

Reliability of a software program to analyse endothelial function.

A comparison of manual and semi-automatic measurement of brachial artery diameter.

An experimental study.

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PREFACE

This report is presented as the thesis to complete the requirements of the Bachelor's Degree in Physiotherapy at Fontys University of Applied Sciences, Eindhoven, The Netherlands. This was an individual assignment that has begun in June 2012 and was completed in May 2013.

As morbidity and mortality increases due to cardiovascular incidents, research should focus in the early diagnosing and preventing of such events. A predictor of early atherosclerosis is the flow mediated dilation which is analyzed using ultrasound scanning techniques and specialized software that analyzes the captured vessels diameter. The aim of the report is to test the reliability of a semi-automatic software that aids in measuring the flow mediated dilation and question its use as a clinimetric tool in the rehabilitation field.

Acknowledgements are given to the thesis supervisor, Steven Onkelinx, for providing subject data and ultrasonic assessment results, from his own flow mediated dilation research in the department of rehabilitation sciences in KU Leuven, Belgium.

Special thanks to my parents for all their support through the years and for granting me the opportunity to be a part of an experience like this.

-Katerina Chamatila

SUMMARY

Background: Endothelial dysfunction is a marker of early stages of atherosclerosis and in general of cardiovascular disease. Assessment of the vascular endothelium can be achieved non-invasively, using the flow-mediated dilatation technique.

Method: For the experiment 21 patients with an inactive cardiovascular disease were employed. Using a B-mode ultrasound with a high resolution (12MHz) transducer, images of the brachial artery diameter were captured before and after induced ischemia. Images were comparatively analyzed and flow-mediated dilatation was measured using a manual and a semi-automatic software program.

Results: Inter-correlation coefficient for the measurement was relatively high, with a p-value of 0.855 for flow mediated dilatation, 0.856 for arterial diameter at baseline and 0.756 for the post ischemic arterial diameter, showing good reliability for the software. The coefficient of variation for flow mediated dilatation was 30.32%, a high value compared to the coefficient of variation for the baseline and pos-occlusion diameters, respectively, 4.59% and 5.78%.

Conclusion: Both techniques show acceptable consistency with the semi-automatic outperforming the manual technique in terms of reliability. However, there are indications of high measure of dispersion when it comes to measuring the flow mediated dilatation with the software.

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Introduction

The endothelial lining of vessels operates as an endocrine organ, playing a great role, amongst others, in regulating the vascular tone.¹⁻³ This regulation is accomplished by the action of vasoactive substances produced, out of which Nitric Oxide (NO) is considered to have the greatest influence on the smooth muscle surrounding the vessels.^{1,3,4} NO is produced by the endothelium as a response to shear stress along the vessel walls and acts to inhibit inflammation, cellular proliferation and thrombosis.^{1,3} Endothelium dysfunction appears with a reduced NO bioavailability and is the feature of early cardiovascular and endocrine metabolic pathologies, such as atherosclerosis.^{1,3-5}

Evaluation of the endothelium can be achieved with invasive and non-invasive procedures.^{1,6,7} Non-invasive estimation of endothelial dysfunction is performed by using algorithms based on risk factors of cardiovascular events, such as the Framingham risk score,^{8,9} by measuring the pulse wave amplitude detected by finger arterial pulse wave amplitude,^{1,7,10} or by assessing the endothelial function by measuring the flow mediated dilation (FMD).^{3,11,12} FMD is the most reliable, non-invasive, method to forecast independent prognostic information since it utilizes a marker of NO bioavailability in vivo.^{2,3,12} FMD is a strong indicator of cardiovascular events in patients with an already established cardiovascular disease and a modest predictor in asymptomatic population.^{9,12,13}

To visualize the vessel diameter of the brachial artery, a non-invasive ultrasound scanning (US) technique is used and the images obtained are analyzed.^{3,14,15} Figure 1 illustrates an example of a longitudinal section of a brachial artery obtained by vascular ultrasound scanning technique using an 11MHz transducer.³ FMD is calculated as the dilation induced by increased blood flow to the tissues, after a supra systolic occlusion, which is expressed in either millimetres or percent change from the resting vessel diameter.^{2,3,13} The percentage increase in the vessel diameter calculated is a marker of vascular endothelial dysfunction.^{1,5}

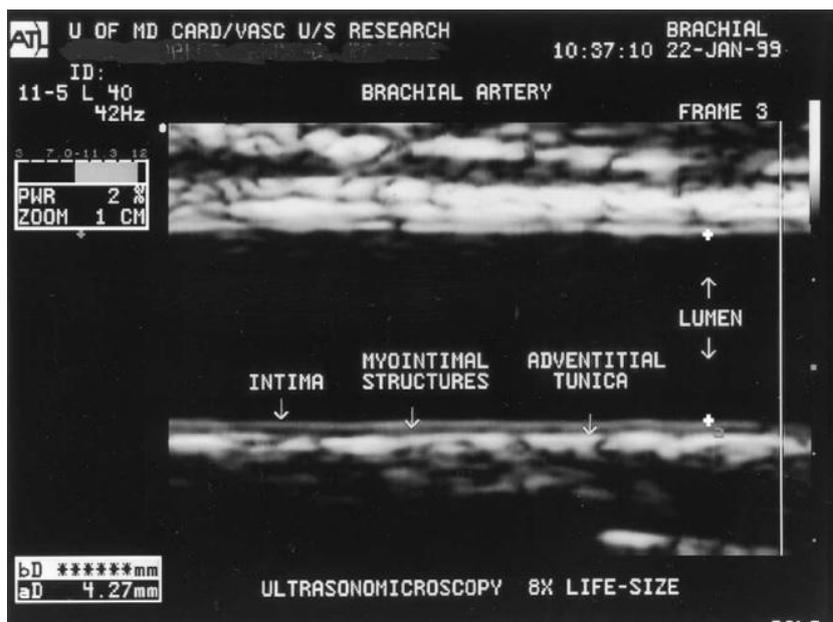


Figure 1. Illustration of brachial artery in a longitudinal section with anatomical landmarks.

Changes in FMD can provide analytical information and can be used to examine mechanisms that alter and/or normalize vascular function or to analyze hemodynamic changes in vivo.^{4,6,13} It has been already shown by research that physical activity, vitamin supplements and medication can greatly improve FMD thus prevent or decrease cardiovascular episodes in healthy and asymptomatic patients with cardiovascular disease.^{13,14,16-18} Using the FMD technique independently and without errors by caregivers would be of a great benefit in the diagnosing and rehabilitation field by increasing insight into the pathophysiology of the dysfunction of the endothelial layer and provide a testing tool to assess the efficacy of different treatment modalities.

There has been identification of methodological and interpretive restrictions regarding FMD measurements,^{3,13} thus further testing should be conducted in order to progress the FMD technique to be used as future clinical tool. Current techniques measuring FMD are highly operator dependent, consequently showing unreliability.^{3,7} One of the difficulties of using ultrasound imaging is to identify clear and defined boundaries between the lumen and the arterial wall, and this can be a difficult task for inexperienced investigators or when using a lower frequency probe.^{3,7,14} Precise work has to be done in order to be able to capture distinguishable vessel margins so that accurate measurements can be achieved.^{3,13,14}

Two techniques are mentioned in the literature to support the vessel diameter calculation after image acquisition with ultrasound imaging.^{6,11,14} Manual measuring of FMD has been the original technique which has been proven to be a time-consuming method, highly operator dependent and significant to observer error. Further, semi-automated, wall tracking and edge detector software were tested and have shown more precise and reproducible measurements of FMD compared to the manual technique.^{6,13,15}

This experiment has aimed to investigate the measurements of the brachial artery diameter, taken manually using the Santosoft Dicom view and semi-automatically by an edge detection software FMDi, in order to standardize the protocol for FMD measurement and to decrease user dependency.

Method

Subject data and ultrasonic assessment results used for the particular research were provided by Steven Onkelinx whom was the leader of a FMD research in the department of rehabilitation sciences in KU Leuven, Belgium. The images used in this experiment were acquired by an experienced technician in the brachial artery ultrasound scanning technique.¹⁰

Subjects

For the purpose of the study 21 patients volunteered all of whom had experienced a coronary artery disease (CAD) in the past but have had not related incident or arrhythmias during nine months prior to the testing. The subjects were part of a maintenance program for patients with cardiovascular disease, participating in sporting activities and abstaining from smoking habits. The study was approved by the biomedical ethical committee of the KU Leuven and written informed consent was obtained from the participants after an explanation of the experimental procedures.

Procedure

To ensure accurate measurements the subjects participating had to avoid ingesting any substances that would affect endothelial function such as any sort of high-fat diet, caffeine, alcohol, vitamin supplements, drugs and medication (when possible) and would have to keep away from exercising for at least 24 hours prior to the testing. On the day of the assessment subjects had to be rested and fasted. All measurements were performed in a dark, quiet and temperature controlled environment in the same position in which the tests took place in order to control orthostatic changes.

Brachial artery ultrasound scanning (BAUS) was used by experienced investigators who have followed a consistent protocol according to Corretti et al.³ The brachial arteries diameters were obtained in a longitudinal fashion by means of a high-resolution, 12MHz, linear array vascular ultrasound transducer (Vivid 7; GE healthcare). Once the subject established resting state, baseline arterial diameter was obtained through two-dimensional images, 2cm over the antecubital fossa. To assess the post-occlusion reflex arterial diameter a blood pressure cuff was placed proximal to the imaging transducer and inflated 50 mmHg above systolic arterial pressure for five minutes to induce a stimulus. The vessel was then imaged for one minute after the occlusion release and the reactive hyperaemia was confirmed with a pulse wave Doppler scanning.

To analyze the images and to measure the vessel diameter Santesoft dicom view 2.1 was used for the manual technique and the edge detection software Flomedi FMDi (2009, Brussels) for the semi automated technique. All analyses were performed by the same investigator who was blinded to the patients' characteristics. The testing of the two techniques took place a week apart to avoid influence from the results. When using the manual technique three diameters were taken for each image by drawing a vertical line from the posterior to anterior tunica intima-media boundaries. For the semi automated technique, after calibration, a discrete region was selected on the vessel and an average diameter of the selected area was given. All the vessel diameters were measured during the end diastole (R wave) of the cardiac cycle.

The measurements acquired for each subject were then used to calculate the FMD using the following formula:

$$FMD (\%) = [(post-ischemic\ diameter - baseline\ diameter) / baseline\ diameter] \times 100.^{5, 14}$$

Data analysis

The data acquired were entered into Excel and analyses were performed with SPSS, version 18, for Windows. The data were normally distributed and a paired t-test was used to determine the significance of the correlation between the pairs ($P < 0.05$). Mean, standard deviation and standard error of the mean (SEM) of the paired differences was shown. The coefficients of variation (CV) were calculated for every subject by dividing the standard deviation for each pair of measurements by their mean values [$CV = (SD/mean) \times 100$] and the mean CV was calculated. Interclass correlation coefficient (ICC) was calculated to assess the correlation of the data acquired from the manual and semi-automatic technique as a measurement of agreement. A P-value of < 0.05 was considered significant.

Results

In total 21 brachial artery images were assessed for the study. The subjects' baseline characteristics from which the images were obtained are shown in Table 1. Mean age of the subjects with inactive coronary artery disease (CAD) was 57.6 years with a mean body mass index (BMI) of 27.27 kg/m².

Table 1.

Baseline characteristics of the CAD patients.

Variable	Unit
Age (years)	57.65 ± 9.87
Height (cm)	173 ± 8.10
BMI (kg/m ²)	27.27 ± 4.49
Sex (male)	19 (90.5%)
Beta-blocker	16 (76.2%)

Continuous variables are expressed as mean ± standard deviation and categorical variables as percentages. BMI, body mass index.

Table 2 demonstrates the reliability statistics of the FMD measurements taken with the two techniques.

The mean values for the FMD was 10.13% with a SD of 8% for the manual and 12.41% and 0.8.16% for the semi-automatic technique. For BBAD and POBAD the mean and standard deviation values were 0.39±0.05 mm and 0.43±0.05 mm for the manual and 0.41±0.06 mm and 0.46±0.05mm for the semi-automatic technique respectively.

The paired sample test has showed significance for the measurements of the paired diameters before and after occlusion and FMD, respectively $p=0.003$, $p=0.001$ and $p=0.026$. Values of the mean and SDs of the paired differences were 0.02±0.03 mm, 0.03±0,04 mm and 2,28±4,35% for BBAD, POBAD and FMD respectively.

The ICC was statistically significant for all measurements; however the ICC for POBAD (0,756) was somewhat lower than for BBAD (0.856) and FMD (0.855). The CV for the different techniques for BBAD (4.59%) was lower than POBAD (5.78%) but CV for FMD had the greatest value (30.32%) compared to the previously mentioned.

Table 2.

Reliability statistics of the FMD measurements acquired with the two software.

	Mean ± SD	CV	ICC*	Mean ± SD of the differences	Sig.(2-tailed)
BBAD					
Manual	0.39 ± 0.05 mm				
Semi-automatic	0.41 ± 0.06 mm	4.59%	0.856	0.02 ± 0.03 mm	0.003
POBAD					
Manual	0.43 ± 0.05 mm				
Semi-automatic	0.46 ± 0.05 mm	5.78%	0.756	0.03 ± 0,04 mm	0.001
FMD					
Manual	10.13 ± 8.00%				
Semi-automatic	12.41 ± 8.16%	30.32%	0.855	2,28 ± 4,35%	0.026

*P<0.05

SD, standard deviation; CV, coefficient of variation; ICC, intraclass correlation coefficient; BBAD, baseline brachial artery diameter; POBAD, post-occlusion brachial artery diameter; FMD, flow-mediated dilation

Discussion

The findings of the experiment suggest that there is a relatively high statistical association with using the manual and semi-automatic technique for measuring the FMD. The results showed substantial reliability for POBAD ($p=0.756$) and almost perfect reliability for BBAD ($p=0.856$) and FMD ($p=0.855$). Conversely, results of CV point towards a high measure of dispersion for the FMD ($CV=30.32\%$), showing a low reproducibility. The high value for the CV for FMD can be attributed to the fact that FMD is a percentage-ratio measure. Small differences in the two parameters (BBAD,POBAD) used can result in an increased calculation of FMD.

There has been no research found comparing the between-techniques reliability using the ICC for the two techniques, however, the CV calculated in previous literature is lower than what has been estimated in the present study. Research by Harris et al.¹⁴ showed lower variability, with CV's of 1.9% for BBAD and POBAD and 15.2% for FMD, stating that the semi-automatic technique shows sensitivity in assessing FMD, comparing to the manual technique, by diminishing human error from the data analysis. Woodman et al.⁶ has performed a study using three methods of analysis, two manual and one semi-automatic, tested by three different observers. The intraobserver CVs were significantly

lower for the semiautomatic, concluding that this technique measures brachial artery diameters more reliably compared to the two manual techniques. Meirelles et al.¹⁵ conducted a study where measurements using the manual technique showed high reliability for same and separate day measurements, and so, it was proposed that the manual technique can be used to analyze and monitor endothelial function. The author also states that, in a clinical setting, manual recording of brachial artery diameter are more practical as they are quicker and easier to obtain.

The experiment followed the guidelines of Corretty et al.³ which state that the assessment of FMD should be performed by a trained individual. Training of the sonographer requires several months and requires hands on training by an experienced individual, such as a radiologist.^{3,19} It is recommended that at least 100 scans and image analyses must be performed before independent FMD assessment.³ In the case of this study, even though the ultrasound measurement were obtained by an experienced technician, the investigator of brachial artery diameter did not possess any previous experience in analyzing techniques and had received only a short introduction to the software operations. There has been inadequate information in research regarding operator experience in image analysis; this could have a major impact on FMD results.

In the majority of the images being analysed, the vessel width had the tendency to increase or decrease throughout the one end to the other, thus diameter greatly differed from one area of the same image to the other. This lateral dilatation can be attributed to the compression of the probe on the vessel during the assessment. This can be avoided by measuring the FMD using cross sectional ultrasound image technique rather than the longitudinal section proposed by the guidelines followed in this study.^{19,20}

During measuring of the brachial artery diameter, there was no use of specific landmarks (i.e. vessels, fascia planes) or positioning of the callipers on a specific region on the image (i.e. middle). It cannot be ensured that the same segments were measured between baseline and hyperaemia phase and between the software. The guidelines of Corretty et al.³ recommend usage of a stereo static probe to assist in maintenance of the image throughout the study but this has not been followed during analyses in the case of this research. Another factor contributing to false measurements could be that, for the observer, it was not clear on which level the contrast and greyscale should be used. In many occasions, due to change in contrast or/and greyscale, the real boundaries were lost causing the vessel diameter to appear wider. Overall, there was not a clear protocol to follow for using the software.

In general, out of the research done on the assessment of FMD, measurement differences can be explained to different factors such as occlusion duration, transducer and cuff placement.¹⁰ Studies suggest that the time to the "true" maximal diameter can vary between individuals from four to five minutes and it is hard to capture due to their short duration.^{2,13,21} For the purposes of this research, measurements were taken one minute post-occlusion so it can be assumed that the images acquired may not represent the peak diameter of the vessel for all subjects, showing inconsistency in the FMD results.

A major limitation of the present study was that the images were not randomized and even though the testing took place a week apart, this may have had a negative influence on the analyzing of the

brachial artery diameters. The failure to randomize can show a decreased validity and reliability in the experimental outcomes.

Further research should focus on creating a protocol for the handling of the software used to assess endothelial function with the FMD technique in order to reduce human interaction and succeeding estimator bias. An inter-observer study, for the semi-automatic software used in the present experiment, is recommended in order to verify reproducibility of the measurements acquired.

The semi-automated technique shows greater reliability compared to the manual technique but both techniques could aid in the clinical field. However, image analysis by using the FMD technique is operator dependent so it directed for appliance in clinical trials and serial measurements. Other techniques assessing endothelial dysfunction, such as the finger arterial pulse wave amplitude (PAT) technique, are recommended to be used in clinical practise since this does not require experience by the user and is more functional compared to the FMD technique.^{1,7,10}

Conclusion

Assessment of FMD with the semi-automatic software program is a reliable technique compared to the original, manual measuring technique. Further research needs to be done to assess the software measurement reproducibility in order to allow the technique to be applied in clinical settings. User protocols for the software should be made available and followed by the investigators to reduce bias.

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APPENDIX

I. Project plan approval.....i

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I. Assessment form project plan

Name: Katerina Chamatila

Student no:2144680

Date: 30.05.2013

Title: : Reliability of a software program to analyze endothelial function. A comparison of manual and semi-automatic measurement of brachial artery diameter. An experimental study

General

- The project plan is according to format **yes / no**
- Spelling and language are correct **yes / no**

Problem description and problem definition (introduction)

- The problem description is sufficiently clearly formulated **yes / no**
- The problem description reflects social and paramedical relevance **yes / no**
- A concrete and relevant research question (or questions) can be formulated based on the problem definition, including possible sub questions **yes / no**

Objective

The objective is:

- Sufficiently clearly and concretely formulated **yes / no**
- Relevant for a selected target group within the (paramedical) professional practice **yes / no**
- Practically feasible **yes / no**
- Achievable within the set time **yes / no**

Project product

The project product:

- Is in line with the problem definition, research question and objective **yes / no**
- Is usable for the selected target group **yes / no**
- Is in line with the client's wishes **yes / no**
- The product requirements are accurately described **yes / no**

Activities/method

Sufficient insight is given into the type of activities and types of sources for the performance of the research and the realization of the product

yes / no

Time schedule

- The time schedule gives a global phasing and time investment for the project as a whole and for the coming weeks an increasingly detailed schedule **yes / no**
- Important moments are recorded in the table (typographically noticeable) (e.g. contact moments, handing-in moments) **yes / no**
- The time schedule gives a global task division of the planned activities **yes / no**

II. Confidentiality statement

Name: Katerina Chamatila

Student No°: 2144680

Title: Reliability of a software program to analyze endothelial function. A comparison of manual and semi-automatic measurement of brachial artery diameter. An experimental study.

Background: Endothelial dysfunction is a marker of early stages of atherosclerosis and in general of cardiovascular disease. Assessing the endothelium can be achieved non-invasively, using the flow-mediated dilatation technique. **Method:** For the experiment 21 patients with an inactive cardiovascular disease were employed. Using a B-mode ultrasound with a 12MHz transducer, images of the brachial artery diameter were captured before and after induced ischemia. Images were comparatively analyzed using a manual and a semi-automatic software program. **Results:** Inter-correlation coefficient for the measurement was relatively high, with a p-value of 0.855 for flow mediated dilatation, 0.856 for arterial diameter at baseline and 0.756 for the post ischemic arterial diameter, showing good reliability for the software. The coefficient of variation for flow mediated dilatation was 30.32%, a high value compared to the coefficient of variation for the baseline and pos-occlusion diameters, respectively, 4.59% and 5.78%. **Conclusion:** Both techniques show acceptable consistency with the semi-automatic outperforming the manual technique in terms of reliability. However, there are indications of high measure of dispersion when it comes to measuring the flow mediated dilatation with the software

-
1. By signing this Statement, the Fontys Paramedic University of Applied Sciences in Eindhoven commits itself to keep any information concerning provided data and results obtained on the basis of research of which is taken cognizance as part of the above practical research project and of which it is known or can be reasonably understood that said information is to be considered secret or confidential, in the strictest confidence.
 2. This confidentiality requirement also applies to the employees of the Fontys Paramedic University of Applied Sciences, as well as to others who by virtue of their function have access to or have taken cognizance of the aforesaid information in any way.
 3. The above notwithstanding, the student will be able to perform the practical research project in accordance with the statutory rules and regulations.

Student:

Name: Katerina Chamatila



(signature)

Date:30.05.2013

Supervisor:

Name: Steven Onkelinx



(signature)

Date:30.05.2013

Coordinator: for receipt

Name: Chris Burtin



(signature)

Date: 30.05.2013

III. Conveyance of Rights Agreement

AGREEMENT

Pertaining to the conveyance of rights and the obligation to convey/return data, software and other means

The undersigned:

1. Ms. Katerina Chamatila
[full name as stated in passport], residing at 5623 EX, Eindhoven, The Netherlands
[postal code, place of residence] at the Raedecker straat 14
[street and house number], hereinafter to be called “**Student**”

and

2. Fontys Institute trading under the name Fontys University of Applied Sciences, Rachelsmolen 1, 5612 MA Eindhoven, hereinafter to be called “**Fontys**”

CONSIDERATION

- A. Student is studying at the Fontys Paramedic University of Applied Sciences in Eindhoven and is performing or will perform (various) activities as part of his/her studies, whether or not together with third parties and/or commissioned by third parties, as part of research supervised by the lectureship of Fontys Paramedic University of Applied Sciences. The aforesaid activities will hereinafter be called “**Lectureship Study Activities**”. At the time of the signing of this Statement, the Lectureship of Fontys Paramedic University of Applied Sciences supervises in any case the studies listed in Appendix 1, but this list is not an exhaustive one and may change in the future.
- B. It is of essential importance to Fontys Paramedic University of Applied Sciences that (the results of) the Lectureship Study Activities can be further developed and applied without any restriction by Fontys Paramedic University of Applied Sciences and/or used for the education of other students. Fontys wishes in any event – but not exclusively – (i) to be able to share with and/or convey to third parties (the results of) the Lectureship Study Activities, (ii) to publish these under its own name, where the Student may be named as co-author providing that this is reasonable under the circumstances, (iii) to be able to use these as a basis for new research projects.
- C. In case intellectual ownership rights and/or related claims on the part of Student will be/are attached to (the results of) the Lectureship Study Activities, parties wish – taking into account that which was mentioned under (B) – Fontys Paramedic University of Applied Sciences to be the only claimant with regard to said rights and claims. The Student therefore wishes to convey all his/her current and future intellectual property rights as well as related claims concerning (results of) the Lectureship Study Activities to Fontys, subject to conditions to be specified hereafter;
- D. Student furthermore wishes to enter into the obligation – again taking into account that which was mentioned under (B) – to convey all data collected by him/her as part of the (results of) the Lectureship Study Activities to Fontys and not to retain any copies thereof, and also to return all data, software and/or other means previously provided by Fontys as part of (the results of) the Lectureship Study Activities, such as measuring and testing equipment, to Fontys without retaining copies thereof, all the above being subject to conditions to be specified hereafter.

AGREE THE FOLLOWING

Conveyance of intellectual property rights

1.1 Student herewith conveys to the Fontys Paramedic University of Applied Sciences all his/her current and future intellectual property rights and related claims concerning (the results of) the Lectureship Study Activities, for the full term of these rights.

1.2 Intellectual property rights and/or related claims are understood to refer to, in any case – but not limited to – copyright, data bank law, patent law, trademark law, trade name law, designs and model rights, plant breeder's rights, the protection of know-how and protection against unfair competition.

1.3 The conveyance described under 1.1 shall be without restriction. As such, the aforesaid conveyance shall include all competences related to the conveyed rights and claims, and said conveyance shall apply to all countries worldwide.

1.4 Insofar as any national law requires any further cooperation on the part of Student for the conveyance mentioned under 1.1, Student will immediately and without reservation lend such cooperation at first request by Fontys Paramedic University of Applied Sciences

1.5 Fontys accepts the conveyance described under 1.1.

Waiver of personal rights

2.1 Insofar as permitted under article 25 'Copyright' and any other national laws that may apply, Student waives his/her personal rights, including – but not limited to – the right to mention Student's name and the right to oppose any changes to (the results of) the Lectureship Study Activities. If and insofar as Student can claim personality rights pursuant to any national laws notwithstanding the above, Student will not appeal to said personality rights on unreasonable grounds.

2.2 In deviation from that which was stipulated under 2.1, the Fontys Paramedic University of Applied Sciences may decide to mention the name of Student if this is reasonable in view of the extent of his/her contribution and activities.

Compensation

Student agrees that he/she will receive no compensation for the conveyance and waiver of rights as described in this Statement.

Guarantee concerning intellectual property rights

Student declares that he/she is entitled to the aforesaid conveyance and waiver, and declares that he/she has not granted or will grant in future, license(s) for the use of (the results of) the Lectureship Study Activities in any way to any third party/parties. Student indemnifies Fontys from any claims by third parties within this context.

Obligation to convey/return data, software and other means

5.1 At such a time as Student is no longer performing any Lectureship Study Activities and/or is no longer a student at Fontys, Student is obliged to convey to Fontys all data, in the widest sense of the word, collected by him/her as part of (results of) the Lectureship Study Activities, including – but not

limited to – studies and research results, interim notes, documents, images, drawings, models, prototypes, specifications, production methods, process descriptions and technique descriptions.

5.2 Student guarantees not to have kept any copies in any way or form of the data meant under 5.1.

5.3 Student is obliged to return to Fontys all data, software and other means provided to him/her by Fontys as part of the Lectureship Study Activities, and guarantees not to have kept copies in any way or in any form, of the provided software and/or other means.

5.4 Student agrees that if he acts and/or proves to have acted contrary to the obligations mentioned under 5.1 up to and including 5.3, (a) he/she shall be liable for all and any damages incurred or to be incurred by Fontys, and (b) that this will qualify as fraud and that Fontys can apply the appropriate sanctions hereto. The sanctions to be applied by Fontys may consist of, among other things, the denying of study credits, the temporary exclusion of the Undersigned from participation in examinations, but also the definitive removal of the registration of the Undersigned as a student at Fontys.

Waiver

Student waives the right to terminate this Agreement.

Further stipulations

7.1 Insofar as this Agreement deviates from the Student Statute, this Agreement shall prevail.

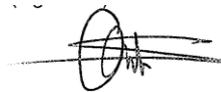
7.2 This Agreement is subject to Dutch law. All disputes resulting from this statement will be brought before the competent judge in Amsterdam.

Student:

**Fontys Institute
trading under the name Fontys Hogescholen
Supervisor:**

Name: Katerina Chamatila

Name: Steven Onkelinx



(signature)

(signature)

Date: 30.05.2013

Date: 30.05.2013

Place: Eindhoven, The Netherlands

Place: Eindhoven, The Netherlands

I, Ms. M.H. de Waard, sworn translator for the English language registered at the Court in Groningen, the Netherlands, and registered in the Dutch Register of Sworn Translators and Interpreters (Rbtv) under nr. 2202, herewith certify the above to be a true and faithful translation of the attached Dutch document into the English language.

Groningen, 23 May 2012,

[M.H. de Waard]