Microvascular Sex and Age-related Day-to-day Variability in Healthy Subjects

- Statistical Analysis plan

NCT-number: 06009939

Document revision history

Issue	Summary of change
01	New document

Statistical program
The statistical analysis will be conducted in IBM SPSS (SPSS, Chicago, IL).

Statistical analysis of Day-to-day variability
Median values from the baseline data of each measurement will be extracted for all parameters. The maximum value during the post-occlusive reactive hyperemia (PORH) will also be extracted for all parameters. See Table 1 and Table 2 for more details regarding which values will be included in the data analysis.

Table 1. Values extracted from the EPOS data of each measurement.

Time period	Conventional perfusion	Speed-resolved-perfusion -Low speed	Speed-resolved-perfusion -Medium speed	Speed-resolved-perfusion -High speed	Oxygen saturation	Vessel diameter	RBC tissue fraction
Baseline of foot (10 minutes)	Median	Median	Median	Median	Median	Median	Median
Occlusion of foot (5 minutes)	No value extracted	No value extracted	No value extracted	No value extracted	No value extracted	No value extracted	No value extracted
PORH in foot (5 minutes)	Peak value	Peak value	Peak value	Peak value	Peak value	Peak value	Peak value
Baseline of arm (10 minutes)	Median	Median	Median	Median	Median	Median	Median
Occlusion of arm (5 minutes)	No value extracted	No value extracted	No value extracted	No value extracted	No value extracted	No value extracted	No value extracted
PORH in arm (5 minutes)	Peak value	Peak value	Peak value	Peak value	Peak value	Peak value	Peak value

Table 2. Values extracted from the PSI-X camera of each measurement.

Time period	Conventional perfusion	Speed-resolved-perfusion -Low speed	Speed-resolved-perfusion -Medium speed	Speed-resolved-perfusion -High speed	Oxygen saturation
Baseline of foot (10 minutes)	Median	Median	Median	Median	Median
Occlusion of foot (5 minutes)	No value extracted	No value extracted	No value extracted	No value extracted	No value extracted
PORH in foot (5 minutes)	Peak value	Peak value	Peak value	Peak value	Peak value
Baseline of arm (10 minutes)	Median	Median	Median	Median	Median
Occlusion of arm (5 minutes)	No value extracted	No value extracted	No value extracted	No value extracted	No value extracted
PORH in arm (5 minutes)	Peak value	Peak value	Peak value	Peak value	Peak value

To identify potential sex- and age-related variability, Wilcoxon test will be conducted for non-normally distributed data and t-test for normally distributed data. To compare the data collected from the foot to the arm, Kruskal-Wallis or Anova will be used.

Since multiple comparisons between groups will be conducted, Bonferroni corrections will be done to reduce the risk of Type 1 error.

Statistical analysis of Vasomotion data

Table 3 describes the values from each measurement which will be included in the flowmotion analysis. The columns describe the different flowmotion signal types, the rows describe the different groups which will be compared.

Table 3. Values extracted for the flowmotion analysis, from each measurement.

	Cardiac	Respiratory	Myogenic	Neurogenic	Endothelial
Baseline of foot (10 minutes)	Mean-energy over time	Mean-energy over time	Mean-energy over time	Mean-energy over time	Mean-energy over time
Occlusion of foot (5 minutes)	Mean-energy over time	Mean-energy over time	Mean-energy over time	Mean-energy over time	Mean-energy over time
PORH in foot (5 minutes)	Mean-energy over time	Mean-energy over time	Mean-energy over time	Mean-energy over time	Mean-energy over time
Baseline of arm (10 minutes)	Mean-energy over time	Mean-energy over time	Mean-energy over time	Mean-energy over time	Mean-energy over time
Occlusion of arm (5 minutes)	Mean-energy over time	Mean-energy over time	Mean-energy over time	Mean-energy over time	Mean-energy over time
PORH in arm (5 minutes)	Mean-energy over time	Mean-energy over time	Mean-energy over time	Mean-energy over time	Mean-energy over time

Flowmotion analysis is described in depth in the following articles:

- Fredriksson et al. 2021, *Vasomotion analysis of speed resolved perfusion, oxygen saturation, red blood cell tissue fraction, and vessel diameter: Novel microvascular perspectives.*
- Hultman et al. 2023, *Flowmotion imaging analysis of spatiotemporal variations in skin microcirculatory perfusion.*

Please see references for further details.

For the time being, the different flowmotion signals will be compared on a group level.

Significance level

Significance level is set to $p < 0.05$.

Method for dealing with missing data

The goal is to have 48 complete set of data collection for the variability analysis and recruitment to the study will continue until this is achieved. Flowmotion analysis is more sensitive to artefacts, which most likely will be discovered late in the process once the recruitment to the study already is complete. Therefore we expect to exclude some measurements and most likely will not reach 48 complete sets of flowmotion signals.

Motivation to the number of study-participants

12 participants in each group (men and women, age 20-30 years or 50-60 years), which adds up to a total of 48 participants, is chosen due to the tradition within the study field. In addition, we do not know if there is a difference among the four groups, which is why we chose a smaller study population.

Method for dealing with outliers

Outliers in the collected data caused by movement (and thereby considered artifacts) will be handled using the algorithm presented in reference 2 above.

Visual presentation of data

Data as well as our findings will be presented with boxplots or regression plots.