The stroke volume response to exercise in healthy volunteers: a test-retest pilot study

Trial registration: None

Danish title: Ændringer i hjertets slagvolumen hos raske under muskelarbejde

Protocol no.: 1.2

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1. Abstract

Background

Changes in cardiac output, driven by increased heart rate and stroke volume, are crucial for oxygen delivery during acute exercise and influence maximal oxygen uptake (VO_2max). Thoracic electrical bioimpedance provides a non-invasive alternative to traditional invasive methods by estimating stroke volume through impedance changes during ventricular filling and emptying. While the texchnical precision of the method is well-established, the biological variability of the cardiac output response to exercise remains to be established, requiring further investigation for use in acute and long-term training assessments.

Methods

Inclusion: 12 healthy individuals (six males, six females)

Exclusion: Known ischaemic heart disease, known heart failure, symptoms of disease within 2 weeks prior to the study, known malignant disease, claudication, pregnancy, unstable cardiac arrhythmic disease, renal or liver dysfunction, known chronic lung disease

Design: Test-retest study of thoracic electrical bioimpedance-based stroke volume

Intervention: Acute exercise bout on a bicycle ergometer

Statistical design: Rest-to-exercise changes, as well as between-day smallest real difference, coefficient of variation and intraclass correlation coefficient

Sample size: Since this is a pilot study, the sample size is set at 12 in accordance with current recommendations.

Regulatory considerations

This study will be submitted for approval by Regional Scientific Ethical Committee and the Knowledge Centre for Data Reviews.

Perspective

The results are relevant for designing future studies in which stroke volume is measured and compared in response to various interventions at rest and during exercise.

2. Background

Changes in cardiac output due to increased heart rate and stroke volume constitute a fundamental aspect of the cardiorespiratory response to acute exercise, ensuring sufficient oxygen delivery to working tissues (1). Indeed, the increase in stroke volume represents an important limiting factor in maximal oxygen uptake (VO_2max), i.e. maximal aerobic capacity, and an enhancement in the stroke volume response to acute exercise is furthermore the primary adaptation to aerobic exercise training (2). While measurement techniques that estimates stroke volume via electrodes placed on the thoracic cage to measure impedance changes associated with ventricular filling and emptying, are available, with well-established tehnical precision (3,4), the biological variability of this response remains to be established by test-retest studies in healthy adults. However, studies on its test-retest reliability remain scarce, with existing investigations limited to a study on healthy children and children with congenital heart disease during cycling (5,6) and another on patients with chronic heart failure undergoing a six-minute walk test (7). In order to assess both acute changes in stroke volume and long-term adaptations over an extended training period—particularly in evaluating the effect of training on the rest-to-exercise increase in stroke volume—it is essential to establish the day-to-day biological viariability of the response.

3. Aim

To assess the test-retest reliability of the stroke volume response to incremental exercise in healthy volunteers.

4. Methods

4.1 Study design and recruitment

Participants will be recruited through advertisements on notice boards at Rigshospitalet and at the University of Copenhagen and by advertisements in newspapers, magazines, social media. We have created a specific social media account which is used on different social medias to share knowledge and potentially recruit participants. It will not be possible to comment or tag people in our posts, and potentially interested participants will only be able to contact our research group via encrypted Region H mail. An e-mail address and telephone number will be provided by which interested participants can contact the research group. To ensure that individual is eligible, we will contact the participant by telephone and ensure that he or she is within the required age

range and interested in the study. No data collection will take place at this stage. Furthermore, an e-mail address will be obtained so that written information about the project will be forwarded by one of the study coordinators, and a meeting time will be set up where the participant is informed in detail about the study if he or she is still interested after having read the written information with a consideration time of at least 24 hours; if not interested, he or she is encouraged to cancel the information meeting by telephone or e-mail. If interested, the meeting will be held in an undisturbed meeting room at the Centre for Physical Activity Research. The participant may bring an assessor during the meeting. At the end of the meeting, the participant will be asked if he/she is ready to decide whether to participate in the study. The participant will have up to three days to provide oral and written consent after the information meeting. Only after the participant has signed the consent form, and if deemed eligible, the participant is booked for the study day.

4.2 Eligibility criteria

Inclusion criteria

- Men and women
- 18-70 years

Exclusion criteria – healthy controls

- Known chronic lung disease
- Known ischaemic heart disease
- Known heart failure
- Symptoms of disease within 2 weeks prior to the study
- Known malignant disease
- Claudication
- Pregnancy
- Unstable cardiac arrhythmic disease
- Renal or liver dysfunction

4.3 Informed consent

If the inclusion criteria are fulfilled, written information about the project will be forwarded by e-mail, and a meeting will be set up where the subject is informed in detail about the study. The subject will be informed that he or she may bring an assessor

('bisidder') during the interview. The meeting will take place in an undisturbed room and information regarding the study will be provided by one of the research group participants with a health science background. Because the written information has been forwarded by e-mail in advance, he or she may provide oral and written informed consent immediately after the meeting if he or she should wish to do so. The subject will have up to three days after the interview to sign the consent form. The subject will be informed both written and orally about the project's substance, potential risks and benefits before consent is given.

4.4 Preliminary testing

After providing oral and written informed consent. Stroke volume, as well as heart rate and arterial blood pressure will be measured at rest while sitting and while. This will be followed over a a cardiopulmonary exercise test (CPET), during which stroke volume and heart rate are continuously measured. The study group will not access the participant's electronic health record.

4.5 Outcomes

Co-primary

- Between-day smallest real difference (SRD) for stroke volume during upright rest
- Between-day coefficient of variance (CV) for for stroke volume during upright rest

Secondary

- Between-day SRD for stroke volume during supine rest
- Between-day CV for for stroke volume during supine rest
- Between-day SRD for stroke volume at 60% of maximal workload (W_{max}) during CPET
- Between-day CV for stroke volume at 60% of W_{max} during CPET

Exploratory

- Between-day SRD for stroke volume at 40% of W_{max} during CPET
- Between-day CV for stroke volume at 40% of W_{max} during CPET
- Between-day SRD for stroke volume at 80% of W_{max} during CPET
- Between-day CV for stroke volume at 80% of W_{max} during CPET
- Between-day SRD for stroke volume at W_{max} during CPET
- Between-day CV for stroke volume at W_{max} during CPET

- Between-day intraclass correlation coefficient (ICC) for stroke volume during upright rest
- Between-day ICC for stroke volume during supine rest
- Between-day ICC for stroke volume at 60% of W_{max} during CPET
- Between-day ICC for stroke volume at 40% of W_{max} during CPET
- Between-day ICC for stroke volume at 80% of W_{max} during CPET
- Between-day ICC for stroke volume at W_{max} during CPET
- Between-day SRD for cardiac output (measured as the product of stroke volume and heart rate) during upright rest
- Between-day CV for for cardiac output during upright rest
- Between-day ICC for for cardiac output during upright rest
- Between-day SRD for cardiac output during supine rest
- Between-day CV for for cardiac output during supine rest
- Between-day ICC for for cardiac output during supine rest
- Between-day SRD for cardiac output at 60% of W_{max} during CPET
- Between-day CV for cardiac output at 60% of W_{max} during CPET
- Between-day ICC for cardiac output at 60% of W_{max} during CPET
- Between-day SRD for cardiac output at 40% of W_{max} during CPET
- Between-day CV for cardiac output at 40% of W_{max} during CPET
- Between-day ICC for cardiac output at 40% of W_{max} during CPET
- Between-day SRD for cardiac output at 80% of W_{max} during CPET
- Between-day CV for cardiac output at 80% of W_{max} during CPET
- Between-day ICC for cardiac output at 80% of W_{max} during CPET
- Between-day SRD for cardiac output at W_{max} during CPET
- Between-day CV for cardiac output at W_{max} during CPET
- Between-day ICC for cardiac output at W_{max} during CPET
- Between-day SRD for heart rate (measured with the CPET equipment) during upright rest
- Between-day CV for for heart rate during upright rest
- Between-day ICC for for heart rate during upright rest
- Between-day SRD for heart rate during supine rest
- Between-day CV for for heart rate during supine rest
- Between-day ICC for for heart rate during supine rest

- Between-day SRD for heart rate at 60% of W_{max} during CPET
- Between-day CV for heart rate at 60% of W_{max} during CPET
- Between-day ICC for heart rate at 60% of W_{max} during CPET
- Between-day SRD for heart rate at 40% of W_{max} during CPET
- Between-day CV for heart rate at 40% of W_{max} during CPET
- Between-day ICC for heart rate at 40% of W_{max} during CPET
- Between-day SRD for heart rate at 80% of W_{max} during CPET
- Between-day CV for heart rate at 80% of W_{max} during CPET
- Between-day ICC for heart rate at 80% of W_{max} during CPET
- Between-day SRD for heart rate at W_{max} during CPET
- Between-day CV for heart rate at W_{max} during CPET
- Between-day ICC for heart rate at W_{max} during CPET
- Uright-to supine changes in stroke volume, heart-rate and cardiac output and arterial blood pressure at rest
- Changes in stroke volume, heart-rate, cardiac output, and arterial blood pressure from upright rest to exercise at 40%, 60%, 80% and 100% of W_{max}.

4.6 Experimental procedures

Overall design

12 healthy individuals (6 males, 6 females). The study consists of two study days. Separation between the study days are at least 48 hours and maximum 10 days.

Before entering the study: Informed written and oral consent.

Study days: The two study days are identical, and consist of stroke volume, heart rate and non-invasive blood pressure measurements during upright rest, in the supine position, and during incremental exercise (CPET).

Maximal aerobic capacity

The participants will undergo a maximal graded exercise test on a bicycle ergometer for evaluation of cardiovascular function and determination of maximal oxygen consumption (VO_2peak). The VO_2peak is assessed during an incremental bicycle ergometer exercise test. The test is initiated by a warmup of 5 min at an intensity

depending on the fitness level of the participant followed by incremental steps of 5-20 watt per minute until exhaustion. The subject's limit of tolerance will be used as the criterion for the finishing the test or if either below 60 rounds per minute (RPM) or any medical circumstances. The greatest 30-sec averaged VO_2 is taken as the VO_2 peak and respiratory exchange ratio (RER) will be noted at this point. The highest achieved heart rate is determined maximal heart rate. Ventilation and expired gases will be measured during the entire test with an indirect calorimetric system, and heart rate will be assessed simultaneously. Saturation will be measured throughout the test, at will be reported as the change from beginning to termination of exercise. Rate of perceived exhaustion (RPE) will be measured immediately after termination of exercise using a 6-20 Borg scale. The participant is verbally encouraged to perform maximum during the test. On study day 2, the exact same workload as during study day 1 will be targeted.

Thoracic electrical bioimpedance

Stroke volume is continuously measured using commercially available technology (PhysioFlow®, Manatec, Type PF05L1, Paris, France). In accordance with the manufacturer's instructions, following gentle skin preparation, six electrodes (Skintact FS-50) are applied: two on the neck, two at the xiphisternum, and one on each side of the chest. The bioimpedance method calculates stroke volume by analysing changes in transthoracic impedance during cardiac ejection. Heart rate is determined based on the R-R interval duration, using the first derivative of the electrocardiogram. Signal quality is assessed via a colour graph, and artifact detection is performed by the PhysioFlow® device. Following an initial calibration period of 20 seconds, continuous measurements are recorded, with stroke volume measurements derived from 10-second artifact-free averages. The PhysioFlow® software requires the input of participant information, including sex, age, height, weight, and systolic and diastolic blood pressures.

4.6 Biological material and biobank

No biological material will be obtained.

4.7 Statistical procedures

General considerations

All statistical analyses will be performed using R statistical software. A two-tailed $p<0.05$ will be considered statistically significant. Data will be presented as mean

(standard deviation (SD)) or mean [95% confidence interval, lower limit (LL), upper limit (UL))]. Within-day differences of stroke volume will be tested using paired t-tests. The p-value will be Bonferroni corrected.

Reliability

Reliability estimates the amount of random error created by variability in the measured variable. Absolute reliability will be presented with Bland-Altman plots and limits of agreement (LOA) and SRD, estimating the difference there will be between two measurements in 95% of the occasions. This will determined by a one-way analysis of variance (ANOVA) to assess the standard deviation within participants (SDw), and SRD was subsequently estimated with the following formula:

$$SRD = (T - quantile\ with\ appropriate\ degress\ of\ freedom) \cdot \sqrt{2 \cdot SDw^2}$$

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Furthermore, CV will serve as a relative reliability estimate. CV estimates in percentages, how much of the variance is caused by measurement error:

$$CV = \frac{SDw}{Mean} \cdot 100$$

Based on the distribution of the estimates of mean and residual variance from a linear mixed model, the distribution of the CV to obtain 95% confidence intervals for the CV will be simulated.

Sample size considerations

Given that the effect size is unknown for the method used for measuring stroke volume, the sample size is set at 12, as recommended for pilot studies, as this provides the optimal information about feasibility and gains in the precision about the mean and variance for use in the design of the subsequent studies (8).

5. Ethical considerations

5.1 Significance, novelty and expected impact.

This study evaluates a physiological response, i.e., the stroke volume response to exercise, by a non-invasive method important for investigating mechanisms of cardiorespiratory fitness in future studies. By determining the test-retest reliability, we will be able to qualify the magnitude of expected physiologically relevant changes, for

example after an exercise intervention in future studies. The results are thus relevant for the understanding of basic exercise physiology and for designing future studies with interventions that aim to affect cardiorespiratory fitness.

5.2 General information

This study will be conducted in accordance with the regional ethical committee and the Declaration of Helsinki. Informed consent will be obtained from all study participants before enrolment and baseline testing. The study falls under the Danish Data Protection Decree ('Databeskyttelsesforordningen') and the Danish Data Protection Law ('Databeskyttelsesloven'), and all data will be handled in accordance with these, including submission to the Knowledge Centre for Data Reviews (Videnscenter for Dataanmeldelser). The protocol will also be submitted for approval to the Regional Ethical Committee of Copenhagen.

Confidentiality of the subjects will be maintained by assigning subjects a study number, keeping identifiers separate from the data and storing data in a locked file and secure computer database. Scientific reports generated from the study will not contain information that would identify the participants.

5.3 Risk of adverse events during the study

The risk of discomfort or dizziness during the exercise protocols is minimal.

5.4 Reporting of adverse events during the study

There are no anticipated adverse events associated with enrolment in the study. However, in the unlikely event an adverse event does occur, all adverse events (AEs) will be recorded on adverse event form (Appendix A). These forms will include a description and classification of the event, date of onset, date resolved, whether the event was serious or not (ICH criteria), relationship of the event to the study (1=none, 2=unlikely, 3=possible, 4=probable, 5=definitely), action taken, and whether the study was suspended or not. All serious adverse events (SAEs), regardless of causation, will be reported to the Regional Ethical Committee of Copenhagen.

5.5 Ensuring integrity and privacy of the participant

All sensitive information obtained during the study will be handled in accordance with the Danish law for protection of personal data ("lov om behandling af

personoplysninger") and the Danish health law ("sundhedsloven"). Both "Databeskyttelsesforordningen" and "databeskyttelsesloven" will be respected.
The study will be submitted to the Danish Data Protection Agency for approval.

5.6 Compensation

Participants will be reimbursed for documented transportation up to a maximum of 1000 DKK.

5.7 Insurance

The study falls under the Danish worker's compensation law ('Lov om Arbejdsskadesikring') as it includes healthy volunteers The Danish Patient Insurance Association ('Patientskadeerstatningen') will cover any injury that may occur to the participants due to the study program.

6. Study location, feasibility, and organization

6.1 Facilities available

All experiments will be conducted at dedicated facility at CFAS, Rigshospitalet. The investigators have vast experience with exercise studies and high-level expertise with all the described techniques. All equipment necessary for a successful completion of the study is available, and all techniques are well established. Therefore, the study is feasible.

6.2 Funding

The costs associated with the study are covered by the Centre for Physical Activity Research at Rigshospitalet. It is PI Ronan Berg who have taken the initiative to conduct this study. The PI's salary is paid by the Department of Clinical Physiology and Nuclear Medicine, Rigshospitalet (50%) and Centre for Physical Activity Research, also Rigshospitalet (50%). None of the involved researchers or any third parties have any private or financial interests in the study.

7. Study results

7.1 Novelty and importance

This study provides fundamental knowledge on the limitations of exercise performance in humans.

7.2 Dissemination of study results

Positive as well as negative or inconclusive results of the study will be disseminated through publication in international peer-reviewed journals.

8. Time Schedule

As soon as the protocol is approved this study will be initiated. It is estimated that the study period will encompass approximately 12 months from 1. April 2025, to March 31, 2026. Data analysis and subsequent scientific writing for publication will be performed directly hereafter.

9. References

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Appendix A. Adverse event reporting guidelines

Reporting of adverse reactions (in health scientific research projects not involving medicinal products)

Serious unexpected adverse reactions or events:
The chief investigators must immediately inform the committee if suspected unexpected serious adverse reactions or serious events occur during the project. The report must include comments on any outcomes for the concerned trial.

Reporting must take place no later than 7 days after the sponsor or the chief investigator became aware of any such adverse reactions or events.

In case of serious adverse reactions or serious events resulting from the project, the chief investigator must make available any information requested by the committee.

The report can be made using a particular form prepared by the committee system. The form is in Danish and downloadable from (http://www.dnvk.dk/English/Reporting%20of%20adverse%20reactions.aspx). The form and attachments can be submitted electronically to the regional research ethics committee using digital signature.

Annual report:
Once every year and throughout the trial period, the chief investigator must submit a list of all serious expected and unexpected adverse reactions and all serious events having occurred in the period. Enclosed with the report must be an assessment of the trial subjects' safety.

The reported material can be in either Danish or English.

The report must be made using a form prepared by the committee system. The form is in Danish and downloadable from (http://www.dnvk.dk/English/Reporting%20of%20adverse%20reactions.aspx).

The form and attachments can be submitted electronically to the regional research ethics committee using digital signature.