

The Acute Effect of Ischemic Preconditioning on Microvascular Function in  
Tibialis Anterior in Smokers and Non-smokers; Contributions of The  
Autonomic Nervous System

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Gruppe 10101:  
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# Forord

Dette speciale er udarbejdet af Gustav Vad Thomsen og Magnus Rosenkilde Henriksen på kandidatuddannelsen i Idræt, School of Medicine and Health ved Aalborg Universitet, udarbejdet i perioden februar 2019 til juni 2019. Specialet tager sit udgangspunkt i begrebet iskæmisk prækonditionering, specifikt denne behandlingsforms indflydelse på mikrovaskulær reaktivitet og belyse bidraget fra det autonome nervesystem, i både rygere og ikke-rygere. Menneskekroppen og dens komplekse funktioner har altid været et interessepunkt for os begge, hvoraf vi igennem Idrætsuddannelsen har beskæftiget os med forskellige former for optimering af præstation og sundhed, samt forebyggelse af skader mm. Vi har derfor fundet det interessant at tage udgangspunkt i problematikken omkring rygning, da det er et samfundets største og dyreste problemer i forhold til sundhed. Igennem kandidatuddannelsen har vi primært beskæftiget os med iskæmisk prækonditionering og dens anvendelsesmuligheder, hvorfor vi i dette speciale laver en sammenkobling mellem netop rygning og iskæmisk prækonditionering.

Ifølge WHO er tobaksepidemien en af de største helbredstrusler verdensbefolkningen nogensinde har mødt, med direkte indflydelse på mere end syv millioner dødsfald hvert år på verdensplan. På verdensplan findes der i dag omkring 1,1 milliard rygere, hvoraf 80% kommer fra lav- og mellemindkomstlande hvor byrden af tobaksrelaterede sygdom og død er størst<sup>1</sup>. Rygning fortsætter dog stadig med at være den enkeltstående faktor som forårsager de største helbredsmæssige komplikationer i Danmark<sup>2</sup>. Ydermere ses der for første gang i 20 år i stigning i antallet af rygere i Danmark, en stigning fra 21% i 2016 til 23% i 2018<sup>3</sup>. Som nævnt er konsekvensen af længerevarende rygning i de værste tilfælde død, dog er konsekvenserne generelt funktionsnedsættelse af hjerte-karsystemet, selv som følge af let rygning. Disse funktionsnedsættelser kan, hvis gjort i tide, omvendes. Ifølge forskning kan iskæmisk prækonditionering være en mulig metode til at omvende konsekvenserne af rygning, da utallige undersøgelser har vist positiv indflydelse på hjerte-karsystemet. Hvilket leder til netop formålet med dette speciale.

Vi vil gerne rette en stor tak til vores vejledere gennem hele kandidatuddannelsen Ryan Godsk Larsen og Andrew James Thomas Stevenson for yderst kompetent og motiverende vejledning. Desuden rettes en stor tak til vores forsøgspersoner for deres tid og indsats.

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Aalborg Universitet, juni 2019  
Kandidatuddannelsen i Idræt

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<sup>1</sup> [WHO](#)

<sup>2</sup> [Sundhedsstyrelsen](#)

<sup>3</sup> [Sundhedsstyrelsen](#)

# The Acute Effect of Ischemic Preconditioning on Microvascular Function in Tibialis Anterior in Smokers and Non-smokers; Contributions of The Autonomic Nervous System

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Smoking is recognized as a major risk factor for developing cardiovascular diseases. Smoking causes a reduction in nitric oxide (NO) together with an unbalance in the autonomic nervous system (ANS). The changes lead to an attenuated ability to regulate vascular tonus in order to meet various metabolic demands. Research indicates that ischemic preconditioning (IPC) has positive effects on both NO and the ANS. IPC consists of multiple cyclical periods of short periods of restriction in blood supply to the implicated tissue and subsequent reperfusion. The effect of IPC on microvascular function and the contribution of the ANS in smokers and non-smokers has not yet been investigated. Hence, the aim of this study was to determine the effect of IPC on microvascular function in young healthy smokers and non-smokers and elucidate the contribution of the ANS. Eighteen young healthy smoking ( $n = 7$ ) and non-smoking ( $n = 11$ ) males went through controlled counter balanced crossover design over two sessions. The participants received either IPC (250 mmHg) or sham conditioning (20 mmHg) at the first session and vice versa at the second session. Each session consisted of three measurement blocks (Baseline, Post, and Post 1-hour). At each measurement block, microvascular function and the contribution of the ANS was measured. Microvascular function was assessed as the contraction-induced hyperemic responses to single brief contractions of the dorsiflexor muscles using near-infrared spectroscopy ( $HbO_2$ ). The contribution of the ANS was assessed via heart rate variability (high frequency and low frequency domains) using electrocardiographic recordings. IPC or sham conditioning were performed between Baseline and Post.  $HbO_2$  time-to-peak and  $HbO_2$  relative peak were increased for both groups and conditionings at Post and Post 1-hour compared to Baseline (all  $p$ 's  $< 0.001$  for both time-to-peak and relative peak). No change for the high frequency domain was found for both groups

and conditionings (all  $p$ 's  $\geq 0.065$ ). A significant increase in the low frequency domain was found for both groups and conditionings at Post and Post 1-hour (all  $p$ 's  $\leq 0.027$ ). Microvascular function was not affected acutely by IPC. Neither were there any differences found between smokers and non-smokers. IPC did not alter the balance of the ANS. Smokers and non-smokers did not exhibit different autonomic tone at any time. Inactivity seems to play a pivotal role in explaining the changes found in this study. In the future the intensity and duration of the physical demand should be considered when studying differences between smokers and non-smokers. Furthermore, the dosage of IPC seems to be important for achieving microvascular adaptations.

**Key words.** Cigarette smoking, contraction-induced hyperemia, vasodilation, heart rate variability, ischemia, endothelium-dysfunction.

## Introduction

Cigarette smoking has long been recognized as a major risk factor for developing cardiovascular impairments and diseases, thus being a main health threat and cause of death worldwide<sup>1</sup>. The negative effects of smoking can be detrimental and if not treated, they can lead to chronic diseases and worst-case scenario, death. Therefore, the need of finding a method to possibly reverse these effects seems attractive.

Cigarette smoking is associated with dose-related impairments of endothelium-dependent arterial dilation equivalent to endothelial dysfunction, even in young asymptomatic adults<sup>2</sup>. In continuation, cigarette smoking causes a reduction in nitric oxide (NO) generation, resulting in reduced endothelium-dependent vasodilation<sup>3</sup>. Release of the endothelial vasoactive substance NO together with prostaglandins is important in the development and maintenance of cardiovascular functions by controlling vascular smooth muscle tone<sup>4</sup>. Smooth

muscle tone regulates blood flow, which is well known to play an important role in order to meet variations in metabolic demands<sup>5</sup>.

The regulation of blood flow is additionally influenced by the autonomic nervous system (ANS). The ANS consists of sympathetic and parasympathetic nerve fibers and has either excitatory or inhibitory influences<sup>6</sup>. In regard to heart rate and blood vessel tone, an increase in sympathetic activity is known to result in increased heart rate and vasoconstriction, while an increase in parasympathetic activity result in decreased heart rate and vasodilation.

Cigarette smoking is shown to affect sympathetic activity by two pathways; particulate matter and nicotine<sup>6</sup>. The particulate matter of the cigarette smoke reduces the generation of endothelium derived NO locally in the smooth musculature, which leads to impaired baroreflex sensitivity, thereby reducing the ability to downregulate sympathetic activity<sup>2</sup>. Nicotine in cigarette smoke stimulates nicotinic receptors located in the medulla, which causes a decrease in the central production of NO and thereby allowing for an increased sympathetic activity<sup>6</sup>.

Different methods have been applied in order to counterbalance these effects of smoking, such as antioxidants<sup>7</sup> and exercise<sup>8,9</sup>. Antioxidants (vitamin A, C and E) have been used to reduce the concentration of reactive oxygen species (ROS) in order to increase the NO availability for maintaining normal vascular function<sup>7</sup>. The evidence of a beneficial effect of antioxidants unfortunately points to little or no benefit<sup>7</sup>. Exercise on the other hand is shown to increase the NO production<sup>8</sup> and is able to decrease sympathetic activity and increase parasympathetic activity through stimulation of baroreceptors<sup>9</sup>. However, not all smokers are capable of performing demanding exercise.

Research indicates that ischemic preconditioning (IPC) has positive effects on both NO<sup>10</sup> and the ANS<sup>11,12</sup>. IPC consists of multiple cyclical short (typically five minutes) periods of tissue ischemia by arterial restriction and subsequent reperfusion. IPC is a cheap and easily applicable non-invasive and minimal-demanding method which seemingly has no negative effects. The effects of IPC have been investigated in many different populations such as young healthy adults, healthy elderly or groups with different reduced functions or diseases, such as coronary artery disease<sup>10,13–15</sup>.

Positive effects on vascular function have been found when applying IPC. Kimura et al. (2007) tested the effect of repetitive IPC over a four-week period. Their protocol consisted of six daily conditionings of five minutes of occlusion and reperfusion. This resulted in augmented acetylcholine-induced endothelium-dependent vasodilation measured as an increase in forearm blood flow. This effect was suggested to be a result of an increase of endothelial vasoactive substance NO<sup>10</sup>. Further, is repetitive IPC shown to increase skin microcirculation after one week of daily IPC<sup>16</sup>. Following a single session of IPC, consisting of three cycles of occlusion and reperfusion. Enko et al. (2011) found increased parasympathetic activity through heart rate variability (HRV) analysis of electrocardiographic (ECG) recordings, reflected by a higher high frequency (HF) domain. This increase was accompanied by reduced vasomotor tone, reflected by an increase in arterial diameter acutely following three sessions of IPC<sup>11</sup>. Further, is capillary blood flow found to be increased after a single session of IPC<sup>17</sup>. The combined results of these studies may indicate that IPC is able to positively affect the endothelium-dependent vasodilation and the ANS activity.

Casey et al. (2013) suggested that the reduction in vasodilatory response to a contraction in healthy elderly compared to healthy young adults is partly due to a decreased NO bioavailability and a decreased ability to blunt sympathetic vasoconstriction<sup>18</sup>. These traits are similar to those of smokers<sup>6</sup>. Moro et al. (2011) found increased flow-mediated dilation among young healthy adults, healthy elderly and hypertensive elderly after three cycles of five minutes of occlusion followed by five minutes of reperfusion. A greater increase in flow-mediated dilation was found for the healthy elderly compared to the healthy young adults<sup>13</sup>, why a greater response to IPC among smokers compared to healthy may be found.

The response to a brief single muscle contraction is an often-used method to assess vascular function and can reflect the ability of the microvasculature to adapt to metabolic demands. The response is characterized by an immediate intensity dependent increase in blood flow and is thought to be a due to a simultaneous increase in vasodilation<sup>19</sup>. The exact mechanism responsible for this response is however unclear<sup>20</sup>. The method eliminates the intervening effects of prolonged and multiple contractions which allows unhindered assessment of the acute reactivity of the vasculature<sup>21,18</sup>. Another method used to evaluate

vascular function is the vascular occlusion test (VOT). The test takes advantage of the response following cuff release making it possible to assess the relative speed of re-oxygenation (slope 2). A direct way to assess this response in the microvasculature of the two methods is near-infrared spectroscopy (NIRS), a non-invasive methodology that has been used to assess the microvascular function of smokers and other populations<sup>22-25</sup>. With NIRS, it is possible to assess the local changes in hemodynamics by measuring local oxygen delivery and consumption reflected by level of oxyhemoglobin (HbO<sub>2</sub>)<sup>26</sup>, with HbO<sub>2</sub> reflecting the balance between both oxygen supply and consumption. With this method, microvascular function will be defined as the reactivity (time-to-peak) and peak response (relative peak).

The acute effect of IPC on microvascular function in smokers is yet to be elucidated, hence the aim of this study was to investigate the effect of ischemic preconditioning on microvascular function in young healthy smokers and non-smokers and elucidate the contribution of the ANS. Specifically, the following hypotheses were tested: 1) Improved microvascular function, measured as decreased HbO<sub>2</sub> time-to-peak and increased HbO<sub>2</sub> relative peak following ischemic preconditioning compared to baseline, and a greater improvement for smokers compared to healthy adults. 2) Increased parasympathetic activity, measured as a higher high frequency domain in the power spectral analysis of ECG, following ischemic preconditioning compared to baseline, and a greater increase for smokers compared to healthy adults.

## Methods

### Participants

Eleven healthy young males (mean  $\pm$  SD; age 25.00  $\pm$  2.73 years; height 1.81  $\pm$  0.03 m; weight 83.50  $\pm$  12.27 kg) and seven healthy asymptomatic young smoking males (age 26.00  $\pm$  2.04 years; height 1.92  $\pm$  0.06 m; weight 94.00  $\pm$  15.75 kg) volunteered to participate in the study. To participate in the study, the participants did not: 1) have any exposure to occlusion training or ischemic preconditioning seven days prior to participation, 2) perform intensive physically demanding activity 24 hours prior to participation, 3) suffer from sleep apnea, 4) have any disorders of the cardiovascular and microvascular system or in the extremities, 5) suffer from hyper- or hypotension, 6) use cardiovascular agents, sedatives or stimulants, 7) have a drug addiction or

dependency, 8) consume high oxidant nutrients or herbal/vitamin supplements 48 hours before each session, 9) consume caffeine 24 hours before each session, and further 10) did not smoke 8 hours before each session for the participants classified as smokers.

Participants answered the standard NHIS current smoking variable definition questionnaire to determine if they were current smokers<sup>27</sup>. The questionnaire was supplemented by a calculation of smoker degree by the pack-year equation; very light (0 to 4 pack-years), light (5 to 9 pack-years), or moderate (10 to 19 pack-years)<sup>2</sup>.

Prior to participation, the participants gave their written consent and were informed about possible discomforts. Ethical approval for the study was given by the Scientific Ethics Committee for Nordjylland (Reference No. N-20170081). The study was performed in accordance with the Declaration of Helsinki.

### Design and experimental protocol

A controlled counterbalanced crossover design was used to investigate the acute effect of ischemic preconditioning (IPC) on microvascular function and elucidate the contribution of the autonomic nervous system (ANS) as well as investigate how smoking affects this context.

In order to do so, participants went through two sessions with a 1-2-week washout period in between. Both sessions were performed at the same time of the day, starting in the morning between 7 and 9 am, with participants fasting for a minimum of 12 hours prior to participation. The participants were randomized to receive either IPC or sham conditioning at their first session and vice versa at the second session (Figure 1). Participants' weight and height were obtained at the beginning of the first session, as well as the calculation of degree of smoking.

The length of each session was approximately two and a half hours. During the experimental session, participants were positioned lying supine on a massage table. Each session consisted of three blocks; baseline, post- and post 1-hour measurements (Figure 1). The initial phase of 15 minutes before the measurement blocks involved the fitting of equipment, and time for the participants' heart rate to stabilize at resting levels while lying down. All measurement blocks included evaluation of the ANS by electrocardiography (ECG), followed by assessment of microvascular function through a contraction-induced hyperemia (CIH) test while measuring changes in muscle oxygenation using

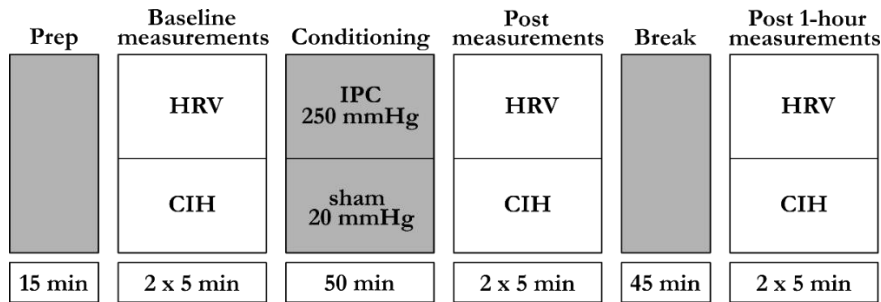


Figure 1. Experimental protocol with, HRV being heart rate variability, CIH being contraction-induced rapid hyperemia test and IPC being ischemic preconditioning.

near-infrared spectroscopy (NIRS). Between the baseline and post measurements, the participants received either IPC or sham conditioning. The post and post 1-hour measurements were separated by a 45-minute break. The participants were allowed to sit or stand upright during the break in order to avoid discomforts. The participants were instructed back into lying position for the last ten minutes of the break.

### Ischemic preconditioning (IPC) and sham conditioning

The conditioning protocol consisted of five cycles of alternating 5-minutes of cuff inflation and 5-minutes reperfusion<sup>28</sup>. The conditioning was performed with a tourniquet cuff (61 cm tourniquet cuff, VBM, Sulz am Neckar, DE) attached on the left thigh just above the knee joint (Figure 2). The conditioning was performed on the same extremity as microvascular function was measured, making it local IPC. The tourniquet cuff was inflated to 250 mmHg for the IPC and 20 mmHg for the sham conditioning. Participants were instructed not to move during the conditioning. The placement of the tourniquet cuff was marked with a semi-permanent marker and remained visible throughout the entire participation period.

### Near-infrared spectroscopy (NIRS)

Microvascular function was assessed using NIRS. The system consisted of an Oxymon system (Mk III, Artinis Medical Systems, Elst, NL). The Oxymon was setup with a probe and three lasers transmitting light in the near infrared spectrum. The lasers transmitted light in the wavelengths 764 and 860 nm. The probe was combined with a receiver optode with a source-detector distance of 40 mm. Data were sampled at 2 Hz. Before each session, the Oxymon system was warmed up for 10 minutes and calibrated.

The probe was placed on the skin covering the muscle belly of tibialis anterior of the left lower leg (Figure 2)<sup>24</sup>. The targeted area was shaved and disinfected before attaching the probe. Before attaching the probe with fixomull tape, the placement was marked with a semi-permanent marker and remained visible throughout the entire participation period.

### Vascular occlusion test (VOT)

The participants vascular occlusion test (VOT) was measured during the IPC protocol using NIRS. During the IPC protocol NIRS measured levels of Oxyhemoglobin (HbO<sub>2</sub>) and deoxyhemoglobin (HHb) and continuously calculated the tissue saturation index (TSI) which is defined as  $[HbO_2 / (HbO_2 + HHb)]$ . VOT was measured during reperfusion following 5 minutes of cuff occlusion. Five measurement points for each participant was conducted. The participants MVR was defined as the upgoing TSI slope (slope 2) which appeared immediately in the start of the reperfusion period<sup>29</sup>.

### Contraction-induced hyperemia (CIH)

Microvascular function of the participants was determined while performing a CIH test using a customized footplate. The footplate was connected to a force transducer (SSM-AJ-1000, Interface, Scottsdale, US) and force data were sampled at 500 Hz. Participants were placed lying supine on a massage table with the left foot placed in the footplate in a 120° angle of the ankle joint and affixed with a velcro strap (Figure 2). A pillow was placed below the knee for support. To test the microvascular function, the participants performed five brief (~1s) maximal isometric contractions of the dorsiflexors<sup>30,31</sup>, with a one-minute rest between each contraction. Participants were instructed to press the heel into the footplate while pulling the toes towards themselves as quickly as possible on the “go” signal and relax as quickly as possible on the “stop” signal.

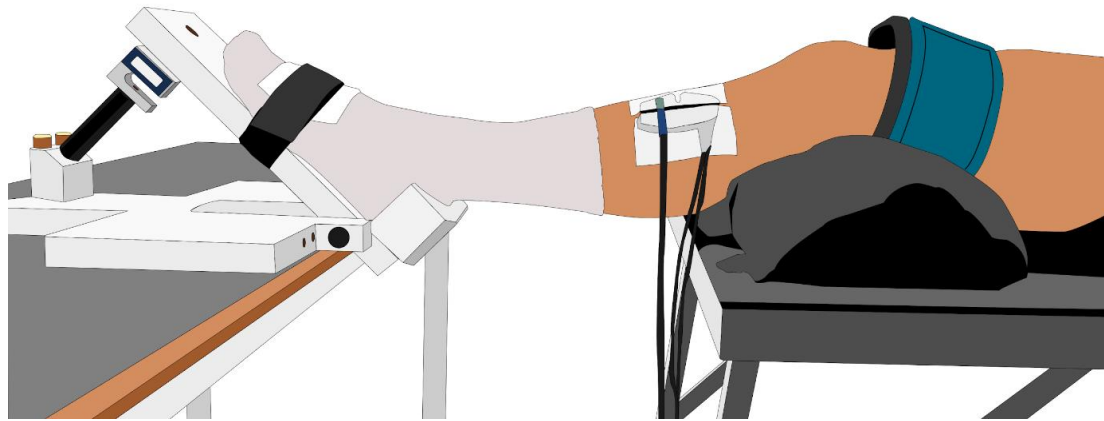


Figure 2. Illustration of the experimental setup with the specialized footplate, near-infrared spectroscopy probe attached to the tibialis anterior and the blood pressure cuff placed above the knee joint.

### Heart rate variability (HRV)

HRV was used to assess ANS activity. The HRV analyses were defined by ECG recordings, via a shimmer system (Shimmer, Dublin, IE) using Consensus software (v1.50, Shimmers, Dublin, IE) with a sampling frequency of 512 Hz. The shimmer system consisted of a shimmer with five leads connected to electrodes (Neuroline 720, Ambu, Copenhagen, DK). The shimmer was applied around the torso just beneath the chest with a belt strap. The electrodes were placed in accordance with the recommendations from the Shimmer company<sup>32</sup>. Two electrodes were placed on the clavicle, one on right and one on the left. The next two were placed on the anterior superior iliac spine of the pelvis, likewise one each on the right and left side. The last electrode was placed at V<sub>5</sub> between the 6th and 7th rib on the left side. The ECG were recorded for five minutes during each of the baseline, post, and post 1-hour measurements and the participants were instructed to lie completely still during the recordings. The electrode placements were marked with a semi-permanent marker and remained visible throughout the entire participation period.

### Data analysis

#### Contraction-induced hyperemia (CIH)

The data recorded with NIRS during the CIH test were processed using Matlab (R2018b, Mathworks, Natick, US). For analysis, time-to-peak and relative peak were identified for oxyhemoglobin (HbO<sub>2</sub>), Total hemoglobin (THb) and tissue saturation index (TSI) during each contraction.

Relative peak and time-to-peak was determined by defining background and identifying the absolute peak (Figure 3). Background was defined using the mean values of the period five seconds before contraction until the start of the contraction. Absolute peak was defined by identifying the local maximum in the period from start to 30 seconds following contraction. Time-to-peak was defined as the elapsed time between start contraction and the identified absolute peak.

The binary force transducer data, collected during the CIH test, were converted into newton. Hereafter, maximal force, force duration and force area under curve (AUC) were defined for each contraction. Force background was defined as the beginning four seconds (2000 values) of the collected force data before the contraction (Figure 4). Additionally, the standard deviation of background was calculated for later use. Maximal force was defined as local maximum in the contraction period. The force onset of the contraction was determined by using the defined analysis window, spanning from one second before until the point of maximal force. The start of contraction was defined as the point where the force surpassed the background plus ten Standard deviations. The end of contraction was defined as the time when the force was below 10% of the maximal force. The duration of the contraction was defined as the time elapsed from start to the end of the contraction.

Means for HbO<sub>2</sub>, THb, TSI, maximal force, force duration and force AUC of the five contractions of each CIH block respectively, were used for the statistical analysis

## Ischemic Preconditioning and Microvascular Function in smokers and non-smokers

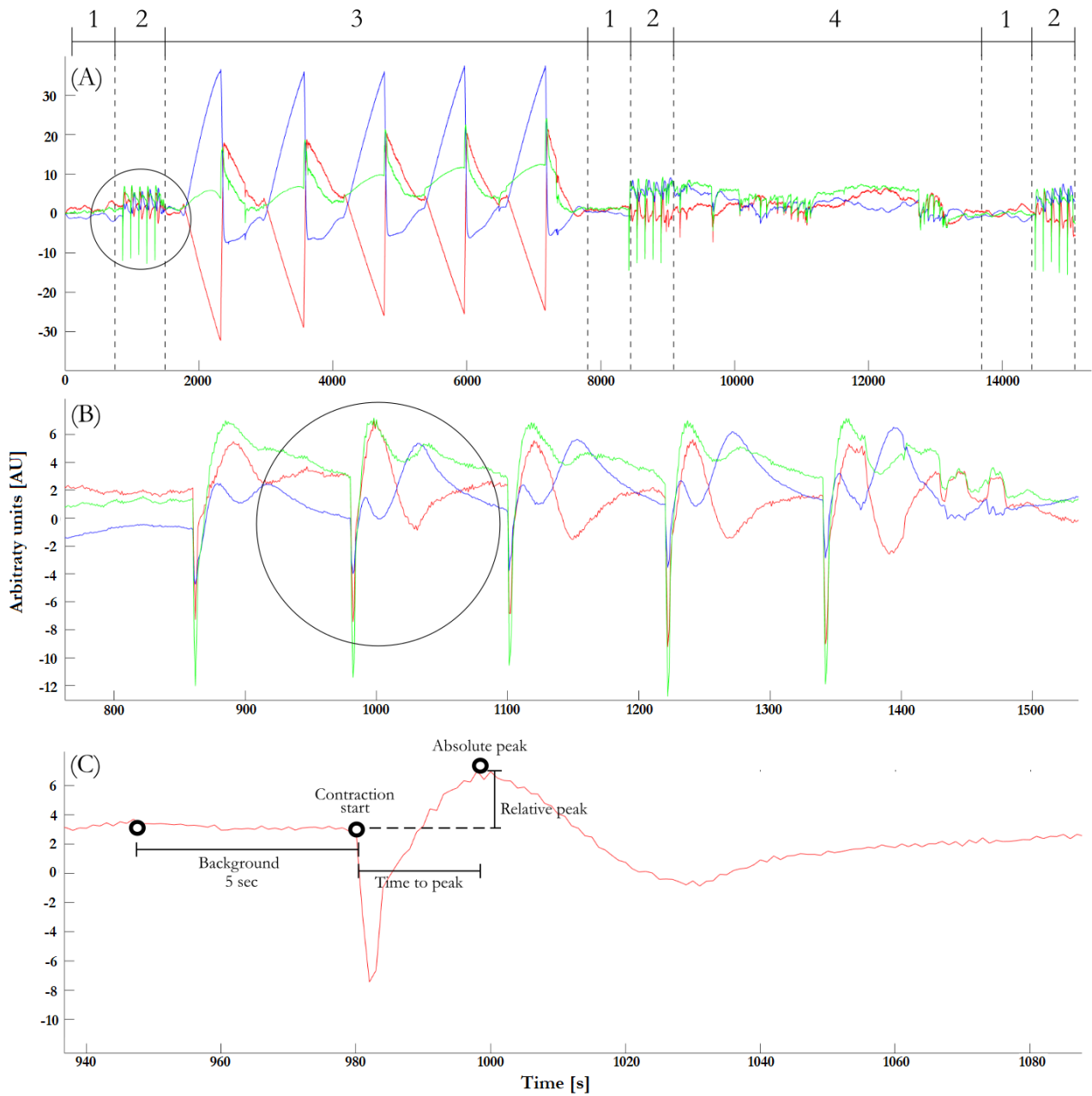


Figure 3. Illustrations of near-infrared spectroscopy (NIRS) data from a single random participant. The green lines represent total hemoglobin (THb), red lines represent oxyhemoglobin (HbO<sub>2</sub>), and blue represent deoxyhemoglobin (HHb). (A) Representation of an experimental session, plotting the entire time lapse. With 1 being electrocardiography recordings, 2 being the contraction-induced hyperemia (CIH) test, 3 being the ischemic preconditioning (IPC) or sham conditioning (an IPC session is plotted in this example), and 4 being the 45-minute break. (B) Representation of the five contractions during the CIH test. (C) Representation of a single contraction of the CIH test and how data were analyzed for HbO<sub>2</sub>.

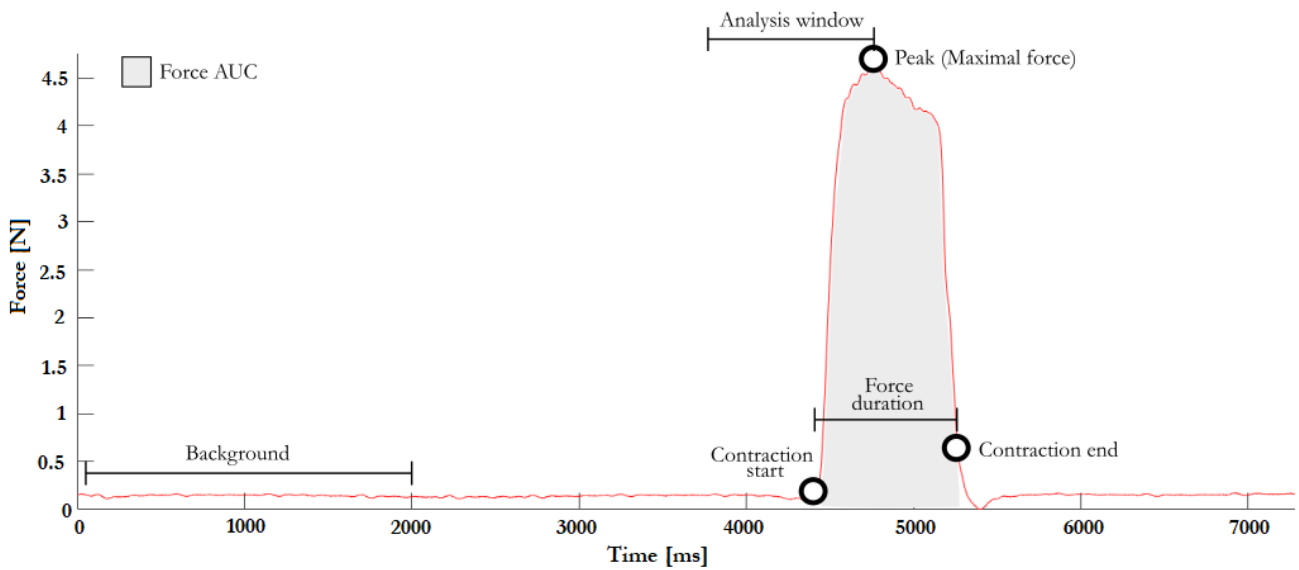


Figure 4. Illustration of force data from a single random contraction and how data were analyzed.

### Vascular occlusion test (VOT)

The data recorded with NIRS during the IPC were processed using Matlab (R2018b, Mathworks, Natick, US) To determine the rate of reperfusion (our measure of microvascular responsiveness) the upgoing slope appearing immediately after cuff deflation was identified (Figure 5). The beginning of the slope was defined as the lowest point prior to cuff release. The first 10 seconds of the following reperfusion period was then used to calculate the rate of reperfusion. The rate of reperfusion was defined as:  $\frac{TSI_{10seconds} - TSI_{minimum}}{10\ seconds}$

Absolute numbers of TSI during each IPC protocol cycle respectively, were used for the statistical analysis.

### Heart rate variability (HRV)

All ECG data were processed using Matlab (R2018b, Mathworks, Natick, US) and later analyzed using Kubius HRV (3.1, Kubius, Kuopio, FI). ECG data were recorded during the entire experimental session. The defined 5-minute HRV measurement periods (baseline, post and post 1-hour) were defined using noted times and by visually comparing the ECG data to the time-synched NIRS data graphs. R-R intervals

were then calculated for every HRV period respectively using the Pan Tomkins Matlab application<sup>33</sup>. Artifact correction was initially made in Matlab and was executed by removing misplaced r-waves by visual assessment. If the correction in Matlab was not sufficient, a threshold, varying between very low (0.45 s) to medium (0.25 s), was applied. The thresholds used were defined according to the visual nature of the R-R interval tachogram. For example, the “very low” correction level will find all intervals that are larger/smaller than 0.25 seconds compared to the average locally. The correction replaces the artifacts with values that are interpolated using a cubic spline interpolation<sup>34</sup>. For analysis, mean heart rate during the HRV-period was noted. For spectral estimation, the autoregressive (AR) model was used alternative to the fast fourier transform (FFT). AR was used as it is thought to be the appropriate use for short data sequences<sup>35</sup>. Using the AR values for low frequency domain (LF), the high frequency domain (HF) and the ratio (LF to HF) were noted. LF is defined in the interval from 0.04 to 0.15 Hz and HF is defined in the interval from 0.15 to 0.4 Hz.

## Ischemic Preconditioning and Microvascular Function in smokers and non-smokers

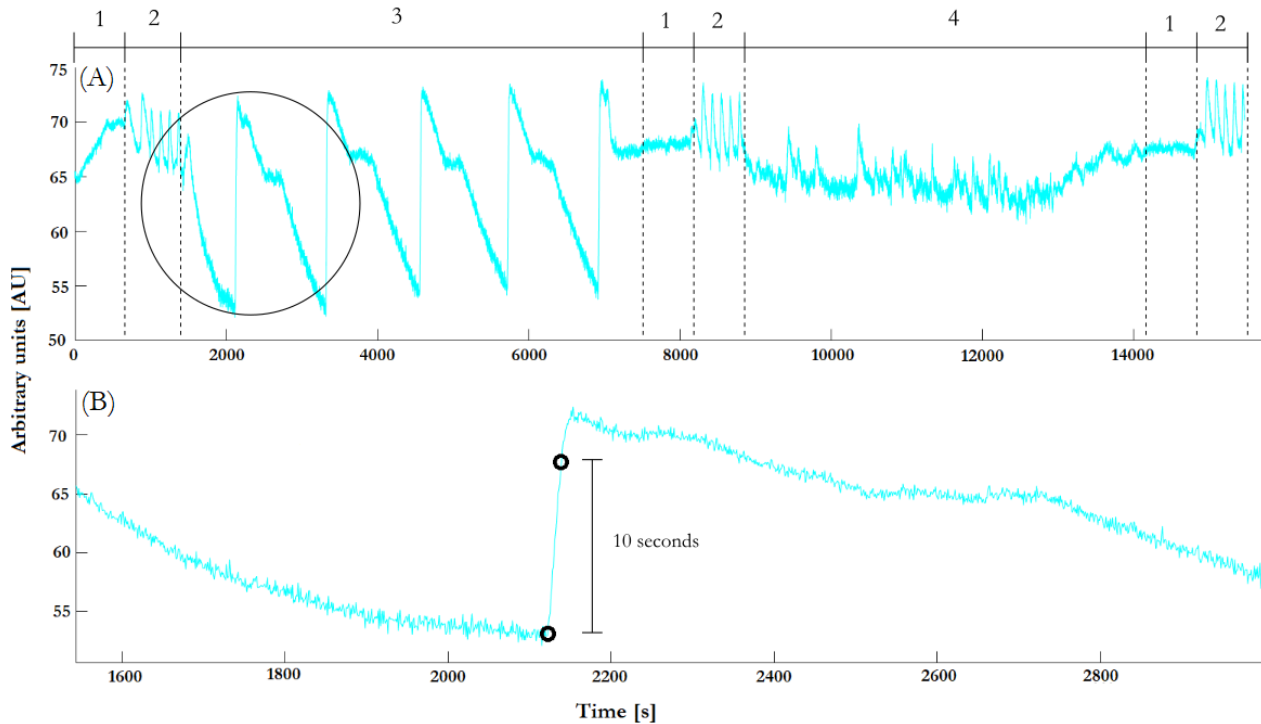


Figure 5. Illustrations of tissue saturation index (TSI) from near-infrared spectroscopy (NIRS) data from a single random participant. (A) Representation of an experimental session, plotting the entire time lapse. With 1 being electrocardiography recordings, 2 being the contraction-induced hyperemia (CIH) test, 3 being the ischemic preconditioning (IPC) or sham conditioning (an IPC session is plotted in this example), and 4 being the 45-minute break. (B) Representation of a single slope 2 (after cuff release) and how data were analyzed.

### Statistics

Statistical analyses were conducted using SPSS (v.25, IBM, USA). Between group differences regarding age, height and weight were assessed using independent-samples t-tests. For all parameters, including CIH (HbO<sub>2</sub>, maximal force, force duration and force AUC) and HRV (heart rate, LF, HF and LF:HF ratio), separate three-way mixed model analyses of variance (ANOVAs) were used to compare means with one between-subjects factor of group (non-smokers, smokers) and two within-subjects factors of time (Baseline, Post, Post 1-hour) and condition (IPC, sham conditioning). If a three-way interaction was present, two-way mixed model ANOVAs were conducted for each condition separately with group and time as the factors. To

### Results

#### Participants

No participants were excluded throughout the experimental period. For the analysis of the contraction-induced hyperemia (CIH) test, as well as for the force analysis, all data for the participants were included. Data from one participant from the non-smoker group was not analyzed for the vascular

correct for multiple two-way mixed ANOVAs, a Bonferroni correction was applied. If any two-way interactions or main effects were found, a Bonferroni post-hoc test was used to compare group, time and conditioning differences, depending on the location of the interaction or main effect. For VOT, a two-way repeated measures analyses of variance (ANOVAs) was used to compare means of within subjects' factors of time (Baseline, Post, Post 1-hour) and group (smokers, non-smokers). If a two-way interaction or main effects were found, Bonferroni post-hoc tests were used to compare time and condition differences. The level of significance was set as  $p \leq 0.05$ . If the assumption of sphericity was not met, Greenhouse-Geisser corrected degrees of freedom were used. Unless otherwise stated, all data are presented as means  $\pm$  standard deviation (SD) occlusion test (VOT) due to inadequate data, resulting in data from 17 participants being included in the analysis. Data from two participants from the non-smoker group were not analyzed for the heart rate variability (HRV) test due to inadequate data sampling, resulting in data from 16 participants being included in the analysis. No significant differences were found comparing age ( $T_{16} = 0.5, p = 0.627$ ) and

Table 1. Characteristics of participants.

Characteristics	Non-smokers (n = 11)	Smokers (n = 7)	Group differences (p-value)
Age (years)	25.00 ± 2.73	26.00 ± 2.04	$p = 0.627$
Height (m)	1.81 ± 0.03	1.92 ± 0.06	$p = 0.002$
Weight (kg)	83.50 ± 12.27	94.00 ± 15.75	$p = 0.347$
Pack-years		4.00 ± 1.84	

Values represent means ± SD.

weight ( $T_{16} = 0.1$ ,  $p = 0.347$ ) between the smoking and non-smoking group, although a significant difference was found comparing height ( $T_{16} = 3.8$ ,  $p = 0.002$ ) with the smokers being taller than the non-smokers (Table 1). All sessions were performed according to the protocol.

#### Contraction-induced hyperemia (CIH)

*Oxyhemoglobin (HbO<sub>2</sub>) time to peak (TTP)*. Figure 6A and B shows no significant three-way interaction between conditioning, time and group was identified ( $F_{2,32} = 1.0$ ,  $p = 0.383$ ). A significant interaction between time and group was identified ( $F_{2,32} = 4.1$ ,  $p = 0.026$ ). Despite the significant interaction, a post-hoc simple main effects analysis revealed no significant pairwise differences in HbO<sub>2</sub> time-to-peak between the smoking and non-smoking groups for either the Baseline, Post, or Post 1-hour measurements across both conditions (all  $p$ 's > 0.171). No significant interaction between conditioning and group was identified ( $F_{1,16} = 2.6$ ,  $p = 0.125$ ). No significant interaction between time and conditioning was identified ( $F_{2,32} = 1.3$ ,  $p = 0.278$ ). A significant main effect was identified for

time ( $F_{2,32} = 10.4$ ,  $p < 0.001$ ). A post-hoc analysis revealed that, regardless of group and conditioning, Baseline was lower compared to Post ( $p < 0.001$ ) and Post 1-hour ( $p < 0.001$ ). No significant main effect was identified for conditioning ( $F_{1,16} = 1.9$ ,  $p = 0.183$ ).

*Oxyhemoglobin (HbO<sub>2</sub>) relative peak*. Figure 6C and D shows no significant three-way interaction between conditioning, time and group was identified ( $F_{2,32} = 2.7$ ,  $p = 0.080$ ). No significant interaction between time and group was identified ( $F_{2,32} = 3.0$ ,  $p = 0.064$ ). No significant interaction between conditioning and group was identified ( $F_{1,16} = 0.0$ ,  $p = 0.841$ ). No significant interaction between time and conditioning was identified ( $F_{2,32} = 0.1$ ,  $p = 0.880$ ). A significant main effect was identified for time ( $F_{2,32} = 26.7$ ,  $p < 0.001$ ). A post-hoc analysis revealed that, regardless of group and conditioning, Baseline was lower compared to Post ( $p < 0.001$ ) and Post 1-hour ( $p < 0.001$ ). No significant main effect was identified for conditioning ( $F_{1,16} = 0.5$ ,  $p = 0.513$ ).

## Ischemic Preconditioning and Microvascular Function in smokers and non-smokers

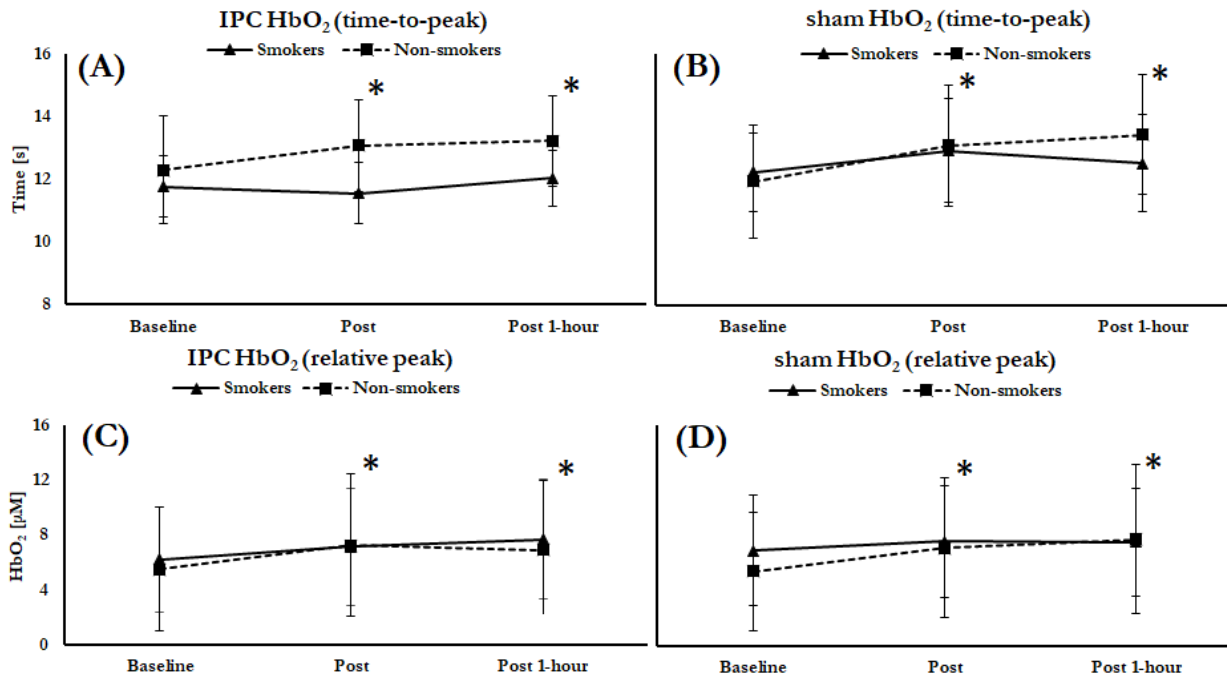


Figure 6. Comparison of mean time-to-peak ( $\pm$  SD) for HbO<sub>2</sub> [A, B]. Comparison of mean relative peak ( $\pm$  SD) for HbO<sub>2</sub> [C, D]. \* shows significant difference from Baseline for both smokers and non-smokers [A, B, C, D].

**Maximal force.** Figure 7A and B shows no significant three-way interaction between conditioning, time and group was identified ( $F_{2,32} = 0.6, p = 0.548$ ). No significant interaction between time and group was identified ( $F_{2,32} = 0.8, p = 0.474$ ). No significant interaction between conditioning and group was identified ( $F_{1,16} = 0.0, p = 0.873$ ). A significant interaction between time and conditioning was identified ( $F_{2,32} = 4.3, p = 0.023$ ). Despite the significant interaction, a post-hoc simple main effects analysis revealed no significant pairwise differences in maximal force between the IPC and sham conditioning for either the Baseline, Post, or Post 1-hour measurements across both groups (all  $p$ 's > 0.055). No significant main effect was identified for time ( $F_{2,32} = 0.5, p = 0.628$ ). No significant main effect was identified for conditioning ( $F_{1,16} = 0.4, p = 0.549$ ).

**Force duration.** Figure 7C and D shows no significant three-way interaction between conditioning, time and group was identified ( $F_{2,32} = 3.0, p = 0.064$ ). No significant interaction between

time and group was identified ( $F_{2,32} = 0.1, p = 0.872$ ). No significant interaction between conditioning and group was identified ( $F_{1,16} = 1.0, p = 0.332$ ). No significant interaction between time and conditioning was identified ( $F_{1,422,22,748} = 1.5, p = 0.245$ ). A significant main effect was identified for time ( $F_{2,32} = 3.6, p = 0.039$ ). A post-hoc analysis revealed no main effect (all  $p$ 's > 0.119). No significant main effect was identified for conditioning ( $F_{1,16} = 1.9, p = 0.187$ ).

**Force area under curve.** Figure 7E and F shows no significant three-way interaction between conditioning, time and group was identified ( $F_{2,32} = 0.8, p = 0.466$ ). No significant interaction between time and group was identified ( $F_{2,32} = 0.7, p = 0.489$ ). No significant interaction between conditioning and group was identified ( $F_{1,16} = 0.8, p = 0.392$ ). No significant interaction between time and conditioning was identified ( $F_{2,32} = 1.4, p = 0.268$ ). No significant main effect was identified for time ( $F_{1,503,24,041} = 0.1, p = 0.844$ ). No significant main effect was identified for conditioning ( $F_{1,16} = 1.7, p = 0.212$ ).

## Ischemic Preconditioning and Microvascular Function in smokers and non-smokers

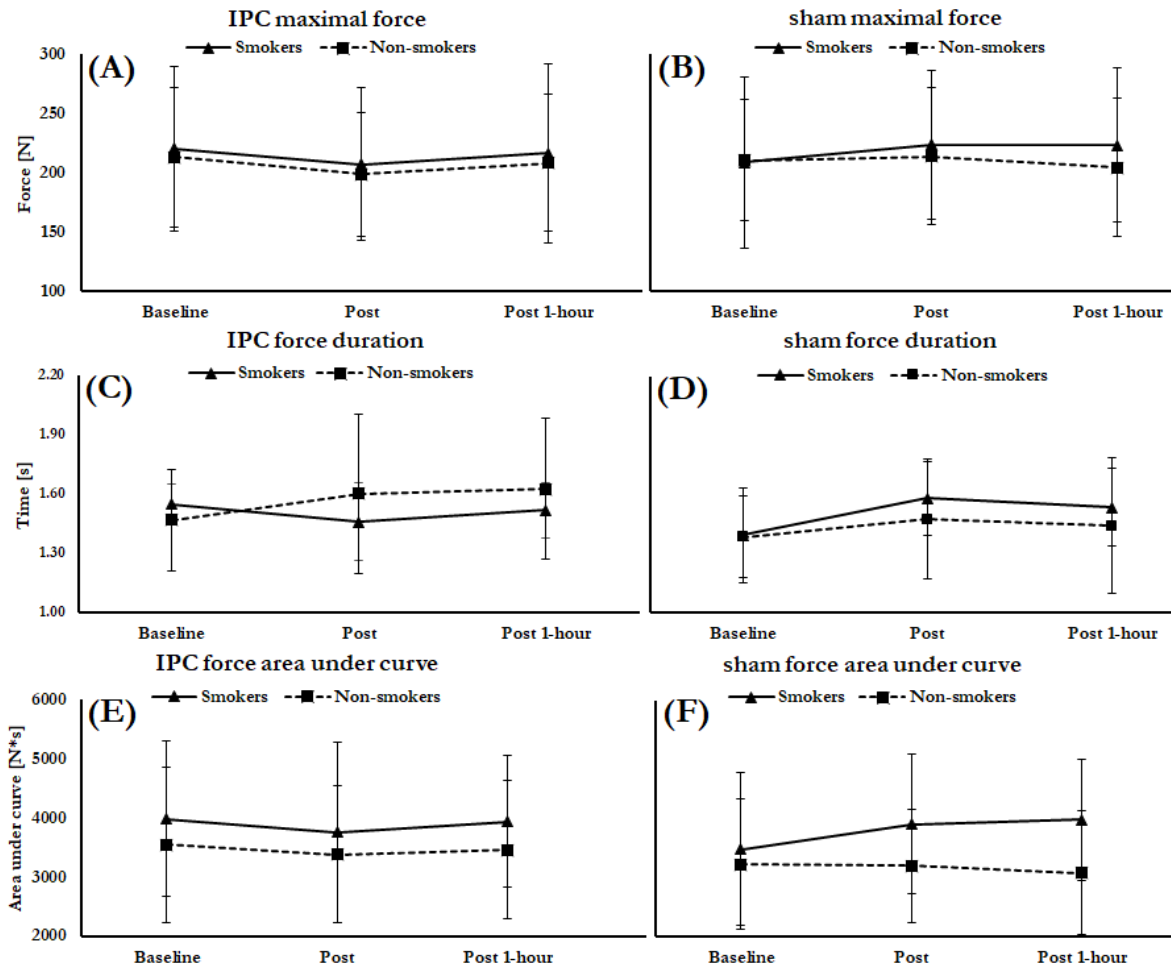


Figure 7. Comparison of mean maximal force [A, B], mean duration [C, D] and mean AUC [E, F] ( $\pm$  SD).

### Vascular occlusion test (VOT)

Figure 8 shows no significant two-way interaction between time and group was identified ( $F_{4,60} = 1.2, p = 0.305$ ). No significant main effect was identified for time ( $F_{2,223,33,347} = 2.3, p = 0.108$ ).

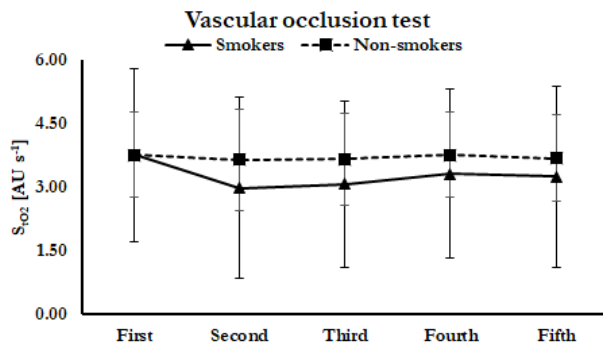


Figure 8. Comparison of the effect of IPC on microvascular responsiveness after cuff release between smokers and non-smokers for all the cuff releases (first-fifth).

### Heart rate variability (HRV)

**Heart rate.** Figure 9A and B shows no significant three-way interaction between conditioning, time and group was identified ( $F_{2,28} = 1.2, p = 0.315$ ). A significant interaction between time and group was identified ( $F_{2,28} = 5.7, p = 0.009$ ). Despite the significant interaction, a post-hoc simple main effects analysis revealed no significant pairwise differences in heart rate between the smoking and non-smoking groups for either the Baseline, Post, or Post 1-hour measurements across both conditions (all  $p$ 's > 0.562). No significant interaction between conditioning and group was identified ( $F_{1,14} = 3.0, p = 0.101$ ). No significant interaction between time and conditioning was identified ( $F_{1,156,16,181} = 0.1, p = 0.784$ ). A significant main effect was identified for time ( $F_{1,435,20,093} = 24.0, p < 0.001$ ). A post-hoc analysis revealed, that regardless of group and conditioning, Baseline was higher compared to Post ( $p = 0.001$ ) and Post 1-hour ( $p < 0.001$ ). No

## Ischemic Preconditioning and Microvascular Function in smokers and non-smokers

significant main effect was identified for conditioning ( $F_{1,14} = 0.4, p = 0.538$ ).

*Low frequency domain (LF).* Figure 9C and D shows no significant three-way interaction between conditioning, time and group was identified ( $F_{2,28} = 2.9, p = 0.072$ ). No significant interaction between time and group was identified ( $F_{2,28} = 0.5, p = 0.695$ ). No significant interaction between conditioning and group was identified ( $F_{1,14} = 1.2, p = 0.296$ ). No significant interaction between time and conditioning was identified ( $F_{2,28} = 1.6, p = 0.211$ ). A significant main effect was identified for time ( $F_{1,353,18,947} = 6.4, p = 0.014$ ). A post-hoc analysis revealed, that regardless of group and conditioning, Baseline was lower compared to Post ( $p = 0.001$ ) and Post 1-hour ( $p = 0.027$ ). No significant main effect was identified for conditioning ( $F_{1,14} = 1.2, p = 0.296$ ).

*High frequency domain (HF).* Figure 9E and F shows no significant three-way interaction between conditioning, time and group was identified ( $F_{2,28} = 0.4, p = 0.711$ ). No significant interaction between

time and group was identified ( $F_{2,28} = 0.4, p = 0.652$ ). No significant interaction between conditioning and group was identified ( $F_{1,14} = 0.8, p = 0.399$ ). No significant interaction between time and conditioning was identified ( $F_{2,28} = 2.0, p = 0.150$ ). No significant main effect was identified for time ( $F_{2,28} = 3.0, p = 0.065$ ). No significant main effect was identified for conditioning ( $F_{1,14} = 0.7, p = 0.412$ ).

*Ratio (low frequency domain to high frequency domain).* Figure 9G and H shows no significant three-way interaction between conditioning, time and group was identified ( $F_{2,28} = 0.5, p = 0.591$ ). No significant interaction between time and group was identified ( $F_{2,28} = 2.2, p = 0.132$ ). No significant interaction between conditioning and group was identified ( $F_{1,14} = 0.9, p = 0.370$ ). No significant interaction between time and conditioning was identified ( $F_{2,28} = 0.5, p = 0.612$ ). No significant main effect was identified for time ( $F_{2,28} = 1.2, p = 0.320$ ). No significant main effect was identified for conditioning ( $F_{1,14} = 2.5, p = 0.137$ ).

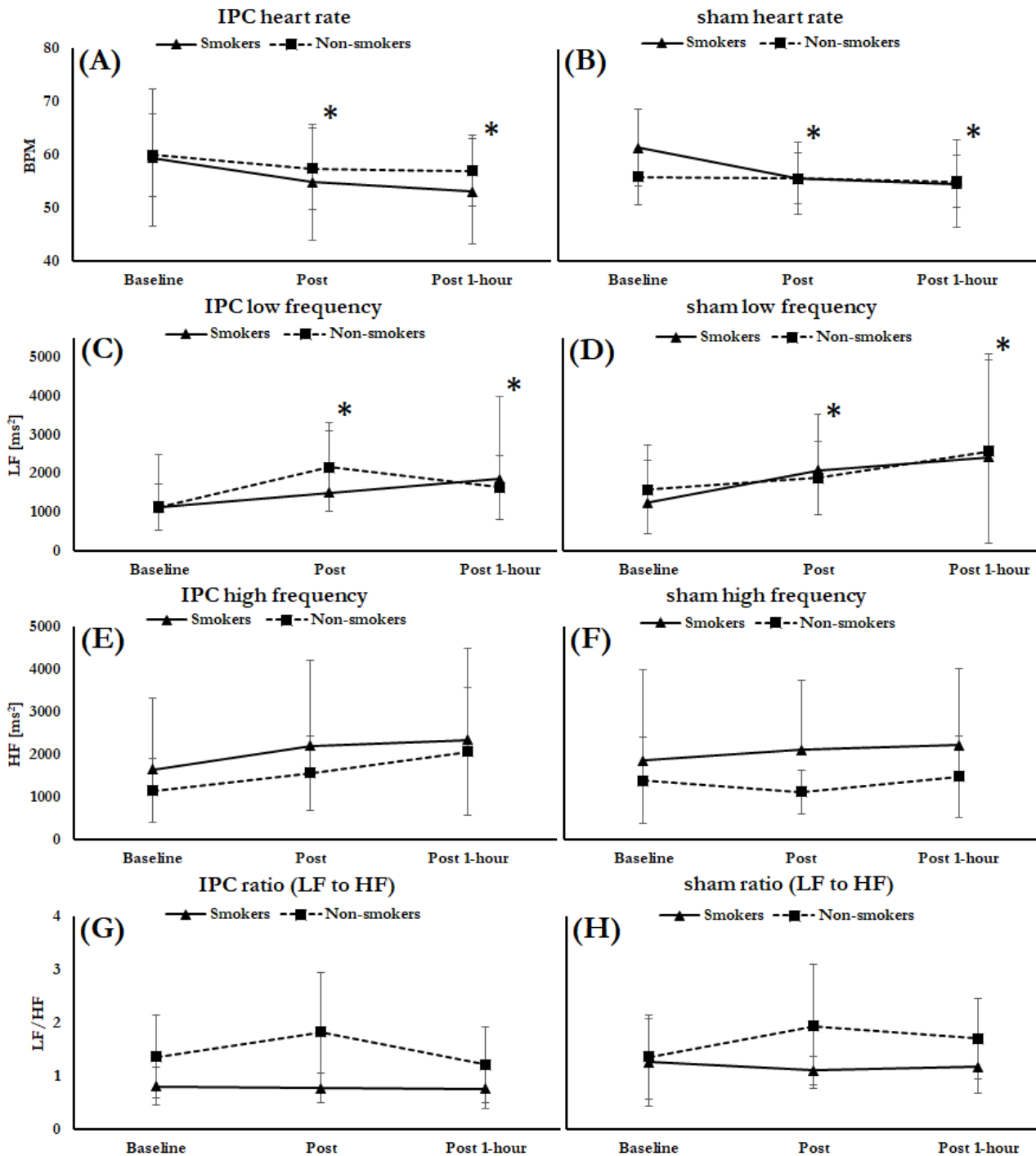


Figure 9. Comparison of mean heart rate ( $\pm$  SD) [A, B]. Comparison of mean HRV ( $\pm$  SD) for LF and HF respectively [C, D, E, F]. Comparison of ratio (LF to HF) ( $\pm$  SD) [G, H]. \* shows significant difference from Baseline for both smokers and non-smokers [A, B, C, D].

## Discussion

The aim of this study was to investigate the effect of ischemic preconditioning (IPC) on microvascular function and elucidate the contribution of the autonomic nervous system (ANS) on possible changes in sympathetic and

parasympathetic activity in healthy young smokers and non-smokers. The main findings of this study were that oxyhemoglobin (HbO<sub>2</sub>) time-to-peak and relative peak were significantly increased from Baseline to Post and Post 1-hour for both IPC and sham conditioning. These findings contradict with

the first hypothesis that microvascular function would improve through faster time-to-peak and increased relative peak in HbO<sub>2</sub> following IPC and the smokers would have greater improvement, as the increases were found across both groups and conditionings. The invalidation of the first hypothesis was supported by the results of the vascular occlusion test (VOT), as no significant changes were identified. This indicates that the IPC had no effect on microvascular function. In opposition to the second hypothesis that parasympathetic activity (higher HF domain) would increase following IPC and the smokers would have greater improvement, no increase in the high frequency domain (HF) for the IPC was found compared to the sham conditioning across both groups. However, a significant increase in the low frequency domain (LF), for both groups and conditionings, was identified at Post and Post 1-hour compared to baseline.

*Microvascular function.* In this study, a decreased time-to-peak parameter was interpreted to reflect a greater microvascular reactivity. Contrary to our first hypothesis, we found an increase in time-to-peak for HbO<sub>2</sub> (Figure 6A and B), which at first glance would seem to reflect that the microvascular reactivity was impaired for smokers and non-smokers. These findings were present for both the IPC and sham conditioning, which is why another explanation other than IPC as the cause of the impaired microvascular reactivity is needed.

During the protocol, the only shared state across both sessions, is the relatively long period of inactivity, which has been shown to affect vascular function<sup>36,37</sup>. Lewis et al. (2017) found that flow mediated dilation and blood flow peak velocity were both decreased in the brachial artery after being supine for 30 minutes<sup>36</sup>. Vranish et al. (2018) investigated the effect of inactivity on both the macrovascular function with FMD and the microvascular function with hyperemic response<sup>37</sup>. They found that microvascular function was more exposed to the effects of inactivity as they found that a mere amount of 10 minutes of sitting was enough to impair the hyperemic response in the leg to five minutes of occlusion. This was only present in the microvasculature, as no changes in FMD were found<sup>37</sup>. The participants of this study were supine for more than 60 minutes at a time, which might explain the increased time-to-peak in HbO<sub>2</sub> across both groups and conditionings.

Regarding the same hypothesis, an increase in relative peak for HbO<sub>2</sub> would reflect an enhanced O<sub>2</sub>

delivery compared to O<sub>2</sub> consumption<sup>26</sup>. An increase from Baseline to Post and Post 1-hour was present (Figure 6C and D), though for both groups and conditionings which invalidates the hypothesis. The larger relative peak may suggest an enhanced delivery of O<sub>2</sub> during the Post and Post 1-hour measurements, which could indicate a larger workload during the CIH test.

The CIH test is highly intensity dependent, which has been established by many<sup>38,18,21</sup>, and the increased response is due to an increase in the acute metabolic demand in the contracting muscle<sup>18</sup>. In order to determine whether the increase in O<sub>2</sub> delivery was due to a larger workload during the CIH test, force data were included. Maximal force reflects the intensity of the contractions and was used to determine whether the intensity differed during the CIH test. Credeur et al. (2015) demonstrated that force intensity is a large determinant for the vasodilatory response following a contraction<sup>38</sup>. The results for maximal force showed no differences between group, conditioning and time (Figure 7A and B). In extension, to certify the homogeneity of the contractions, force duration was analyzed. No differences were found in force duration between group, conditioning and time (Figure 7C and D), which clarifies the similarity between contractions. Force area under the (AUC) is the direct expression of the workload performed during the CIH test. The results of force AUC showed no differences between group, conditioning, and time (Figure 7E and F), hence supporting the conclusion that workload was not different at the Baseline, Post and Post 1-hour measurements. The increase in HbO<sub>2</sub> relative peak is therefore not likely to be a result of larger workload, which is why it is more likely that the increase in HbO<sub>2</sub> at Post and Post 1-hour indicates a lower resting O<sub>2</sub> consumption. Jeffries et al. (2018) found that resting metabolism tended to be lower following one week of IPC<sup>39</sup>. However, this is only a possible explanation for the increase in HbO<sub>2</sub> following IPC and not sham conditioning, why further research to find the exact mechanism is needed.

To assess the effect of IPC on microvascular reactivity with another model than that of a single muscle contraction the VOT was included. The tissue saturation index (TSI) was used to evaluate the resaturation response following the five minutes of occlusion<sup>29</sup>. Because of the nature of the VOT, it was only possible to assess this during the IPC protocol for smokers and non-smokers and not for the sham conditioning. The result of the VOT showed no differences between the groups and no effect of time (Figure 8), which is why the effect of a single session

of IPC does not seem to be different in smokers compared to non-smokers on the microvascular function.

*Autonomic nervous system.* The second hypothesis that IPC would increase parasympathetic activity was based on the findings of Enko et al. (2011), who found increased parasympathetic activity, reflected by a higher HF domain. This increase was accompanied by reduced vasomotor tone, reflected by an increase in arterial diameter acutely following three sessions of IPC<sup>11</sup>. The smokers were expected to exhibit a greater increase compared to non-smokers, as smokers tend to have increased sympathetic activity<sup>2,6</sup>.

Contradictory to our hypothesis, no increase in the HF domain following IPC was found across both groups and conditionings (Figure 9E and F). However, an increase in the LF domain was present at Post and Post 1-hour compared to Baseline for both groups and conditionings (Figure 9C and D). An increase in LF would suggest higher sympathetic activity<sup>40</sup>. Hughes et al (2017) found that an increase in sympathetic activity can attenuate the contraction-induced vasodilatory response, shown evident by a decrease in blood flow and vascular conductance response<sup>41</sup>. In compliance with the NIRS measurements, time-to-peak for HbO<sub>2</sub> was prolonged at Post and Post 1-hour, reflecting an attenuated vasodilatory response. However, the increase in LF was present for both groups and conditionings, which is why the explanation behind the results might come from the relatively long period of inactivity.

Inactivity is not typically associated with an increase in sympathetic activity, but rather with an increase in parasympathetic activity. Increased parasympathetic activity could be reflected by a decrease in heart rate, which is present at Post and Post 1-hour across both groups and conditionings (Figure 9A and B). The heart rate is regulated by the ANS through the release of neurotransmitters, where sympathetic and parasympathetic activity increases and decreases the heart rate, respectively. It is proposed by some that the increase in LF domain could reflect an increase in parasympathetic and not only sympathetic activity<sup>42</sup>.

A change in the LF/HF ratio would be expected, if the alterations were caused by a change in autonomic tone. No changes in LF/HF ratio were found (Figure 9G and H), which raises the question as to whether the increase in the LF domain can be attributed to a factor other than an increased parasympathetic activity. HRV analysis is profoundly

dependable on heart rate, which is clarified by the name. This dependence can be a hindrance as the overall HVR spectral power is also highly affected by changes in heart rate. Tsuji (1996) exhibited a negative correlation between a rise in heart rate and the overall spectral power for both the LF and HF domain<sup>43</sup>. An increase in both LF and HF would therefore be expected if a fall in heart rate was present. However, only an increase in LF was present. The decrease in heart rate could consequently be the cause of the change in the LF domain. The sensitivity of the HRV model could also explain the results of Enko et al. (2011) as they, similar to this study, had a significant decrease in heart rate from Pre to Post measurements. This consideration should therefore be taken into account when interpreting the results of HRV in the future.

*Smokers.* It was expected that the smokers would exhibit a greater response to the IPC protocol for all tests of microvascular function. No differences, in any test, were found between the groups. This might propose that the smokers included in this study were not affected sufficiently by smoking to observe any differences between the groups. The smokers were defined as very light smokers (4.0 pack-years) which, according to a study by Celermajor et al. (1993), is associated with impairment of the endothelium-dependent arterial dilation<sup>2</sup>. The participants of the current study did, however, not exhibit any impairments compared to non-smokers in the tests conducted in this study.

Similar to this study, Findlay et al. (2013) found no differences in young healthy smokers (2.3 pack years) compared to healthy non-smokers assessed by FMD. However, when performing a 10-minute handgrip exercise, the smokers exhibited an impaired ability to adapt to increasing metabolic demands<sup>44</sup>. This suggests that the CIH test did not elicit sufficient stimuli to reveal possible microvascular impairments in the smoking group. Supporting this, Doonan et al. (2011) found that impairments in macrovascular function amongst young healthy smokers (2.9 pack-years) was not notable at rest. An impairment was found when evaluating the time following exercise to exhaustion<sup>45</sup>. This indicates that impairments in the macrovasculature in smokers are more detectable when testing in more demanding settings, which might also be evident in the microvasculature shown in this study.

The combined results of these studies suggest that the reason for no differences in any test between smokers and non-smokers in the current study was likely due to the participants being at rest.

Furthermore, the metabolic requirements of the microvascular function tests may not have been sufficient. Therefore, the effect of IPC cannot be dismissed if investigated using other methods.

*Methodological considerations.* To investigate microvascular function and the contribution of the ANS on young healthy smokers and non-smokers, NIRS and ECG were used, respectively. These methods are not able to provide any information regarding possible humoral mechanisms. Blood samples would enable a more detailed analysis of the effects of IPC. Blood samples were collected for the non-smokers and will be used in the future for a larger study but were not included in the analysis due to the time barrier of the current study.

Another relevant consideration is the dosage of IPC. The majority of IPC studies use a repetitive approach where the participants receive daily treatments for as much as up to a month<sup>39,10,16</sup>, whereas this study only implicates a single session of IPC. This design was chosen to investigate the minimum dosage, combined with a time barrier for the current study. Future studies should focus on investigating the effect of repetitive IPC on microvascular function on smokers and non-smokers.

*Conclusion.* Microvascular function was not affected acutely by ischemic preconditioning (IPC), as the same changes following conditioning in HbO<sub>2</sub> time-to-peak and HbO<sub>2</sub> relative peak were present for the IPC as in the sham conditioning. Neither were there any differences found between smokers and non-smokers. Additionally, no changes in resaturation response between smokers and non-smokers were found after cuff releases in the vascular occlusion test. IPC did not alter the balance of the autonomic nervous system, as the same changes were found for the IPC and sham conditioning. Smokers and non-smokers did not exhibit different autonomic tone at any time. Inactivity seems to play a pivotal role in the increased HbO<sub>2</sub> time-to-peak and increased HbO<sub>2</sub> relative peak, as it is the only common factor between both groups and conditionings. Furthermore, it seems to be of great importance to elicit a physical demand greater than that of a single contraction to demonstrate differences between healthy young smokers and non-smokers. Finally, using a repetitive IPC approach should be considered, as the dosage of IPC seems to be important for achieving microvascular adaptations.

### Conflicts of interest

The Authors have no conflicts of interest to declare.

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# The Acute Effect of Ischemic Preconditioning on Microvascular Function in Tibialis Anterior in Smokers and Non-smokers; Contributions of The Autonomic Nervous System

Arbejdsblad

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Idræt 10. Semester

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Arbejdsbladet er supplerende materiale til artiklen

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## 1. Litteratursøgning

Litteraturen til dette specialeprojekt blev søgt online på relevante databaser gennem Aalborg Universitet. Indsamlingen af litteraturen blev foretaget gennem en tredelt proces; indledende ustruktureret søgning indenfor interesseområdet, struktureret søgning via søgestrategien PICO og slutteligt en gennemgang af de benyttede artiklers referencelister via sneboldmetoden. Under PICO søgningen blev der taget udgangspunkt i P (problem), I (intervention) og C (kontekst). O (udfald) blev udeladt, da vi ønskede at få alt litteraturen indenfor området og ikke blot litteratur med et bestemt udfald.

Den indledende ustrukturerede søgning tog udgangspunkt i interessen for *iskæmisk prækonditionering og rygning*. Søgningen havde til formål at finde nuværende forskning indenfor området, da vi med viden fra vores tidligere projektet havde set potentiale i dette emne. Søgningen blev foretaget databasen Scopus med udgangspunkt i følgende keywords; *ischemic preconditioning, IPC, remote ischemic preconditioning, smooking, vascular dysfunction, autonomic nervous system*.

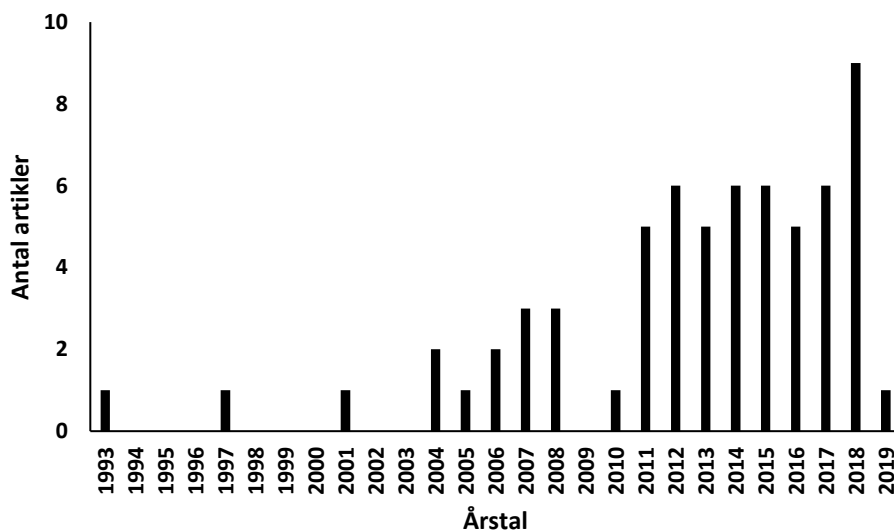
Via gennemlæsning af abstract fra de øverste relevante artikler på Scopus, fandt vi flere keywords som senere benyttes til den strukturerede PICO søgning:

<b>Problem</b>	<b>Intervention</b>	<b>Kontekst</b>
vascular function	Ischemic preconditioning	smoking
vascular dysfunction	IPC	cigarette smoke
microvascular function	ischemia	healthy
microvascular dysfunction	RIPC	young
vascular reactivity	remote ischemic preconditioning	recovery of function
microvascular reactivity	ischemic conditioning	NIRS
endothelial function	RLIPC	near-infrared spectroscopy
endothelial dysfunction	remote limb ischemic preconditioning	HRV
endothelial-dependent vasodilation	physical activity	heart rate variability
vasodilation	contraction-induced hyperemia	ANS
hyperemia	contraction-induced rapid vasodilation	autonomic nervous system
hyperemic response	FMD	sympathetic nervous system
	flow-mediated dilation	parasympathetic nervous system
	single contraction	sympathetic activity
	physical activity	parasympathetic activity
	exercise	

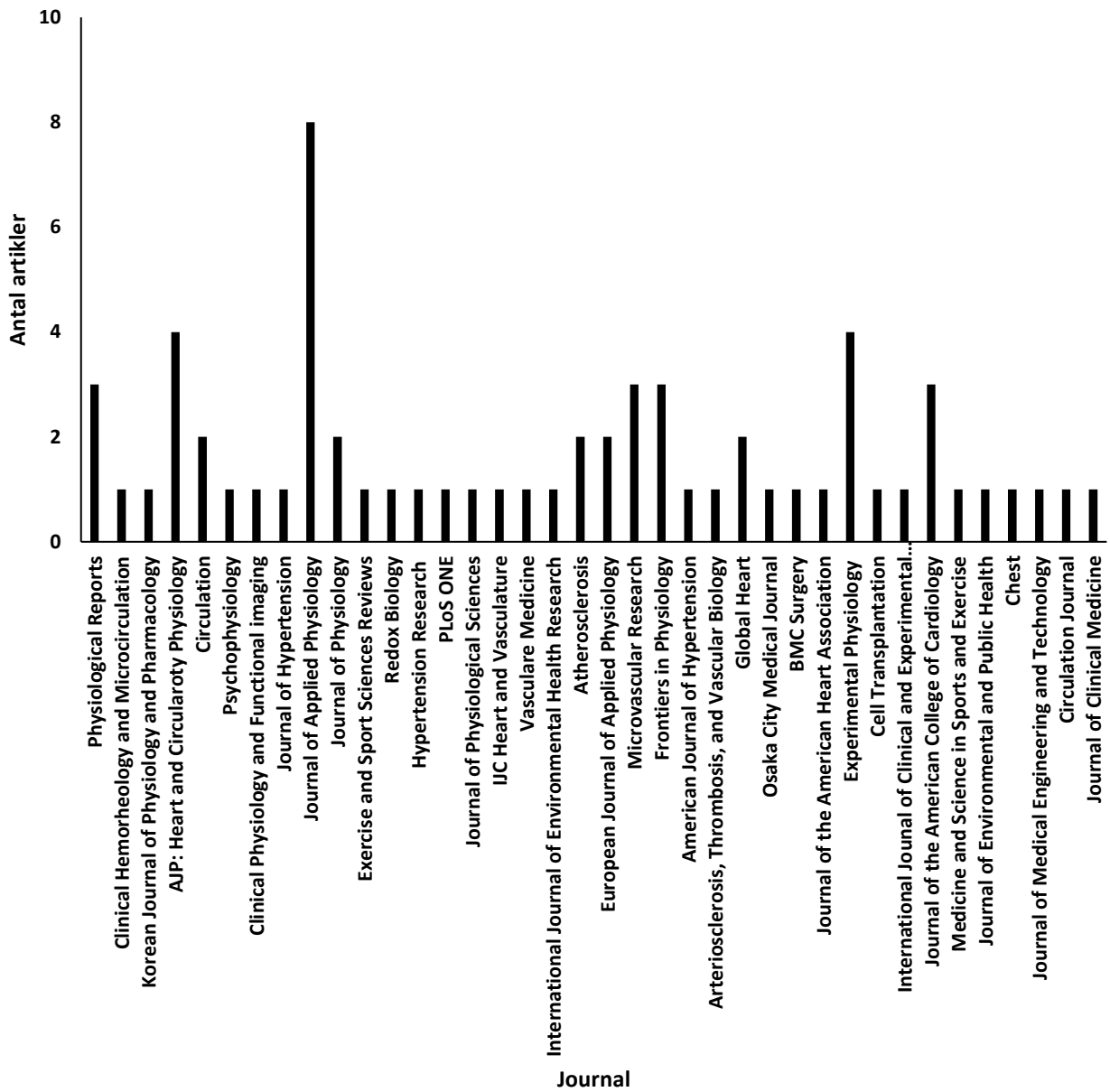
Følgende søgning blev foretaget på Scopus:

*(“vascular function” OR “vascular dysfunction” OR “microvascular function” OR “microvascular dysfunction” OR “vascular reactivity” OR “microvascular reactivity” OR “endothelial function” OR “endothelial dysfunction” OR “endothelial-dependent vasodilation” OR “vasodilation” OR “hyperemia” OR “hyperemic response”) AND (“Ischemic preconditioning” OR “IPC” OR “ischemia” OR “RIPC” OR “remote ischemic preconditioning” OR “ischemic conditioning” OR “RLIPC” OR “remote limb ischemic preconditioning” OR “physical activity” OR “contraction-induced hyperemia” OR “contraction-induced rapid vasodilation” OR “FMD” OR “flow-mediated dilation” OR “single contraction” OR “physical activity” OR “exercise”) AND (“smoking” OR “cigarette smoke” OR “healthy” OR “young” OR “recovery of function” OR “NIRS” OR “near-infrared spectroscopy” OR “HRV” OR “heart rate variability” OR “ANS” OR “autonomic nervous system” OR “sympathetic nervous system” OR “parasympathetic nervous system” OR “sympathetic activity” OR “parasympathetic activity”)*

Søgningen resulterede i 75.152 artikler, hvoraf titel og abstract blev gennemgået for de første 200 artikler efter sortering efter relevans. Heraf blev 55 artikler anset som værende relevante. Yderligere 64 artikler blev fundet via sneboldmetoden eller fri søgning på specifikke problematikker eller metoder. De 119 artikler blev gennemlæst og et review blev nedskrevet på artikler vi fandt relevante (jvf. Afsnit 2. Reviewtabeller).



Figur x. Frekvensanalyse af udgivelsesårstal for den gennemgåede relevante litteratur.



Figur x. Frekvensanalyse af udgivelsesjournal for den gennemgÅede relevante litteratur.

## 2. Reviewtabeller

Ordliste			
ANS	Autonomic nervous system	MC	Muscle compression
BOLD	Blood-oxygenation-level-dependent	NO	Nitric oxide
DOMS	Delayed onset muscle soreness	PAT	Pulse arterial tonometry
ECG	Electrocardiography	PRH	Peak reactive hyperemia
EDV	Endothelium-dependent vasodilatation	RIC	Remote ischemic conditioning
FMD	Flow mediated dilation	RIPC	Remote ischemic preconditioning
HRV	Heart rate variability	ROS	Reactive oxygen species
HF	High frequency	ROV	Rapid onset vasodilation
IR	Ischemia/reperfusion	SNS	Sympathetic nervous system
LF	Low frequency	VOT	Vascular occlusion test

Author, year, title			
Aim	Population	Methods	Conclusion/use
<b>Abdul-Ghani S., Fleishman A., Khaliulin I. et al., 2017</b> , Remote ischemic preconditioning triggers changes in autonomic nervous system activity: implications for cardioprotection			
Aims to identify cardiovascular changes associated with autonomic nervous system (ANS) activity during RIPC and prior to index ischemia.	Male mice.	RIPC (four cycles of 5 min inflation and 5 min deflation. ECG HRV (HF and LF) Heart rate blood flow	Higher heart rate increased sympathetic activity and decreased blood flow during RIPC. Provides possible explanations for the changes.
<b>Alhejily W., Aleksy A., Martin B.J. and Anderson t.j., 2014</b> , The effect of ischemia-reperfusion injury on measures of vascular function			
The objective of our study was to assess the effect of ischemia-reperfusion injury on microvascular function including peripheral arterial tonometry (PAT) hyperemic index	Men and Women free of vascular disease non-smoking	FMD - Ultrasound hyperemic velocity time integral (microvascular function) PAT	15 min I/R caused endothelial dysfunction in the macrovasculature but not the microvasculature.  There're differences between the macro- and microvasculature
<b>Aulakh A., Randhawa P. Singh N. et al., 2017</b> , Neurogenic pathways in remote ischemic preconditioning induced cardioprotection: Evidences and possible mechanisms			
The present review focuses on the potential involvement of neurogenic pathways in mediating remote ischemic preconditioning- induced cardioprotection.	Rats, mice, rabbits and humans	Involvement of neurogenic signaling in RIPC by blocking different signalers.  Different mechanism	RIPC induces the local release of chemical mediators that activate the sensory nerve endings and convey signals to the brain. Thereafter, the efferent nerve endings convey the cardioprotective signals the myocardium to induce cardioprotection.

Arbejdsblad

<b>Bailey, T. G., Birk, G. K., Cable, N. T., 2012, Remote ischemic preconditioning prevents reduction in brachial artery flow-mediated dilation after strenuous exercise</b>			
The aim of the present study was to examine the effect of RIPC on brachial artery endothelial function (measured as FMD) after strenuous running	healthy moderately trained men	FMD - ultrasound RIPC	a strenuous running bout is associated with a decrease in brachial artery endothelium-dependent function, which is prevented by RIPC of the lower limbs before strenuous running exercise. they found that an impairment in FMD can be altered in a positive direction by RIPC
<b>Barua R., Ambrose J., Eales-Reynolds L. et al., 2001, Dysfunctional endothelial nitric oxide biosynthesis in healthy smokers with impaired endothelium-dependent vasodilatation</b>			
Assessment of EDV in healthy smokers and nonsmokers.	Male healthy smokers and nonsmokers.	FMD of brachial artery. Serum (NO, eNOS)	Demonstrate an association between decreased NO production and reduced EDV. Cigarette smoking was associated with reduced EDV, NO generation, and eNOS activity.
<b>Berntson G., Bigger T., Eckberg D. 1997 et al., Heart rate variability: origins, methods, and interpretive caveats</b>			
			LF occurs in the interval from 0.04 to 0.15 Hz and reflects activity of both the sympathetic and parasympathetic parts of ANS
<b>Bond, B., Williams, C. A., Barker, A. R. et al., 2017, The reliability of a single protocol to determine endothelial, microvascular and autonomic functions in adolescents</b>			
This study determines the within-day reliability and between-day reliability of a single protocol to non-invasively assess these outcomes in adolescents.	12- to 15-year-old adolescents	FMD – ultrasound (macrovasculature) PRH -ultrasound (microvasculature) HRV	data indicate suitable within-day reliability and between-day reliability of these measures
<b>Carter S., Faulkner A., Rakobowchuk M. et al., 2014, The role of prostaglandin and antioxidant availability in recovery from forearm ischemia-reperfusion injury in humans</b>			
Assess the effect of ischemia-reperfusion injury on endothelial function and role of prostaglandins.	Healthy men and women	FMD - ultrasound. Ischemia-reperfusion injury (20 min upper arm inclusion) Vascular function (blood flow, blood velocity)	Results demonstrate ischemia–reperfusion injury causes endothelial dysfunction. The role of prostaglandins.
<b>Carter, J. B., Banister, E. W., Blaber, A. P., 2003, Effect of endurance exercise on autonomic control of heart rate</b>			
reviews the effect of endurance exercise on autonomic control of heart rate	male female old	HRV Heart rate	Endurance training increases HRV, increases parasympathetic activity and decreases sympathetic activity in the human heart at rest Athletes have a lower resting heart rate and a more rapid recovery of heart rate following exercise, due to enhanced parasympathetic activity produced by long-term endurance training.

Arbejdsblad

<p><b>Casey, D. P., Walker, B. G., Ranadive, S. M. et al., 2013</b> Contribution of nitric oxide in the contraction-induced rapid vasodilation in young and older adults.</p>			
<p>We tested the hypothesis that reduced nitric oxide (NO) bioavailability contributes to the attenuated peak and total vasodilation following single-muscle contractions</p>	<p>young and older people non-smokers</p>	<p>blood flow heart rate blood pressure single muscle contraction NO inhibitor</p>	<p>Nitric oxide (NO) is a fast-acting vasodilator substance that can be generated from both the endothelium and skeletal muscle cells during contraction. Therefore, NO is an appealing candidate that might explain some of the rapid vasodilator response following a single-muscle contraction.</p> <p>age-related impairments in contraction-induced rapid vasodilation are, in part, due to alterations in endothelial function and blunted NO signaling in healthy older adults. When</p> <p>NO bioavailability with aging may contribute to the blunted rapid vasodilation through one of two ways: 1) less direct vasodilation via cGMP-induced smooth muscle cell relaxation; and/or 2) decreased ability to blunt sympathetic vasoconstriction (e.g., functional sympatholysis)</p> <p>NO bioavailability might prove to be useful in reversing the age-related decline in muscle blood flow during exercise, particularly at the onset of muscle contractions.</p>
<p><b>Celermajer D., Sorensen K., Georgakopoulos D. et al., 1993</b>, Cigarette Smoking Is Associated with Dose-Related and Potentially Reversible Impairment of Endothelium-Dependent Dilatation in Healthy Young Adults</p>			
<p>Testing the hypothesis that endothelial dysfunction might be present in smokers and dose dependency. Further, if it-is reversible.</p>	<p>Lifelong nonsmokers. current smokers and former smokers. All young men and women.</p>	<p>Ultrasound (vessel diameter at rest and during reactive hyperemia - endothelial-dependent dilatation) FMD Categorization of smokers</p>	<p>Cigarette smoking is associated with dose-related and potentially reversible impairment of endothelium-dependent arterial dilatation in asymptomatic young adults, consistent with endothelial dysfunction.</p>
<p><b>Clifford P., Kluess H., Hamann J. et al., 2006</b>, Mechanical compression elicits vasodilatation in rat skeletal muscle feed arteries</p>			
<p>These experiments tested the hypothesis that rapid vasodilatation is a consequence of release of a vasoactive substance from the endothelium because of mechanical deformation of the vasculature during contraction.</p>	<p>Rats</p>	<p>Testing the effect of duration and number of compressions on vasodilatation.</p>	<p>The magnitude of dilatation was not affected by increasing the duration of compression but was enhanced by increasing the number of compressions. Dilatation is mediated by both endothelium-dependent and -independent signaling pathways.</p>

<b>Clifford, P. S., Tschakovsky, M.E., 2008, Rapid Vascular Responses to Muscle Contraction</b>			
This article examines recent advances in our understanding of rapid vascular responses to skeletal muscle contraction including the response to a single contraction and to changes in intensity during repeated contractions	Human Canine	single muscle contraction	Results of in vivo experiments in the human forearm or canine hind limb have revealed that skeletal muscle blood flow is elevated "immediately" (within 1 s) after the release of a brief contraction  Whether the immediate increase is a consequence of vasodilation or the muscle pump has been a difficult question to resolve. The pattern of continued increase in blood flow observed in vivo (15,22) and shown in Figure 1B argues against the muscle pump effect and in favor of rapid vasodilation.
<b>Creelius A. R., Kirby B. S., Luckasen G. J. et al., 2013 Mechanisms of rapid vasodilation after a brief contraction in human skeletal muscle</b>			
they sought to determine the underlying signaling mechanisms of muscle contraction-induced rapid vasodilation in humans.	young healthy adults	Single Dynamic Forearm Contractions  Arterial Catheterization, Arterial Blood Pressure, and Heart Rate  Forearm Blood Flow and Vascular Conductance  Vasoactive Drug Infusions	Rapid vasodilation occurs after a brief skeletal muscle contraction, and, In the present study, we demonstrated that K <sup>+</sup> -stimulated vascular hyperpolarization and the subsequent vasodilation significantly contribute to contraction-induced rapid vasodilation, as do NO and PGs, in combination. Collective blockade of these pathways nearly abolishes this phenomenon in humans, thus remarkably explaining the vast majority of rapid vasodilation
<b>Credeur, D. P., Holwerda, S. W., Restaino, R.M. et al., 2015, Characterizing rapid-onset vasodilation to single muscle contractions in the human leg</b>			
The purpose of this study was to characterize ROV following single muscle contractions	young men	heart rate Blood pressure ROV - leg	single muscle contractions in the leg produce a rapid, robust, and intensity-dependent vasodilation in humans  ROV in the leg was greater than ROV in the forearm  mechanical cuff compressions also induce a rapid hyperemic response in the leg, these were short in duration and lower in magnitude compared with single muscle contractions of the leg.  presence of a rapid and robust vasodilation to single isometric muscle contractions in the leg that is largely independent of mechanical factors

Arbejdsblad

<b>Cunniffe, B., Sharma, V., Cardinale, M. et al., 2017</b> , Oxygenation response to vascular occlusion: implications for remote ischaemic preconditioning and physical Performance			
This preliminary study aimed to establish the dose–response effect of different occlusion pressures on skeletal muscle oxygenation and blood flow in healthy males	healthy males	NIRS	Muscle oxygenation properties are influenced by choice of limb occluded and findings show that tissue ischaemia can be induced at much lower absolute pressures than traditionally used in RIPC studies. Blood flow and muscle oxygenation may be enhanced for at least 15 min following the last occlusion. Introduction
<b>Dezfulian C., Taft M., Corey C. et al., 2017</b> , Biochemical signaling by remote ischemic conditioning of the arm versus thigh: Is one raise of the cuff enough?			
Tested whether the number of cycles of RIC and its localization (arm versus thigh) determines biochemical signaling and cytoprotecting.	Healthy men and women	Blood flow, tissue perfusion, concentrations of the circulating protective mediator nitrite, and platelet mitochondrial function.	These data demonstrate that localization and “dose” of RIC does not affect cytoprotecting and further elucidate the mechanisms by which nitrite contributes to RIC-dependent protection.
<b>Dhindsa M., Sommerlad S., Devan A. et al., 2008</b> , Interrelationships among noninvasive measures of postischemic macro- and microvascular reactivity			
The aim of the study was to determine the interrelationships among different non- invasive measures that have been used to assess micro- and macrovascular reactivity.	Healthy men and women	1) flow-mediated dilatation (FMD), 2) changes in pulse wave velocity between the brachial and radial artery (ΔPWV), 3) hyperemic shear stress, 4) reactive hyperemic flow, 5) reactive hyperemia index (RHI) assessed by fingertip arterial tonometry, 6) fingertip temperature rebound (TR), and 7) skin reactive hyperemia.	In more than 75% of cases, vascular reactivity measures were not significantly associated. We concluded that associations among different measures of peripheral micro- and macrovascular reactivity were modest at best. These results suggest that different physiological mechanisms may be involved in changing different measures of vascular reactivity.
<b>Doonan, R.J., Hausvater, A., Scallan, C. et al., 2010</b> , The effect of smoking on arterial stiffness			
The purpose of this systematic review was to assess the effect of acute, chronic and passive smoking on arterial stiffness, as well as the effect of smoking cessation on arterial stiffness	review	arterial stiffness measured by aortic pulse wave velocity (PWV) and augmentation index (AIx)	Acute, chronic and passive smoking all have a detrimental effect on arterial stiffness, although the role of chronic smoking in increasing arterial stiffness is slightly more controversial. Chronic smoking was also shown to have a role in sensitizing arterial response to acute smoking. However, whether arterial stiffness is reversed after smoking cessation, and the timeline in which this occurs could not be determined from the available literature.

Arbejdsblad

<b>Doonan R., Scheffler P., YU A. et al., 2011, Altered Arterial Stiffness and Subendocardial Viability Ratio in Young Healthy Light Smokers after Acute Exercise</b>			
The purpose of this study was to estimate the effect of smoking on arterial stiffness and subendocardial viability ratio, at rest and after acute exercise in young healthy individuals	young healthy male light smokers.	Arterial stiffness and hemodynamic.  Arterial stress test.	Chronic and acute smoking appears to diminish the vascular response to physical stress. This can be seen as an impaired 'vascular reserve' or a blunted ability of the blood vessels to accommodate the changes required to achieve higher workloads. These changes were noted before changes in arterial stiffness or subendocardial viability ratio occurred at rest. Even light smoking in young healthy individuals appears to have harmful effects on vascular function, affecting the ability of the vascular bed to respond to increased demands
<b>Duval, W. L., 2005, Endothelial dysfunction and antioxidants</b>			
Reviews the effect of antioxidant vitamins A,C and E	Review	endothelial function	Unfortunately, the weight of the evidence points to little or no benefit of this therapy in reducing cardiovascular outcomes, and there appears to be no important niche for antioxidant vitamins in cardiovascular therapy.
<b>Enko, K.i, Nakamura, K., Yunoki, K., et al. 2011, Intermittent arm ischemia induces vasodilatation of the contralateral upper limb</b>			
we hypothesized that RIPC by intermittent arm ischemia changes the autonomic balance and induces vasodilatation of the distant organ's artery. The purpose of this study was to determine the validity of this hypothesis. We	healthy volunteers	RIPC HRV	Intermittent arm ischemia was accompanied by vasodilatation of another artery and enhancement of parasympathetic activity (upregulation in HF). It may be associated with the mechanism of RIPC.
<b>Ejiri, K., Miyoshi, T., Kohno, K. et al. 2019, Tobacco smoking protective effect via remote ischemic preconditioning on myocardial damage after elective percutaneous coronary intervention: Subanalysis of a randomized controlled trial</b>			
The aim of this study was to investigate the effect of RIPC on pMD following PCI in smokers	smoking and nonsmoking adults over 20 years who was awaiting elective Percutaneous Coronary Intervention	RIPC	These results suggest that RIPC might improve Periprocedural myocardial damage after PCI in smokers

<b>Findlay, B. B., Gupta, P., Szijgyarto, I. C., 2013</b> , Impaired brachial artery flow-mediated vasodilation in response to handgrip exercise-induced increases in shear stress in young smokers			
The purpose of the present study was therefore to compare the brachial artery FMD of young healthy smokers and non-smokers in response to handgrip exercise and Reactive hyperemia-mediated increases in shear stress.	healthy non-smoking and healthy smoking males	FMD (ultrasound) macrovasculature reactive hyperemia muscle contraction	It was found that handgrip exercise-, but not reactive hyperemia-FMD was impaired in the smokers. This suggests that handgrip exercise-FMD may provide a sensitive test for detecting endothelial dysfunction early in the pathological process. From a functional perspective, impaired FMD responses to exercise-induced increases in shear stress may have negative implications for perfusion  smokers have impaired FMD during simple muscle contraction exercise, but not during fmd caused by occlusion
<b>Flouris, A. D., Dinas, P. C., Tzatzarakis, M. N., 2014</b> , Exposure to secondhand smoke promotes sympathetic activity and cardiac muscle cachexia			
the aim of this experimental study was to investigate, for the first time, the immediate impact of secondhand smoke on cardiac autonomic control and myocardial integrity	healthy never-smokers	HRV Kubios Blood samples	In conclusion, this is the first study to demonstrate that a 1 h second hand smoke exposure at bar/restaurant levels suppress HRV  second hand smoke=more sympathetic activity
<b>Hausenloy, D. J., Yellon, D. M., 2008</b> Preconditioning and postconditioning: Underlying mechanisms and clinical application			
The purpose of this article is to provide an overview of the different forms of 'conditioning', their evolution and underlying mechanisms, and their emergence as clinical therapy	review	IPC RIPC Ischemic postconditioning	protecting effect of IPC and how it is mediated.  general overview of the application possibilities of IPC
<b>Hamann J., Buckwalter J., Clifford P., 2004</b> , Vasodilatation is obligatory for contraction-induced hyperaemia in canine skeletal muscle			
Testing the hypothesis that the rapid increase in blood flow to active skeletal muscle with the onset of exercise is attributable to vasodilatation because of smooth muscle hyperpolarization	Dogs	Ultrasound (blood flow) Blood samples Contraction through electrical stimulation of the muscle.	The increase in blood flow immediately following a single muscle contraction is due to vasodilatation, presumably because of smooth muscle hyperpolarization.
<b>Hodges, G. J., Stewart, D. G., Davison, Paul J., 2018</b> Episodic bouts of hyperaemia and shear stress improve arterial blood flow and endothelial function			
we investigated the effects of 6 weeks of brief repeated bouts of occlusion on endothelial-dependent dilation, brachial blood flow, vascular conductance, and arterial stiffness.	healthy male participants	endothelial-dependent dilation (FMD), brachial blood flow vascular conductance arterial stiffness.	We found that FMD responses, forearm blood flow, and vascular conductance increased, while pulse wave velocity also improved (i.e. decreased), with these responses progressively enhanced after 3 and 6 weeks of stimulation. Most importantly, these changes only occurred in the treated experimental limb and not in the untreated contralateral control limb

Arbejdsblad

<b>Hughes W., Kruse N., Casey D., 2017</b> , Sympathetic nervous system activation reduces contraction-induced rapid vasodilation in the leg of humans independent of age			
determine whether enhanced sympathetic vasoconstriction contributes to the attenuated contraction-induced rapid onset vasodilation response observed in the leg of older adults	young (24 ± 1 years) and older (67 ± 1 years) adults	single-leg knee extensions Ultrasound (artery diameter and blood velocity)	Sympathetic stimulation reduces contraction-induced rapid vasodilation in the leg of young and older adults similarly.
<b>Hughes, W. E., Ueda, K., Treichler, D. P. et al., 2015</b> Rapid onset vasodilation with single muscle contractions in the leg: Influence of age			
we aimed to examine whether potential age-related impairments in rapid vasodilation following a single muscle contraction were greater in the leg compared to the forearm. Methods	young and older men and women	single muscle contractions ROV ultrasound Heart rate blood pressure	aging blunts contraction-induced rapid vasodilation in the leg across exercise intensities and is largely independent of muscle mass  age-related impairments in contraction-induced vasodilation do not appear to be different between limbs (arm vs. leg)  The primary mechanism(s) and their inter- actions which contribute to the substantially attenuated rapid hyperemic and vasodilator response in the leg of older adults remains unknown
<b>Iannetta D., Inglis E., Soares R. et al., 2018</b> , Reliability of microvascular responsiveness measures derived from near-infrared spectroscopy across a variety of ischemic periods in young and older individuals			
Examine the reliability of the near-infrared spectroscopy (NIRS)-derived oxygen saturation (StO <sub>2</sub> ) reperfusion slope, a measure of microvascular responsiveness, to four different vascular occlusion tests of different durations in young and older participants.	Young (29 ± 5 yr) and older (67 ± 4 yr) men	Test-to-test reliability (30 s, 1, 3- and 5-min occlusions)  Day-to day reliability (two visits separated by 1-2 weeks)	NIRS-derived StO <sub>2</sub> reperfusion slope, has good reliability across a range of occlusion durations with the strongest reliability during longer occlusion durations.
<b>Jeffries O., Waldron M., Pattison J. et al., 2018</b> , Enhanced local skeletal muscle oxidative capacity and microvascular blood flow following 7-day ischemic preconditioning in healthy humans			
Examine the effects of 7 consecutive days of bilateral lower-limb IPC on local skeletal muscle oxidative capacity and microvascular blood flow	Healthy young men	Repeated IPC NIRS (muscle oxidative capacity and microvascular blood flow)	Repeated bouts of IPC over 7 consecutive days increased skeletal muscle oxidative capacity and microvascular muscle blood flow. These findings are consistent with enhanced mitochondrial and vascular function following repeated IPC.

<p><b>Jones, H, Hopkins, N, Bailey, T. G. et al. 2014</b>, Seven-day remote ischemic preconditioning improves local and systemic endothelial function and microcirculation in healthy humans</p>			
<p>The purpose was to examine the hypothesis that daily IPC leads to improvement in endothelial function and skin microcirculation not only in the arm exposed to IPC but also in the contralateral arm</p>	<p>healthy males</p>	<p>FMD ultrasound repeated IPC 7-days forearm microcirculation (cutaneous vascular conductance (CVC)</p>	<p>7 days of daily exposure to IPC leads to local (intervention arm) and systemic (contralateral arm) improvements in conduit artery endothelial function and elevation of resting skin microcirculation, which are present beyond the late phase of protection of IPC.</p> <p>repeated IPC increased resting microcirculation by approximately 30%, a clinically relevant improvement, especially because the increase was similar in a remote vascular bed not exposed to IPC.</p>
<p><b>Joyner J. and Casey W., 2015</b>, Regulation of Increased Blood Flow (Hyperemia) to Muscles During Exercise: A Hierarchy of Competing Physiological Needs</p>			
<p>This review focuses on how blood flow to contracting skeletal muscles is regulated during exercise in humans.</p>	<p>review</p>	<p>contraction-induced vasodilation  contraction-induced hyperemia</p>	<p>answers following questions: What is the range of oxygen consumption in humans? How is the oxygen delivery generated to meet the demands of the contracting muscles? What fraction of cardiac output goes to skeletal muscle during exercise? What are peak values for skeletal muscle blood flow? How is blood pressure regulated when blood flow to contracting skeletal muscles is very high? What are the local blood flow responses to muscle contraction, and what mechanisms cause them? How does the sympathetic nervous system control blood flow to both inactive and contracting skeletal muscles?</p>
<p><b>Kimura, M., Ueda, K., Goto, C., 2007</b> Repetition of ischemic preconditioning augments endothelium-dependent vasodilation in humans: Role of endothelium-derived nitric oxide and endothelial progenitor cells</p>			
<p>To determine the effects of late phase and repetition of IPC on vascular function in humans, we measured vascular responses to acetylcholine (ACh), an endothelium-dependent vasodilator</p>	<p>young healthy men</p>	<p>forearm blood flow NO repeated IPC 4 weeks</p>	<p>repetition of IPC augmented endothelial function through an increase in NO production</p> <p>Repetition of IPC may be a simple, safe, and feasible therapeutic technique for endothelial protection of peripheral vessels. Furthermore, this technique has the potential for improving endothelial function as a new treatment for cardiovascular disease associated with endothelial dysfunction.</p>

Arbejdsblad

<b>Kirby, B. S., Carlson, R. E., Markwald, R. R. et al, 2007, Mechanical influences on skeletal muscle vascular tone in humans: Insight into contraction-induced rapid vasodilatation</b>			
they tested the general hypothesis that mechanical deformation of forearm blood vessels via acute increases in extravascular pressure elicits a rapid vasodilatation in the human forearm.	young healthy adults	contraction-induced rapid vasodilatation	acute increases in extravascular pressure elicit a rapid vasodilatation in the human forearm. This mechanically induced vasodilatation peaks within 1–2 cardiac cycles, and thus is dissociated from that normally observed in response to brief muscle contractions. Our collective findings indicate that mechanical influences contribute largely to the immediate vasodilatation (first cardiac cycle) observed in response to a brief, single contraction
<b>Kitami M., Ali M., 2012, Tobacco, Metabolic and Inflammatory Pathways, and CVD Risk</b>			
The purpose of this review is to distill the literature and provide a comprehensive but concise overview of the pathways that connect tobacco use, and particularly smoking, with the development of cardiovascular disease.	Review	Cardiac output vasomotor function Cardiac remodeling Endothelial dysfunction (oxidative stress, endothelial cells)	Overview of how smoking leads to cardiovascular diseases. Data over smokers and smoking related deaths worldwide.
<b>Kobayashi M., Takemoto Y., Norioka N. et al., 2015, Vascular Functional and Morphological Alterations in Smokers during Varenicline Therapy.</b>			
Determine whether smoking cessation using varenicline therapy improves FMD, nitroglycerin-induced vasodilatation	Smokers and healthy adults	Ultrasound (FMD)	Smoking cessation with varenicline therapy significantly increased FMD. It appears to improve vascular function in smokers, which depends on endothelial function rather than on vascular smooth muscle function or changes in vascular structure.
<b>Kraemer, R., Lorenzen, J, Kabbani, M., et al. 2011, Acute effects of remote ischemic preconditioning on cutaneous microcirculation - A controlled prospective cohort study</b>			
aim of this study is to evaluate the acute microcirculatory effects of remote ischemic preconditioning on a distinct cutaneous location at the lower extremity which	healthy subjects	RIPC Microcirculation hemoglobin concentration in tissue by spectrometric technique	Remote ischemic preconditioning effects cutaneous tissue oxygen saturation, arterial capillary blood flow and postca- pillary venous filling pressure at a distinct location of the lower extremity  significant increase of the cutaneous capillary blood flow
<b>Lambert E., Thomas C., Hemmes R. et al., 2016, Sympathetic nervous response to ischemia-reperfusion injury in humans is altered with remote ischemic preconditioning</b>			
Evaluate sympathetic nerve response by direct microneurographic recordings, finger reactive hyperemia, noradrenaline, NO, and ROS release to forearm IR injury in healthy humans and determined whether RIPC altered these responses.	Healthy	RIPC Ischemia-reperfusion injury Muscle sympathetic nerve activity (microneurography) Ischemic reactive hyperemie index (RHI) NO	RIPC attenuated ischemia-induced sympathetic activation, prevented the production of an erythrocyte marker of oxidative stress and the reduction of NO availability, and ameliorated RHI.

<b>Lau, J. K., Roy, P., Javadzadegan, A., 2018, Remote Ischemic Preconditioning Acutely Improves Coronary Microcirculatory Function</b>			
The aim of this study was to assess the effect of RIPC on markers of microcirculatory function	Clinically stable patients	RIPC	RIPC leads to improvement in coronary microcirculatory function
<b>Lewis N., Bain A., Wildfong K. et al., 2017, Acute hypoxaemia and vascular function in healthy humans</b>			
assessed brachial FMD at baseline and after 30 min of mild and moderate normobaric hypoxia or normoxia.	Healthy young adults	Rest FMD Hypoxia Blood flow velocity	Both mild and moderate levels of hypoxia, there was a graded impairment in FMD. Findings from the normoxic control study suggest that the decline in FMD during acute hypoxia also appears to be influenced by 30 min of supine rest/inactivity in between FMD assessments.
<b>Li S., Ma C., Shao G. et al., 2015, Safety and feasibility of remote limb ischemic preconditioning in patients with unilateral middle cerebral artery stenosis and healthy volunteers</b>			
The aim of the present study was to assess whether upper arm ischemic preconditioning is feasible and safe in patients with unilateral middle cerebral artery stenosis compared to healthy volunteers.	Patients with unilateral MCA stenosis and healthy adults	RIPC (five cycles of 5-min inflation and reperfusion)  NIRS	RIPC has no significant effect on the heart rate, oxygenation index, or mean flow velocity in patients with unilateral MCA stenosis or healthy volunteers. However, healthy volunteers showed a reduction in blood pressure 30 min following reperfusion of the last cycle. Limb ischemic preconditioning was found to be safe and well tolerated in both patients and healthy volunteers.
<b>Lim S., Hausenloy D, 2012, Remote ischemic conditioning: From bench to bedside</b>			
provide an overview of RIC, the potential underlying mechanisms, and its potential as a novel therapeutic strategy for protecting the heart and other organs from acute IR injury.	Review		Overview of different mechanism. A little about the cardiac system.
<b>Liu Z., Yang W., Fu x. et al., 2015, Remote ischemic precondition prevents radial artery endothelial dysfunction induced by ischemia and reperfusion based on a cyclooxygenase-2-dependent mechanism</b>			
Determine whether RIPC protects endothelial function of radial artery in human against IR and whether Cyclooxygenase (COX)-2 involves in this effect	Healthy nonsmoking adults	FMD COX-2 inhibitor IPC IR injury	RIPC prevents radial artery endothelial dysfunction induced by IR. This protective effect of RIPC in the late phase is mediated by a COX-2-dependent mechanism
<b>Loukogeorgakis S., Panagiotidou A., Broadhead M. et al., 2005, Remote ischemic preconditioning provides early and late protection against endothelial ischemia-reperfusion injury in humans: Role of the autonomic nervous system</b>			
The aim of this study was to characterize the time course and neuronal mechanism of RIPC of the vasculature in humans.	Healthy adults	IR injury FMD RIPC Autonomic ganglion blocker	Remote ischemic preconditioning in humans has two phases of protection against endothelial IR injury; an early (short) and late (prolonged) phase, both of which are neuronally mediated

<b>Maguire M., Weaver T., Damon B., 2007, Delayed blood reoxygenation following maximum voluntary contraction</b>			
To characterize the total hemoglobin concentration ([THb]) and oxyhemoglobin saturation (%HbO <sub>2</sub> ) time courses after brief dorsiflexion maximal voluntary contractions (MVC) and to determine whether these responses varied by gender.	Healthy adults	3-s dorsiflexion MVC NIRS	There are three phases to the post-MVC oxygen supply–demand coupling: 1) rising oxygen demand relative to supply; 2) rising oxygen supply relative to demand; and 3) restoration of precontraction oxygen supply–demand matching. These processes are unaffected by gender
<b>McLay K., Fontana F., Nederveen J. et al., 2016, Vascular responsiveness determined by near-infrared spectroscopy measures of oxygen saturation</b>			
Evaluate whether NIRS can be used as a measure of vascular responsiveness by establishing a correlation between NIRS-derived StO <sub>2</sub> reperfusion slope and FMD	Healthy young adult men	FMD NIRS	Significant correlation between the NIRS-derived measure of slope 2 StO <sub>2</sub> and ultrasound-derived %FMD. The data support the notion that NIRS-derived measures of StO <sub>2</sub> obtained directly at the microvascular level are reflective of the microvascular reperfusion and reactivity as measured via FMD.
<b>McLay K., Nederveen J., Pogliaghi S. et al., 2016, Repeatability of vascular responsiveness measures derived from near-infrared spectroscopy</b>			
Examine the test-to-test reliability (variability between repeated tests within a single day) and day-to-day reliability of the NIRS-derived measure slope 2 StO <sub>2</sub> , and compare it to the widely used FMD measurement	Healthy young men	NIRS FMD	NIRS-derived slope 2 StO <sub>2</sub> can be used as a reliable measure of vascular reactivity.
<b>Messere A., Tschakovsky M., Seddone S. et al., 2018, Hyper-oxygenation attenuates the rapid vasodilatory response to muscle contraction and compression</b>			
Isolate local tissue oxygenation changes from mechanical compression in the attenuation of compression-induced hyperemia	Healthy men	MC NIRS Ultrasound	The present study provides direct evidence of the novel role of tissue oxygenation as a modulator of the rapid dilatory mechanisms underlying the hyperemic responses to both muscle contraction and compression
<b>Middlekauff, H. R. Park, J. Moheimani, R. S., 2014 Adverse effects of cigarette and noncigarette smoke exposure on the autonomic nervous system: Mechanisms and implications for cardiovascular risk</b>			
This review summarizes the detrimental effects of cigarette and noncigarette emission exposure on autonomic function, with particular emphasis on the mechanisms of acute and chronic modulation of the sympathetic nervous system.	review	HRV cigarette smoke	smoking cessation as brief as 7 days has been associated with increased HRV (more HF), consistent with a shift toward the restoration of normal sympathetic balance  Acute and long-term exposure to cigarette smoke, in either active or passive smokers, leads to acute and chronic changes in the balance of the autonomic nervous system, resulting in sympathetic predominance

<b>Moro, L., Pedone, C., Mondì, A., 2011, Effect of local and remote ischemic preconditioning on endothelial function in young people and healthy or hypertensive elderly people</b>			
We designed this study to verify whether older age affects remote preconditioning in an experimental framework comparing local and remote preconditioning of young, healthy elderly and elderly hypertensive subjects	healthy young people healthy elderly people hypertensive elderly people	FMD	remote preconditioning is as effective as local preconditioning and, as percent change, greater in healthy elderly than in young people, but to some extent impaired in hypertensive elderly.
<b>Morris, P. B., Ference, B. A., Jahangir, E., et al. 2015 Cardiovascular Effects of Exposure to Cigarette Smoke and Electronic Cigarettes: Clinical Perspectives from the Prevention of Cardiovascular Disease Section Leadership Council and Early Career Councils of the American College of Cardiology</b>			
This review considers selected updates on the genetics and epigenetics of smoking behavior and associated cardiovascular risk, mechanisms of atherogenesis and thrombosis, clinical effects of smoking and benefits of cessation, and potential impact of electronic cigarettes on cardiovascular health	review	cigarette smoke NO	Exposure to cigarette smoke and secondhand smoke (CSE/SHS) causes endothelial cell activation, dysfunction, injury, and death, leading to insudation of lipids and inflammatory cells.  smoking causes larger morbidity
<b>Nosarev, A. V., Smagliy, L. V., Anfinogenova, Y. et al., 2015, Exercise and NO production: relevance and implications in the cardiopulmonary system</b>			
This article reviews the existing knowledge about the effects of physical exercise on nitric oxide (NO) production in the cardiopulmonary system	Human and Animals	NO	Evidence suggests the possible existence of the exercise amount/effort thresholds pivotal for the regulation of NO production. Physical activity significantly improves functioning of the cardiovascular system through the increase in NO bioavailability, potentiation of antioxidant defense, and decrease in the expression of ROS-forming enzymes
<b>Ramos Gonzalez, M., Caldwell, J. T., Branch, P. A. et al., 2018, Impact of shear rate pattern on post-occlusive near-infrared spectroscopy microvascular reactivity</b>			
aim of the present study was to determine the impact of acute changes in shear rate patterns, in particular retrograde shear rate, on microvascular function in 15 healthy, young men and women as determined via the post-occlusive near-infrared spectroscopy (NIRS) microvascular reactivity response	healthy individuals	NIRS microvascular reactivity	By increasing retrograde shear rate in the leg via the 75 mm Hg cuff-inflation method, we observed a significant increase in post-occlusive NIRS-derived microvascular reactivity. The primary findings were that the total post-occlusive microvascular reactivity response, as described by the area under the TSI% post-occlusion response curve, was significantly increased in response to the retrograde shear.

<b>Rosenberry, R., Munson, M., Chung, S., et al., 2018.</b> Age-related microvascular dysfunction: novel insight from near-infrared spectroscopy			
Can near-infrared spectroscopy (NIRS)-derived post-occlusion tissue oxygen saturation recovery kinetics be used to study age-related impairments in microvascular function?	young and elderly	NIRS	NIRS-derived indices of microvascular function were markedly reduced in elderly compared with young participants  In conclusion, we believe NIRS-derived post-occlusive tissue oxygen saturation kinetics can serve an important role in clinical vascular biology and assessment.
<b>Ryan, H., Troscclair, A., Gfroerer, J, 2012,</b> Adult current smoking: Differences in definitions and prevalence estimates - NHIS and NSDUH, 2008			
To compare prevalence estimates and assess issues related to the measurement of adult cigarette smoking in the National Health Interview Survey (NHIS) and the National Survey on Drug Use and Health (NSDUH)	USA	smoker definitions	In comparisons between NHIS and NSDUH, NSDUH consistently yielded higher national overall and subpopulation estimates of current cigarette smoking among adults than NHIS and, among current smokers, lower estimates of daily smoking  Our study provides further information on how different smoking definitions between two national surveys may impact the overall and subpopulation prevalence estimates observed for some smoking behaviors
<b>Ryan, T. E., Erickson, M. L., Brizendine, J. T., et al. 2012</b> Noninvasive evaluation of skeletal muscle mitochondrial capacity with near-infrared spectroscopy: correcting for blood volume changes			
The aim of this study was to measure skeletal muscle mitochondrial capacity using blood volume-corrected NIRS signals that represent oxygenated hemoglobin/myoglobin (O <sub>2</sub> Hb) and deoxygenated hemoglobin/myoglobin (HHb)	healthy and injured	NIRS	In conclusion, we measured mitochondrial capacity using NIRS and assessed the reliability and reproducibility of resting muscle oxygen consumption and the recovery rate of muscle oxygen consumption after exercise. A blood volume correction has been developed and applied to arterial occlusion measurements of oxygen consumption. Without correcting for blood volume changes, the metabolic exchange between O <sub>2</sub> Hb and HHb may be masked by blood changes under the NIRS probe. We demonstrate three methods for correcting blood volume changes
<b>Salahuddin S., Prabhakaran D., Roy A., 2012,</b> Pathophysiological mechanisms of tobacco-related CVD			
Outlines the various postulated pathophysiological mechanisms involved in tobacco-related cardiovascular disease	Review (93 artikler)	Role of components in the smoke in cardiovascular disease.  Mechanisms.	Cigarette smoking is a leading preventable risk factor for the development and progression of cardiovascular disease. Tobacco exerts its deleterious cardiovascular effects through multiple mechanisms. Endothelial dysfunction increased oxidative stress, and induction of a hypercoagulable state appear to be the key pathobiological mechanisms involved

<b>Siafaka A., Angelopoulos E., Kritikos K. et al., 2007</b> , Acute effects of smoking on skeletal muscle microcirculation monitored by near-infrared spectroscopy			
Characterize the acute effects of cigarette smoking on microcirculatory parameters of young, healthy smokers, as measured with NIRS, and to compare them with healthy nonsmokers.	Young healthy smokers and nonsmokers.	NIRS (O <sub>2</sub> consumption) Acute effect of smoking. Occlusion protocol	Smoking acutely affects microcirculatory function. It seems promising for the prospective evaluation of the effects of long-term exposure to cigarette smoke
<b>Singh, D., Vinod, K., Saxena, S. C. 2014</b> Sampling frequency of the RR interval time series for spectral analysis of heart rate variability			
In this paper, we shall analyse the RR interval time series from selected subjects for different sampling frequencies to compare the error introduced in selected frequency-domain measures of HRV at a constant frequency resolution for a specific duration of electrocardiogram (ECG) data.		HRV	HRV measures have been linked with manifold pathophysiological and psychopathological conditions such as cardiovascular (CV) disease, diabetes, anxiety disorders, smoking, obesity, lack of physical exercise and attentional deficit  low frequency (LF) component around 0.1 Hz (in a range between 0.04 and 0.15 Hz) and whose changes in power have been related to the sympathetic activity on the basis of pharmacological and clinical experiments [2, 11], and a high frequency (HF) component, in synchrony with respiration rate (in the range between 0.15 and 0.4 Hz), which is considered to be an expression of the respiration disturbances mediated by the vagal activity [12].
<b>Soares R., George M., Proctor D. et al., 2018</b> , Differences in vascular function between trained and untrained limbs assessed by near-infrared spectroscopy			
Examine whether differences in vascular responsiveness associated with training status would be more prominent in the trained limb (leg) than in the untrained limb (arm) microvasculature	Untrained and trained healthy men.	VOT NIRS (slope 2)	The present study suggests that the vascular adaptations induced by lower limb endurance exercise training are more prominent in the trained limb than in the untrained limb microvasculature
<b>Soares, R. N., Murias, J. M., 2018</b> Near-infrared spectroscopy assessment of microvasculature detects difference in lower limb vascular responsiveness in obese compared to lean individuals			
This study aimed to evaluate whether the near-infrared spectroscopy (NIRS) combined with a vascular occlusion (VOT) assessment was capable of detecting differences in vascular responsiveness within the microvasculature of the lower limb between lean and obese individuals.	lean and obese individuals	NIRS	In conclusion, the present study showed that differences in vascular responses exist within the microvasculature of the lower limb between obese individuals and those with normal weight. These differences were represented by a reduced Slope 2 and area under the curve of oxygen saturation observed in the obese individuals compared to the lean control group.

<b>Spyers-Ashby, J.M., Bain, P.G., Roberts, S.J., 1998</b> , A comparison of fast fourier transform (FFT) and autoregressive (AR) spectral estimation techniques for the analysis of tremor data			
This review outlines the theory of spectral estimation techniques based on the fast Fourier transform (FFT) and autoregressive (AR) model and their application to the analysis of human tremor data	review	HRV	The inherent performance limitations of FFT-based spectral estimation when analysing short data segments can often be eliminated by the appropriate use of AR techniques
<b>Thijssen, D. H. J., Atkinson, C. L., Ono, K., et al., 2014</b> , Sympathetic nervous system activation, arterial shear rate, and flow-mediated dilation			
The aim of this study was to examine the contribution of arterial shear to changes in flow-mediated dilation (FMD) during sympathetic nervous system (SNS) activation in healthy humans.	healthy men	FMD heart rate lower body negative pressure (SNS stimuli)	SNS stimulation using 10 min of lower body negative pressure decreased brachial artery FMD
<b>Thijssen D., de Groot P., Kooijman M. et al., 2006</b> , Sympathetic nervous system contributes to the age-related impairment of flow-mediated dilation of the superficial femoral artery			
Assess whether acute changes in sympathetic activity alter FMD in the leg	Healthy young and healthy older men	Maximal cycling exercise (reduction of sympathetic activity) Cold pressor test (increase sympathetic activity) Nitroglycerin (endothelium-dependent vasodilation) FMD.	in older men, FMD of the femoral artery is impaired. Local attenuation of the sympathetic responsiveness partly restores the FMD in these subjects. In contrast, in young subjects, acute modulation of the sympathetic nervous system activity does not alter flow-mediated vasodilation in the leg
<b>Thijssen D., Maxwell J., Green D. et al., 2016</b> , Repeated ischaemic preconditioning: A novel therapeutic intervention and potential underlying mechanisms			
This review discusses the effects of repeated exposure of tissue to ischaemic preconditioning on cardiovascular function, the attendant adaptations and their potential clinical relevance	Review		Provide an overview of the literature pertaining to the impact of repeated IPC on cardiovascular function, related to both local and remote adaptation, as well as potential clinical implications
<b>Towse, T. F., Slade, J. M., Ambrose, J. A., 2011</b> , Quantitative analysis of the postcontractile blood-oxygenation-level-dependent (BOLD) effect in skeletal muscle			
This study examined the relationship between these transient changes by measuring anterior tibial artery flow (Doppler ultrasound), anterior muscle SI (3T, one-shot echo-planar images, TR/TE // 1,000/35), and muscle blood volume and hemoglobin saturation [near-infrared spectroscopy (NIRS)] in the same subjects after 1-s-duration maximum isometric ankle dorsiflexion contractions.	subjects healthy	BOLD NIRS ultrasound	the magnitude of the postcontractile increase in blood flow following a single 1-s contraction is greater in physically active subjects  there is a striking similarity of BOLD data and NIRS DATA

<b>Tsuji, H., Venditti, F. J., Manders, E. S. et al., 1996, Determinants of heart rate variability</b>			
clinical determinants and normative values of heart rate variability measures have not been studied systematically in a large community-based population.	healthy subjects 30-90 years	HRV	there are many clinical variables associated with reduced or increased heart rate variability. The impact of age and heart rate must be taken into account when evaluating heart rate variability.
<b>van den Munckhof I., Riksen N., Seeger J. et al., 2013, Aging attenuates the protective effect of ischemic preconditioning against endothelial ischemia-reperfusion injury in humans</b>			
Examine the impact of advanced age on the ability of IPC to protect against endothelial dysfunction due to IR injury.	Healthy young men and healthy older men.	FMD (endothelial function) IR injury IPC (three cycles of 5 min inflation and 5 min deflation)	Older age is associated with an abolished effect of IPC to protect against endothelial dysfunction after IR in the brachial artery.
<b>Vranish, J. R., Young, B. E., Stephens, Brandi Y. et al., 2018, Brief periods of inactivity reduce leg microvascular, but not macrovascular, function in healthy young men</b>			
they sought to investigate the effect of a brief (10 min) bout of sitting on popliteal artery FMD and reactive hyperaemia	healthy young men	inactivity FMD reactive hyperemia	We demonstrate that a mere 10 min of sitting is sufficient to impair leg microvascular function (reactive hyperaemia). conduit artery vasodilatation (flow-mediated dilatation) was unaffected, indicating maintained
<b>Watanabe, S., A., E., Watanabe, M. 2013</b> Simultaneous Heart Rate Variability Monitoring Enhances the Predictive Value of Flow-Mediated Dilatation in Ischemic Heart Disease			
we investigated the autonomic nervous system-related regulatory factors that control endothelial function in ischemic heart disease (IHD) patients and evaluated the relationship between endothelial function and autonomic nervous system.	ischemic heart disease (IHD) patients	FMD Ultrasound HRV	Endothelial function is well correlated with autonomic nervous system activity.  An in-depth study of the interaction between the cardiovascular autonomic nervous system and endothelial function can help clarify the detailed mechanisms involved in the development and progression of cardiovascular disease
<b>Zagidullin N., Scherbakova E., Safina Y. et al., 2016, The Impact of Remote Ischemic Preconditioning on Arterial Stiffness and Heart Rate Variability in Patients with Angina Pectoris</b>			
Study the impact of RIPC in patients with stable angina pectoris and compare it with healthy individuals with respect to arterial stiffness and heart rate variability.	Healthy adults and coronary heart disease patients.	RIPC (three cycles of 5 min) Arterial stiffness and pulse wave velocity HRV	Ischemic preconditioning reduces not only systolic blood pressure, but also reduces central systolic blood pressure and improves arterial compliance and heart rate modulation reserve.

### 3. Etisk protokol

## **FORSØGSPROTOKOL**

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### **Projekttitle**

Effekten af iskæmisk prækonditionering på blodkarfunktion hos rygere og ikke-rygere

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## Forsøgsprotokol

### *Baggrund*

Iskæmisk prækonditionering (IPC) er en metode, hvor man ved gentagne korte afklemninger (5 minutters varighed ved hjælp af en blodtryksmanchet)<sup>1,2</sup> af blodgennemstrømningen til et ben eller en arm kan opnå beskyttende effekter på forskellige typer væv i kroppen, herunder hjerte-, skelet- og glat muskulatur. IPC har traditionelt været anvendt i forbindelse med behandling af hjertetilfælde og under operationer. Metoden er blevet benyttet til at forebygge skader på organer og muskelvæv, der opstår under længerevarende iskæmi<sup>3</sup>. IPC har vist at kunne beskytte skeletmuskulaturen mod den skade, der opstår i forbindelse med uvant og belastende excentrisk muskelarbejde<sup>2</sup> samt beskytte kroppens blodkar imod skader efter længerevarende iskæmi<sup>4</sup>.

Normal karfunktion er vigtig for at kunne regulere blodgennemstrømningen i forhold til de varierende metaboliske krav, som opstår f.eks. under fysisk aktivitet. Overgangen fra hvile til arbejde resulterer i en mangedobling af muskelvævet's energiomsætning og dermed en øget efterspørgsel efter ilt (O<sub>2</sub>) i vævet. Således er karfunktionen central for at O<sub>2</sub>-transporten til muskelvævet kan matche O<sub>2</sub>-kravet lokalt og dermed understøtte musklens energiomsætning under fysisk arbejde<sup>5,6</sup>. Karfunktion er hovedsageligt bestemt af det inderste lag af blodkarrene, som består af endothelceller. Aktiviteten i endothelcellerne bestemmer om blodkarrene skal trække sig sammen (vasokonstriktion) eller udvide sig (vasodilation) og kontrollerer dermed blodgennemstrømningen i forskellige væv i kroppen. En population med kendt svækket endotelfunktion er rygere<sup>7,8</sup>. Den svækkede endotelfunktion hos rygere er kendetegnet ved nedsat tilgængelighed af molekyler som fremmer vasodilation, eks. nitrogenoxid (NO)<sup>8,9</sup>. Endvidere har rygere øget sympatisk nerveaktivitet, der forårsager øget vasokonstriktion<sup>10</sup>. Samlet set betyder det, at rygere med svækket endotelfunktion har nedsat blodgennemstrømning af muskelvæv under fysisk aktivitet.

IPC er traditionelt blevet benyttet som en metode til at beskytte væv, men har også vist at have positiv indvirkning på kroppens karfunktion. De positive effekter inkluderer en opregulering af molekyler som fremmer vasodilation (eks. NO) samt øget parasympatisk nerveaktivitet, som hæmmer den vasokonstriktive effekt fra det sympatiske nervesystem. Det kan dermed forventes, at en intervention bestående af IPC vil forårsage en forbedring af karfunktionen hos unge mennesker. Ydermere vil IPC kunne forventes at modvirke den nedsatte karfunktion hos unge rygere. Effekten af IPC på den vaskulære funktion er undersøgt på raske unge mænd med positive resultater<sup>11</sup>, men effekten af IPC på karfunktion er endnu ikke blevet undersøgt hos unge rygere. Potentielt kan IPC bruges forebyggende eller som rehabiliterende behandlingsmetode rettet imod rygere og andre populationer med nedsat karfunktion. Karfunktionen i de små kar (mikrovaskulær funktion) kan vurderes ved brug af nærinfrarød spektroskopi (NIRS). Med NIRS er det muligt at bestemme hvor meget ilt og blod, der er i muskelvævet, og hvordan det ændrer sig under muskelarbejde<sup>12,13</sup>.

## **Strategi for litteratursøgning**

Baggrunden for studiet er fundet blandt peer-reviewed artikler, offentligt tilgængeligt materiale og Aalborgs Universitets egen forskning. Der er fundet 75 artikler og anvendt 13 kilder (se afsnittet Referenceliste) til dette studie, som danner basis for forskningen inden for karfunktion og iskæmisk prækonditionering.

Litteraturen er fundet ved litteraturgennemgang af søgemaskinerne PubMed og Scopus. Følgende søgeord er anvendt i kombinationer til gennemgang af litteraturen; 'microvascular function', 'ischemic preconditioning', 'autonomic nervous system' og 'nitrogen oxide'. Der er anvendt studier inden for dyre- og humanforskning, som er udarbejdet inden for de sidste 20 år.

## **Formål**

Dette studie ønsker at undersøge hvilken indflydelse iskæmisk prækonditionering har på mikrovaskulær reaktivitet og belyse bidragene fra det autonome nervesystem og biomarkører for vasodilation, hos både rygere og ikke-rygere. Specifikt testes hypoteserne:

1: Som følge af IPC vil mikrovaskulær reaktivitet blive forbedret.

2: Som følge af IPC vil forbedringer i mikrovaskulær reaktivitet være større hos rygere sammenlignet med ikke-rygere.

Resultaterne fra projektet vil generere ny viden omkring sammenhængen mellem iskæmisk prækonditionering og mikrovaskulær reaktivitet, herunder forskelle mellem rygere og ikke-rygere. Desuden vil projektet belyse bidraget fra det autonome nervesystem samt biomarkører for vasodilation, til mulige ændringer i mikrovaskulær reaktivitet.

## **Forsøgspersoner**

Til forsøget rekrutteres i alt 80 forsøgspersoner fordelt på 2 grupper: I) mandlige ikke-rygere, II) mandlige rygere. Forsøgspersonerne indgår i et crossover studie, hvor alle forsøgspersoner tilfældigt modtager enten iskæmisk prækonditionering i første session og "falsk" konditionering i anden session eller omvendt.

Forsøgspersoner rekrutteres via opslag på Aalborg Universitet, og på hjemmesiden [www.forsog.dk](http://www.forsog.dk) (opslag vedlagt denne forsøgsprotokol) og på Facebook. Følgende inklusions- og eksklusionskriterier er gældende for alle grupper:

### *Inklusionskriterier:*

1. Raske mænd i alderen 21-34 år
2. Normalvægtige (Body Mass Index mellem 18,5 og 30 kg/m<sup>2</sup>)
3. Ikke-rygere  
*eller*
4. Rygere (defineres som nuværende eller tidligere fast ryger og nuværende "festryger". Dette indbefatter også snus samt andre tobaks- og/eller nikotinprodukter)

*Eksklusionskriterier:*

1. Eksponering til iskæmisk prækonditionering eller okklusionstræning (en træningsform, der benytter lave vægtbelastninger kombineret med delvis aflukning af blodgennemstrømningen) syv dage før forsøgsdeltagelse.
2. Historik med søvnapnø
3. Lidelser i ekstremiteterne eller i det kardio- og mikrovaskulære system.
4. Forhøjet eller for lavt blodtryk.
5. Brug af kardiovaskulære medikamenter, stimulanser eller beroligende medicin.
6. Addiktiv adfærd, defineret som misbrug af hash, opioider eller andre euforiserende stoffer
7. Manglende evne til at samarbejde

Endvidere bliver forsøgspersonerne bedt om at afholde sig fra indtagelse af koffein, og undgå anstrengende fysisk aktivitet 24 timer inden hver forsøgsgang samt i at afstå fra indtagelse af fødevarer med højt antioxidantindhold 48 timer inden hver forsøgsgang. Desuden vil forsøgspersonerne blive instrueret i at være fastende 12 timer inden forsøgsdeltagelse. Gruppen bestående af rygere vil også blive instrueret i at afholde sig fra rygning 12 timer inden forsøgsdeltagelse.

## ***Forsøgsdesign og metoder***

### **Forsøgsdesign**

Forsøgspersonerne skal gennemføre en forsøgsprotokol, der har til hensigt at undersøge effekten af iskæmisk prækonditionering på mikrovaskulær reaktivitet og belyse bidraget fra det autonome nervesystem samt undersøge hvordan rygning påvirker denne sammenhæng. Ydermere tages der blodprøver for at kortlægge eventuelle biomarkører, som kan bidrage til ændringer i mikrovaskulær reaktivitet. Blodprøver til bestemmelse af biomarkører foretages i begyndelsen og slutningen af forsøget. Der foretages ligeledes måling af hjertets elektriske aktivitet i hvile til bestemmelse af hjertefrekvens variation, voluntære kontraktioner af dorsiflexion-musklerne samt iskæmisk prækonditionering eller "falsk" konditionering, bestående af kortvarig forhindring af blodtilførsel til det ene underben ved brug af en blodtryksmanchet placeret omkring låret.

Forsøget forløber over 2 forsøgssessioner, én gang med iskæmisk prækonditionering og én gang med "falsk" konditionering. Hver forsøgssession hver varer ca. 2 timer. Sessionerne udføres over en periode på 1-2 uger.

I forbindelse med forsøget skal forsøgspersonerne gennemgå nedenstående tests og procedurer:

- I) Biomarkører vha. blodprøve
- II) Måling af hjertets elektriske aktivitet
- III) Tests af mikrovaskulær reaktivitet
- IV) Iskæmisk prækonditionering eller "falsk" konditionering

## Metoder

I det efterfølgende beskrives de anvendte metoder. Tilsvarende eksperimentelle procedurer er tidligere beskrevet og godkendt ved Den Videnskabetiske Komite for Region Nordjylland (fx N-20130029, N-20180083).

### Biomarkører

Biomarkører er målbare indikatorer for normale biologiske og patogene tilstande, såvel som farmakologiske eller ikke-farmakologiske reaktioner på behandlingsintervention eller strategier. Biomarkøren bør være til stede ved baseline, og dens niveauer skal vise ændring som reaktion på behandling eller anvendt strategi. De ideelle biomarkører skal være lette at måle og kvantificere, og vigtigst af alt, bør de nøje korrelere med parametre målt for andre effekter (fx. Præstationsmål). Der findes ingen enkelt ideal biomarkør. Mange forskellige biomarkører er krævet for at øge sensitivitet og specificitet i reaktivitet. Ændringsmønstret for biomarkører med kendt effekt på karfunktion vil blive undersøgt for at give en bedre karakterisering for effekten af IPC, som benyttes i studiet, sammenlignet med baseline-niveauer. Blodprøver vil blive taget af laboranter eller videnskabeligt personale, som har gennemført og bestået et kursus i blodprøvetagning. Forsøgspersonerne skal sidde eller ligge i en behagelig position, mens blodprøverne bliver taget. Analyser af blodprøverne bliver lavet ved brug af massespektrometri, som projektgruppen har indgående erfaring med. Disse analyser indbefatter ikke detaljeret DNA-sekventering.

### Måling af hjertets elektriske aktivitet

Hjertets elektriske aktivitet (dvs. elektrokardiogram, EKG) vil blive målt, mens kroppen er i hvile. Dette udføres ved at påsætte elektroder på overkroppen. Målingerne bliver foretaget, mens forsøgspersonen ligger på ryggen og slapper af, og varer ca. 5-10 minutter.

### Tests af mikrovaskulær reaktivitet

Blodgennemstrømning og energiomsætning i muskelvævet bestemmes ved brug af nærinfrarød spektroskopi (NIRS)<sup>12,13</sup>. Denne metode er baseret på absorption af nærinfrarødt lys og muliggør kvantificering af iltmætningen af hæmoglobin/myoglobin i muskelvævet. NIRS-systemet, som består af optiske sensorer, fastgøres til huden over musklerne, hvilket muliggør målinger af ændringer i iltmætningen i muskulaturen under og efter muskelarbejde<sup>12,13</sup>. NIRS-målingerne foretages i laboratoriet under og efter kontraktioner af musklerne samt under og efter afklemning af blodforsyningen til muskulaturen, hvilket er standardprocedure for disse målinger<sup>12,13</sup>. Muskelarbejdet består af kortvarige (1-5 sek.) maksimale isometriske kontraktioner af dorsiflexormusklerne. Disse kontraktioner udføres med foden spændt fast til en pedal med en kraftmåler. Metoderne til undersøgelse af blodgennemstrømning og energiomsætning er således alle non-invasive teknikker.

### Iskæmisk prækonditionering og falsk konditionering

Afklemning af blodforsyningen til dorsiflexor-musklerne gøres ved at placere en oppustelig trykmanchet rundt om forsøgspersonens lår og øge trykket i manchetten til 250 mmHg i fem cyklusser af 5 min med øget tryk og 5 min uden tryk, hvilket er standardprocedure for iskæmisk prækonditionering<sup>1,2</sup>. Under falsk konditionering vil trykket i manchetten blot blive øget til 20 mmHg, hvilket ikke resulterer i afklemning af blodforsyningen.

## ***Forskningsbiobank***

Ved forsøget tages i alt 4 blodprøver på hver 18 mL med henblik på at undersøge proteinsammensætningen i blodet og for at kunne relatere fundene til afklemning af blodgennemstrømning.

I forbindelse med studiet vil der blive oprettet en forskningsbiobank, og det forventes, at studiet vil være med til at øge forståelsen af mulige virkninger fra afklemning af blodgennemstrømning. Prøverne vil få et løbenummer, som gør, at forsøgspersonen ikke kan identificeres ud fra dette nummer. Der er således tale om en pseudo-anonymisering som anmeldes til Datatilsynet. Når forsøgspersonens data og materiale er samlet, vil alt data pseudo-anonymiseres, og nøglen til at oversætte tilbage opbevares på forskningsenheden og har kun relevans, hvis en forsøgsperson trækker sig fra studiet eller at der er manglende data, som skal genskabes.

Prøverne vil blive indsamlet, behandlet og analyseret af projektgruppen under Institut for Medicin og Sundhedsteknologi ved Aalborg Universitet i henhold til reguleringen af Datatilsynet.

Prøverne opbevares i forskningsbiobanken, indtil de skal analyseres. Prøverne opbevares i op til 15 år, og eventuelt overskydende materiale gemmes, så eventuelle nye resultater efterfølgende kan valideres. Efter 15 år destrueres overskydende materiale.

Det er muligt, at forskergruppen kan se at prøverne kan anvendes i forbindelse med et andet studie – fx til sammenligning med blodprøver fra andre patienter. Hvis dette bliver tilfældet, vil forskergruppen tage kontakt til forsøgspersonerne og spørge om de vil deltage i et eventuelt nyt studie, som vil kræve et nyt mundtligt og skriftligt samtykke fra forsøgspersonen og en ny informationssamtale. Derudover skal videnskabsetisk komité også godkende og give tilladelse til nye forsøg og andre studier, som ikke er beskrevet i dette materiale.

## ***Risici, bivirkninger og ulemper***

Der er ingen kendte risici eller bivirkninger forbundet med de metoder, der anvendes i projektet. Metoderne anvendes rutinemæssigt på Institut for Medicin og Sundhedsteknologi, Aalborg Universitet samt på mange forskningsinstitutioner over hele verden.

### **Blodprøver**

Blodprøver tages fra en blodåre i albueleddet og blodprøvetagning vil blive udført af trænet personale. I forbindelse med blodprøvetagning er der altid en lille risiko for at der kan opstå en blødning i musklen. Hvis dette skulle ske, vil det behandles med is, massage og/eller udstrækning og vil forsvinde efter få dage. For at minimere risikoen for infektion, vil der blive benyttet sterile procedurer.

### **Iskæmisk prækonditionering**

Der kan forekomme akut ubehag ved oppustning af manchetten rundt om låret. For at minimere eventuelt ubehag vil forsøgspersonen gradvist blive tilvænnet denne procedure. Hvis forsøgspersonen ønsker det, vil luften straks kunne lukkes ud af manchetten.

### Nærinfrarød spektroskopi (NIRS)

Der er ingen risici forbundet med brug af nærinfrarød spektroskopi til måling af energiomsætning og blodgennemstrømning i muskelvævet.

### Måling af elektrisk aktivitet i hjertet

Måling af hjertets elektriske aktivitet ved brug af elektroder påsat overkroppen er standard procedure og ikke forbundet med risici.

### ***Statistik***

Der anvendes variansanalyser (ANOVA) til at undersøge forskelle i parametre relateret til vaskulær og metabolisk funktion. Gruppe (ikke-rygere og rygere) og konditionering (iskæmisk prækonditionering og sham/falsk konditionering) indgår som faktorer i analyserne. For alle statistiske analyser fastsættes signifikans-niveauet til  $p < 0.05$ . Statistiske analyser laves ved hjælp af SPSS software (SPSS Inc., Chicago, IL, USA). Antallet af forsøgspersoner i hver gruppe er estimeret med en forventning om en effekt-size på 0.25, en statistisk power på 90% og et signifikans-niveau på 5%, hvilket indikerer at det er nødvendigt at rekruttere 36 forsøgspersoner til hver gruppe for at kunne påvise statistiske forskelle for denne type variansanalyse (dvs. 'repeated measures ANOVA within-between interaction') (GPower). Som et konservativt estimat og med forventning om frafald, rekrutterer vi 40 forsøgspersoner til hver gruppe og dermed 80 forsøgspersoner i alt.

### ***Etiske overvejelser***

Der er ingen umiddelbare fordele for de involverede frivillige forsøgspersoner ved deltagelse, men undersøgelsen vil være med til at klarlægge væsentlige spørgsmål omkring sammenhængen mellem det autonome nervesystem, biomarkører og mikrovaskulær funktion hos rygere og ikke-rygere samt hvordan iskæmisk prækonditionering (IPC) påvirker mikrovaskulær funktion i disse grupper.

Den eventuelle risiko for bivirkninger hos disse unge, raske individer er minimale i forhold til de nye informationer, som forsøget forventes at give i forhold til hvordan basal vaskulær funktion kan påvirkes ved IPC. Endvidere er det muligt, at IPC-metoden på sigt kan implementeres i kliniske sammenhænge i forbindelse med undersøgelse af patofysiologiske tilstande i blodkar.

Forsøget afvikles i overensstemmelse med Helsinki-deklarationen, og den mundtlige information til forsøgspersonerne vil følge retningslinjerne i den vedlagte skriftlige information.

### ***Forsikring***

Forsøgspersonerne er dækket af Patientforsikringen.

### ***Personlige data***

Efter forsøget gemmes data. Disse data er reelt alene anvendelige for tolkningen af nærværende forsøg og vil derfor være uinteressante for tredje part.

Data opbevares og gemmes i overensstemmelse med databeskyttelsesreglerne og anden relevant lovgivning.

Forsøget registreres via intern registrering i AAU's Art. 30 Fortegnelse.

## ***Økonomi***

Projektet er initieret af lektor Ryan Godsk Larsen, lektor Andrew Stevenson og lektor Allan Stensballe, Institut for Medicin og Sundhedsteknologi, Aalborg Universitet.

Det finansieres af Institut for Medicin og Sundhedsteknologi med kr. 50.000. Beløbet administreres af Institut for Medicin og Sundhedsteknologi.

Projektdeltagerne har ikke økonomiske forbindelser til eksterne virksomheder eller andre parter, som kunne have interesse i projektet.

## ***Vederlag til forsøgspersoner***

Forsøgspersonerne vil ikke modtage vederlag for deres deltagelse i forsøget.

## ***Publicering af resultater***

Projektets resultater vil blive offentliggjort uanset udfaldet af projektet.

## ***Tidsplan***

Projektet startes efter godkendelse fra Videnskabsetisk Komite og forventes afsluttet i december 2023.

## ***Retningslinjer for mundtlig information og informeret samtykke***

### **Indkaldelse af mulige forsøgspersoner**

Når mulige forsøgspersoner henvender sig til kontaktpersonen på opslaget med henblik på deltagelse i forsøget, skal følgende oplyses:

- At der er tale om en anmodning om deltagelse i et videnskabeligt forsøg
- Formålet med forsøget
- At det er frivilligt at deltage, og at forsøgspersonen når som helst kan trække sit tilsagn om deltagelse tilbage, uden at dette vil påvirke forsøgspersonens nuværende eller fremtidige behandling
- At forsøgspersonen har ret til betænkningstid, før der afgives samtykke til deltagelse i forsøget, og at forsøgspersonen har ret til at medbringe en bisidder, når den mundtlige information gives. Forsøgspersonen vil få udleveret skriftet "Forsøgspersonens rettigheder i et sundhedsvidenskabeligt forskningsprojekt", som indeholder oplysninger omkring tavshedspligt, aktindsigt og klageadgang
- At materialet "Deltagerinformation" fremsendes pr. brev til forsøgspersonen, således at denne kan få oplysninger om forsøget inden informationssamtalen.
- Til slut aftales et tidspunkt og sted for informationssamtalen

### **Informationssamtalen**

Til informationssamtalen reserveres et egnet lokale, f.eks. et mødelokale, hvor samtalen kan gennemføres uforstyrret. Der kan evt. serveres kaffe, te og/eller sodavand. Selve informationssamtalen skal afholdes af den projektansvarlige eller en seniorforsker, der har fået bemyndigelse til dette.

Samtalen skal indeholde følgende oplysninger/spørgsmål:

- Det er frivilligt at deltage, og forsøgspersonen kan når som helst trække sit tilsagn om deltagelse tilbage, uden at dette vil påvirke hans/hendes nuværende eller fremtidige behandling
- Forsøgspersonen har ret til betænkningstid før samtykket afgives, og forsøgspersonen har ligeledes ret til at medbringe en bisidder, når han/hun modtager den mundtlige information.
- Forsøgspersonen spørges, om han/hun ønsker, at der er en bisidder til stede.
- Formålet med forsøget oplyses, og det forklares, hvordan forsøget skal udføres. Der tages udgangspunkt i "Deltagerinformation", som forsøgspersonen har modtaget inden informationssamtalen.
- Forsøgspersonen spørges, om han/hun er sund og rask, og om han/hun er bærer af en smitsom sygdom
- Forsøgspersonen spørges, om vedkommende er dansk statsborger. Hvis svaret er nej, spørges vedkommende, om han/hun har en gyldig arbejdsstilladelse.
- Skriftet "Forsøgspersonens rettigheder i et sundhedsvidenskabeligt forskningsprojekt" udleveres, og det forklares, at skriftet indeholder oplysninger omkring tavshedspligt, aktindsigt og klageadgang.
- Forsøgspersonen spørges om, at han/hun har gennemlæst "Deltagerinformation". Hvis dette ikke er tilfældet, beder vi forsøgspersonen gennemlæse denne.
- Når det er sikret, at forsøgspersonen har gennemlæst deltagerinformationen, spørges forsøgspersonen, om han/hun har spørgsmål til forsøget?
- Herefter gives forsøgspersonen en demonstration i laboratoriet og div. måleudstyr og dets anvendelse i forsøget fremvises.
- Forsøgspersonen gøres opmærksom på, at han/hun har ret til betænkningstid, før samtykket afgives (vær opmærksom på, at Den Nationale Videnskabsetiske Komité anbefaler, at man så vidt muligt skal give 24 timers betænkningstid!).
- Forsøgspersonen gøres igen opmærksom på, at det er frivilligt at deltage, og at han/hun når som helst trække dit tilsagn om deltagelse tilbage, uden at dette vil påvirke nuværende eller fremtidige behandling.
- Forsøgspersonen oplyses om, at såfremt han/hun ikke ønsker at gøre brug af betænkningstiden, kan samtykket afgives herefter.

- Der aftales tidspunkt og sted for forsøgets afholdelse.
- Til slut informeres der om, hvem der er kontaktperson for projektet (det vises, at navnet fremgår af "Deltagerinformation"), og at denne person til hver en tid kan kontaktes, hvis der er yderligere spørgsmål.

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## 4. Etisk protokol - deltagerinformation

### Deltagerinformation

**Forsøgets titel:** Effekten af iskæmisk prækonditionering på blodkarfunktion hos rygere og ikke-rygere

Vi vil spørge, om du vil deltage i et sundhedsvidenskabeligt forsøg, der udføres ved Aalborg Universitetshospital og Institut for Medicin og Sundhedsteknologi, Aalborg Universitet.

Før du beslutter, om du vil deltage i forsøget, skal du fuldt ud forstå, hvad forsøget går ud på, og hvorfor vi gennemfører forsøget. Vi vil derfor bede dig om at læse denne deltagerinformation grundigt.

Du vil blive inviteret til en samtale om forsøget, hvor denne deltagerinformation vil blive uddybet, og hvor du kan stille de spørgsmål, du har om forsøget. Du er velkommen til at tage et familiemedlem, en ven eller en bekendt med til samtalen.

Hvis du beslutter dig for at deltage i forsøget, vil vi bede dig om at underskrive en samtykkeerklæring. Husk, at du har ret til betænkningstid, før du beslutter, om du vil underskrive samtykkeerklæringen.

Det er frivilligt at deltage i forsøget. Du kan når som helst og uden at give en grund trække dit samtykke tilbage.

#### Formål med forsøget

Kroppens evne til at kunne transportere ilt til musklerne via blodbanen er afgørende for at vores muskler kan arbejde.

Med dette forsøg ønsker vi at undersøge, hvordan gentagne afklemninger af blodgennemstrømningen til underbenet påvirker funktionen i blokarrene hos henholdsvis rygere og ikke-rygere. Teknikken med afklemning af blodgennemstrømningen kaldes også iskæmisk prækonditionering. Mere viden omkring afklemningsmetodens virkning kan forhåbentlig på sigt bidrage til forbedring af forebyggelsen og behandlingen af personer med nedsat karfunktion.

#### Hvem kan deltage i forsøget?

Du kan deltage i forsøget, hvis du er:

1. rask, mand og i alderen 21-34 år
2. normalvægtig (Body Mass Index mellem 18,5 og 30 kg/m<sup>2</sup>)
3. ikke-ryger  
*eller*
4. rygere (defineret som nuværende eller tidligere fast ryger og nuværende "festryger". Dette indbefatter også brug af snus samt andre tobaks- og/eller nikotinprodukter)

Du kan ikke deltage i forsøget, hvis du blandt andet udøver okklusionstræning (en træningsform, der benytter lave vægtbelastninger kombineret med delvis aflukning af blodgennemstrømningen), lider af søvnapnø, har lidelser i arme, ben, har en kredsløbssygdom, lider af forhøjet eller lavt blodtryk, bruger medicin for hjertet, stimulanser eller beroligende, er misbruger af hash eller andre euforiserende stoffer.

Du vil blive bedt om at afholde dig fra indtagelse af koffein (fx kaffe og te), og undgå anstrengende fysisk aktivitet 24 timer inden hvert besøg. Du må heller ikke indtage fødevarer med et højt indhold af antioxidanter (fx bær, granatæble, mørk chokolade mm.) 48 timer inden hvert besøg. Desuden skal du være fastende 12 timer inden forsøgsdeltagelse. Hvis du er ryger må du ikke ryge 12 timer inden forsøgsdeltagelse.

### **Hvordan foregår forsøget?**

Du skal deltage i 2 forsøgssessioner, der hver varer ca. 2 timer. Sessionerne udføres over en periode på 1-2 uger.

I forbindelse med forsøget skal du gennemgå nedenstående tests og undersøgelser:

#### Blodprøve

I begyndelsen og slutningen af hver session vil der blive foretaget en blodprøve på 18 mL blod; i alt 4 blodprøver i forsøgene.

#### Måling af hjertets elektriske aktivitet

Vi måler den elektriske aktivitet i dit hjerte med overfladeelektroder, som sættes på din overkrop. Målingerne foregår, mens du ligger på ryggen og hviler dig.

#### Bestemmelse af blodgennemstrømning

Vi måler blodgennemstrømningen i dit ben ved brug af målinger med infrarødt lys, hvor lysfølsomme sensorer kæbes på huden over dine muskler. Målingerne foretages, mens du ligger på en briks med foden placeret i en pedal samtidigt med, at du foretager kortvarige sammentrækninger af musklerne.

#### Afklemning af blodgennemstrømning

Ved hjælp af en blodtryksmanchet, som blæses op omkring dit ene lår, vil vi over en periode på 50 minutter skiftevis afklemme blodgennemstrømningen i dit ben og åbne op for gennemstrømningen igen. Hver afklemning har en varighed på 5 minutter efterfulgt af 5 minutter uden afklemning. Du skal også ligge ned og slappe af under disse undersøgelser.

### **Biologisk materiale**

Ved forsøget tager vi i alt 4 blodprøver på 18 mL hver med henblik på at undersøge proteinsammensætningen i dit blod og for at kunne relatere fundene til afklemning af blodgennemstrømning.

Der oprettes en forskningsbiobank, hvor prøverne opbevares, indtil de skal analyseres. Prøverne vil få et løbenummer, som gør, at du ikke kan identificeres. Prøverne opbevares i op til 15 år, og eventuelt overskydende materiale gemmes, så eventuelle nye resultater efterfølgende kan valideres. Efter 15 år destrueres overskydende materiale.

Det er muligt, at forskergruppen ønsker at anvende dine prøver i forbindelse med et andet studie – fx til sammenligning med væv fra andre forsøgspersoner. Hvis dette bliver

tilfældet, så vil forskergruppen tage kontakt til dig og spørge, om du vil deltage i det nye studie, som vil kræve en ny informationssamtale og et nyt skriftligt samtykke fra dig. Derudover skal Den Videnskabetiske Komité også godkende og give tilladelse til det nye forsøg.

### **Risici, bivirkninger og ulemper**

Der er ingen kendte risici eller bivirkninger forbundet med de metoder, der anvendes i forsøget. Metoderne anvendes rutinemæssigt på Aalborg Universitetshospital og Institut for Medicin og Sundhedsteknologi på Aalborg Universitet og ved mange forskningsinstitutioner over hele verden.

Blodprøverne vil blive udført af trænet personale og der er minimal risiko forbundet med denne procedure. Dog kan der i forbindelse med blodprøvetagning opstå en blødning i muskelen. Dette behandles med is, massage og/eller udstrækning og vil forsvinde efter få dage. For at undgå risikoen for infektion, vil der blive benyttet sterile procedurer.

Der kan forekomme akut smerte ved oppustning af manchetten rundt om låret. For at minimere eventuelt ubehag vil du gradvist blive tilvænnet denne procedure. Hvis du ønsker det, vil luften straks blive lukket ud af manchetten.

Forsøgene med test af blodkarfunktion, ved brug af nærinfrarødt lys, er ikke forbundet med risici.

Måling af hjertets elektriske aktivitet er standard procedure og ikke forbundet med risici.

Der kan være risici ved forsøget, som vi endnu ikke kender. Vi beder dig derfor om at fortælle, hvis du oplever problemer med dit helbred, mens forsøget står på. Hvis vi opdager bivirkninger, som vi ikke allerede har fortalt dig om, vil du naturligvis blive orienteret med det samme, og du vil skulle tage stilling til, om du ønsker at fortsætte i forsøget.

### **Nytte ved deltagelse**

Der vil ikke være fordele for dig ved at deltage i forsøget, men forsøget bidrager med væsentlig viden omkring sammenhængen mellem karfunktion og afklemning af blodgennemstrømningen hos rygere og ikke-rygere. Endvidere er det som nævnt muligt, at afklemningsmetoden på sigt kan anvendes til forebyggelse eller behandling af personer med nedsat karfunktion.

### **Udelukkelse fra og afbrydelse af forsøg**

Reagerer du efter forsøgslederens vurdering uventet på forsøgets procedurer, eller viser du dig på anden vis ikke egnet til videre deltagelse i forsøget, kan forsøget til ethvert tidspunkt afsluttes. Forsøget som helhed vil blive stoppet, hvis det skulle vise sig, at du generelt ikke tolererer procedurerne i forsøget eller finder forsøget for udmattende.

### **Persondata**

Efter forsøget gemmer vi de data, vi har opsamlet under forsøget. Disse data er reelt alene anvendelige for tolkningen af nærværende forsøg og vil derfor være uinteressante for tredje part.

Data opbevares og gemmes i overensstemmelse med databeskyttelsesreglerne og anden relevant lovgivning.

### **Oplysninger om økonomiske forhold**

Forsøget er initieret af lektor Ryan Godsk Larsen, lektor Andrew Stevenson og lektor Allan Stensballe fra Institut for Medicin og Sundhedsteknologi, Aalborg Universitet.

Forsøget finansieres med kr. 50.000 af Institut for Medicin og Sundhedsteknologi. Beløbet administreres af Institut for Medicin og Sundhedsteknologi.

Forskerne bag forsøget har ikke økonomiske forbindelser til eksterne virksomheder eller andre parter, som kunne have interesse i projektet.

Du vil ikke modtage økonomisk kompensation for din deltagelse i forsøget.

### **Adgang til forsøgsresultater**

Projektets resultater vil blive offentliggjort uanset udfaldet af projektet.

Forsøget er godkendt af "Den Videnskabetiske Komité for Region Nordjylland", sagsnummer N-20190015.

Vi håber, at du med denne information har fået tilstrækkeligt indblik i, hvad det vil sige at deltage i forsøget, og at du føler dig rustet til at tage beslutningen om din eventuelle deltagelse. Vi beder dig også om at læse det vedlagte materiale "Forsøgspersonens rettigheder i et sundhedsvidenskabeligt forskningsprojekt".

Hvis du vil vide mere om forsøget, er du meget velkommen til at kontakte undertegnede.

Med venlig hilsen

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## Forsøgspersonens rettigheder i et sundhedsvidenskabeligt forskningsprojekt

Som deltager i et sundhedsvidenskabeligt forskningsprojekt skal du vide at:

- din deltagelse i forskningsprojektet er helt frivillig og kan kun ske efter, at du har fået både skriftlig og mundtlig information om forskningsprojektet og underskrevet samtykkeerklæringen
- du til enhver tid mundtligt, skriftligt eller ved anden klar tilkendegivelse kan trække dit samtykke til deltagelse tilbage og udtræde af forskningsprojektet. Såfremt du trækker dit samtykke tilbage påvirker dette ikke din ret til nuværende eller fremtidig behandling eller andre rettigheder, som du måtte have
- du har ret til at tage et familiemedlem, en ven eller en bekendt med til informationssamtalen
- du har ret til betænkningstid, før du underskriver samtykkeerklæringen
- oplysninger om dine helbredsforhold, øvrige rent private forhold og andre fortrolige oplysninger om dig, som fremkommer i forbindelse med forskningsprojektet, er omfattet af tavshedspligt
- opbevaring af oplysninger om dig, herunder oplysninger i dine blodprøver og væv, sker efter reglerne i databeskyttelsesforordningen, databeskyttelsesloven samt sundhedsloven
- der er mulighed for at få aktindsigt i forsøgsprotokoller efter offentlighedslovens bestemmelser. Det vil sige, at du kan få adgang til at se alle papirer vedrørende din deltagelse i forsøget, bortset fra de dele, som indeholder forretningshemmeligheder eller fortrolige oplysninger om andre
- der er mulighed for at klage og få erstatning efter reglerne i lov om klage- og erstatningsadgang inden for sundhedsvæsenet. Hvis der under forsøget skulle opstå en skade, kan du henvende dig til Patienterstatningen, se nærmere på [www.patienterstatningen.dk](http://www.patienterstatningen.dk).

(Dette tillæg udgives af det videnskabsetiske komitéssystem og kan vedhæftes den skriftlige information om det sundhedsvidenskabelige forskningsprojekt. Spørgsmål til et konkret projekt skal rettes til projektets forsøgsansvarlige. Generelle spørgsmål til forsøgspersoners rettigheder kan rettes til den komité, som har godkendt projektet.)

## 5. Forsøgsprotokol

### **Forberedelse før forsøgspersonerne kommer**

#### **Samtykkeerklæring**

- Samtykkeerklæringen ligger klar til at blive underskrevet.
- Udføres kun under første forsøgsgang.

#### **Randomisering**

- Kuverterne ligger klar.
  - To med IPC, to med sham.
  - Når en kuvert bliver trukket, bliver den lagt til side. Efter alle kuverter er blevet trukket, genbruges de.
- Udføres kun under første forsøgsgang.
  - Den omvendte konditionering benyttes under anden forsøgsgang.

#### **Karakteristika af forsøgspersonerne**

- Målebånd er fastgjort til væggen.
- Vægten står klar.
- Computeren står klar med dataark åbnet.
- Udføres kun under første forsøgsgang.

#### **Near-infrared spectroscopy (NIRS)**

Udstyr der ligges klar:

- Computeren og NIRS-apparatet tændes.
- Skruetrækker.
- Fixomull tape.
- Saks.
- Engangsskraber.
- Alkoholswabs.
- Sprittusch.

Opret ny fil:

- Systemet opvarmes indtil "status" lyser grønt (ca. 5-10 min).
- Start "OxySoft DAQ 2.1.6".
- Tryk "File" → "New".
- Tryk "Projekt" → "DAQ Management Wizard".
- Udfyld "Name" → "IPC MG".
- Tryk "Browse" og gem i mappen "Magnus og Gustav NIRS".
- Tryk "Next".
- Under "Optode template" vælges "1 channel TSI QCF"
  - $DPF = 4$ ,  $d(\text{Nom})(\text{mm}) = 40$ ,  $d(\text{Slope})(\text{mm}) = 4$ ,  $k = 1.55$ ,  $h(1/\text{mm } 1/\text{nm}) = 0.00063$ .
- Tryk "Next" og tryk "Next" igen.
- Sample rate sættes til 2 Hz.

- Tryk "Next".
- Vælg "Do not start measurement..".
- Tryk "Finish".
- Sæt flueben i alle kasserne bortset fra "HbDiff".

Kalibrering:

- Fjern det blå stykke plastik under optoden.
- Tryk "DAQ" → "Absolute Calibration".
- Tryk "Proceed"
  - Gentages indtil alle processerne er gennemført.
- Tryk "Save".
- Gem som fx "FP1test1".
- Tryk "Exit"
- De to dele af NIRS proben skrues sammen.
  - Det andet hul fra venstre på den side der skal have kontakt med huden.
  - Den skal stikke ud, så der er ca. 1-2 mm.

### Contraction-induced hyperemia (CIH)

Udstyr der ligger klar:

- National Instruments board.
  - Kobles til Output på Amplifieren og til PC via USB.
- Fodpedalen.
  - Kobles til Input på Amplifieren.

Opstart af LabView2018:

- Tryk på ikonet i proceslinjen.
- Åben "IPC – Continuous Input.vi".

### Heart rate variability (HRV) og photoplethysmography (PPG)

Udstyr der ligger klar:

- Shimmer oplades fuldt dagen før.
- Alkoholswabs.
- 5 stk elektroder.
- 5 ledninger.
- Expansionboard med PPG.
- Elastikbælte.

Opret forbindelse til shimmer (i docken):

- Åben "Consensus".
- Tryk "Launs now" under basic.
- Tryk "LIVE DATA".
- Tænd for shimmeren.
  - Skal blinke blå og grøn.

- Tryk på "LIVE DATA ikonet" og derefter "play ikonet".
  - Skal være henholdsvis blå og grøn.
  - Overall Packets skal være 100% og shimmeren lyser blå.

### **Ischemic preconditioning (IPC) og sham conditioning**

- Blodtryksmanchet.

### **Udførelse af forsøget**

#### **Samtykkeerklæring**

- Samtykkeerklæringen underskrives og arkiveres.
- Udføres kun under første forsøgsgang.

#### **Randomisering**

- Forsøgspersonen trækker en kuvert og behandlingen noteres i dataarket.
- Udføres kun under første forsøgsgang.

#### **Karakteristika af forsøgspersonerne**

- *Alder*: Alderen noteres i dataarket.
- *Højde*: Forsøgspersonen placeres med ryggen mod væggen med hælene helt ind til væggen. Højden noteres i dataarket.
- *Vægt*: Forsøgspersonen vejes og vægten noteres i dataarket.
- *Ugentlig aktivitetsniveau*: Gennemsnitlig ugentlig aktivitet i timer noteres i dataarket.
- *Rygning*: Gennemsnitlig antal cigaretter om dagen og antal år som ryger noteres i dataarket.
- Udføres kun under første forsøgsgang.

#### **Near-infrared spectroscopy (NIRS)**

##### *Måling:*

- Proben fastgøres til muscledelly af tibialis anterior med to stykker fixomull tape.
- Tryk "start".
  - Knappen er en lille grøn cylinder.
- Tryk på "DAQ".
- Tryk "receiver gain" → "all receiver gain high".
- Tjek at ingen af kanalerne lyser rød, ellers rettes proben.
- Tryk "start recording".
- Efter hele protokollen er gennemført trykkes "stop recording".

#### **Contraction-induced hyperemia (CIH)**

##### *Instruks:*

- Pres hælen mod pladen og træk med tæerne. Det er vigtigt det kun er foden du laver bevægelsen i, således du kan mærke at du bruger musklerne på skinnebenet.
- Jeg vil tælle ned fra fem og derefter sige "træk". Her skal du trække så hurtigt og kraftigt som muligt.
- Efter ét sekund siger jeg "slap af". Her skal du slappe af i fodleddet så hurtigt som muligt. Indtil da, holder du spændingen i trækket.

*Måling:*

- Forsøgspersonens venstre fod fastspændes til fodpedalen.
- Pude foldes under knæet.
- Der ventes til NIRS målingen er stabil.
- Tryk "Run" – hvid pil øverst.
- Ti sekunder før første kontraktion trykkes på "Log to file [F10]".
  - Når der optages, lyser denne knap grøn.
- Gennemgang af kontraktioner.
  - Der foretages fem kontraktioner af ét sekunds varighed med ét minuts pause mellem hver kontraktion.
- Efter sidste kontraktion trykkes på "Log to file [F10]" og derefter "stop [esc]".
- Tryk på "Stifinder" i proceslinjen".
- Vælg "Document" → "Ryan".
- Den nyeste fil overføres til mappen "Magnus og Gustav" og omdøbes til fx "FP1\_1\_1".

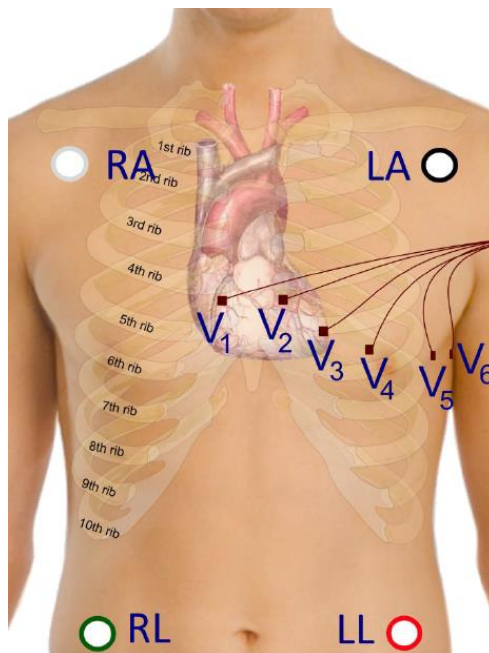
**Heart rate variability (HRV) og photoplethysmography (PPG)**

*Instruks:*

- Vi kommer til at optage i fem minutter. I disse fem minutter er det meget vigtigt, at du ligger fuldstændig stille, ellers kan det lave forstyrrelser på optagelsen.

Placering af elektroder:

- Elektroder placeres som angivet på nedenstående billede. Elektroden til ribbenene, benyttes V<sub>5</sub>.



*Måling:*

- Shimmeren tages ud af docken og fastgøres til bæltet, der er placeret lige under brystkassen.
- Ledningerne isættes således:
  - Hvid = RA (right arm).
  - Sort = LA (left arm).
  - Grøn = RL (right leg).
  - Rød = LL (left leg).
  - Brun = Vx (V<sub>s</sub>, ribben).
- Expansionboard med PPG tilkobles og tapes fast til øret med to stykker fixomull.
- Signaler tjekkes.
  - Ext\_Exp\_A6.
  - ECG\_LL-RA\_24BIT.
  - Hvis signalerne ikke er gode, korrigeres der.
- Tryk "START TO PC".
  - Dette gøres samtidig med NIRS.
- Når målingen er færdig, tryk "STOP TO PC" og på "play ikonet".
- Tryk "MANAGE DATA".
- Vælg mappen "Kathrine\_1".
- Tryk på "synkroniserings tegnet" på den nyeste fil.
- Tryk "Done" når synkroniseringen er på 100%.
- Klik flueben ved ønskede fil.
- Tryk på to på pile i nederste venstre hjørne.
- Sæt "File Format" til ".mat".
- Tryk "EXPORT".
  - Exporteres til den ønskede mappe.

**Ischemic preconditioning (IPC) og sham conditioning**

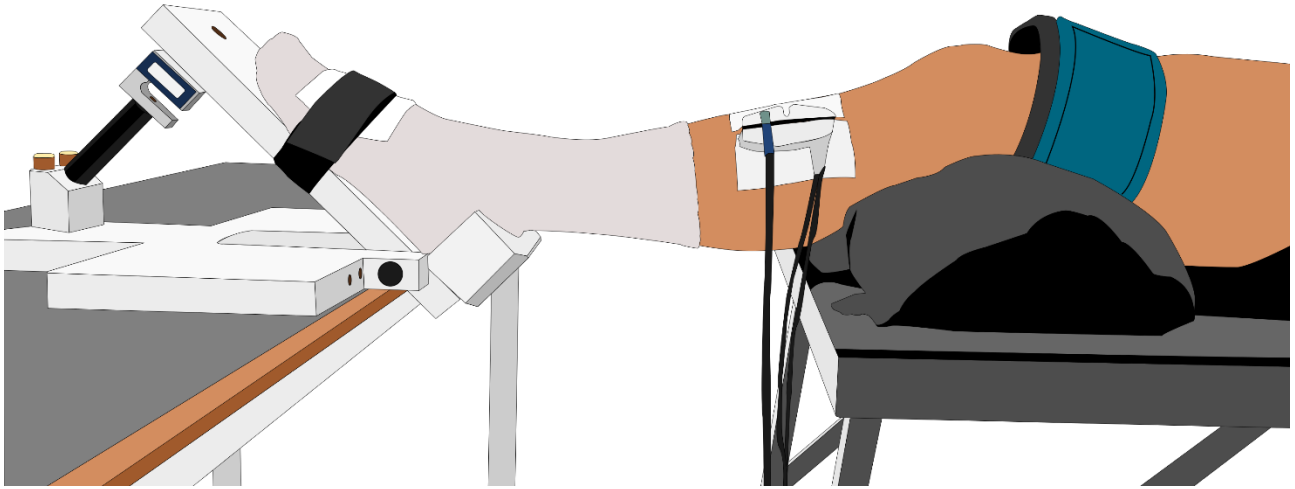
*Instruks:*

- Vi placerer denne blodtryksmanchet omkring dit lår. Den vil vi puste op i fem minutter, hvorefter vi lukker luften ud i fem minutter. Dette gentager vi fem gange. Det er vigtigt at du ligger så stille som muligt undervejs.
- Vi undersøger forskellige tryks indvirkning, så det vil komme til at føles forskelligt i dag og næste gang.
- Første oppustning kan godt føles en smule ubehageligt, men det går hurtigt over.

*Måling:*

- Blodtryksmanchetten placeres omkring låret, lige over knæet.
- Blodtryksmanchetten pumpes op til 250 eller 20 mmHg i fem minutter.
  - Kontroller hele tiden at trykket holdes.
- Luften lukkes herefter ud i fem minutter.
- Gentages fem gange.
- Herefter fjernes blodtryksmanchetten igen.

## Forsøgsopstilling



## Udstyrsliste

- Målebånd (til højde)
- Vægt
- Computer til persondata
- NIRS-apparat (Oxymon system Mk III, Artinis Medical Systems, Elst, NL)
  - Computer
- Skruetrækker
- Fixomull tape
- Saks
- Sprittusch
- Engangsskraber
- Alkoholswabs
- Fodpedal
  - Force transducer (SSM-AJ-1000, Interface, Scottsdale, US)
  - Computer med LabView2018
  - National Instruments board amplifier
- Shimmer (Dublin, IE)
  - Dock
  - 5 ledninger
  - Expansionboard med PPG
  - Elastikbælte
- Elektroder (Neuroline 720, Ambu, Copenhagen, DK)
- Blodtryksmanchet (61 cm, VBM, Sulz am Neckar, DE)