thus, developing an easy-to-apply, early detection method of sarcopenia is greatly beneficial. Microvascular function is an essential component in muscle aging, and near-infrared spectroscopy (NIRS) is a well-established tool for assessing microvascular function with the use of an arterial occlusion. Investigating whether a simpler method of application can assess microvascular function without the use of an occlusion could hold future implications for the development of an easy-to-apply, early detection method of sarcopenia. The purpose of the present study was to utilize NIRS to investigate if an arm lift procedure (ALP) was capable of detecting aging-related differences in microvascular function without the use of an arterial occlusion.

25 healthy volunteers (M=14, F=11) participated in the study and 24 participants were included in the final data analysis. One group of young participants (n=13, age 27+7 yr), and one group of older participants (n=12, age 65+4 yr). Total hemoglobin concentration was monitored in the right m. flexor digitorum superficialis using a portable NIRS device at three different elevations: at the level of the heart, 30° below- and 30° above the level of the heart, for a total of 7 intervals. Aging-related differences in the results of the first 4 intervals were investigated using a general linear model repeated measures (GLM-RM).

The GLM-RM showed no significant difference between groups ($F=0.376$, $p=0.546$). [Mean (SD)] response to the first up- and down positions respectively; -1.13 (2.6) μM and 4.59 (4.33) μM for the young participants, and -5.58 (11.56) μM and 7.77 (9.1) μM for the older participants. The older group of participants had an unexpectedly low pulse pressure (Mean:1.51 mmHg lower), and great values of maximal oxygen consumption (Mean±SD; 41.7±7.63 ml/kg/min), reflecting that the older group had great cardiovascular fitness. As cardiorespiratory fitness is an important variable for the decline in microvascular function associated with age, this could contribute to explain the lack of significant findings in this study.

In conclusion, the ALP in combination with NIRS failed to highlight aging-related differences in microvascular function without the use of an arterial occlusion. Based on a multitude of weaknesses in the study a definitive conclusion that the ALP holds no future relevance for the development of an easy-to-apply, early detection method of sarcopenia could not be drawn.

Abstrakt

Sarkopeni utgjør seriøse konsekvenser for de påvirkede individene. Nåværende metoder for diagnostisering av sarkopeni er tungvint og krever avansert utstyr og kyndig personell, og ingen metode for tidlig oppdagelse av syndromet eksisterer. Utvikling av en slik tidlig deteksjonsmetode vil være høyst nyttig. Mikrovaskulær funksjon er en essensiell komponent I muskelaldring, og nær-infrarød spektroskopi (NIRS) er en veletablert metode for å evaluere mikrovaskulær funksjon i kombinasjon med en arteriell okklusjon. Å undersøke om en metode som unngår bruken av en arteriell okklusjon er i stand til å fremheve aldrings-relaterte forskjeller i mikrovaskulær funksjon kan ha implikasjoner for utviklingen av en lettvint tidlig deteksjonsmetode av sarkopeni. Dermed er hensikten med denne studien å bruke NIRS til å undersøke om en armløfts prosedyre (ALP) er i stand til å fremheve aldringsrelaterte forskjeller i mikrovaskulær funksjon, uten bruk av en arteriell okklusjon.

25 friske frivillige (menn=14, kvinner=11) deltok i prosjektet, og 24 av deltakerne var inkludert i den endelige dataanalysen. Det var en gruppe yngre deltakere (n=13, alder 27±7 år) og en gruppe eldre deltakere (n=12, alder 65±4 år). Total hemoglobin konsentrasjon ble monitorert i høyre m. Flexor digitorum superficialis ved hjelp av en bærbar NIRS enhet i tre forskjellige elevasjoner: på høyde med hjertet, 30° ovenfor- og 30° nedenfor hjertets nivå, for totalt 7 intervaller. Aldringsrelaterte forskjeller i resultat mellom gruppene i de 4 første intervallene ble undersøkt ved hjelp av en generell lineær modell (GLM-RM).

GLM-RM testen viste ingen signifikante forskjeller mellom gruppene (F=0.376, p=0.546). [Gj.snitt (SD)] respons til den første opp- og ned posisjonen respektivt; -1.13 (2.6) μM and 4.59 (4.33) μM for de yngre, og -5.58 (11.56) μM and 7.77 (9.1) μM for de eldre deltakerne. Gruppen av eldre deltakere hadde et uforventet lavt pulstrykk (Gj.snitt 1.51 mmHg lavere), i tillegg til utmerkede verdier for maksimalt oksygenopptak. Dette reflekterer at den eldre gruppen hadde utmerket kardiorespiratorisk form. Ettersom kardiorespiratorisk form er en viktig variabel for nedgangen i mikrovaskulær funksjon assosiert med aldring, kan dette være med på å forklare mangelen på statistisk signifikante funn i denne studien.

For å konkludere, ALP i kombinasjon med NIRS var ikke i stand til å fremheve aldringsrelaterte forskjeller i mikrovaskulær funksjon uten bruk av en arteriell okklusjon. Basert på et mangfold svakheter i studien kan ikke en definitiv konklusjon om at ALP ikke holder noen fremtidig relevans for utviklingen av en lettvint tidlig deteksjonsmetode av sarkopeni trekkes.

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I am very grateful to the 25 delightful people volunteering to participate in this project, enabling the collection of data for my master thesis. I want to thank my fellow colleagues Mani Izadi and Andrea Spirka for a wonderful collaboration, for everything they have taught me and for the precious memories that never will be forgotten. I am eternally grateful for all the support provided by my supervisor, Dr. Mireille Van Beekvelt. Her expertise in the field and the insight she provided is what enabled me to carry through with this project. Thank you!
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1. Introduction

There is currently a substantial increase in the absolute number of people reaching old age in our society, posing a range of complications for the affected individuals as well as for the surrounding communities (30). Advancing age is accompanied by a range of deleterious effects on the human body (16, 33, 6), and associated with aging is a progressive decline in multiple physiological processes leading to an increased risk of cardiovascular disease (35). These changes include neurological degeneration, changes to the heart and arterial system as well as changes to the pulmonary system (35, 2, 4). Furthermore, accompanying old age is a steady decline in muscle size, strength, and physical function (18). This decline can lead to sarcopenia, a geriatric syndrome which is characterized by a progressive and generalized loss of skeletal muscle mass and strength, at an accelerated rate compared to normal, expected aging (7). Sarcopenia is a shared risk factor for many highly prevalent diseases in older persons (23, 25), and strongly correlates with physical disability, poor quality of life as well as death (7).

Most cases of sarcopenia go undetected, as screening tools are inaccurate and the effectiveness of such screening on relevant outcomes have not been proven (29). Because of this, a case finding approach is usually practiced. This involves looking for sarcopenia whenever relevant symptoms are reported, such as falls, muscle weakness, muscle wasting or difficulties performing daily activities (29). Diagnosis of sarcopenia currently requires accurate measurement of muscle mass and physical performance and can only be detected once the syndrome has advanced to a crippling level (31). Because the current diagnosis methods of sarcopenia require accurate measurements, expensive equipment is required and can only be performed at certain facilities such as hospitals (31). For people with already advanced muscle weakness and muscle wasting it can be quite cumbersome to travel to such a facility. Thus, the development of an easy-to-apply detection method of sarcopenia that can be carried out at home could be greatly beneficial for this demographic. Furthermore, as the development of sarcopenia is a steady decline over a prolonged time period, detecting the syndrome early in its development would be extremely beneficial and would enable preventative action to halt the decline in physical function (22, 32). Currently, no such method of an easy-to-apply, early detection method of sarcopenia is available, and further research is needed (22, 32).

The development of sarcopenia is however multifactorial and complex, which complicates the pursuit for such a method of detection. Various age-related factors such as neuromuscular

degeneration, changes in muscle protein turnover and hormone levels, inflammation, oxidative stress, and lifestyle factors are all involved in the development of sarcopenia (30). Furthermore, aging is associated with a decreased function of the microvasculature, including increased vascular stiffness, impaired microvascular reactivity and decreased vascular density (33, 3). The microcirculation provides the basis for oxygen- and nutrient delivery to the tissue, removal of waste products and carbon dioxide, as well as transvascular exchange and fluid economy (3, 12). Most organs, tissues and cells depend on adequate perfusion from the microcirculation, and microvascular function is essential to fulfill this vital role (12). Thus, muscle cell survival depends on adequate microvascular function and perfusion (12). As microvascular function is known to decrease with age (33, 3), this highlights microvascular function as an important factor contributing to the decline of the human skeletal muscle associated with aging, and thus as an important variable to further investigate in the search of an easy-to-apply, early detection method of sarcopenia (3).

Near-infrared spectroscopy (NIRS) has in recent years emerged as a valuable, well-established tool for measuring blood flow, oxygen saturation and oxidative capacity in skeletal muscle tissue (24, 27). Furthermore, NIRS has been used to noninvasively measure microvascular function by observing the NIRS signals of total hemoglobin (tHb) during reactive hyperemia following the release of an arterial occlusion (15). Investigating whether this aging-related difference in microvascular function achieved by observing differences in the tHb response can be detected by combining NIRS with a different method that circumvents the use of an occlusion could hold some future relevance for the development of an easy-to-apply, early detection method of sarcopenia. To highlight any aging-related differences in microvascular function however, a stimulus was needed to trigger a response that could be investigated. It has already been demonstrated by Willingham et al. that altering the perfusion pressure in the limb can serve as such a stimulus (15). They utilized NIRS in combination with a limb-lift technique and arterial occlusions to investigate whether different perfusion pressures in the lower limb would lead to different reperfusion rates (15). The different perfusion pressures were achieved by positioning the lower limb in three different elevations: baseline at the level of the heart, 30cm- and 60cm above baseline. They demonstrated the ability of NIRS measures of reactive hyperemia to detect changes in perfusion pressure and reperfusion time in the lower limb. In other words, altering the perfusion pressure by placing the limb in different elevations proved to be sufficient stimulus to

stress the microvasculature enough to alter reperfusion time (15). This highlights the limb lift procedure as sufficient stimulus to trigger a response that could be investigated in the present study. If the limb lift procedure is capable of detecting aging-related differences in the microvasculature, the utility of this procedure could hold some implications for the development of an easy-to-apply, early detection method of sarcopenia in the future (15).

Thus, the purpose of the present study was to investigate whether an arm lift procedure (ALP) was capable of detecting aging-related differences in microvascular function between young and older participants. This was achieved by monitoring tHb concentration changes in the upper limb in three different elevations without the use of an arterial occlusion, using NIRS. If the ALP is capable of detecting aging-related differences in tHb concentration changes it would indicate the ability of the ALP to assess aging-related differences in microvascular function without the use of an arterial occlusion, which in turn could hold some future implications for the development of an easy-to-apply, early detection method of sarcopenia.

It was hypothesized that the older participants would have a more pronounced response to the stimulus, and that a more rapid adaptation to the stimulus would occur in the microvasculature of the young participants based on the assumption of better microvascular function within the young group (3, 33). Furthermore, it was hypothesized that every interval of each position in the ALP would result in a similar response to the previous intervals for that position based on the assumption that the tHb concentration would plateau after a given time in each position during rest.

2 Materials and Methods

2.1 Participants

A total of 25 (14M, 11F) healthy volunteers participated in the project. There were two participant groups based on age. The group of younger participants (n=13) ranged from 19 to 37 years of age (Mean±SD; 27±7 yr), and the group of older participants (n=12) ranged from 60 to 82 years of age (Mean±SD; 65±4 yr). The participant characteristics of both groups are presented in table 1. The inclusion criteria included age between 18-40 years or 60 years or above, and being recreationally physically active. Exclusion criteria included BMI>30, diabetes, uncontrolled hypertension, smoking, metabolic disease, history with cardiovascular- or pulmonary disease, presence of acute disease, and medication use that would affect the hemodynamic responses to the testing protocol.

All participants submitted a written, informed consent before any testing was performed. The study was conducted with the approval of the institutional review board at the Norwegian University of Science and Technology (NTNU) (Trondheim, Norway). The testing period spanned from early January till the end of February 2022.

Participants were volunteers recruited through information posters situated in the city of Trondheim, Norway, and some were recruited from a local gym. The majority of participants were volunteers recruited from organized Facebook groups for recreational physical activity in Trondheim.

2.2 Study design

Participants reported to the laboratory on two different days separated by 48 hours to ensure sufficient recovery between test days. The first day consisted of a maximal oxygen consumption (VO2max) test, and the second day consisted of an arm lift procedure (ALP). All participants were instructed to abstain from alcohol and caffeine for at least 12h, and vigorous exercise for at least 24h before their scheduled testing. This was a single-site study taking place in a laboratory in Trondheim, Norway. All tests were performed in a quiet, temperature-controlled room (20-22 degrees Celsius).

Participant information including age, gender, height, weight, tobacco and alcohol habits, as well as menopause status was documented. Participants were asked about exercise habits, frequency, duration and type of exercise. Furthermore, skinfold thickness on the m. flexor digitorum superficialis (FDS), body fat percentage, systolic- and diastolic blood pressure values, as well as maximal oxygen uptake values were obtained for all participants.

All participants abstained from alcohol and vigorous exercise for at least 24 hours, and all participants except 3 abstained from caffeine for at least 6 hours before the testing. The main outcome variable measured was total hemoglobin concentration (tHb) measured in μM. Participant height was measured using a wall-mounted stadiometer (Seca 222, Seca GmbH & Co, Hamburg, Germany). Blood pressure (BP) was manually measured using an automatic blood pressure monitor (OSZ 5 easy, Welch Allyn, Jungingen, Germany).

2.3 Near-infrared Spectroscopy (NIRS)

Skeletal muscle oxygenation (tHb concentration) was monitored in the right m. FDS using a continuous-wave near-infrared spectrophotometer (Oxymon MKIII, Artinis Medical Systems, Netherlands). Measuring the changes in light absorption at different wavelengths allows for the measurement of oxygenated- and deoxygenated hemoglobin, and thus tHb concentration changes could be monitored by utilizing NIRS (1). A portable NIRS device (Portamon, Artinis Medical Systems, Netherlands) was placed longitudinally on the muscle belly of FDS on the right arm. Hair was removed from the optode site, and the skin was cleaned using alcohol cleaning pads prior to placement. The Portamon device was secured to the forearm using broad strips of tape to prevent any movement of the device. The device was covered with an optically dense black piece of fabric to minimize extraneous light from interfering with the optode. The middle source-detector distance was utilized, measuring at a depth of 35mm. The LED-transmitters measured at wavelengths of 761 nm and 845 nm, corresponding to the measurement of deoxygenated- and oxygenated hemoglobin. Concentration changes were obtained using the modified Beer-Lambert law (36). Data was acquired using data acquisition software (Oxysoft, Artinis Medical Systems, BV, The Netherlands). Acquired data was stored digitally on a computer in movement lab III at St. Olavs Hospital, Trondheim, Norway.

The NIRS measurements are highly affected by adipose tissue thickness (20). To account for this, skinfold thickness was measured at the NIRS measurement site on the muscle belly of FDS using a skinfold caliper (Holtain, Crymmych, UK). Two measurements were made, and the average of the two results was reported. Additionally, body fat percentage was measured using a bio impedance analysis (Inbody 770, BIOSPACE, Seoul, Korea) in a fasted state.

2.4 Experimental protocol

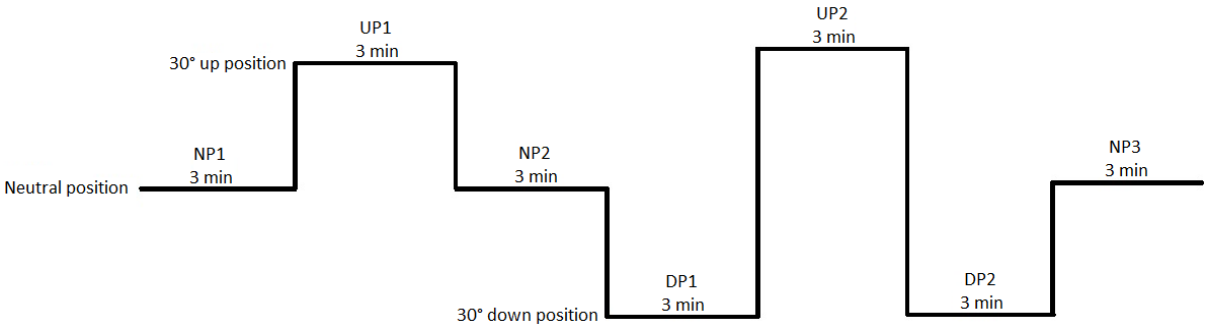
2.4.1 The Arm Lift Procedure

The arm lift procedure (ALP) consisted of positioning the arm in three different elevations for three-minute intervals. The participant was seated comfortably in an adjustable examination bed, with a 10–20-degree bend in the backrest. The arm was positioned on a custom-made arm-lift device, consisting of a plank and rope system to manually position the plank in the correct elevation. The plank was fixed to a horizontal metal pole in the proximal part, with free range of motion in the sagittal plane in the distal part of the plank. A rope was attached to the distal part of the plank, which was manually pulled in order to elevate and lower the plank into the three different positions. The examination bed was elevated to the point where the shoulder joint was situated slightly above the plank, slightly behind the pivot point. The arm was placed on the plank, with the shoulder and elbow in an extended position at the level of the heart, with the forearm pronated. The arm was positioned at a slight angle outward from the body, at a 20-degree angle. The arm was resting on 2 Styrofoam cushioning pads in order to elevate the NIRS sensors above the plank and to avoid pressure on sensitive bony parts to enable full relaxation of the arm. One cushion at the wrist, and one cushion just distal to the elbow joint. Additionally, a third, softer cushion was used proximal to the elbow joint in order to prevent discomfort in the elbow. The participant was instructed to sit as still as possible and avoid talking throughout the procedure.

There was a total of three different positions. The first was the neutral position (NP), where the arm was elevated to be perpendicular to the ground at a 20-degree angle from the torso in the transverse plane, at the level of the heart. The second position was the upright position (UP), where the arm was elevated to 30 degrees above relative to the NP. The final position was the

downward position (DP), where the arm was lowered to 30 degrees below relative to the NP. There was a total of 7 intervals, each lasting 3 minutes. There were 3 repetitions of the NP, and 2 repetitions of the UP and DP. The entire protocol totaled 21 minutes. The protocol is demonstrated in figure 1.

Figure 1. Demonstration of the ALP protocol.



2.4.2 Maximal oxygen consumption test

A maximal oxygen consumption (VO2max) test was conducted in order to characterize the cardiorespiratory fitness of all participants. The test was conducted on an electro-magnetically braked cycle ergometer (Lode Excalibur Sport, Lode B. V., Groningen, Netherlands). VO2max values were obtained using open-circuit indirect calorimetry (Oxycon Pro, Jaeger GmbH, Hoechberg, Germany). The open-circuit indirect calorimetry was calibrated using a 3-liter calibration syringe (Hans Rudolph Inc, Kansas City, MO, USA). To define an appropriate starting resistance for the VO2max test a lactate profile test (LPT) was conducted just prior to the VO2max test. The LPT is designed to define the participants' onset of blood lactate accumulation (OBLA), where the work rate at which OBLA was reached was used as the starting work rate for the VO2max test. The participants started with a warm-up period of 10 minutes at low resistance on the cycle ergometer. The LPT was performed continuously in 4-minute periods of exercise with an increase in resistance with every period. Blood lactate was manually measured after every period using a lactate analyzer (Lactate Pro LT-1710/1730, Arkray, Kyoto, Japan). The work rate at which blood lactate reached 4 mmol/L was used as the starting point for the VO2max test. The LPT started at different resistance levels and had different incremental increases in resistance based on age and gender. For the young group men started at 100 watts

(w) of resistance with 25 w increments, and females started at 95 w with 20 w increments. For the older group men started at 95 w of resistance with 20 w increments, while females started at 75 w with 15 w increments.

Between the LPT and VO2max test there was 5-10 minutes of active recovery. The VO2max test was performed continuously with an incremental resistance increase every minute. The increments were different based on age and gender, with the same incremental values as for the LPT. The test was conducted until exhaustion and was terminated if the participant was not able to maintain a cadence of >60 rpm. All participants received verbal encouragement from the test personnel.

2.5 Data analysis:

The raw data collected from NIRS was exported, processed and analyzed using custom scripts in MATLAB (version R2021b) and Excel (Microsoft Excel for Office 365 MSO, Microsoft COP., Redmond, WA, USA). All statistical analysis was performed using IBM SPSS 27 software (SPSS Inc., Chicago, IL, USA). For all statistical tests $p < 0.05$ was considered significant. The NIRS data measured from m. FDS was converted to mean values for every 10 seconds in MATLAB. To express the individual response in each position, the average of the last 6 data values of tHb saturation during the last minute of each position was used. The tHb response is expressed as a delta value from the first neutral position. The first neutral position is adjusted to always be 0 μm for each participant.

2.5.1 Investigating similarity of intervals

All data was tested for normality using the Shapiro-Wilk test of normality.

Statistical analysis was performed on all 7 ALP positions to determine if the protocol caused an effect over time, or if the response was similar for each position regardless of being the first, second or third interval for that position. Non-parametric test “Related samples” was utilized to investigate whether the mean response to DP1 differed from DP2, and if UP1 differed from UP2 with statistical significance. A repeated measures analysis of variance (ANOVA, GLM-RM) was used to determine if there were statistically significant differences between the tHb responses to NP1, NP2 and NP3 for all participants. The delta values of tHb between NP1, NP2 and NP3 were tested in the GLM-RM.

2.5.2 Investigating group differences in response to the ALP

To investigate if there was an aging-related difference between the group responses to the first 4 positions, a general linear model repeated measures (GLM-RM) was performed on the first interval of each position. Because the hypothesis that every interval of the same position would be similar was broken, the relative change from the preceding neutral position was investigated. To account for the small change that occurs from the first neutral position to the second neutral position, UP1corr and DP1corr were calculated using the compute variable function in SPSS. This was achieved by subtracting the preceding neutral position (NP2) from DP1, and the preceding neutral position (NP1) from UP1. Group was added as a between-subjects factor in order to compare the group responses to highlight aging-related differences.

2.5.3 Investigating relevance of Pulse Pressure

It was investigated if pulse pressure (PP) was related to the response in UP1corr and DP1corr in the results from the GLM-RM. A bivariate correlation test was run, utilizing the Pearson correlation coefficient to investigate the relationship between PP and the NIRS data from UP1corr and DP1corr. PP was manually calculated for all participants using excel by subtracting their diastolic BP from their systolic BP.

An independent samples T-test was run for PP with group as the grouping variable to investigate if there were group differences in mean PP.

3. Results

A total of 25 volunteers participated in the project. A total of 24 participants were included in the final data analysis, one subject was excluded from data analysis because of an error in the data processing. There was no missing data. There were no adverse events.

Table 1: Participant Characteristics

Variable	Young	Older	P-value
n	13	11	-
Male/Female	8/5	6/5	-
Age *(years)*	27 ± 7	65 ± 4	<0.001
Height (cm)	177.7 ± 7.6	169 ± 5.3	0.005
Weight (kg)	73.3 ± 7.7	68 ± 10	0.15
BMI	23.2 ± 1.5	23.7 ± 2.5	0.55
Skinfold thickness FDS (mm)*	8.9 ± 2.7	7.7 ± 3.5	0.365
Body fat (%)	18.5 ± 5.4	23.3 ± 6.7	0.063
SBP (mmHg)	116 ± 10.9	114.8 ± 11.6	0.761
DBP (mmHg)	73.5 ± 8.1	73.6 ± 6.9	0.975
VO2max (ml/m/kg)*	51.27 ± 9.8	41.7 ± 7.63	0.015

Data reported as mean ± SD. n = sample size.
**Skinfold thickness FDS: Skinfold thickness measured at the m. Flexor digitorum superficialis directly at the measurement location of the NIRS sensor.*
BMI, Body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure.
**VO2max is expressed as milliliters per minute, per kilogram of bodyweight.*

3.1 Investigating similarity of intervals

When testing the data for normality, the Shapiro-Wilk test showed that the data for UP1, UP2, DP1 and DP2 was not normally distributed. However, the test showed that NP1, NP2 and NP3 were all normally distributed, as shown in table 2.

Table 2: Test of normality

Position	Statistic	Sig. (P-value)
NP1	-	-
UP1	0.547	<0.01
NP2	0.965	0.540
DP1	0.770	<0.01
UP2	0.495	<0.01
DP2	0.897	0.018
NP3	0.954	0.327

NP, Neutral position; UP, upright position; DP, downward position

When investigating whether the response in UP1 differed from UP2, and in DP1 from DP2 respectively, the non-parametric test "related samples" showed that they differed from each other with statistical significance, as shown in Table 3. A non-parametric test was utilized because UP1, UP2, DP1 and DP2 were not normally distributed, as shown in table 2.

Table 3*:* Result from non-parametric tests "Related Samples" that shows UP1&2 and DP1&2 are sig. different from each other.

Position		
	Standardized test statistic	**Sig.**
UP1 and UP2	2.657	0.008
DP1 and DP2	2.8	0.005

Sig. expressed as asymptotic sig. (2-sided)

When investigating whether all 3 neutral positions lead to a similar response, the GLM-RM showed that they all differed from each other with statistical significance, as shown in Table 4 and 5. It demonstrates the difference between NP1, NP2 and NP3, showing an increasing trend of delta values.

Table 4: GLM-RM Results showing differences between neutral positions

Position			***95% Confidence Interval***	
	Mean	**Std. Error**	**Lower Bound**	**Upper Bound**
NP1	-	-	-	-
NP2	1.193	.471	0.220	2.167
NP3	3.356	.942	1.409	5.304

Expressed as mean delta values of micromole (µM) between NP1, NP2 and NP3.
NP, Neutral position.

Table 5: Post-hoc from GLM-RM showing that NP1 differs from NP2, and that NP2 differs from NP3 with statistical significance

Position		
	F-value	**Sig.**
NP1 vs. NP2	6.431	0.018
NP2 vs. NP3	5.814	0.024

Sig. Is significance level (p-value)

Because each position was not similar to the previous iterations of the same position, as shown in table 3, 4 and 5, an average of each position could not be taken and then compared, breaking the hypothesis that all intervals of each interval would be similar to the previous ones. Subsequently, the first 4 positional changes were utilized to compare the result between groups, corrected for the differences between the first and second neutral position.

3.2 Group differences in response to the ALP

The GLM-RM test run for UP1corr and DP1corr with group set as the between-subjects factor showed that there was not a statistically significant difference (p = 0.546) in the response in tHb concentration changes based on group, as presented in table 6. It showed that there was an effect of position for both UP1corr and DP1corr which demonstrates a response to the stimulus, but no significant difference between groups was found, indicating that the effect was similar in both groups.

Table 6: Results from GLM-RM showing mean response to UP1 and DP1 corrected for the difference between NP1 and NP2, and the between-subjects factor showing no significant difference between the groups.

Position			***Between-subjects factor***	
	Group	**Mean ± SD**	**F**	**Sig.**
UP1corr	y	-1.13 ± 2.6	0.376	0.546
	o	-5.58 ± 11.56		
DP1corr	y	4.59 ± 4.33		
	o	7.77 ± 9.1		

Expressed as mean ± SD values of μM.
Group y, young participants; group o, older participants.
UP1corr, first upward position corrected for the difference between first and second neutral position.
DP1corr, first downward position corrected for the difference between first and second neutral position.

3.3 Pulse pressure

The bivariate correlation test run in SPSS showed that there was a weak, non-significant negative correlation between PP and the UP1corr results for the young group (r = -0.127), and a moderate, non-significant negative correlation for the older group (r = -0.467). Furthermore, it showed that there was a weak, non-significant correlation between PP and the DP1corr for the young group (r = 0.315), and a moderate, non-significant correlation for the older group (r = 0.446). The results from the bivariate correlation test are presented in table 7.

Table 7: Results from bivariate correlation showing Pearson correlation coefficient between Pulse Pressure and UP1corr and DP1corr, split by group.

	Pulse Pressure		
	Group	**r-value**	**Sig.**
UP1corr	y	-0.127	0.680
	o	-0.467	0.148
DP1corr	y	0.315	0.315
	o	0.446	0.169

r-value, Pearson correlation coefficient.
r-value is expressed as the correlation between UP1corr and Pulse Pressure, and DP1corr and Pulse Pressure.
UP1corr, first upward position corrected for difference between first and second neutral position.
DP1corr, first downward position corrected for difference between first and second neutral position.
Sig. is 2-tailed significance level (p-value).
Absolute magnitude of observed correlation coefficient cutoff points:
0.00-0.10 = Negligible correlation
0.10-0.39 = Weak correlation
0.40-0.69 = Moderate correlation
0.70-0.89 = Strong correlation
0.90-1.00 = Very strong correlation

The independent samples t-test run for pulse pressures showed that there was no statistically significant difference between the groups. The older participants had a slightly lower PP, with a mean difference of 1.51 mmHg and a p-value of 0.570. Values are presented in table 8.

Table 8: Results from independent samples t-test run for pulse pressure with group set as the grouping variable.

Variable					*95% Confidence Interval*	
	Mean Young	**Mean Older**	**Mean diff.**	**Sig.**	**Lower Bound**	**Upper Bound**
PP	42.69	41.18	1.51	0.570	-3.91	6.94
SD	5.09	7.67				

PP, pulse pressure; SD, Standard deviation
Mean values expressed as millimeters of mercury (mmHg).
95% Confidence Interval of mean difference result
Sig. is 2-tailed significance level (p-value).

4. Discussion

This study investigated whether NIRS in combination with different levels of limb elevation achieved by the ALP was capable of detecting aging-related differences in tHb concentration changes in young and older persons without the use of an occlusion in the upper limb. The main finding of the study was that there was no statistically significant difference in tHb concentration changes between the groups in response to the stimulus created by the ALP. Subsequently, the study failed to prove that the ALP in combination with NIRS is capable of investigating aging-related differences in microvascular function without the use of an occlusion.

There was a small, non-significant difference in the response between groups as shown in the mean values presented in table 6, but the difference could however not be attributed to aging-related factors, as shown by the GLM-RM with group set as a between-subjects factor.

4.1 Total peripheral resistance, pulse pressure and cardiorespiratory fitness

Total peripheral vascular resistance (TPR) refers to the sum of the resistance in the peripheral vasculature in the systemic circulation which creates blood pressure (BP) and blood flow (38, 21). TPR is affected by different factors related to the cardiovascular system, and most notably arterial stiffness and reduced arterial elastic properties (14). These factors are known to increase with age which in turn leads to an increase in TPR as the body ages (39). Thus, based on the increased resistance, the BP is subsequently increased in order to deliver blood to the tissue (39). In other words, to compensate for the increased resistance created by increased arterial stiffness and decreased arterial elastic properties it can be expected to see an increase in systolic blood pressure (sBP) with advancing age (5). This leads to an aging-related increase in pulse pressure (PP), the difference between dBP and sBP, which is used as a raw index of arterial stiffness (14). The aging-related increase in BP is mainly attributable to an increasing sBP while maintaining a similar diastolic blood pressure (dBP), subsequently resulting in a greater PP (39). As the force required to supply the tissue in the limb with blood increases with age it was expected to find aging-related differences in tHb concentration in response to the stimulus created by the ALP in this study. Because the results from the GLM-RM showed no significant differences in the response, it was further investigated whether PP differed between the two groups using an independent samples t-test. The test showed that there was no significant difference in PP between the groups, where the older group had a slightly lower PP (Mean; 1.51 mmHg lower)

comparatively to the young group. This is the opposite of what one would expect and could indicate that the group of older participants investigated in this study was a poor representation of a normal population of older persons in terms of cardiovascular fitness status and vascular health. The older participants were mainly recruited from organized groups related to outdoor physical activity which could have created a bias towards them having great cardiovascular fitness compared to their peers. Interestingly, previous research has shown no significant differences in the lower limb reperfusion rate between young and older adults of similar fitness level (34, 9, 26). The authors suggested that fitness level is a very important factor contributing to reduced microvascular function in older adults, and that aging might not be the main factor contributing to this decline. The unexpectedly low PP of the older participants in this study could indicate that they had a very high level of cardiovascular fitness, and thus could contribute to explain the lack of significant findings in this study.
Important to highlight is the fact that the correlation tests did not find any significant correlation between PP and the test results, which indicates that PP alone is not responsible for the lack of significant findings in terms of aging-related differences in the response to the stimulus.

The results from the VO2max test further highlight the fact that the group of older participants in this study had great cardiovascular fitness relative to their peers. Based on categories presented as normative values derived from multiple sources (19), the VO2max scores for the group of older participants in this study (Mean±SD; 41.7±7.63 ml/m/kg) falls within the categorization of having an excellent cardiorespiratory fitness for males. The VO2max scores for the group of young participants in this study (Mean±SD; 51±9.8 ml/m/kg) are categorized as having good cardiorespiratory fitness for males (19). Based on this categorization of cardiorespiratory fitness level an argument can be made that the older group has better cardiorespiratory fitness, relatively speaking, compared to the young group in this study (19). This is further exemplified by the fact that this categorization is for males, whereas the values required to reach a certain category is somewhat lower for females, and the mean VO2max values presented are for both males and females in both groups. Thus, based on the knowledge that cardiorespiratory fitness is an important factor in the decline of microvascular function associated with age, the unexpectedly excellent cardiovascular fitness level of the older group could contribute to explaining the lack of significant findings of aging-related differences in microvascular function in this study (34, 9).

4.2 Poor protocol design

The protocol of the ALP was designed with the hypothesis that each interval of every position respectively would result in similar tHb concentration changes. If this hypothesis was true, an average of the different intervals of each position could have been calculated and then compared in the data analysis. This would have utilized all the data gathered in the protocol. However, the non-parametric test "related samples" showed that DP1 differed from DP2-, and that UP1 differed from UP2 with statistical significance, as shown in table 3. Furthermore, the first GLM-RM with post-hoc test results presented in table 4 and 5 showed that NP1, NP2 and NP3 were all different from each other with statistical significance, with delta values that were increasing for every repetition, 1.93 μM from NP1 to NP2, and 3.35 μM from NP2 to NP3. This result highlighted that the ALP protocol caused an effect over time, meaning that every position change in the protocol was dependent on all the preceding position changes. Thus, an average of every interval of the same position could not be calculated and used as the main variables in the data analysis. This led to the decision of comparing the response to only the first 4 intervals (NP1, UP1, NP2 and DP1) as it was possible to account for the impact of the preceding position changes by adjusting the neutral positions to the baseline of 0 μM.

This could have been circumvented if a different protocol had been utilized, where a randomized order would have accounted for the impact of previous intervals, and thus would allow for the entire protocol to be included in the end analysis comparing the responses. As this oversight in protocol design inhibited the ability to utilize all the data gathered, it resulted in being a limitation in this study.

Additionally, the NIRS signal was reset to zero for NP1 for all participants during the data processing, and thus a resting steady state level of tHb was assumed. This might however not have been the case for some participants, where the values could have been higher or lower compared to their real resting steady state level of tHb. Consequently, this can have affected the results.

4.3 Limitations

4.3.1 Test duration

The protocol test duration ended up being a limitation. As the NIRS measurements are highly affected by even small movement in the muscles (55) the participants were instructed to sit as still as possible without talking. This in combination with the protocol duration of 21 minutes proved to be difficult as participants became very sleepy and struggled to stay awake for the entirety of the protocol. This could have negatively impacted the measurements as falling asleep affects systemic variables like heart rate, breathing frequency and BP which could all have negatively affected the NIRS measurements (10, 13). Furthermore, the participants tended to struggle sitting completely still without movement for the entire 21 minutes. Consequentially, small movements in the legs, torso, shoulders and neck were occurring occasionally. Participants were not struggling with keeping their right arm where the NIRS sensor was situated still, yet small disturbances were occurring to the NIRS signal as a result of movement of other body parts. This could have negatively affected the NIRS measurements. However, when judging whether the protocol duration could be shortened for future studies by reducing the duration of every position interval, it became clear that the entire 3-minute intervals should be kept because the NIRS signal of tHb did not have a tendency to plateau towards the end of the interval as hypothesized.

4.3.2 The ALP construction and anatomical differences

The custom-made arm lift device can be improved. The setup of the device made it difficult to set a standardized position for every individual participant because of anatomical differences between them. Because the protocol requires such a large range of motion of the limb, it was difficult to find a standardized position that would fit every participant without compromise. Different limb length, weight of the limb, torso length and posture of the upper torso were anatomical differences that required slightly different height of the examination bed, slightly different angles of the limb and in the backrest of the examination bed for each individual participant. Further work should be done to develop a device that allows for a standardization of position across all participants. Furthermore, the lack of a standardized position combined with anatomical differences between participants presented difficulties finding a comfortable position for all participants. This resulted in five (2 young, 3 older) participants reporting difficulties

keeping their muscles in the limb and shoulder fully relaxed for the entire duration of the protocol, leading to small levels of muscle contraction. Several factors are known to affect the NIRS signal during muscle contraction, including heterogeneity of muscle blood flow and oxygen utilization, capillary recruitment and volume distribution of blood and muscle fibers (11). Additionally, the relative contribution of myoglobin and hemoglobin to the NIRS signal from the tissue can change depending on oxygen delivery (17). As a consequence, this muscle contraction throughout the protocol could have had a negative impact on the accuracy of the NIRS measurements of tHb concentration for these specific participants. (24, 11).

4.4 Potential limitations when utilizing NIRS

The NIRS measurements have several limitations as an instrument of measurement (24). Adipose tissue at the site of measurement influences the NIRS signals because of its effect on the light scattering properties of the tissue (24, 8). This was accounted for by measuring skinfold thickness at the measurement location on the m. FDS on the forearm, as well as total body fat percentage. The results from these measurements showed normal values for both groups, as presented in table 1, which indicates that the negative effect of adipose tissue on the reliability of the NIRS signal was not detrimental for the NIRS signals in this study. Furthermore, skin perfusion can influence the NIRS signal, as both oxy- and deoxyhemoglobin from the skin may confound the muscle signal (24). However, the contribution of skin perfusion to the NIRS signal is mainly attributable to an increase in body temperature (24), which was accounted for by having a temperature-controlled room and by having the participant in a resting position. Additionally, melanin in the skin absorbs light in the near-infrared range and can therefore influence the NIRS signal, and thus should be accounted for by utilizing physiological calibration to the signal (28). In this study there was very small variation in skin complexion between participants, and thus no calibration of signals was needed.

4.5 Future relevance of the ALP

The GLM-RM showed that there was a small, non-significant group difference in the response to the stimulus created by the ALP, yet the response could not be attributed to aging-related differences as presented in table 5. However, this could serve as an indicator that the ALP is capable of creating a substantial stimulus through altering perfusion pressure, and it could be relevant to further investigate the usefulness of the method. As the previously mentioned limitations to the study could explain the lack of significant findings, running a similar study while rectifying these limitations could highlight the ability of the ALP to detect aging-related differences in microvascular function without the use of an occlusion when combined with NIRS. If further work is done with the ALP, it could hold the potential of furthering the development of an easy-to-apply, early detection method of sarcopenia.

5. Conclusion

In conclusion, the current study failed to highlight the ability of the ALP in combination with NIRS to highlight aging-related differences in microvascular function by monitoring and comparing tHb concentration changes between young and older participants. Based on the fact that the group of older participants had great cardiorespiratory fitness, as well as a multitude of limitations related to the protocol and participant selection in the study, a definitive conclusion that the ALP holds no future relevance for the development of an easy-to-apply, early detection method of sarcopenia should not be drawn. Future studies should further investigate the effect of altering perfusion pressure in the limb to highlight aging-related differences in microvascular function in order to validate this method of application.

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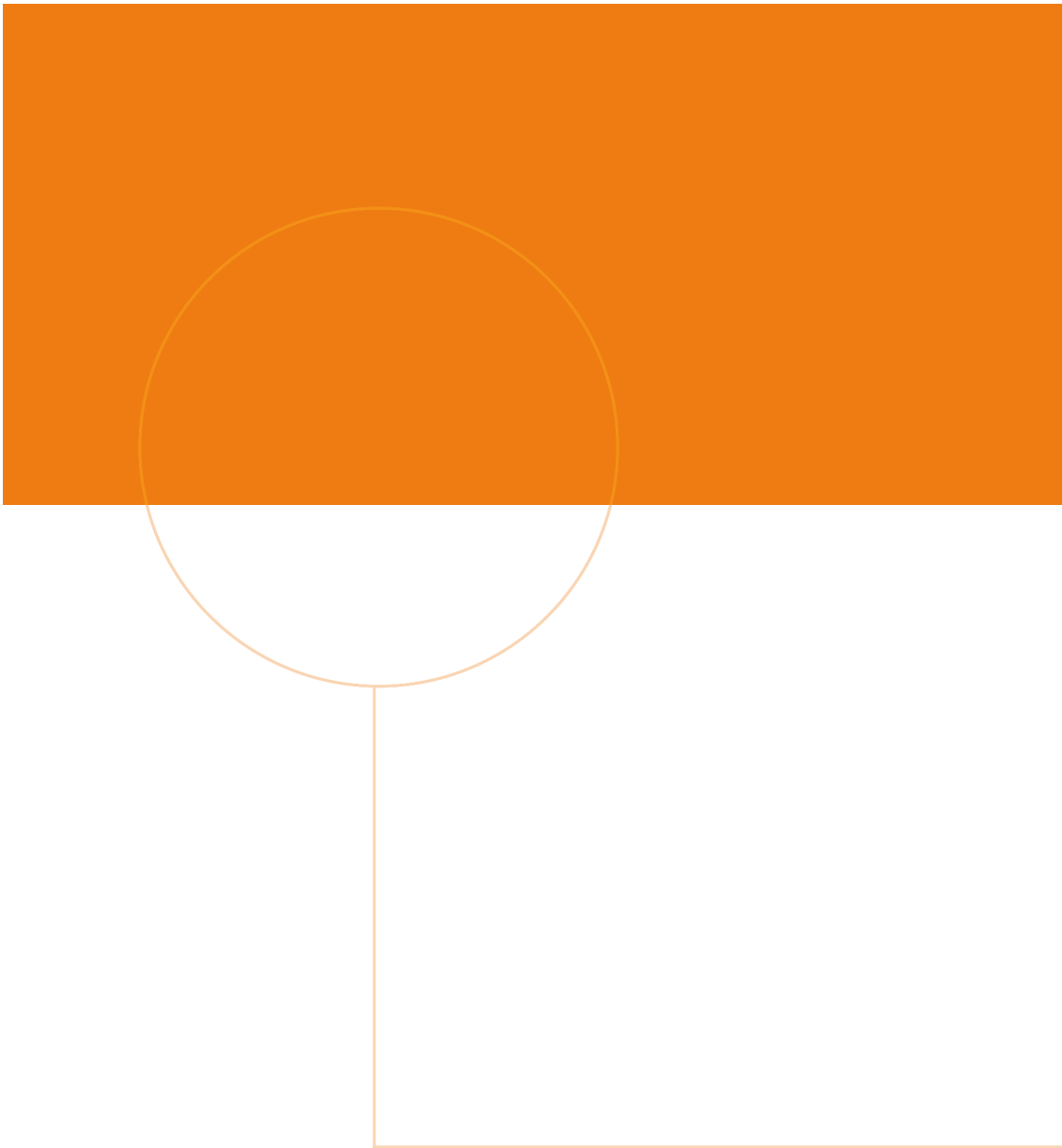

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