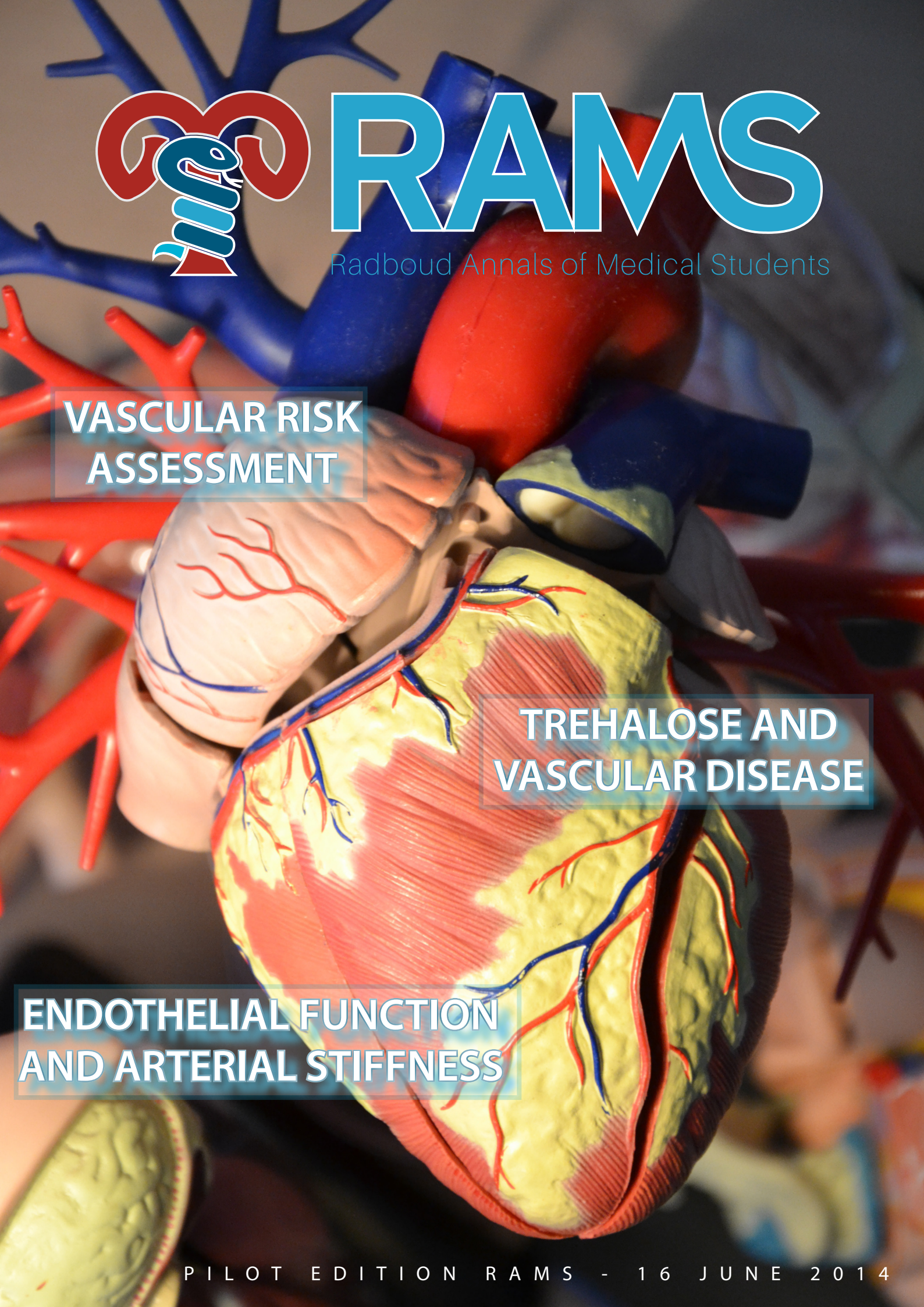


RAMS

Radboud Annals of Medical Students

VASCULAR RISK ASSESSMENT

TREHALOSE AND VASCULAR DISEASE

ENDOTHELIAL FUNCTION AND ARTERIAL STIFFNESS

PILOT EDITION RAMS - 16 JUNE 2014

RAMS

General board of RAMS

RAMS is directed by the general board, consisting of four medical students: Tessa Schoot (chair), Lars Gallée (vice-chair), Barov Sanaan (treasurer) and Josianne Luijten (public relations). As board of the foundation they frequently meet to make sure all activities of RAMS run smoothly. Moreover, they are in close contact with the Supervisory Board and the Editorial Board. If you have any questions on general, promotional or financial subjects, you can contact the general board of RAMS via vice-voorzitter.rams@ru.nl or check our website www.ramsresearch.nl.

Editorial board

In contrast to the Board of RAMS, the Editorial board determines the content of RAMS. Currently this Editorial board consists of five members: Sander Groen in 't Woud, Michiel Schoenaker, Rick Verstegen, Bas Vreugdenhil and David Wolthuis. Together they decide which articles are getting published in RAMS. Furthermore they decide the subjects of other non-reviewed articles as well. Finally they are also responsible for the lay-out of this journal.

Editors

The content of RAMS is devided into two parts: publication of scientific research and other interesting medical articles. The editors are responsible for any kind of articlues that are not reviewed by our reviewers. They provide the content for example with interviews, columns, short messages and more. Our group of editors consists of: Jeroen Hol, Anna van Boekel, Mieke Peters, Lysanne van Silfhout, Kim Cortenbach and Marije Kaan.

Reviewers

This is the biggest group of our team. RAMS can count on the support of twelve reviewers that are trained by professors and teachers of the Radboudumc. Those professionals have developped a few masterclasses which they taught to the students of RAMS. With the help of these masterclasses and their own specific knowledge the reviewers judge the scientific articles that have been sent to our Editorial board. They review those articles within a complete anomymity of the article's author(s).

COLOPHON

Edition	Pilot
Date of publication	16th of june 2014
Place of publication	Nijmegen

CONTACT

89 SOOS - Radboud Annals of Medical Students
Geert Grooteplein Noord 21
6525 EZ Nijmegen
www.ramsresearch.nl

GENERAL BOARD

Tessa Schoot	(Chair)
Lars Gallée	(Vice-chair)
Barov Sanaan	(Treasurer)
Josianne Luijten	(Public Relations)

EDITORIAL BOARD

Sander Groen in 't Woud
Michiel Schoenaker
Rick Verstegen
Bas Vreugdenhil
David Wolthuis

EDITORS

Anna van Boekel
Kim Cortenbach
Jeroen Hol
Marije Kaan
Mieke Peters
Lysanne Silfhout

DESIGN

Marc van Buul
Mieke Peters
Bas Vreugdenhil

SUPERVISORY BOARD

dr. Janielle van Alfen-van der Velden
Lotte Eijkenboom (Assessor Medical Faculty)
mr. Bob de Jonge
dr. Jur Koksma
dr. Dirk Schubert

Preface

Two years ago we came up with the crazy idea to create a scientific journal made by students. After criticism by the Inspectorate of Education on the value of scientific education at our faculty, the opportunity presented itself to actually establish this journal and thereby improve our own curriculum. After consulting researchers, professors and editors of scientific journals to explore our possibilities, we were able to lay the foundations of Radboud Annals of Medical Students (RAMS). In May 2014 the first board, editors and reviewers were installed - all students medicine, biomedical sciences and dentistry. This big team of talented students is proud to present you this first pilot edition of RAMS.

The goal of RAMS is to educate all students of the Faculty of Medical Sciences of the Raboud University Nijmegen about the ins and outs of medical research. Like every other scientific journal, we strive to publish high-quality papers written by students. Thus, this is the perfect opportunity for you to learn to write a journal-proof paper and to obtain your first publication at the same time! Also as reviewer or members of the editorial board you can acquire lots of knowledge and experience in the field of medical research. Hopefully, RAMS will be an education tool with great learning possibilities for every participating student.

We invite all students to submit their medical-scientific papers, essays and research proposals. Our editors and reviewers will assess all submissions and the best ones will be published in this journal. Do you want to publish in the next edition? Check the guidelines for authors at www.ramsresearch.nl and submit!

We can assure you: this is just the start. We hope that in a few years' time all medical students know RAMS, submit papers and dream of their first publication in RAMS. Let's go!

Michiel Schoenaker and Tessa Schoot

Index

From the Editorial Board

Dear reader,

We proudly present to you the pilot edition of RAMS – The Radboud Annals of (bio)Medical Students. A new journal at your faculty, made by students for students. This journal is different from other journals for students. Different because we aim at both educating and entertaining, different because it will be implemented in the educational system as well. RAMS aims at enhancing the students' experience with research. Experience you can gain by reading the journal, publishing in it, or by working with us on the next editions.

One of the key aims of the journal is that we want to be innovative – as a journal run by students we have a constant flow of new members with new ideas. The fact that we are not attached to any associates allows us to try new features whenever we want. Nothing is impossible on forehand, so share all ideas you have.

This pilot edition is meant to show you the possibilities of a journal at our faculty. From now on, there is an easy way to gain experience with publishing your research – whether it is a review made for your bachelor internship, original research conducted for an honours internship abroad or something completely different. The articles in this edition prove that it is possible to write a scientific article during your studies already. Now it is up to you to get involved and send in your contribution for the next edition.

In this edition, we present three different articles from different backgrounds. The first article is an evaluation of clinical risk assessment tools in atrial fibrillation. In this meta-analysis, the authors demonstrate a good understanding of critical appraisal and their final conclusion provides valuable tools for further research. In our second article, the authors included forty-eight from a larger cohort study to evaluate the relationship between two vascular parameters. Both this article and the third publication in this journal report on research done in the US: a clear sign that medical students from the Radboud university medical center are active in learning about research all over the world. The topic of the third article is close to that of the first two, since the authors of this work aimed to evaluate the effect of trehalose on endothelial dysfunction and arterial stiffness.

We do, however, aim to publish more than just medical research, such as interviews or popular articles. An example is the interview with Viola Klück, who went to Boulder to do an Honours internship. With similar content we hope to inspire you to start your research career as well. If you already did research you like to publish or wrote an interesting essay (scientific or not) that could be interesting for our readers, do not hesitate and submit it to submit.rams@ru.nl .

Having said that, we would like to invite you to enjoy this wonderful pilot edition and we hope you will have just as much fun reading it as we had composing it. We would appreciate it very much to hear your opinion about this edition, so please do not hesitate to contact us. All the information about us and the journal is available online at ww.ramsresearch.nl.

On behalf of the editorial board,

Sander Groen in 't Woud

RAMS
Radboud Annals of Medical Students

Vascular Aging: Endothelial Function and Arterial Stiffness

Manon Heldens (1), Takashi Tarumi (2,3), Muhammad Ayaz (2,3), Rosemary Parker (2), Cynthia Tinajero (2), Candace Hill (2), Benjamin Y Tseng (2,3), Jie Liu (2,3) and Rong Zhang (2,3)

(1) Geriatric Medicine, Radboud University Nijmegen Medical Center, Nijmegen, Netherlands (2) Institute for Exercise and Environmental Medicine, Texas Health Presbyterian Hospital Dallas, Dallas, TX (3) University of Texas Southwestern Medical Center, Dallas, TX

Corresponding Author: Manon Heldens, BSc (heldensmanon@gmail.com)

ABSTRACT:

VASCULAR AGING: AN ASSOCIATION BETWEEN ENDOTHELIAL FUNCTION AND ARTERIAL STIFFNESS

BACKGROUND: Endothelial dysfunction and arterial stiffness, two hallmarks of vascular aging, are both related to a number of cardiovascular risk factors and diseases. However, the relationship between these two vascular parameters is not well understood.
OBJECTIVE: The purpose of this study was to test the hypothesis that 1) endothelial dysfunction is associated with an increase in arterial stiffness and 2) endothelial dysfunction and arterial stiffness are associated with ageing.
METHODS: 48 healthy subjects, divided into three age groups of young, middle-aged and older subjects, participated in this study. Endothelial function was measured by brachial artery flow mediated dilation (FMD). Central and peripheral arterial stiffness were assessed by aortic pulse wave velocity (aPWV) and brachial pulse wave velocity (bPWV), respectively.
RESULTS: Flow mediated dilation, measured as the percentage change from baseline to maximal dilation, was inversely correlated with age ($r=-0.751$, $p<0.001$). No relation was found between changes in shear rate and advancing age. Notably, after normalizing FMD for shear rate, we observed a significant correlation between FMD and age ($r=-0.577$, $p=<0.001$). In addition, aPWV showed a positive relationship with age ($r=0.632$, $P<0.001$) and with FMD ($r=-0.404$, $p=0.004$). No relationship was found between bPWV and age.
CONCLUSION: Our findings suggest that in healthy individuals , declines in endothelial function and increases in arterial stiffness with age are correlated and may be mediated by common underlying mechanisms.

WHAT IS KNOWN: There is evidence linking nitric oxide (NO) to the regulation of arterial stiffness. But research on the relationship between endothelial function and arterial stiffness is highly limited to studies involving subjects with cardiovascular risk factors or diseases and regarding this, the evidence is controversial.

WHAT IS NEW: In this study we emphasized on healthy and normal ageing patients to investigate the relationship between endothelial function and arterial stiffness. The results indicate that, even in healthy individuals, a decline in flow mediated endothelial function is associated with an increase in central arterial stiffness with age.

KEYWORDS: Endothelial function, Nitric Oxide, Arterial Stiffness, FMD, PWV, Vascular aging

Introduction

A reduced bio-availability of nitric oxide (NO) in resistance and conduit vessels, known as endothelial dysfunction, is associated with advancing age[1-4]. Since NO is an anti-atherogenic molecule, this leads to a vascular phenotype more prone to atherogenesis.[5]. Indeed, endothelial dysfunction has been demonstrated to be a key event in the development of atherosclerotic cardiovascular disease,[6] the leading cause of morbidity and mortality in the USA, and a predictor of clinically obvious vascular pathology. Over the past twenty years clinical and experimental studies have demonstrated that endothelial dysfunction is associated with cardiovascular risk factors such as diabetes,[7] smoking,[8] hypertension,[9] hypercholesterolemia,[10, 11] ageing,[2] and with cardiovascular diseases such as coronary artery disease.[12]
Besides endothelial dysfunction, increased arterial stiffening, another parameter for vascular aging, is a coexisting event in patients with these cardiovascular risk factors and outcomes.[13-15] Both animal and human studies have investigated the relationship between these two vascular parameters and this has given evidence linking NO to the regulation of arterial stiffness. In a recent study, inhibition of NO production by administration of the NO-synthase inhibitor L-NAME in spontaneously hypertensive rats induced aortic stiffening.[16] Moreover it is demonstrated that a number of therapeutic interventions that improve endothelial function also reduce arterial stiffness.[17] These observations have led to the hypothesis that endothelium derived NO is an important regulator of arterial stiffness.
Endothelial dysfunction is a highly investigated area, but to date the research investigating the relationship between endothelial function and arterial stiffness is mostly limited to studies involving subjects with cardiovascular risk factors or diseases[18-23] and regarding this, the evidence is controversial.[24] In healthy, normotensive and normal ageing patients, the relationship is not adequately defined. Therefore, the present study was performed to investigate this association between arterial stiffness and endothelial function and the relationship of these vascular parameters with ageing in a group of healthy individuals. To our knowledge there is only one study describing the relationship between endothelial function and arterial stiffening in a group of healthy subjects, however this study did not focus on the effects of ageing on either of these parameters.[25]
Arterial stiffness was assessed by measuring aortic/central (carotid-femoral) pulse wave velocity (PWV), considered as the "gold standard" method to evaluate arterial stiffness, and brachial/peripheral (carotid-radial) PWV (bPWV). Endothelial function was obtained by using flow-mediated dilation (FMD), the most widely used non-invasive test

to measure this vascular parameter. This describes the vasodilatory response of a vessel to elevations in blood-flow associated with increases in shear stress, induced by a 5-minute period of reactive ischeamia caused by temporary arterial occlusion.[26] Because of the suggestion that shear stress can differ with age,[27, 28] and in this way can influence the extent of the vasodilatory response of the brachial artery, we also compared the association between shear rate, an adequate surrogate measure of shear stress, and age. By doing this we normalized the effect of change in shear stress on FMD outcomes and were able to perform a more reliable comparison between endothelial function and aging.
First we aimed to compare FMD and PWV with age. Then, we investigated the relationship between FMD and PWV. We hypothesized that endothelial dysfunction is associated with increases in arterial stiffness and that both these parameters are related with an increase in age.

Methods

Subjects

All patients included in this cross-sectional study were participants of the Arterial aging, Brain Perfusion and Exercise (ABC) study, an ongoing, single-center, cohort study. The objectives of the ABC-study are to determine the effects of exercise and aging on vascular parameters, brain blood flow and cognitive function.
The subject characteristics are shown in table 1. A total of forty-eight healthy, sedentary volunteers were included in this sub-study and were stratified based on age into three different groups: young adults (n=13, 4 males and 9 females, 20-39 yr), middle-aged adults (n=21, 12 males and 9 females, 40-59 yr), and older adults (n=14, 3 males and 11 females, 60-80 yr). All subjects were non-smokers, non-obese, normotensive(<140/90 mmHg) and free of cardiovascular, pulmonary and metabolic disease. All subjects were sedentary, defined as <90 minutes of exercise a week. All procedures were approved by Texas Health Presbyterian Hospital Institutional Review Boards, IRB at UT Southwestern. Written informed consent was obtained from all subjects prior to participation in the study.

Study design

Measurements for brachial artery FMD and aortic and brachial PWV were performed during one visit under standardized conditions, in a quiet, dimly-lit, and temperature controlled room, after a resting period of at least 20 min and at least 24 hours of abstinence of caffeine and alcohol.

Study procedure

Asessment of endothelial function and arterial stiffness
Subjects were in supine position and asked to extend the left arm, in an abduction of approximately 80°. The arm was immobilized using foam. Heart rate was continuously monitored using a three-lead ECG system (Hewlett Packard, USA) and brachial blood pressures were taken on the contralateral arm with an automated sphygmomanometer (Sun Tech Medical Inc, Morrisville, NC). A rapid inflation/deflation pneumatic cuff was positioned on the left arm, distal to the elbow to provide a stimulus for forearm ischemia, following established guidelines for assessing FMD.[6] To obtain high resolution B-mode ultrasound images of the brachial artery a 3-12 MHz linear array transducer of a colour coded ultrasonography system (CX-50, Philips, Healthcare, The Netherlands) was used. The probe was placed 10-20 cm above the antecubital fossa and stabilized using a clamp. Probe placement was carefully adjusted to maintain optimal imaging. Continuous Doppler blood flow velocity signals were recorded at the lowest insonation angle possible (always <60°) and were recorded during baseline and during the first 15 seconds after release of the cuff.
After a period of at least 20 minutes of rest, baseline images and velocity measurements were collected for 3 minutes. Then the pneumatic arm cuff was inflated to a suprasystolic pressure (>225 mm Hg), for 5 minutes. At least 30 seconds before deflation, brachial diameter and velocity recordings were restarted. The increase in blood flow (reactive hyperaemia) was recorded during the first 30 seconds after cuff deflation. The change in vessel diameter was recorded from 30-210 seconds, in serial 20 seconds segments at approximately 21 Hz.
After the measurement of FMD, pulse wave velocity (PWV) was measured to assess arterial stiffness using applanation tonometry with a Sphygmocor instrument (SphygmoCor Px, AtCor Medical, Australia) with methods previously established.[29] Brachial and aortic PWV were measured between the right common carotid artery and the right radial and left femoral artery, respectively. PWV index was calculated as the ratio between the distance and the time needed for the pressure wave to travel between the measurement sites and was expressed in meters per second.

Data analysis
The echo-Doppler signal was taken directly from the ultrasound machine and encoded and stored as a digital DICOM file on the PC. Subsequent data analysis of the brachial artery diameter was performed using edge-detection and wall-tracking, commercially available software(Brachial Analyzer 5.9.0, Medical Imaging Applications, LLC, Coralville, IA). In short, the software allows the user to identify a region of interest (ROI) on the portion of the image where the vessel walls are most clear. The arterial wall borders were detected by an optimal graph search-based segmentation that uses a combination of pixel density and image gradient as an objective function. Each sequence of images was reviewed and interactively edited when needed to ensure that diameter measurements were always calculated from the intima-lumen interface at the distal and proximal vessel wall, during the diastolic phase of the cardiac cycle. All measurements were performed by a single investigator (MH). Analyses from the brachial analyser software were transversed to sigma-plot and peak changes in vessel diameter and time to peak changes in diameter during flow mediated vessel dilation were determined using a smoothing function. The time course of diameters and velocities were determined using a 3-sec moving average. The peak dilation of post-occlusion was determined as the highest 3-sec average and was presented as a percent change from baseline diameter (peak FMD; %).
FMD was calculated as the absolute change in millimetre from baseline to peak diameter and as the percentage rise of the peak diameter from the preceding baseline diameter. The time to peak diameter was calculated from the point of cuff deflation to the maximum post-deflation diameter.
Using Xcelera 1.2 L4 SP2 2007 software (Philips, Best, The Netherlands) peak systolic flow velocity and mean flow velocity integrated over a cardiac cycle in baseline and deflation were determined by taking the average of 10 consecutive cardiac cycles. For deflation, the data segment(10-15 cardiac cycles) representing the maximum velocity during hyperaemia were considered, excluding the first cardiac cycle. From the blood flow velocity estimates and diameter measurements, local shear rate was calculated, using the following equation.
Shear rate (s^{1}) = 4 x mean blood velocity (cm/s) / diameter (cm)
Shear rate values are reported as the mean±SD from 0-15 seconds

after cuff deflation.

Statistics

Statistics were performed with the use of commercially available software (SigmaPlot 12.0, Point Richmond, CA). One-way ANOVA was used to examine the significance of differences between age-groups. When a significant main effect was present, the Holm-Sidak method was used for post hoc testing.
A linear regression model was used to examine the relation between FMD, PWV, SR and age on the one hand and between FMD and PWV on the other. Pearson's product-moment correlation analyses were used to further calculate these correlations. Data are expressed as means±SD. P<0.05 was considered to be statistically significant.

Results

Brachial artery FMD and shear rate
The mean values of FMD and PWV measurements are shown in table 2. Mean FMD, presented as the absolute change from baseline, was significantly higher (p<0.001, 95-CI: 0.19-0.25) in the young (0.29±0.10 mm) and middle-aged subjects (0.23±0.16 mm) than in old subjects (0.14±0.06 mm). There was a declining trend in absolute FMD between young and middle-aged subjects, but the difference between these groups was not significant (p=0.161, 95-CI 0.21-0.28). Mean percentage FMD (%FMD) differed significantly (p<0.001, 95-CI: 5.29-6.79) between all groups (young: 9.04±2.11%, middle-aged: 5.62±2.09%, old: 4.07±1.33%). Mean time till peak diameter was significantly higher (p=0.011, 95-CI: 58.17-74.12) in the old(82.1±42.8s) than in the middle-aged(73.5±25.6s) and young subjects(48.7±9.4s). There was no post-hoc significant difference (p=0.343, 95-CI: 9.03-10.16) in shear rate between the different age groups (young: 10.3±2.71, middle-aged: 8.55±1.79, old: 9.93±1.46).
Using stepwise linear regression models, we found a significant negative relationship between age and absolute FMD (r=-0.544, p<0.001) and age and %FMD (r=-0.751, p=<0.001). Concerning the correlation between age and time till peak deflation, Pearsons product showed a weak (r=0.391), but significant (P=0.00603) positive correlation.
With ageing, shear rate did not decline significantly(r=-0.108, p=0.476). When we corrected %FMD for shear rate and investigated the correlation of this corrected %FMD with age we found a significant inverse correlation coefficient of -0.577. These results are illustrated in figure 1A-D.

Aortic and brachial pulse wave velocity
Aortic PWV was significantly higher (p<0.001, 95-CI: 6.8-7.63) in the old age group than in the young and middle-aged age group. (young: 6.01±0.91, middle-aged: 7.58±1.41, old: 7.88±1.31 m/s). Our analysis did not show any significant difference (p=0.985, 95-CI: 7.77-8.42) in bPWV across the different age groups (young:8.07±0.9 , middle-aged: 8.12±1.46, old: 8.15±0.81 m/s)With a positive Pearson correlation coefficient of 0.623 our analysis revealed a significant(p=<0.001) relationship between age and aPWV. There was no significant correlation between bPWV and age (r=0.126, p=0.393). This is illustrated in figure 2A and 2B.

Correlations between FMD and PWV
Our analysis revealed a significant and inverse correlation between %FMD and aPWV, with a correlation coefficient of -0.404(p<0.05). There was no significant correlation between %FMD and bPWV (r=0.036). This is illustrated in figure 3A and 3B.

Discussion

Arterial stiffness is not only regulated by the structural elements of the vessel wall and related to arterial distending pressure but also by the smooth muscle tone, regulated by nitric oxide (NO). It has been shown that removal of the vascular endothelium in animals alters arterial stiffness,[30, 31] leading to the suggestion that endothelial derived mediators play an important role in the regulation of arterial stiffness in vivo. Nitric oxide is one of these endothelium derived mediators that possibly contribute to the regulation of arterial stiffness. The bioavailability of NO in both the resistance and conduit vessels is known as a key determinant of endothelial function and declines with advancing age. This study was designed to examine the effects of age on brachial artery endothelial function, measured by flow mediated dilation (FMD), and on central and peripheral arterial stiffness, measured by aortic pulse wave velocity (aPWV) and brachial pulse wave velocity (bPWV), respectively. Furthermore, an important aim was to examine the relationship between endothelial function and central and peripheral arterial stiffness in a group of healthy, sedentary individuals.
The most important finding of the present study is that aortic PWV was significantly and inversely correlated with flow-mediated dilation in the brachial artery. In addition we found a significant, age-related decline in both absolute and percentage FMD. We also found an inverse increasing relationship between aPWV and age. When we studied the flow mediated dilation parameters in detail we found a significant difference in the time to peak diameter index after deflation between the age groups. In older subjects this parameter was significantly longer than in middle-aged and younger subjects. When we measured shear rate, a surrogate measure for shear stress that causes the vessel to dilate, we did not see a significant difference between the three age groups. After normalization of %FMD for this shear rate the correlation between age and FMD changed from -0.751 to -0.577, which were both significant.
Together, these data show that an advance in age is related to a decline in endothelial function. Concerning arterial stiffness there is a relationship between advancing age and an increase in central arterial stiffness, but for peripheral stiffness this is not the case. Furthermore, our data suggest that endothelial function plays an important role in the process of arterial stiffness in vivo and this emphasizes the importance of NO as a regulator of this process.
Decline in endothelial function with advancing age is a consistent finding in prior studies.[1-4] The mechanisms are still uncertain, but may be related to the age-related turnover of endothelial cells. With ageing the endothelial cells detach from the vessel wall which may result in uncovered areas. Endothelial cell genesis may repair the uncovered area but they exhibit a selective loss in responses that involve cell membrane receptors linked to the activation of nitric oxide synthase (NOS). The result is a reduced bioavailability of NO that causes endothelial dysfunction.[32] The turnover of cells is stimulated by risk factors such as high cholesterol, smoking, hypertension and diabetes. These risk factors may work together with the negative impact of ageing setting up the scene for development of vascular disease.
There is an ongoing debate about whether or not to normalize FMD for shear stress, or its surrogate measure of shear rate. Several studies have demonstrated that exposure to shear stress leads to diameter increases in a dose-dependent fashion.[33, 34] When distinct FMD responses are observed between groups or individuals, it still is unclear whether this is attributable to different biological variability

in endothelial function or a difference in the magnitude of reactive hyperaemia induced shear stress. Since shear rate did not change significantly with an advance in age in our study, and a significant correlation between FMD and age still existed after normalization for shear rate, we excluded the possibility that differences in the magnitude of reactive hyperaemia would be interpreted as changes in FMD with age.

To explore the relationship between arterial stiffness and age we assessed aortic and brachial PWV, which provides an accurate measure of arterial stiffness. The age associated augmentation was much stronger in aortic pulse wave velocity than in brachial pulse wave velocity. Such different effects of ageing on the stiffness of central versus peripheral arteries may be related to their distinct roles in hemodynamic regulation. The cushion functions that damp fluctuations in blood flow that are present in central arteries are not present in the peripheral arteries. Because of this, the peripheral arteries do not exhibit the same extent of pulsatile changes in diameter and as such they may not undergo the adaption (or wear and tear stress) leading to a loss of elasticity.[35]

When determining the relationship between endothelial function and arterial stiffness, flow mediated dilation emerged as a significant determinant of aortic PWV, suggesting endothelial function declines as central arterial stiffness increases. A number of other studies have extensively investigated the relationship between endothelial function and various indices of arterial stiffness in selected patient groups with cardiovascular risk factors and diseases.[18-22]. To our knowledge this is the only study focused on investigating the age-related correlation between endothelial function and arterial stiffness in healthy individuals. The other study describing this relationship in healthy individuals was performed by McEniery et al. and published in 2006.[25] Their observations also showed a significant correlation between flow mediated dilation and aortic PWV in a group of 89 healthy individuals(age of 41±16 years, r=-0,39, p<0.001). Our data confirm these findings, although in a smaller group of participants.

Strengths and Limitations

A limitation of our study is that our cohort largely consisted of Caucasian individuals. Results may not be applicable to other racial or ethnic groups. However, a prior study suggests that flow mediated dilation is comparable in white and black populations.[36] The size of our study cohort also forms a limitation, whereas the other study by McEniery et al. included 89 subjects to compare %FMD with aPWV,[25] our study only included 48 participants. Besides this a limitation can be found in our gender distribution, in both the young and the old age-group, the number of female participants exceeded the number of male participants. This may have influenced our outcomes, because our results show that females have a smaller FMD response and a lower aPWV in comparison to males. It is shown that smooth muscle sensitivity to nitric oxide is modulated by the hormonal patterns of the menstrual cycle,[37], however we did not track the menstrual cycle in the female participants.

Notwithstanding the above limitations, the present study has several strengths, the most important is the inclusion of elderly participants. Ageing exerts a marked effect on arterial stiffness and endothelial function and by dividing our subjects in a young, middle-aged and old age group we were able to make an association between an advance in age with both vascular parameters. Moreover, this study only contained healthy individuals, whereas previous studies describing the relationship between endothelial function and arterial stiffness mainly included subjects with cardiovascular risk factors and diseases.

Conclusion

Since NO is a potent anti-atherogenic molecule it plays a major role in the development of cardiovascular diseases and it is associated with cardiovascular risk factors.[2, 5, 7, 8, 10-12] Arterial stiffness is also associated with these cardiovascular diseases and risk factors,[13-15] leading to the suggestion that impaired NO-bioavailability may be the link between these two vascular parameters. The results of the present study indicate that, even in healthy individuals, a decline in flow mediated endothelial function is associated with an increase in central arterial stiffness. This describes the importance of NO as the common underlying mechanism in the regulation of endothelial function and arterial stiffness in vivo.

This evidence, linking NO to the regulation of arterial stiffness provides a novel therapeutic target for drugs. Particularly, drugs that can restore vascular NO-bioavailability may attenuate or prevent age-related increases in arterial stiffness and its clinical consequences. In this regard, the mechanisms behind the association of NO and arterial stiffness need to be fully studied and understood. Mechanistic study of the role of NO on arterial stiffness is likely to have an significant impact on both health and disease.

Acknowledgements

To Dr. Jurgen Claassen, for being my supervisor during the first months in Nijmegen and helping me during my first steps in scientific research. To A. Van Abeelen for teaching me the necessary analytical skills. To Dr. Rong Zhang, for answering all my questions and introducing me in the world of vascular ageing It was an honour and pleasure to work for and with such an enthusiastic and skilled scientist. To Annelies Ruijs, for all her work as the coordinator of the Honours Programme. Finally I would like to thank all my colleagues from the labs in Nijmegen and Dallas.

References

1. Black, M.A., et al., Impact of age, sex, and exercise on brachial artery flow-mediated dilation. Am J Physiol Heart Circ Physiol, 2009. 297(3): p. H1109-16.
2. Celermajer, D.S., et al., Aging is associated with endothelial dysfunction in healthy men years before the age-related decline in women. J Am Coll Cardiol, 1994. 24(2): p. 471-6.
3. Gerhard, M., et al., Aging progressively impairs endothelium-dependent vasodilation in forearm resistance vessels of humans. Hypertension, 1996. 27(4): p. 849-53.
4. Herrington, D.M., et al., Brachial flow-mediated vasodilator responses in population-based research: methods, reproducibility and effects of age, gender and baseline diameter. J Cardiovasc Risk, 2001. 8(5): p. 319-28.
5. Vallance, P. and N. Chan, Endothelial function and nitric oxide: clinical relevance. Heart, 2001. 85(3): p. 342-50.

6. Thijssen, D.H., et al., Assessment of flow-mediated dilation in humans: a methodological and physiological guideline. Am J Physiol Heart Circ Physiol, 2011. 300(1): p. H2-12.
7. Schalkwijk, C.G. and C.D. Stehouwer, Vascular complications in diabetes mellitus: the role of endothelial dysfunction. Clin Sci (Lond), 2005. 109(2): p. 143-59.
8. Celermajer, D.S., et al., Cigarette smoking is associated with dose-related and potentially reversible impairment of endothelium-dependent dilation in healthy young adults. Circulation, 1993. 88(5 Pt 1): p. 2149-55.
9. Cosentino, F. and M. Volpe, Hypertension, stroke, and endothelium. Curr Hypertens Rep, 2005. 7(1): p. 68-71.
10. Chowienczyk, P.J., et al., Impaired endothelium-dependent vasodilation of forearm resistance vessels in hypercholesterolaemia. Lancet, 1992. 340(8833): p. 1430-2.
11. Mullen, M.J., et al., Heterogenous nature of flow-mediated dilation in human conduit arteries in vivo: relevance to endothelial dysfunction in hypercholesterolemia. Circ Res, 2001. 88(2): p. 145-51.
12. Suwaidi, J.A., et al., Long-term follow-up of patients with mild coronary artery disease and endothelial dysfunction. Circulation, 2000. 101(9): p. 948-54.
13. Mitchell, G.F., et al., Changes in arterial stiffness and wave reflection with advancing age in healthy men and women: the Framingham Heart Study. Hypertension, 2004. 43(6): p. 1239-45.
14. Benetos, A., et al., Influence of age, risk factors, and cardiovascular and renal disease on arterial stiffness: clinical applications. Am J Hypertens, 2002. 15(12): p. 1101-8.
15. Weber, T., et al., Arterial stiffness, wave reflections, and the risk of coronary artery disease. Circulation, 2004. 109(2): p. 184-9.
16. Isabelle, M., et al., Chronic reduction of nitric oxide level in adult spontaneously hypertensive rats induces aortic stiffness similar to old spontaneously hypertensive rats. J Vasc Res, 2012. 49(4): p. 309-18.
17. Van Bortel, L.M., H.A. Struijker-Boudier, and M.E. Safar, Pulse pressure, arterial stiffness, and drug treatment of hypertension. Hypertension, 2001. 38(4): p. 914-21.
18. Nigam, A., et al., Relation between conduit vessel stiffness (assessed by tonometry) and endothelial function (assessed by flow-mediated dilation) in patients with and without coronary heart disease. Am J Cardiol, 2003. 92(4): p. 395-9.
19. Ramsey, M.W., et al., Endothelial control of arterial distensibility is impaired in chronic heart failure. Circulation, 1995. 92(11): p. 3212-9.
20. Tounian, P., et al., Presence of increased stiffness of the common carotid artery and endothelial dysfunction in severely obese children: a prospective study. Lancet, 2001. 358(9291): p. 1400-4.
21. Aggoun, Y., et al., Impaired endothelial and smooth muscle functions and arterial stiffness appear before puberty in obese children and are associated with elevated ambulatory blood pressure. Eur Heart J, 2008. 29(6): p. 792-9.
22. Treasure, C.B., et al., Beneficial effects of cholesterol-lowering therapy on the coronary endothelium in patients with coronary artery disease. N Engl J Med, 1995. 332(8): p. 481-7.
23. Liang, Y.L., et al., Non-invasive measurements of arterial structure and function: repeatability, interrelationships and trial sample size. Clin Sci (Lond), 1998. 95(6): p. 669-79.
24. Malik, A.R., V. Kondragunta, and I.J. Kullo, Forearm vascular reactivity and arterial stiffness in asymptomatic adults from the community. Hypertension, 2008. 51(6): p. 1512-8.
25. McEniery, C.M., et al., Endothelial function is associated with pulse pressure, pulse wave velocity, and augmentation index in healthy humans. Hypertension, 2006. 48(4): p. 602-8.
26. Harris, R.A., et al., Ultrasound assessment of flow-mediated dilation. Hypertension, 2010. 55(5): p. 1075-85.
27. Thijssen, D.H., et al., Does arterial shear explain the magnitude of flow-mediated dilation?: a comparison between young and older humans. Am J Physiol Heart Circ Physiol, 2009. 296(1): p. H57-64.
28. Samijo, S.K., et al., Wall shear stress in the human common carotid artery as function of age and gender. Cardiovasc Res, 1998. 39(2): p. 515-22.
29. Wilkinson, I.B., et al., Reproducibility of pulse wave velocity and augmentation index measured by pulse wave analysis. J Hypertens, 1998. 16(12 Pt 2): p. 2079-84.
30. Boutouyrie, P., et al., In vivo/in vitro comparison of rat abdominal aorta wall viscosity. Influence of endothelial function. Arterio-

Tables

Table 1: Subject characteristics of the participants divided into a group of young, middle-aged and older adults.

Age categorie	20-39	40-59	60-80	Anova
n	13	21	14	
Gender male/female	4/9	12/9	3/11	
Age, yr	28.5±6.2	50.7±5.7	66.6±5.7	<0.001
Heart rate at rest	65.5±6.8	63.3±8.8	61.6±9.2	0.491
Systolic blood pressure, mmHg	107.9±9.3	113.2±12.5	115±10.2	0.236
Diastolic blood pressure, mmHg	67.5±8.7	72.9±8.7	68.7±9.6	0.197
Mean blood pressure, mmHg	81.0±8.0	86.3±9.6	84.1±8.4	0.246
Height, cm	165.1±8.1	170.8±9.7	164.0±5.6	0.041
Weight, kg	61.3±11.2	79.5±14.1	67.7±10.2	<0.001
BMI, kg/m²	22.4±3.1	27.1±3.8	25.1±3.3	0.002

BMI, body mass index
Values are presented as means± SD, n is number of participants, P<0.05 is significant.

Table 2: Brachial artery characteristics and arterial stiffness parameters

Age categorie	20-39			40-59			60-80			Anova*ºǂ
Gender	man	female	overall	man	female	overall	man	female	overall	overall
n	4	9	13	12	9	21	3	11	14	
Baseline diameter, mm	3.92±0.19	2.77±0.50	3.16±0.67º	4.42±0.46	3.38±0.62	3.98±0.73º	3.61±0.87	3.23±0.17	3.31±0.41º	0.001
Peak diameter, mm	4.30±0.22	3.07±0.55	3.45±0.75º	4.71±0.49	3.52±0.62	4.20±0.81º	3.82±0.98	3.35±0.18	3.45±0.46º	0.003
Absolute change from baseline, FMD mm	0.38±0.03	0.25±0.09	0.29±0.10º	0.30±0.07	0.14±0.06	0.23±0.16º	0.21±0.12	0.12±0.03	0.14±0.06º	<0.001
% change from baseline, %FMD	9.74±0.44	8.73±2.50	9.04±2.11*	6.71±1.61	4.17±1.80	5.62±2.09*	5.5±1.97	3.68±0.86	4.07±1.33*	<0.001
time to peak dilation, sec	48.3±12.7	48.0±8.5	48.7±9.4º	74.2±24.1	72.7±29.0	73.5±25.6º	79.3±73.2	82.9±36.2	82.1±42.8º	0.011
Shear rate 0-15 sec, s^{-1}, 10^2	8.15±1.59	11.3±2.56	10.3±2.71ǂ	7.76±1.29	9.38±2.04	8.55±1.79ǂ	8.55±1.83	10.2±1.37	9.93±1.46ǂ	0.043
Aortic pulse wave velocity m/s	6.68±1.21	5.71±0.62	6.01±0.91º	7.64±1.33	7.49±1.60	7.58±1.41º	8.53±1.29	7.71±1.34	7.88±1.33º	<0.001
Brachial pulse wave velocity m/s	8.41±1.37	7.92±0.80	8.07±0.97	8.35±1.51	7.81±1.43	8.12±1.46	9.02±0.95	7.91±0.61	8.15±0.81	0.985

**post hoc significance between all groups.*
ºpost hog significance between young and old and middle-aged an old,
ǂno post hoc significance between groups
Values are presented as means± SD, n is number of participants, P<0.05 is significant.

Figures

Figure 1: The relationship between age and: %FMD(A), time till peak dilation(B) and shear rate(C) and %FMD normalized for Shear Rate(D).

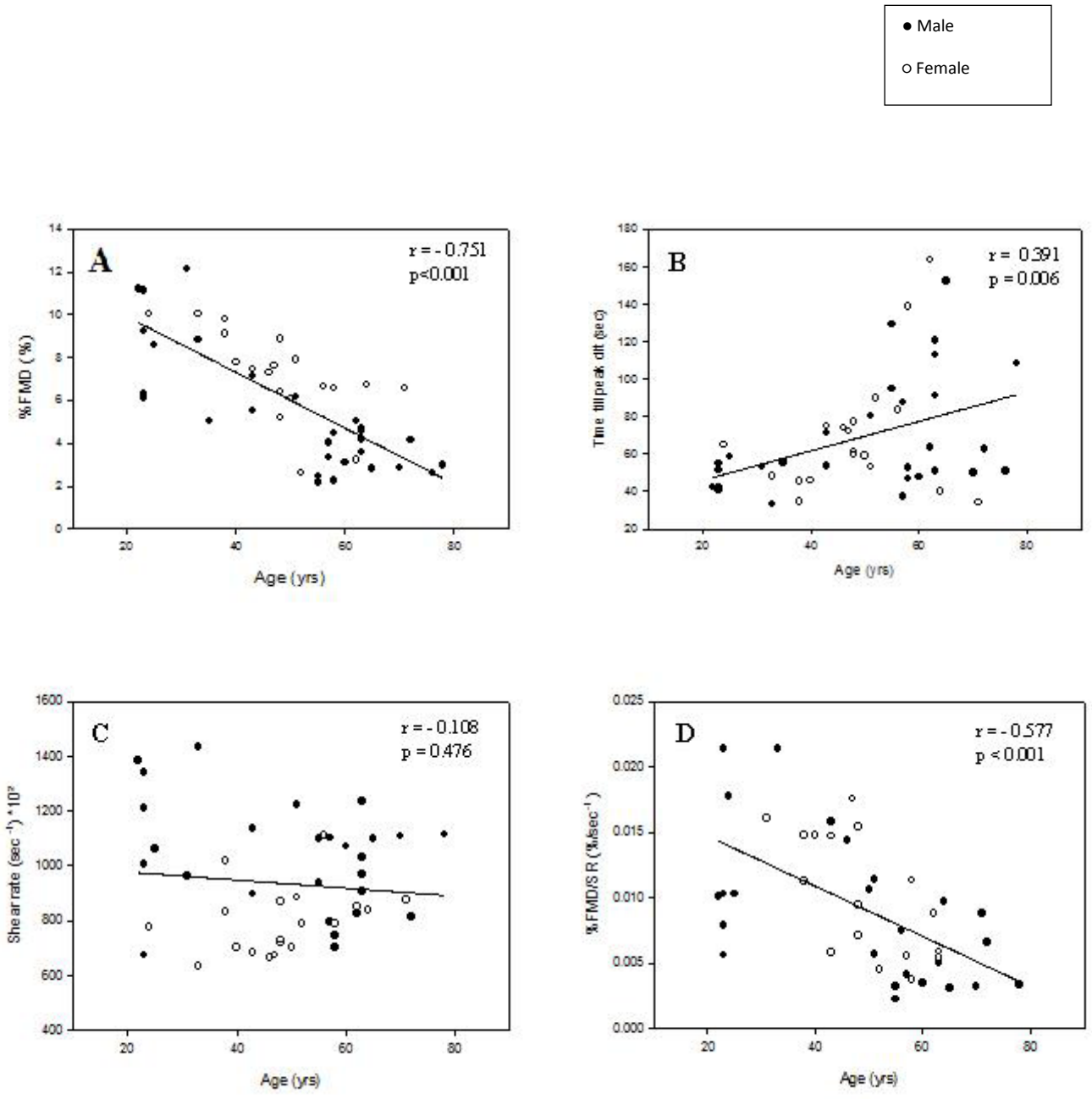


Figure 2: The relationship between age and aPWV (A) and bPWV (B)

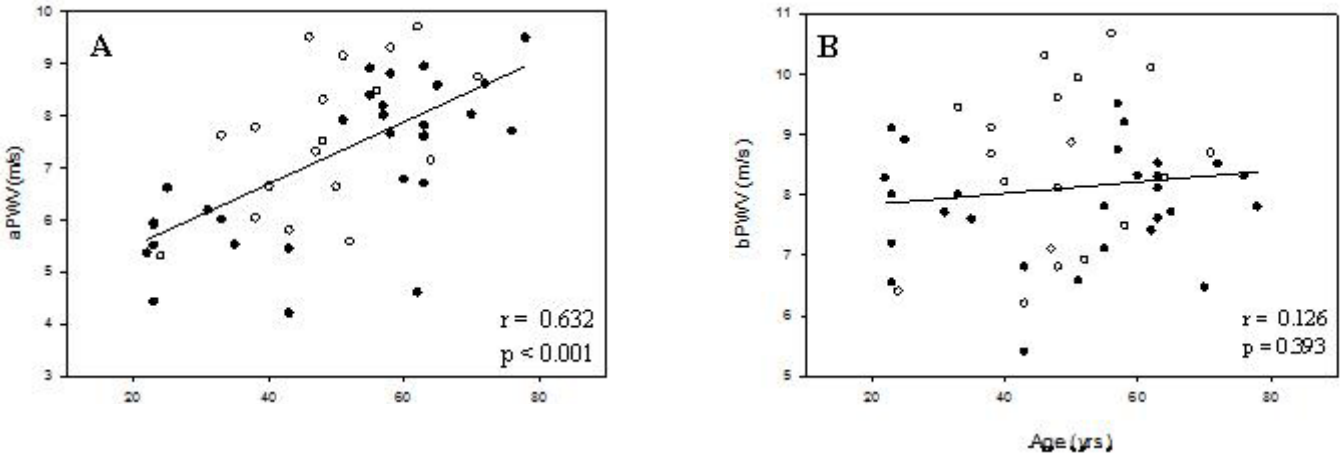
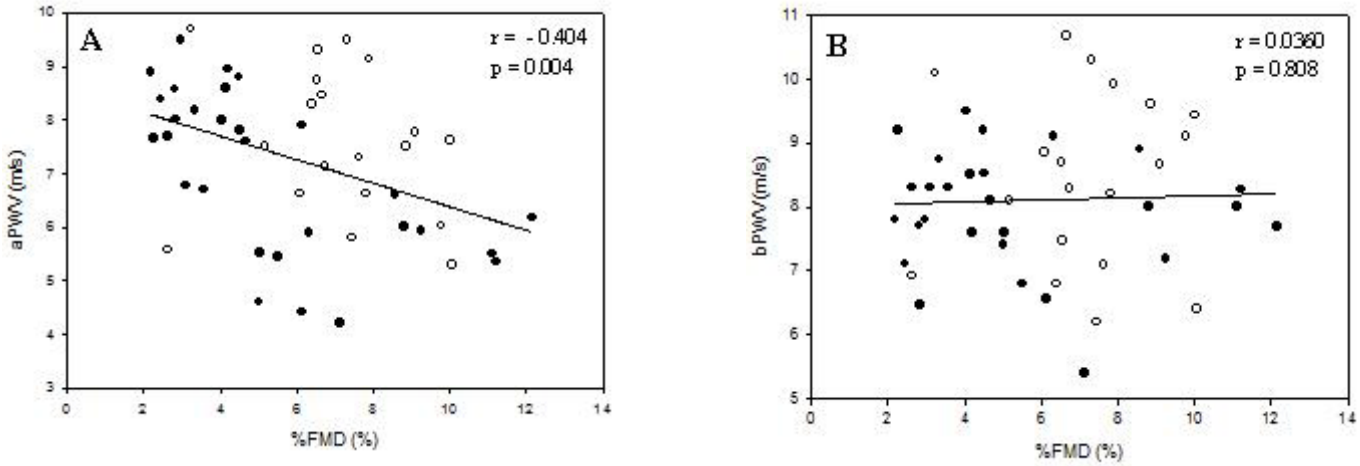


Figure 3: The relationship between %FMD and aPWV(A) and bPWV(B)



Interview Viola Klück

On a warm Friday afternoon, while the preparations for the Kinky Pinky party were up and running, I had an appointment with medical student Viola Klück. Her article on arterial stiffness is published in this first edition of RAMS. Dressed in a pink shirt, completely according to the theme of tonights' party, Viola sits down to the table for an interview.

The Honours Programme gave Viola the opportunity to do research. "During my first year I looked for some challenge besides my study. Furthermore, I was curious about how we find information about diseases and methods of treatment. Therefore, the Honours Programme appeared to be interesting to me." During this programme, she could choose on which department she wanted to do her research. She ended up in the Physiology department, her choice of preference. "In Medicine there are 27 clinical specializations. It's quite difficult to choose your favourite from so many subjects, especially when you just started your study. During the lectures I attended, I found Physiology interesting, that's why I wanted to discover more about it."

That way, she went to the University of Boulder, Colorado, to do the research. Boulder is a city close to the Rocky Mountains. Though this beautiful area and the hot temperatures called out for enjoying the sun the entire day, there also was some work to be done. "My schedule was variable. Every Tuesday there was a labmeeting in which we discussed what experiments we wanted to do that week and in which everybody presented their results so far. On days I had to do an experiment, I set my alarm at six o'clock in the morning and started my experiments at seven. The cell experiments I did, lasted the entire day. It takes some time to process the cells and incubate them. On other days I had time to write or analyse ultrasound scans from blood vessels. I started early in the morning, so sometimes I went home around three or four o'clock in the afternoon, but on other days I worked for about ten hours." Besides the research, she fortunately had some time to relax as well. "I lived in an apartement with two American students. I found this room through some kind of American "kamernet". There was a great number of rooms for rent, so it was quite easy to find one. I had dinner with my roommates on a regular basis and sometimes we went to the cinema. We also developed an addiction to 'Criminal minds' together. With a group of International students I also did some exploration of the area, for example by raft."

Unfortunately, life of a researcher isn't always a bed of roses. Viola told she had some problems at the start of her research with developing a good method of research. "We wanted to try a new method of studying endothelial cells. The incubation time had to be long enough for the substances to incubate, but on the other hand it couldn't be too long because in this scenario the endothelial cells would die. At first, we had to do some fine-tuning on this matter before we found a balance. It was kind of frustrating because this meant that after a few weeks I created a lot of dead endothelial cells but no results yet. When we at last found the right way to do the experiment, I couldn't be happier!"

© Viola Klück, photographed at Radboudumc

At this moment, Viola is a fourth-grade student and she now works as a medical intern. Next to this, unfortunately she finds too little time for other investigations. "The reason I wanted to study medicine was because I wanted to treat people in the first place, not to become a researcher. I find scientific education an important part of the curriculum and I find this very interesting. In the future, I would like to combine doing research with being a physician."

We are looking for:

Reviewers

As a reviewer you will assess papers received by RAMS. Based on your review report, papers will be accepted for publication or rejected. Experience is not obliged, but interest in research is. In a serie of masterclasses, professors and high-class researchers will teach you about the ins and outs of medical-scientific research, after which you will be able to independently work as a reviewer.

What do we offer?

- Experience in medical research.
- Masterclasses by the best teachers.
- Flexible working hours (at home).
- Good addition to your resume.

Who are we looking for?

- Students of medicine, biomedical sciences, dentistry and MMD with interest in scientific research.
- On average 2 hours a week available.
- Available for participation of masterclasses around October 2014 (5 x 2 hours).

Enthousiastic? Send your resume and short motivation to **voorzitter.rams@ru.nl** before August 15. Interviews will be held in the first weeks of September 2014. For questions and more information we can be reached via the same e-mail address, or check **www.ramsresearch.nl**.

RAMS
Radboud Annals of Medical Students

Effect of Trehalose on Arterial Stiffness and Oxidative Stress in Human Vascular Endothelium

Viola Klück (1,2), Rachelle E. Kaplon (2), Thijs M.H. Eijsvogels (1), Maria T.E. Hopman (1) and Douglas R. Seals (2)

(1) Department of Physiology, Radboud university medical center, Nijmegen, The Netherlands (2) Integrative Physiology of Aging Laboratory, University of Colorado, Boulder, CO, USA

Corresponding Author: Viola Klück, BSc (v.kluck@student.ru.nl)

ABSTRACT:

EFFECT OF TREHALOSE ON THE HUMAN ENDOTHELIUM

BACKGROUND: Cardiovascular diseases (CVD) are the most common cause of death worldwide; the majority is associated with the dysfunction of arteries, characterized by impaired endothelial function and arterial stiffness. Oxidative stress is a primary mechanism underlying vascular dysfunction and CVD. Trehalose, a naturally occurring disaccharide, has been shown to have antioxidant effects in different cell and animal models but the effect of trehalose in humans is unknown.
OBJECTIVE: The aim of this study is to evaluate the effect of trehalose on oxidative stress levels in biopsied human endothelial cells and its effect on arterial stiffness in elderly adults.
METHODS: To answer these questions, we developed a novel method to directly measure oxidative stress in endothelial cells harvested from a superficial forearm vein from 7 healthy donors aged 23±2 years. In a subset, whole cell reactive oxygen species (ROS) and mitochondrial superoxide levels were measured in endothelial cells treated with trehalose (2 hour incubation; 100mM). β-stiffness index and carotid artery compliance were evaluated in elderly adults (n=10; aged 65±7) in a 12-week randomized, double-blind, dose response study with oral trehalose supplementation (placebo, 50g or 100g daily).
RESULTS: Trehalose significantly reduced both whole cell ROS and mitochondrial superoxide levels in endothelial cells compared to the control condition (-48%, $p<0.001$ and -26%, $p<0.01$, respectively). Moreover, trehalose supplementation tends to improve β-stiffness index and carotid artery compliance compared to the placebo group.
CONCLUSION: Trehalose can be a promising novel therapeutic strategy in the treatment of CVD. However, the presented results on arterial stiffness are preliminary and more research is needed to further elucidate the effect of trehalose on endothelial dysfunction and arterial stiffness.

WHAT IS KNOWN: Cardiovascular diseases (CVD) are the most common cause of death worldwide. An important factor causing vascular dysfunction resulting in CVD is oxidative stress. Trehalose has been shown to have antioxidant effects in different cell and animal models.

WHAT IS NEW: Trehalose reduces oxidative stress in human endothelial cells and trehalose supplementations tends to improve arterial stiffness in elderly. Therefore it can be a promising novel therapeutic strategy in the treatment of CVD.

KEYWORDS: Trehalose, Endothelium, Oxidative Stress, Arterial Stiffness, Ageing

Introduction

Cardiovascular diseases (CVD) are the most common cause of death worldwide, representing 30% of all global deaths 1. The great majority of CVD deaths are associated with dysfunction and disorders of arteries 2. Although epidemiological studies have discovered many risk factors, such as diabetes, sedentary lifestyle and hypertension, advancing age is the major risk factor for CVD 3. Ageing adversely affects arteries and causes vascular endothelial dysfunction (characterized in part by impaired endothelium-dependent dilation (EDD)) 3-6, which results in reduced compliance and increased arterial stiffness. With age, conduit and resistance arteries develop impaired EDD due to reduced bioavailability of vascular-protective nitric oxide (NO) 7. One of the mechanisms causing this reduction in NO bioavailability is oxidative stress 8.
Oxidative stress can be defined as increased bioactivity of reactive oxygen species (ROS) relative to antioxidant defenses 9. Oxidative stress reduces NO bioavailability via excessive production of superoxide, produced in the mitochondria or by oxidant enzymes such as NADPH oxidase 10. Superoxide can react with NO to form peroxynitrite. Subsequently, peroxynitrite oxidizes tetrahydrobiopterin (BH4) which is an essential cofactor for endothelial nitric oxide synthase (eNOS). The reduction in bioavailability of BH4 also leads to the "uncoupling" of eNOS, which then produces more superoxide and less NO in a vicious cycle that further reduces NO bioavailability (Figure 1) 11, 12. Therefore, normalizing oxidative stress levels in endothelial cells is a promising therapy to improve endothelial dysfunction and large elastic artery stiffening with ageing in humans.
We investigated trehalose, a naturally occurring disaccharide of glucose found in many foods such as mushrooms, honey and baker's yeast. Trehalose is hydrolyzed in the intestinal lumen by trehalase, but a small percentage is absorbed passively into circulation. Trehalose protects proteins from denaturation and acts as a chemical chaperone in vitro 13, 14. In vivo, supplementation with trehalose improves function in animal models of age-related neurological diseases, protects against diet-induced insulin resistance, decreases adipocyte inflammatory signaling and extends longevity 15-17. Although the exact mechanism of action is unknown, trehalose has been reported to induce autophagy, a cellular process of recycling damaged bio-

molecules/organelles that can suppress oxidative stress and inflammation, both in vitro and in vivo 13, 17. Recently, trehalose supplementation in old mice has been shown to restore eNOS and reduce superoxide production to levels observed in young mice 18.
In the present study we tried to illuminate the effects of trehalose on oxidative stress in the endothelial cells from human subjects using a newly developed method. Moreover, we aimed to examine the effect of trehalose on arterial stiffness in a small cohort of elderly. We hypothesize that trehalose can decrease oxidative stress in biopsied human endothelial cells and improve arterial stiffness in elderly adults.

Methods

Arterial Stifness

Study design
We conducted a 12-week randomized, double-blind, dose response study with oral trehalose supplementation. All testing took place at the Clinical and Translational Research Center (CTRC) in the Wardenburg Health Center on the University of Colorado at Boulder campus. The nature, risk and benefits of all study procedures were described to subjects and their written consent was obtained prior to participation in the study. Subjects underwent telephone and CTRC screening with those eligible randomly assigned (using a "RAND()" function in Excel) to 1 of 3 groups: 100 g maltose/350 ml water 1x/d (placebo group), 50 g trehalose + 50 g maltose/350 ml water 1x/d (low-dose group), and 100 g trehalose/350 ml water 1x/d (high-dose group). Maltose was used as a placebo and filler, because it has a similar structure, sweetness and caloric content to trehalose, but does not have effects on physiological function at similar concentrations. Subjects were advised to consume the trehalose drink over the course of the day at their own pace. The investigators involved in the acquisition and analysis of key outcomes were blinded to the trehalose intake status of subjects. CTRC nurses and professional research assistants from our laboratory were informed of the group status of all subjects throughout the study.
All measurements were made under supine, overnight fasted (water only) conditions. Subjects were asked to refrain from non-prescription medications for 48 hours; alcohol, exercise, and prescription medications for 24 hours; and caffeine for 12 hours prior to all study visits as these are factors known to modulate vascular function. Subjects on prescription medications were required to consult their physician and obtain a signed letter of consent prior to participation in the study. Measurements were made at baseline and after 12 weeks of each condition. Figure 2 shows a schematic overview of the study design.

Subjects
Men and women between the ages of 50 and 79 from all ethnic backgrounds were recruited (n=10, placebo n=3, low dose n=2, high dose n=5). Subject characteristics are expressed as mean±SD (Table 1). There are no significant differences in these characteristics between groups. All subjects signed an informed consent. Exclusion criteria were baseline brachial flow-mediated dilation (FMD, a measure of EDD) >7% Δ, body mass index (BMI) >40 kg/m2, unstable weight in the prior 3 months or unwilling to remain weight stable throughout the study, having unstable cardiovascular or metabolic disease or suffering from diabetes. Other exclusion criteria were having past or present alcohol or nicotine dependence or abuse, scoring <23 on the mini-mental state examination, having abnormal blood chemistries for renal and liver function (>1SD outside the normal range), suffering from moderate or severe peripheral artery disease (ankle-brachial index <0.7) or having insufficient health to participate in a VO2 max test. Perimenopausal women were also excluded. All procedures were approved by the University of Colorado Institutional Review Board.

Measurements of arterial stiffness
Two common indices of arterial stiffness were measured: carotid artery compliance, which is the change in cross-sectional area of a vessel per unit of pressure, and β stiffness index, which provides an index of arterial compliance adjusted for distending pressure. Carotid arterial compliance was calculated as described by Armentano et al. [(D1-D0/D0)/(2(P1-P0)) x π x (D0)2] 19 and carotid artery β-stiffness index was calculated using the formula by Harai et al. [ln(P1/P0)]/[(D1/D0)/D0], where P1=carotid systolic blood pressure (BP), P0=carotid diastolic BP, D1=carotid end-systolic diameter, and D0=carotid end diastolic diameter) 20. The common carotid artery diameters and carotid artery BP were sequentially assessed by high-resolution ultrasonography (PowerVision 6000, Toshiba, Inc.) and non-invasive carotid artery applanation tonometry with a pencil-type probe (Noninvasive Hemodynamics Workstation, Cardiovascular Engineering, INC., Norwood, MAs), respectively. AVI images were acquired for subsequent off-line analysis with image analysis software (Vascular Research Tools 5.0, Medical Imaging Applications, LLC).

Data analysis
Statistical analyses were performed with IBM SPSS (version 21). All data are reported as mean±SD. Differences were analyzed by an ANOVA with Bonferroni adjustment and considered to be statistically significant if the P-value was < 0.05.

Oxidative Stress

Current methods to assess oxidative stress in endothelial cells are limited to measuring downstream effected proteins or studying human derived cell lines. In this paper a novel method was used to directly assess oxidative stress in human endothelial cells harvested from an antecubital vein.

Subjects
Healthy men and women between the ages of 20 and 40 were included (n=7). Exclusion criteria were BMI>25 kg/m2 and having unstable cardiovascular or metabolic disease or suffering from diabetes. The average age of the volunteers was 23±2 and the average BMI 21±2. One volunteer donated twice for different experiments. All volunteers signed an informed consent. All procedures were approved by the University of Colorado Institutional Review Board.

Endothelial cell oxidative stress assessment
Up to six sterile J wires (Daig Corp, Minnetonka, Minn) were advanced and retracted one at a time through an 18-gauge catheter placed in an antecubital vein. The obtained cells were isolated, divided in two wells and allowed to adhere to poly-L-lysine–coated slides (Sigma Chemical, St. Louis, Mo).
Cells in one well were treated with trehalose (100 mM for 2 hours), cells in the other well served as a control. Oxidative stress was measured by CellROX or MitoSOX staining for assessment of whole cell ROS production and mitochondrial superoxide production, respectively. Next, the collected cells were fixed with 3.7% formaldehyde. Endothelial cells were stained for Vascular Endothelial-Cadherin (VE-CAD) and nuclei were made visible with DAPI (4',6'-diamidino-2-phenylindole hydrochloride).
For analysis, slides were viewed with a fluorescence microscope

(Eclipse Ni-U, Nikon, Melville, NY), and cell images were captured digitally by a Clara CCD digital camera (Andor Technology, Belfast UK). Endothelial cells were identified by staining for VE-CAD and nuclear integrity. Once endothelial cells with intact nuclei were identified, they were analyzed with Metamorph Software (Universal Imaging Corp, Downingtown, Pa).

Data analysis

Statistical analyses were performed with IBM SPSS (version 21). All data are reported as mean±SD. Changes were analyzed by a two-tailed T-test and considered to be statistically significant if the P-value was < 0.05.

Results

Trehalose reduces ROS levels in human endothelial cells

To investigate whether trehalose ameliorates ROS levels in endothelial cells, we collected live endothelial cells from healthy donors and treated these cells with trehalose. In order to resemble physiological conditions, we did not induce oxidative stress artificially. Whole cell ROS levels in trehalose treated cells, as determined by CellROX staining, decreased 48% compared to control cells (n=5; $p<0.001$). However, only in subjects with relatively high basal values (subjects 1, 2 and 4), mean intensity was significantly decreased compared to control cells ($p<0.01$) (Figure 3a). In subjects with low basal values (mean intensity<200) (subjects 3 and 5) trehalose did not reduce ROS levels.

Trehalose reduces mitochondrial superoxide levels in human endothelial cells

To assess in more detail the ability of trehalose to reduce oxidative stress, we performed a similar experiment and measured mitochondrial superoxide production by treating cells with MitoSOX. Mitochondrial superoxide levels decreased 26% in cells treated with trehalose compared to the control cells (n=3; $p<0.01$). Again, a significant difference between the trehalose and control condition was seen in subjects with elevated basal levels (subjects 6 and 8) (Figure 3b). Mitochondrial superoxide levels in the trehalose treated cells were slightly increased in subject 7 compared to the cells in the control condition.

The effect of trehalose supplementation on arterial stiffness in ageing humans

To determine effects of trehalose on the vasculature in vivo, we conducted two measurements of arterial stiffness in our subjects before and after a 12-week intervention with trehalose. Our placebo group showed an increase in stiffness during our 12-week intervention indicated by an increase in β-stiffness index and a decline in carotid artery compliance (Figure 4). Although differences between groups were not significant (placebo vs. high dose $p=0.26$), the low dose group showed a slightly lower increase in β-stiffness index, while the high dose showed a tendency towards decreased β-stiffness index (Figure 4c) (placebo 1.61±1.11; low dose 1.04±1.97; high dose -0.02±2.07). Both the low dose and high dose group showed a smaller decrease in carotid artery compliance (increase in local arterial stiffness) compared to the placebo group, although the differences did not reach statistical significance (placebo vs. high dose $p=0.11$) (Figure 4d) (placebo -0.019±0.016; low dose -0.003±0.009; high dose -0.002±0.011).

Discussion

Oxidative stress plays a major role in the development of endothelial dysfunction, associated with CVD 8. Since trehalose has been attributed with antioxidant effects, we investigated trehalose in its ability to reduce excess of oxidative stress in endothelial cells from human subjects and to improve arterial stiffness in elderly.

The key finding of this study was that trehalose decreased oxidative stress levels in biopsied human endothelial cells with elevated baseline levels. Both whole cell ROS and mitochondrial superoxide levels were reduced in cells treated with trehalose compared to the cells in control (media treated) condition. These data suggest that trehalose has antioxidant effects in endothelial cells biopsied from human subjects. Moreover, increases in large elastic artery stiffness in a 12-week period tended to be blunted following oral trehalose supplementation versus placebo. This finding suggests that trehalose may be able to reverse age-associated increases in arterial stiffness.

These positive effects may be due to the capacity of trehalose to induce autophagy as described in literature 13, 17. In cardiovascular cells, autophagy acts predominantly as a pro-survival pathway, protecting the cells from oxidative stress 21. Autophagy can involve the direct engulfment of cytoplasmic material into the lysosome (microautophagy), thereby reducing cytoplasmic ROS, or the degradation of damaged cell organelles such as mitochondria (also called mitophagy) 22. This can result in reduced mitochondrial superoxide levels. A more classic autophagy enhancer, rapamycin, has shown to have a maximal effect of inducing autophagy 2 hours after addition 23, indicating that trehalose could also have been inducing autophagy during the 2 hour incubation time used in our experiments.

Although trehalose decreases oxidative stress in subjects with elevated basal levels, trehalose does not further reduce oxidative stress in subjects with already low basal levels. The fact that trehalose does not totally deplete ROS levels is important, because normal ROS levels are required for several intracellular signalling pathways 24. Therefore, trehalose seems an antioxidant that can be safely administered to humans. It also indicates that dysfunction must be present in order for trehalose to have an effect, suggesting trehalose can be more effective treating rather than preventing CVD.

Why we measured an increase of ROS following trehalose treatment in subject 7 is unclear. Except for its ability to induce autophagy, trehalose has many other intracellular functions, for instance participation in stabilization of proteins and membrane structures 25. However, the exact mechanisms of all the different pathways trehalose is involved in are unknown. Possibly, one of the pathways can cause the finding seen in this study. It is also possible that compensatory mechanisms are increasing ROS to maintain basal signaling required for normal cellular homeostasis. Yet the small increase in oxidative stress in the endothelial cells found in subject 7 is unlikely to have any physiological relevant effects.

Reducing excess of oxidative stress seems a potential therapy in treating CVD. However, conventional antioxidants such as Vitamin E or Vitamin C have shown to be incapable of reducing endothelial dysfunction by reducing the concentration of reactive oxygen species in the vessel wall 26. A current theory states conventional antioxidants may not be targeting where they are needed most, namely in the mitochondria, or that they do not reach concentrations where they are effective at sequestering ROS signaling [22]. Subsequently, mitochondrial-targeted antioxidants have been developed and show promising results in rat models 27. Stimulating autophagy may be another way to bypass this problem. Since trehalose was also found to reduce mitochondrial superoxide in the present study, it may be a promising treatment for CVD.

A limitation in our study is that the donors of endothelial cells were all young volunteers. Addressing oxidative stress in an ageing population would have been more clinically relevant. Subsequent studies should evaluate basal differences between young and old volunteers and examine the effect of trehalose in a 12-week intervention study on oxidative stress levels. However, the fact that the effect of trehalose reducing ROS and mitochondrial superoxide can even be seen in some young healthy subjects makes it very promising. Trehalose probably has a greater effect in elderly, because oxidative stress is increased and autophagy impaired in arterial ageing 18.

Although measurements of arterial stiffness show a tendency for trehalose to blunt increases in arterial stiffness when compared to the placebo (maltose) condition, differences were not significant. It is likely that the differences in carotid compliance and β-stiffness index would have reached statistical significance with a larger sample size.

In the study on arterial stiffness we used maltose as placebo and filler in the low dose group. Although no effects of maltose on vasculature have been previously described, it can be argued that maltose has adverse effects on arterial stiffness. Both data from carotid artery compliance and β-stiffness index indicate that arterial stiffness increases in subjects taking 100g maltose daily over a 12-week period. However, others have shown a similar trend in their control group during a 12-week intervention, indicating that this trend can be explained by normal ageing 28. Moreover, the increase in arterial stiffness observed in our subjects is less than can be expected when compared to a data from Hansen et al. 29. Therefore, it is more likely the observed increase in arterial stiffness is due to normal ageing rather than an adverse effect of maltose.

In conclusion, trehalose decreases oxidative stress in human endothelial cells with elevated ROS and may blunt increases in arterial stiffness. Therefore, trehalose is a potential drug in treatment of CVD. However, data concerning the effect of trehalose on arterial stiffness in elderly are preliminary and more research is needed to further elucidate its effect.

Acknowledgements

We would like to thank Gerwen Lammers and Anna van Boekel for their assistance.

References

1. Global status report on noncommunicable disaeses 2010. Geneva: World Health Organization, 2011.
2. Roger VL, Go AS, Lloyd-Jones DM, et al. Heart disease and stroke statistics--2012 update: a report from the American Heart Association. Circulation; 125(1): e2-e220.
3. Lakatta EG, Levy D. Arterial and cardiac aging: major shareholders in cardiovascular disease enterprises: Part I: aging arteries: a "set up" for vascular disease. Circulation 2003; 107(1): 139-46.
4. Yeboah J, Crouse JR, Hsu FC, Burke GL, Herrington DM. Brachial flow-mediated dilation predicts incident cardiovascular events in older adults: the Cardiovascular Health Study. Circulation 2007; 115(18): 2390-7.
5. Yeboah J, Folsom AR, Burke GL, et al. Predictive value of brachial flow-mediated dilation for incident cardiovascular events in a population-based study: the multi-ethnic study of atherosclerosis. Circulation 2009; 120(6): 502-9.
6. Anderson TJ, Charbonneau F, Title LM, et al. Microvascular function predicts cardiovascular events in primary prevention: long-term results from the Firefighters and Their Endothelium (FATE) study. Circulation; 123(2): 163-9.
7. Seals DR, Jablonski KL, Donato AJ. Aging and vascular endothelial function in humans. Clin Sci (Lond); 120(9): 357-75.
8. Ungvari Z, Kaley G, de Cabo R, Sonntag WE, Csiszar A. Mechanisms of vascular aging: new perspectives. J Gerontol A Biol Sci Med Sci; 65(10): 1028-41.
9. Kregel KC, Zhang HJ. An integrated view of oxidative stress in aging: basic mechanisms, functional effects, and pathological considerations. Am J Physiol Regul Integr Comp Physiol 2007; 292(1): R18-36.
10. Galluzzi L, Kepp O, Trojel-Hansen C, Kroemer G. Mitochondrial control of cellular life, stress, and death. Circ Res; 111(9): 1198-207.
11. Landmesser U, Dikalov S, Price SR, et al. Oxidation of tetrahydrobiopterin leads to uncoupling of endothelial cell nitric oxide synthase in hypertension. J Clin Invest 2003; 111(8): 1201-9.
12. Griendling KK, FitzGerald GA. Oxidative stress and cardiovascular injury: Part I: basic mechanisms and in vivo monitoring of ROS. Circulation 2003; 108(16): 1912-6.
13. Sarkar S, Davies JE, Huang Z, Tunnacliffe A, Rubinsztein DC. Trehalose, a novel mTOR-independent autophagy enhancer, accelerates the clearance of mutant huntingtin and alpha-synuclein. J Biol Chem 2007; 282(8): 5641-52.
14. Richards AB, Krakowka S, Dexter LB, et al. Trehalose: a review of properties, history of use and human tolerance, and results of multiple safety studies. Food Chem Toxicol 2002; 40(7): 871-98.
15. Arai C, Arai N, Mizote A, et al. Trehalose prevents adipocyte hypertrophy and mitigates insulin resistance. Nutr Res; 30(12): 840-8.
16. Honda Y, Tanaka M, Honda S. Trehalose extends longevity in the nematode Caenorhabditis elegans. Aging Cell; 9(4): 558-69.
17. Rodriguez-Navarro JA, Rodriguez L, Casarejos MJ, et al. Trehalose ameliorates dopaminergic and tau pathology in parkin deleted/tau overexpressing mice through autophagy activation. Neurobiol Dis; 39(3): 423-38.
18. LaRocca TJ, Henson GD, Thorburn A, Sindler AL, Pierce GL, Seals DR. Translational evidence that impaired autophagy contributes to arterial ageing. J Physiol; 590(Pt 14): 3305-16.
19. Armentano R, Megnien JL, Simon A, Bellenfant F, Barra J, Levenson J. Effects of hypertension on viscoelasticity of carotid and femoral arteries in humans. Hypertension 1995; 26(1): 48-54.
20. Hirai T, Sasayama S, Kawasaki T, Yagi S. Stiffness of systemic arteries in patients with myocardial infarction. A noninvasive method to predict severity of coronary atherosclerosis. Circulation 1989; 80(1): 78-86.
21. Xie Y, You SJ, Zhang YL, et al. Protective role of autophagy in AGE-induced early injury of human vascular endothelial cells. Mol Med Rep; 4(3): 459-64.
22. Kubli DA, Gustafsson AB. Mitochondria and mitophagy: the yin and yang of cell death control. Circ Res; 111(9): 1208-21.
23. Iwai-Kanai E, Yuan H, Huang C, et al. A method to measure cardiac autophagic flux in vivo. Autophagy 2008; 4(3): 322-9.
24. Dalton TP, Shertzer HG, Puga A. Regulation of gene expression by reactive oxygen. Annu Rev Pharmacol Toxicol 1999; 39: 67-101.
25. Iwahashi H, Obuchi K, Fujii S, Komatsu Y. The correlative evidence suggesting that trehalose stabilizes membrane structure in the yeast Saccharomyces cerevisiae. Cell Mol Biol (Noisy-le-grand) 1995; 41(6): 763-9.
26. Duvall WL. Endothelial dysfunction and antioxidants. Mt Sinai J Med 2005; 72(2): 71-80.
27. Graham D, Huynh NN, Hamilton CA, et al. Mitochondria-

targeted antioxidant MitoQ10 improves endothelial function and attenuates cardiac hypertrophy. Hypertension 2009; 54(2): 322-8.
28. Yoshizawa M, Maeda S, Miyaki A, et al. Effect of 12 weeks of moderate-intensity resistance training on arterial stiffness: a randomised controlled trial in women aged 32-59 years. Br J Sports Med 2009; 43(8): 615-8.
29. Hansen F, Mangell P, Sonesson B, Lanne T. Diameter and compliance in the human common carotid artery--variations with age and sex. Ultrasound Med Biol 1995; 21(1): 1-9.

Tables

Table 1: Subjects characteristics

	Placebo	Low dose (50g/d)	High dose (100g/d)
Age, years	64 ± 2	65 ± 9	65 ± 9
Men/Women, n	0 / 3	1 / 1	3 / 2
Body mass, kg	57 ± 11	78 ± 11	77 ± 18
BMI, kg/m^2	22 ± 3	28 ± 2	26 ± 2
Waist:hip ratio	0.77 ± 0.06	0.91 ± 0.09	0.88 ± 0.10
VO_2 peak, ml/kg/min	28 ± 5	26 ± 2	28 ± 6
Brachial SBP, mmHg	133 ± 13	124 ± 21	128 ± 14
Brachial DBP, mmHg	75 ± 8	76 ± 10	71 ± 10
Total Cholesterol, mg/dL	217 ± 34	152 ± 1	184 ± 41
HDL, mg/dL	66 ± 12	49 ± 15	51 ± 25
LDL, mg/dL	133 ± 21	88 ± 11	114 ± 22
Triglycerides, mg/dL	90 ± 9	37 ± 42	99 ± 24
Glucose, mg/dL	91 ± 8	89 ± 6	89 ± 4
Insulin, mU/mL	8 ± 1	11 ± 6	10 ± 3

BMI=body mass index, SBP=systolic blood pressure, DBP=diastolic blood pressure, HDL=high-density lipoprotein, LDL=low-density protein

Figures

Figure 1: Proposed scheme of pathways contributing to vascular dysfunction. Ageing endothelial cells (ECs) show increased levels of oxidative stress characterized by increased superoxide production. Superoxide (O2-) reduces nitric oxide (NO) bioavailability by reacting with NO to form peroxynitrite(ONOO-). Moreover, ONOO- oxidizes BH4 causing a reduction in its availability which leads to uncoupling of endothelial nitric oxide synthase (eNOS), causing eNOS to produces less NO. Reduced NO bioavailability leads to vascular dysfunction. Trehalose may lower oxidative stress in ECs and thereby reverse vascular dysfunction.

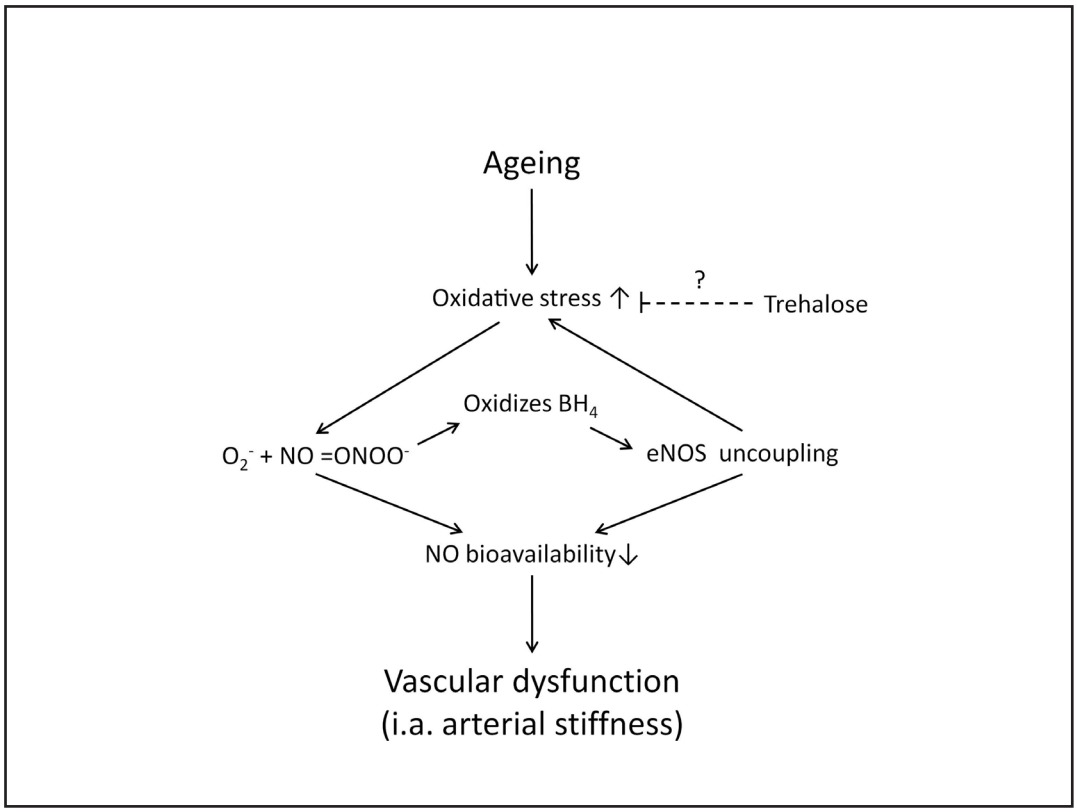


Figure 2: Schematic overview of study design. All subjects underwent screening after obtaining written consent. If inclusion criteria were met, measurements of arterial stiffness were conducted before and after a 12-week intervention of placebo, low dose or high dose study drinks.

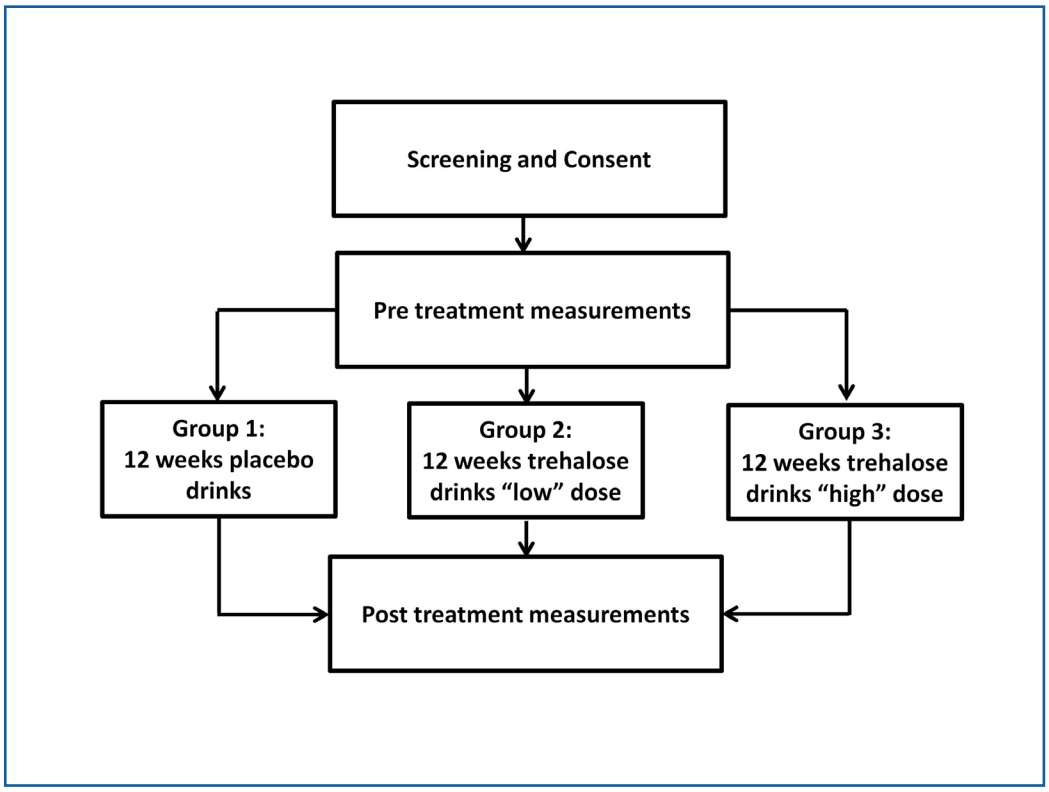


Figure 3: Schematic overview of study design. All subjects underwent screening after obtaining written consent. If inclusion criteria were met, measurements of arterial stiffness were conducted before and after a 12-week intervention of placebo, low dose or high dose study drinks.

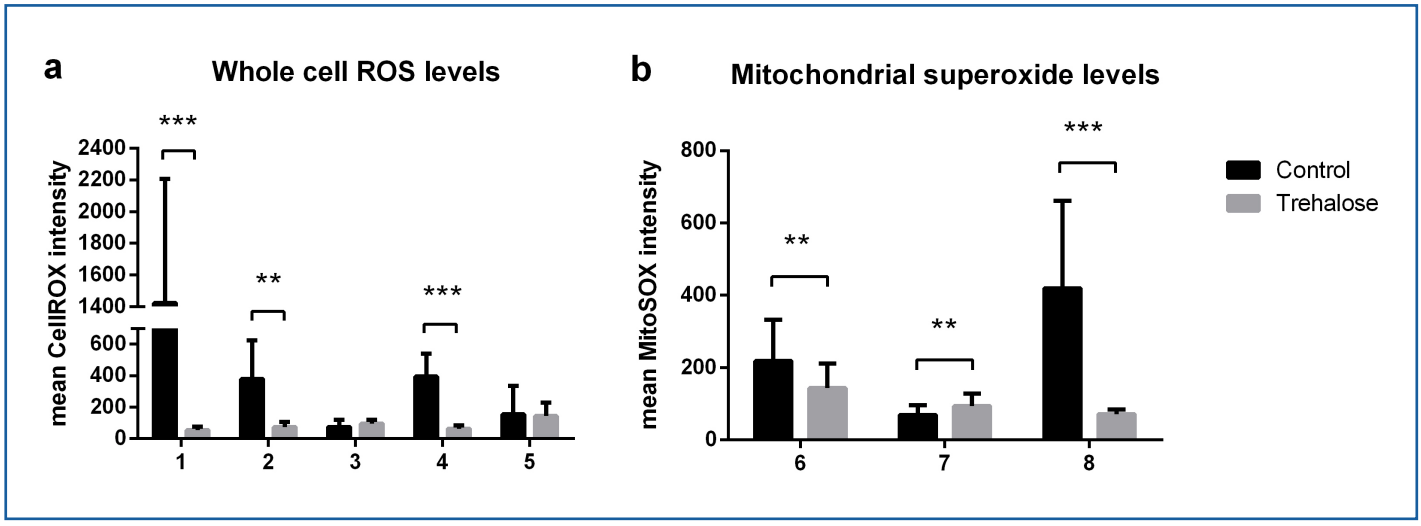
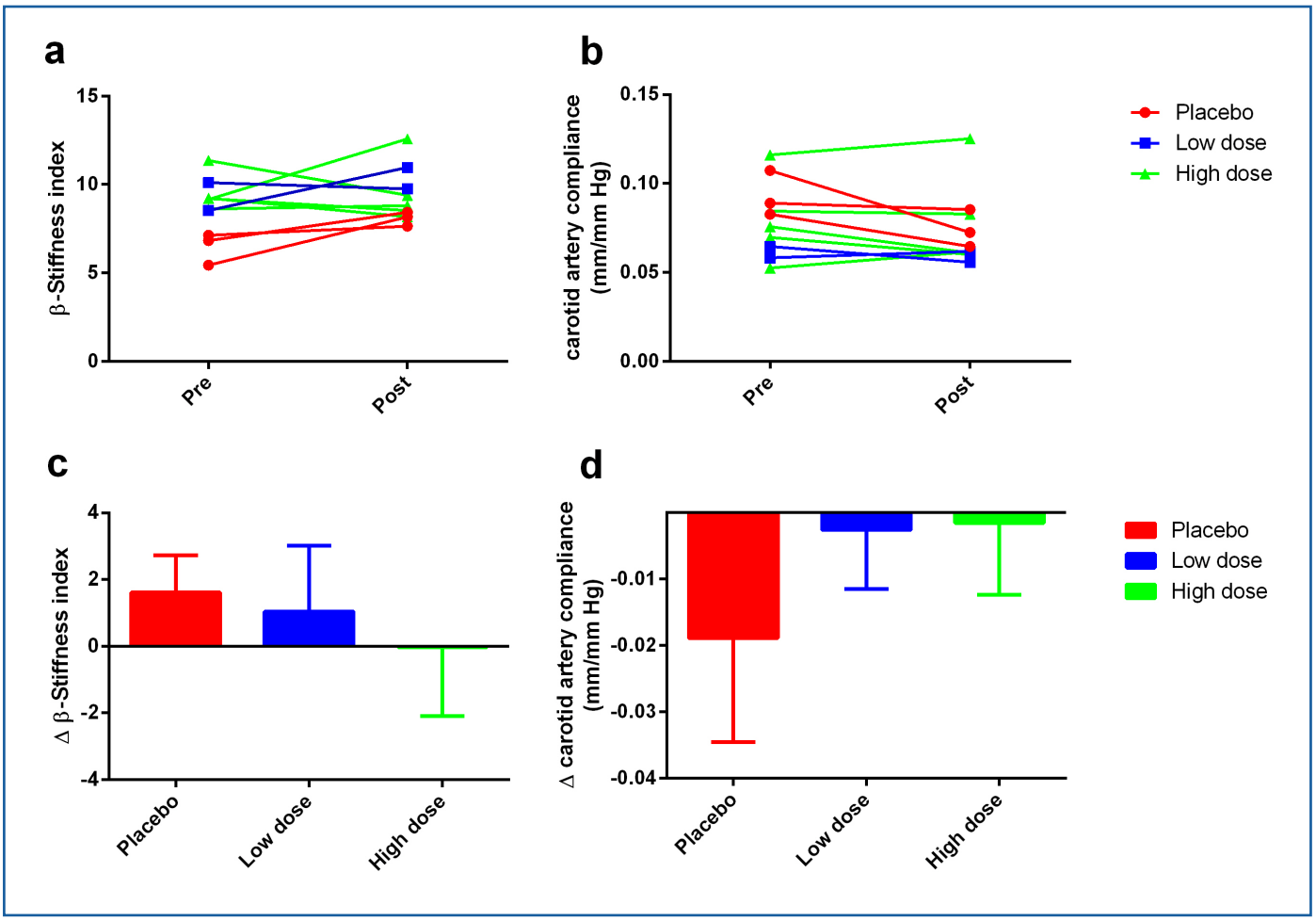


Figure 4: β-stiffness index and carotid artery compliance after a 12-week intervention with placebo (100g maltose), low dose (50g maltose/50 g trehalose) or high dose (100 g trehalose) drinks daily. (a) Individual data on β-stiffness index (b) Individual data on carotid artery compliance (c) Mean differences (post-pre) of β-stiffness index (d) Mean differences (post-pre) of carotid artery compliance.



RAMS
Radboud Annals of Medical Students

The Evaluation of Risk Assessment Tools in Patients with Atrial Fibrillation to Prevent Stroke

Laurens FM Verscheijden (1), Jasper MJ Coolen (1), Jelle Evers (1) and Corne GF Hendriks (1)

(1) Student RadboudUMC

Corresponding Author: Jelle Evers (je.evers@ru.nl)

ABSTRACT:

THE EVALUATION OF RISK ASSESSMENT TOOLS TO PREVENT STROKE BY AF

BACKGROUND: Many clinical risk assessment tools are used to assess the risk of stroke in patients with atrial fibrillation (AF). Using a clinical risk assessment tool with low efficacy leads to avoidable healthcare costs. Several studies have investigated the efficacy of such tools. To date, there is still no review in which most commonly used tools are evaluated systematically for the broadest scope of patients. To ensure a high standard of clinical care to all patients, the best clinical risk assessment tool should be determined. This review aims to contribute to this determination and thus contribute to new guidelines.
OBJECTIVE: The goal of the study was to choose the most efficacious risk assessment tool of those most commonly used in clinical practice: Framingham, SPAF, CHADS2, CHA2DS2-VASc and R2CHADS2 for risk evaluation of stroke in people with AF.
METHODS: Pubmed was searched with the aim to find articles reporting on quality analysis of clinical risk assessment tools. Relevant articles were given critical appraisal . With meta-analysis c-statistics were pooled for each tool. The weighted means were subsequently subjected to t-tests, in which two random tools were compared, to obtain p-values. The p-values were then evaluated on level of significance.
RESULTS: received critical appraisal. Results from 8 articles were included in the meta-analysis. The analysis resulted in pooled c-statistics of 0,665 for CHADS2, 0,652 for CHA2DS2-VASc, 0,659 for SPAF and 0,618 for Framingham. The lowest p-value was 0.22, when comparing Framingham with CHA2DS2-VASc, and the highest was 0.95, when comparing CHADS2 with SPAF.
CONCLUSION: From the resulting p-values we cannot conclude a significant difference between the efficacy of the different clinical risk assessment tools.

WHAT IS KNOWN: Several clinical risk assessment tools are in use to assess the various risk factors for stroke in patients with AF. By means of classification preventive measures can be implemented when a certain risk of stroke is present. Several studies have evaluated the efficacy of clinical risk assessment tools.

WHAT IS NEW: This review compares the most commonly used assessment tools that predict the risk of stroke for the broadest scope of patients with AF. After pooling data from all identified articles, no significant difference was found. This review has thus not clarified which

KEYWORDS: Atrial Fibrillation, Stroke Risk, Classification Systems tool has the largest predictive value.

Introduction

Patients with atrial fibrillation (AF) have a high risk of developing an ischemic cardiovascular accident: stroke. Approximately 35 percent of patients with AF will develop stroke (1). AF is a condition in which the atria contract fast and incoherently. These contractions may result in arterial embolisms which in turn can block the cerebral arteries (2,3). Several risk factors can contribute to classify, and thereby assess, the risk for stroke in patients with AF. By implementing this clinical risk assessment, preventive measures such as administering anticoagulants, can be taken in patients with a given risk for stroke. In patients with a low risk for stroke, preventive administration of anticoagulants is unfavourable and therefore undesired. The risk of complications turns the balance over the preventive effect in these low risk patients. Adequate classification and subsequent risk assessment is therefore imperative (4).

Several clinical risk assessment tools are in use. The most commonly used tools are: Framingham, Stroke Prevention in Atrial Fibrilation (SPAF), CHADS2 [cardiac failure, hypertension, age >74, diabetes, stroke/TIA/thromboembolism (double)], CHA2DS2-VASc [cardic failure, hypertension, age >74 (double), diabetes, stroke/TIA/thromboembolism (double), vascular disease, age 65-74, sex-category (female)] en R2CHAD2 [Renal failure (double), cardiac failure, hypertension, age >74, diabetes, stroke/TIA/thromboembolism (double)] (table 1). CHA2DS2-VASc and R2CHAD2 are the successors of CHADS2, and are designed to assess patients more adequately for their risk for stroke. All clinical assessment tools are easy to use and inexpensive, according to the authors of this review. However, using a clinical risk assessment tool with low efficacy leads to avoidable healthcare costs. These avoidable costs emerge from both patients who develop adverse effects of preventive coagulants, while they should not be treated according to their genuine risk, as well as patients who will get a stroke and are not treated with preventive coagulants, while they should be according to their genuine risk.

Several studies evaluated the efficacy of each of the clinical risk assessment tools mentioned above, however no review is known in which these most commonly used systems are compared for the broadest scope of patients. To contribute to a high standard of clinical care for patients with AF, the most efficacious clinical assessment tool

should be chosen. This review aims to contribute to this search and thus contribute to new guidelines.

Methods

Pubmed was searched with a predetermined search and selection strategy. First, relevant synonyms were formulated for the determinant (clinical risk assessment tools), the domain (patients with AF) and the outcome (stroke). The synonyms were combined in the search strategy (table 2). Secondly, the 490 studies found were screened on title and abstract in accordance with our inclusion criteria. Studies were included when the study population consisted of AF patients and patient were not a member of a specific subpopulation, when c-statistics and one of the clinical risk assessment tools were mentioned. The study had to be focussed on the evaluation of the clinical risk assessment tools. After this selection, 15 relevant articles were included. Thirdly, the full text of these articles were screened. Three articles were subsequently excluded based on the following exclusion criteria: review, no full text or English version available (figure 1).

After this procedure, 12 articles remained which were evaluated individually by two researchers. The articles were evaluated for quality with the aid of a checklist designed by the researchers. This checklist evaluated relevance and validity of the patient selection, the time interval between risk assessment and the diagnosis of stroke, and validity of both the methods used and the results. The complete checklist is available online (5). The checklist was based on QUADAS-2, Offringa et al. (18) and information obtained from a senior investigator (Prof. Rovers, Radboudumc). Differences resulting from this evaluation were discussed together with a third researcher. The result of the critical appraisal is stated in a risk of bias summary. This summary shows in which topics the included studies systematically deviated. In the summary of findings table, the scale of the study population, the c-statistics with according 95% CI, and quality score are stated for each study..

Meta-analysis of the c-statistics was performed with StatsDirect and discussed with a statistician. Only the eight studies which provided 95% CI were included in the meta-analysis. Analysis consists of pooling the c-statistics of each individual risk assessment tool. When pooling the c-statistic data, it was assumed c-statistics were normally distributed. Studies were weighed based on the standard error of the mean, calculated using the 95% CI of the provided c-statistic. A random effects model was used to compare the different risk assessment scores. This model takes both difference between individuals within a study and differences between studies into account. The 95% CI resulting from different studies belonging to the same classification system have minimal overlap, which points to heterogeneity between different studies. Therefore, a random effects-model is more suitable than a fixed effects-model.

To determine whether or not a significant difference was present in the predicted values between two random clinical risk assessment tools, an independent sample t-test was performed. Executing a test in which all of the systems were compared at once would deliver a hard-to-interpret outcome. On top of that, this test would take much longer to execute properly and it would lead to the same outcome and conclusions as the simple-to-use t-tests. Therefore, this test was not performed.

Results

The risk of bias summary summarises the results from the critical appraisal and shows different levels of validity amongst included articles (figure 2). In each study the population was well documented. Selection of patients however, was of more variable quality. In seven studies bias could have resulted from this selection. In one study this could not be determined. In five studies results were gathered in one medical centre or results came from a single country only. Studies that were included in the analysis showed great differences in time interval between clinical risk assessment and stroke as well. Little or no information was available about patients that were lost to follow-up in eleven studies. Clinical risk assessment was impartial and often clear definitions for certain risk factors were stated. Stroke itself is a hard and dichotomous outcome. A clear definition for stroke was stated by the investigators before the studies were performed. In most studies MRI images were evaluated by blinded experts.

Outcomes of each study are shown as a c-statistic. Included studies had no results for the clinical risk assessment tool R2CHADS2. Four out of twelve studies did not report 95% CI's apart from the c-statistics. These studies were not included in the meta-analysis. This resulted in only two studies for SPAF and Framingham included in the meta-analysis. For CHADS2 there were seven included studies and for CHA2DS2-VASc six. C-statistics are close to 0,65 and most 95% CI's do not contain 0,50 (table 3). Different studies give comparable results for the respective clinical assessment tool they focussed on. Only the study of Gage BF et al. show outliners.
In the meta-analysis the following pooled c-statistics were found per clinical risk assessment tool. For CHADS2 a pooled c-statistic of 0,66 (95% CI; 0,59-0,74) for Framingham a pooled c-statistic of 0,62 (95% CI; 0,59-0,65), for CHA2DS2-VASc a pooled c-statistic of 0,65 (95% CI; 0,61-0,70) and SPAF a pooled c-statistic of 0,66 (95% CI; 0,48-0,83) (figure 3).
Comparing the c-statistics stated above the following p-values were calculated. P-value was lowest when comparing Framingham and CHA2DS2-VASc; 0.22. Comparing Framingham and CHA2DS2-VASc; 0.22, comparing Framingham and CHADS2; 0.26, comparing Framingham and SPAF; 0.65, comparing CHA2DS2-VASc and CHADS2; 0.77, comparing CHA2DS2-VASc and SPAF; 0.94 and comparing CHADS2 and SPAF it was 0.95.

Discussion

This review aims to contribute to the guidelines of preventative measures taken to reduce the incidence of stroke. These measures are aimed at the entire population of patients with AF. To accomplish this aim, the most efficacious clinical assessment tool should be pointed out.

By excluding subgroups within populations when selecting studies, a large measure of heterogeneity was prevented. The resulting selection does, however, include populations from different continents. Since a conclusion applicable on the broadest scope of patients was desired, no limitation was made regarding this distribution. Another risk for heterogeneity are different time intervals between classification of clinical risk assessments and confirmation of stroke diagnosis. In three studies included in the meta-analysis, the timespan of this time interval was scored unsatisfactory on the checklist used for critical appraisal. Possibly this led to confounding in the meta-analysis.

Further heterogeneity between studies was present because some studies investigated only patients treated with anticoagulants. Other studies only looked at patients without anticoagulants and there were some studies which investigated both patients with and without anticoagulants. Although clinical assessment tools are designed for patients not currently receiving anticoagulants, it was difficult to include studies with this population. These studies are not frequently executed because of the ethical objections since patients assessed with a high risk should not have been withheld preventive, anticoagulant medication.

Coppens M. et al. have selected patients with a score of 1 for CHADS2. This studies gives a lower c-statistic for CHA2DS2-VASc than other studies. Although this study focuses on a subpopulation, it wasincluded in the meta-analysis, which may have led to confounding. Because of insufficient homogeneity between studies, a meta-analysis with a random effects model was performed(see Methods section). Only studies that mentioned a 95% CI were included in the meta-analysis.

Taken into account that the aim of the study was to give an overview of the clinically most frequently used risk assessment tools, we included all of these tools in our search strategy. After careful selection of articles we concluded no studies were available evaluating the quality of R2CHADS2. The most probable explanation for this would be that R2CHADS2 is a relatively new clinical assessment tool and consequently no studies have yet been performed that met our inclusion criteria.

The outcome used to perform the meta-analysis was the c-statistic. Studies without c-statistic were excluded for this purpose (see Methods section). Since different guidelines are used in various parts of the world, anticoagulant therapy is not started according to the same cut off points. In our review, we should have calculated sensitivity and specificity for every score. Not every study reported sensitivity and specificity for every score. Therefore we chose c-statistics as outcome measure. This measure can be discriminative for each cut off value. Studies reporting on the quality of the clinical assessment tools, but did not report a c-statistic are, however, excluded by this choice.

In our study a risk of bias arises because studies were included that include patients either with or without anticoagulant therapy or included both groups. Studies with a study population receiving anticoagulant therapy will have relatively fewer strokes, given the therapeutic effect of this intervention. If this reduction of strokes occurs predominantly in patients assessed as being high risk patients, this will result in an underestimation of the actual predictive value of the clinical assessment tool used. Differences in anticoagulant use thus lead to confounding of the results that are depicted in the summary of findings table. This is an imperfection of our study.

In our review only studies published on Pubmed were included. Because of practical considerations we had to choose a more specific domain, a more specific outcome, fewer determinants or the use of Pubmed alone. Given the aim of this review we could not suffice exclude some clinical assessment tools or use a more specific domain or outcome. This would compromise the external validity and relevance of this review.

Baruch L. et al. and Lip GY. Frison L. et al. appeared to have used the same dataset (SPORTIF 3). The same patients with atrial fibrillation were included in these studies. The outcomes for the same tools are, however, different. Baruch L. et al. state for CHADS2 a c-statistic of 0.665 and for Framingham 0.64. Lip GY. et al. state a c-statistic for CHADS2 of 0.637 and for Framingham 0.621 respectively. These differences are most likely to be explained due to differences in the length of follow-up. The study of Baruch L. et al. is not included in the meta-analysis because no 95% CI were reported.

Since stroke is a commonly occurring event in patients with AF and no test with a clearly proven superior predictive value, the different clinical assessment tools are all being used in current clinical practice. The reasonably low predictive qualities of the tools are compensated by starting preventive interventions with anticoagulants when only two points are scored on the respective scales (4). The cut-off value is thus being kept low. Therefore a high sensitivity can be calculated. The specificity is reasonably low in that case, because many patients that would never have developed a stroke are treated. These patients are, however, at risk for adverse effects of the preventive interventions.

Conclusion

It could not be clarified which clinical risk assessment tool has the largest predictive value and is, therefore, the most efficacious. No significant difference was found among the individual tools regarding the predictive value for stroke in patients with AF. All tools have a mediocre score. C-statistics were approximately 0,65 for all studies included, indicating the clinical assessment tools have a somewhat superior predictive value over no assessment at all.

The 95% CI show some overlap (figure 3). The Framingham assessment tool appears to deviate most from CHA2DS2-VASc with a p-value of 0.22. Framingham can, based on this information alone, not be advised against since the evidence for such a statement did not show a significant p-value.

Furthermore, CHA2DS2-VASc is a successor of the CHADS2, with the intention to assess patients better for their risk of developing a stroke. However, this does not result from the performed t-test given the p-value of 0,77.

For Framingham and SPAF more relevant and well documented research should be performed. In the meta-analysis of this review only two studies concerning the Framingham and SPAF tools could be included. More studies addressing these clinical assessment tools could possibly prove the inferiority of this tools over the others discussed here.

To decide on the superiority of one tool over the other, more research needs to be performed. Since R2CHADS2 could not be evaluated, for reasons discussed above, no statement concerning all, most frequently used clinical assessment tool can be made. To draw a conclusion on the quality of R2CAHDS2 compared to that of those clinical assessment tools discussed in this review more comparative research should be done into R2CHADS2. The authors of this review stress the need for this research since only then a valid choice for either of these assessment tools can be made.

Given the low c-statistics values for every risk assessment tool, we encourage new investigations to be performed aiming to develop a new, superior, clinical risk assessment tool.

Acknowledgment

We would like to thank Janneke Grutters, investigator at the department of Health Evidence at Radboudumc, for counseling and supporting during the research and writing the review. We would also like to thank Hans Groenewoud, statistician at Radboudumc, for his help with the statistics. Finally we would like to thank Sander Groen in 't Woud and Rick Verstegen, editors of RAMS, for translating our Dutch article.

References

1. StopAfib. Onderdeel van American Foundation for Women's Health; http://www.stopafib.org/stroke.cfm
2. Wolf PA, Abbot RD, Kannel WB, et al. Atrial firbrillation as an independent risk factor for stroke: the Framinham study. Stroke. 1991;22:983-988; http://stroke.ahajournals.org/content/22/8/983.short
3. National Institute of Neurological Disorders and Stroke http://www.ninds.nih.gov/disorders/atrial_fibrillation_and_stroke/atrial_fibrillation_and_stroke.htm
4. Guidelines for the management of atrial fibrillation geschreven door European society of cardiology http://www.escardio.org/guidelines-surveys/esc-guidelines/guidelinesdocuments/guidelines-afib-ft.pdf
5. Evers J, Verscheijden LFM, Coolen JMJ, Hendriks CGF. Critical Appraisal Checklist Prognostic studies. 2014 https://www.dropbox.com/s/rydicv5x5wmjfe7/Checklist.doc
6. Lip GY, Frison L, Halperin JL, Lane DA. Identifying patients at high risk for stroke despite anticoagulation: a comparison of contemporary stroke risk stratification schemes in an anticoagulated atrial fibrillation cohort. Stroke; a journal of cerebral circulation. 2010;41(12):2731-8. Epub 2010/10/23.
7. Lip GY, Nieuwlaat R, Pisters R, Lane DA, Crijns HJ. Refining clinical risk stratification for predicting stroke and thromboembolism in atrial fibrillation using a novel risk factor-based approach: the euro heart survey on atrial fibrillation. Chest. 2010;137(2):263-72. Epub 2009/09/19.
8. Poli D, Lip GY, Antonucci E, Grifoni E, Lane D. Stroke risk stratification in a "real-world" elderly anticoagulated atrial fibrillation population. Journal of cardiovascular electrophysiology. 2011;22(1):25-30. Epub 2010/07/27.
9. Wang TJ, Massaro JM, Levy D, Vasan RS, Wolf PA, D'Agostino RB, et al. A risk score for predicting stroke or death in individuals with new-onset atrial fibrillation in of the American Medical Association. 2003;290(8):1049-56. Epub 2003/08/28.
10. Gage BF, Waterman AD, Shannon W, Boechler M, Rich MW, Radford MJ. Validation of clinical classification schemes for predicting stroke: results from the National Registry of Atrial Fibrillation. JAMA : the journal of the American Medical Association. 2001;285(22):2864-70. Epub 2001/06/13.
11. Guo Y, Apostolakis S, Blann AD, Wang H, Zhao X, Zhang Y, et al. Validation of contemporary stroke and bleeding risk stratification scores in non-anticoagulated Chinese patients with atrial fibrillation. Int J Cardiol. 2013;168(2):904-9. Epub 2012/11/22.
12. Li SY, Zhao XQ, Wang CX, Liu LP, Liu GF, Wang YL, et al. One-year clinical prediction in Chinese ischemic stroke patients using the CHADS2 and CHA2DS2-VASc scores: the China National Stroke Registry. CNS Neurosci Ther. 2012;18(12):988-93. Epub 2012/11/06.
13. Zuo ML, Liu S, Chan KH, Lau KK, Chong BH, Lam KF, et al. The CHADS2 and CHA 2DS 2-VASc scores predict new occurrence of atrial fibrillation and ischemic stroke. J Interv Card Electrophysiol. 2013;37(1):47-54. Epub 2013/02/08.
14. Baruch L, Gage BF, Horrow J, Juul-Moller S, Labovitz A, Persson M, et al. Can patients at elevated risk of stroke treated with anticoagulants be further risk stratified? Stroke; a journal of cerebral circulation. 2007;38(9):2459-63. Epub 2007/08/04.
15. Cha MJ, Lee HS, Kim YD, Nam HS, Heo JH. The association between asymptomatic coronary artery disease and CHADS2 and CHA2 DS2 -VASc scores in patients with stroke. European journal of neurology : the official journal of the European Federation of Neurological Societies. 2013;20(9):1256-63. Epub 2013/04/09.
16. Coppens M, Eikelboom JW, Hart RG, Yusuf S, Lip GY, Dorian P, et al. The CHA2DS2-VASc score identifies those patients with atrial fibrillation and a CHADS2 score of 1 who are unlikely to benefit from oral anticoagulant therapy. Eur Heart J. 2013;34(3):170-6. Epub 2012/09/29.
17. Fang MC, Go AS, Chang Y, Borowsky L, Pomernacki NK, Singer DE. Comparison of risk stratification schemes to predict thromboembolism in people with nonvalvular atrial fibrillation. Journal of the American College of Cardiology. 2008;51(8):810-5. Epub 2008/02/26.
18. Offringa M, Scholten RJPM, Assendelft WJJ. Inleiding evidence-based medicine. Bohn Stafleu van Loghum

Critical appraisal: composed checklist

Patients:
Has the patients population mentioned clear? (2)
Could the selection of patients create bias? (1)
Do the patients deviate from our domain? (1,2)

Time interval:
Could the time interval between classification of the risk and determine an eventual stroke created bias? i.e. was the interval long enough? (1,2)

Method:
Could the type of research created bias? (3)
Is the classification objective and blinded? (2)

Results:
Are the results imprecise? (2)
Has selective loss of results occured? (3)

(1) QUADAS-2
(2) Offringa et al.
(3) Lecture Critical Appraisal Prof. Maroeska Rovers

In articles about diagnostic devises/checklists, often sensitivity and specificity is used. However, this is only possible when the outcome of the test is binary (e.g. sick v.s. not sick). For diagnostic assessments with a continuous outcome an equivalent of the sensitivity and specificity is the c-statistic. In case of the c-statistic, for every possible cut-off value in the continuous outcome, the sensitivity and specificity is calculated. These values are then stated into a diagram where sensitivity is plotted against 1-specificity. When a line is drawn between the points, the area under this curve can be calculated. This is named the c-statistic which has a value between 0,5-1. This means, 0,5: there is no predictive value of the diagnostic device used(e.g. tossing a coin). 1: the diagnostic device predicts the health status perfectly.

Tables

Table 1: Risk factors by each risk assessment tools

Risk factors	risk assessment tools				
	$CHADS_2$	CHA_2DS_2-VASc	R_2CHADS_2	Framingham	SPAF
Hypertension	✓	✓	✓	✓ x0-4 a)	✓
Age between 65 and 75	×	✓	×	✓ x0-10 b)	×
Age above 74	✓	✓ x2	✓	✓ x0-10 b)	✓ c)
Female gender	×	✓	×	✓ x6	✓ c)
Clinical history of heart failure	✓	✓	✓	×	✓
Clinical history of diabetes	✓	✓	✓	✓ x4	×
Clinical history of stroke/TIA/ thromboembolism	✓ x2	✓ x2	✓ x2	✓ x6	✓
Clinical history of vascular disease	×	✓	×	×	×
Clinical history of renal failure	×	×	✓ x2	×	×

a) Framingham score 0 (<120); 1 (120-139); 2 (140-159); 3 (160-179); 4 (>180) for systolic blood pressure (mmHg) b) Framingham score 0 (<60); 1 (60-62); 2 (63-66); 3 (67-71); 4 (72-74); 5 (75-77); 6 (78-81); 7 (82-85); 8 (86-90); 9 (91-93); 10 (>93) for ages (years) c) SPAF combines age >74 and female gender as one risk factor.

Table 2: Brachial artery characteristics and arterial stiffness parameters

Pubmed hits	490 (date: 03/17/2014)
Determinant	risk classification system[Title/Abstract] OR chads2[Title/Abstract] OR SPAF[Title/Abstract] OR cha2ds2 vasc[Title/Abstract] OR r2chads2[Title/Abstract] OR risk classification[Title/Abstract] OR Framingham[Title/Abstract]
Domain	atrial fibrillation[Title/Abstract] OR "Atrial Fibrillation"[Mesh] OR af[Title/Abstract] OR a-fib[Title/Abstract]
Outcome	"Stroke"[Mesh] OR stroke[Title/Abstract) OR cerebrovascular accident[Title/Abstract] OR cva[Title/Abstract] OR cerebrovascular insult[Title/Abstract] OR cvi[Title/Abstract] OR brain attack[Title/Abstract]
Total	((risk classification system[Title/Abstract] OR chads2[Title/Abstract] OR SPAF[Title/Abstract] OR cha2ds2 vasc[Title/Abstract] OR r2chads2[Title/Abstract] OR risk classification[Title/Abstract] OR Framingham[Title/Abstract]) AND ("Stroke"[Mesh] OR stroke[Title/Abstract) OR cerebrovascular accident[Title/Abstract] OR cva[Title/Abstract] OR cerebrovascular insult[Title/Abstract] OR cvi[Title/Abstract] OR brain attack[Title/Abstract]) AND (atrial fibrillation[Title/Abstract] OR "Atrial Fibrillation"[Mesh] OR af[Title/Abstract] OR a-fib[Title/Abstract]))

Table 3: summary of findings-table

Study	*Patients*	*risk assessment tools*	*c-statistic (95%-CI)*	*Quality (GRADE)*	*Anticoagulants*
[6] Lip GY, Frison L	N = 7329	$CHADS_2$ Framingham CHA_2DS_2-VASc	0,637 (0.607-0.674) 0.621 (0.589-0.658) 0.647 (0.613-0.678)	++	Yes
[7] Lip GY, Nieuwlaat R	N = 1084 from the Euro Heart Survey	$CHADS_2$ Framingham SPAF	0.549 (0.435-0.662) 0.586 (0.477-0.695) 0.561 (0.450-0.672)	++	Yes
[8] Poli D, Lip GY	N = 662 'elderly' from Italy	$CHADS_2$ CHA_2DS_2-VASc	0.524 (0.435-0.614) 0.683 (0.606-0.759)	++	Yes
[9] Wang TJ, Massaro JM	N = 868 from the Framingham cohort	$CHADS_2$ SPAF Framingham	0.62 0.62 0.66	+	No
[10] Gage BF, Waterman AD	N = 1733 from seven states of the USA (California, Connecticut, Louisiana, Maine, Missouri, New Hampshire and Vermont)	SPAF $CHADS_2$	0,74 (0,71-0,76) 0,82 (0,80-0,84)	+	No
[11] Guo Y, Apostolakis S	N = 1034 chinese people from the PLA general hospital, Beijing	$CHADS_2$ CHA_2DS_2-VASc	0,72 (0,64-0,81) 0,58 (0,50-0,67)	-	No
[12] Li SY, Zhao XQ	N = 1297 from the Chinese National Prospective Registry	$CHADS_2$ CHA_2DS_2-VASc	0,551 0,532	-	No
[13] Zuo ML, Liu S	N = 528 from the Queen Mary Hospital, Hong Kong	$CHADS_2$ CHA_2DS_2-VASc	0,69 (0,65-0,73) 0,69 (0,65-0,73)	+	Yes
[14] Baruch, L, Gage BF	N = 7329	Framingham $CHADS_2$ SPAF	0.64 0.65 0.61	+	Yes
[15] Cha, MJ, Lee HS	N = 1733 From a Korean cohort	$CHADS_2$ CHA_2DS_2-VASc	0.668 (0.641-0.696) 0.710 (0.683-0.737)	--	No
[16] Coppens M, Eikelboom JW	N = 4670	CHA_2DS_2-VASc	0.587 (0.550–0.624)	+	Yes
[17] Fang, MC, Go AS	N = 13559	SPAF $CHADS_2$ Framingham	0.60 0.58 0.62	++	Yes

Figures

Figure 1: process of searching and selection studies for inclusion

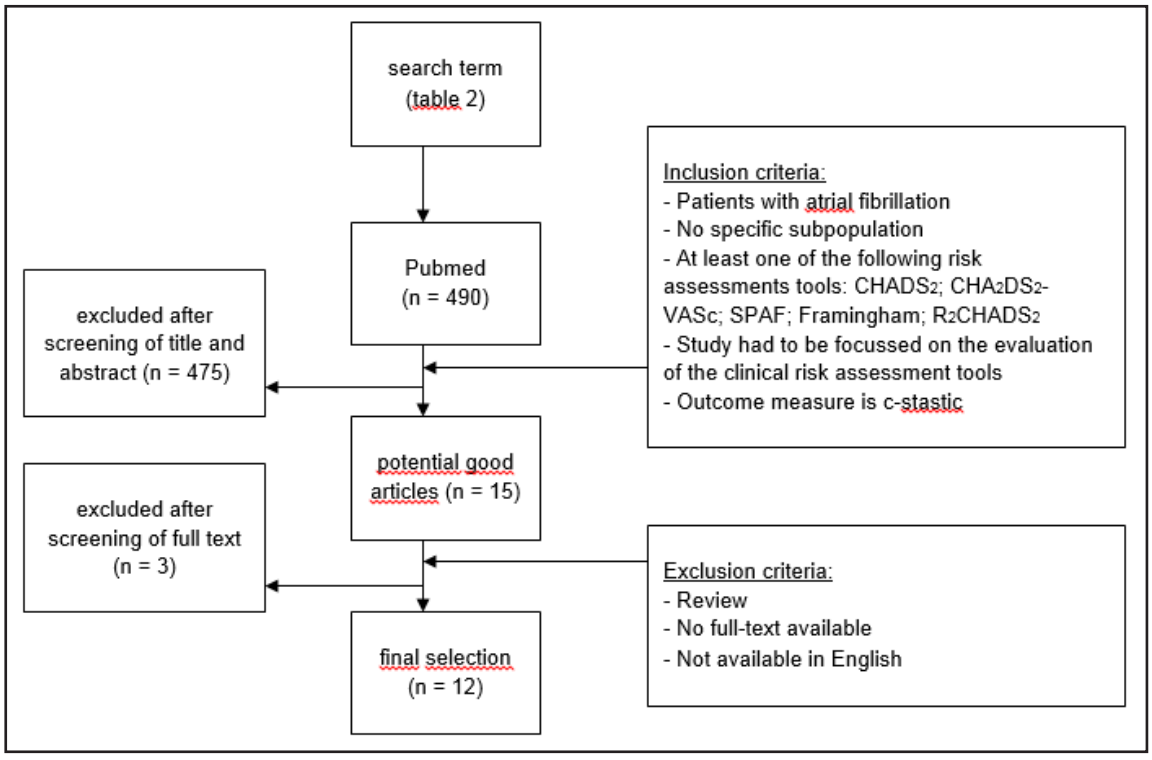


Figure 2: Risk of bias summary

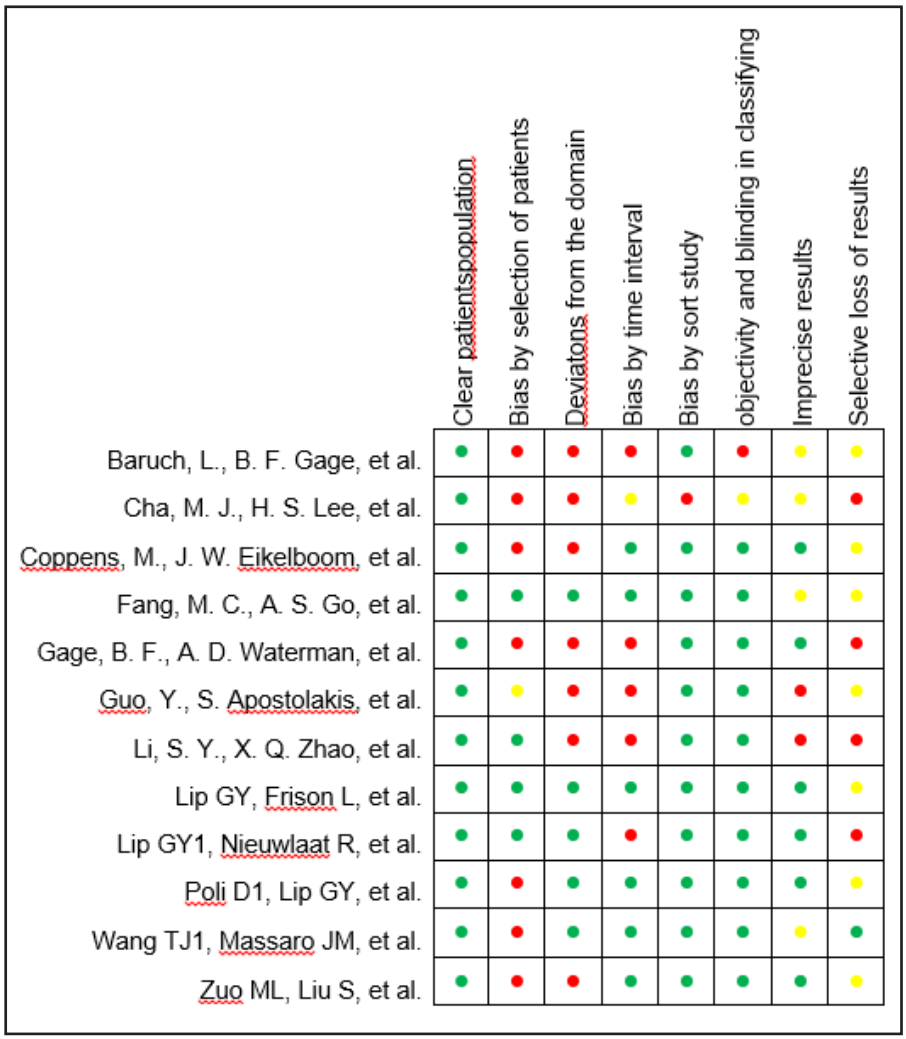
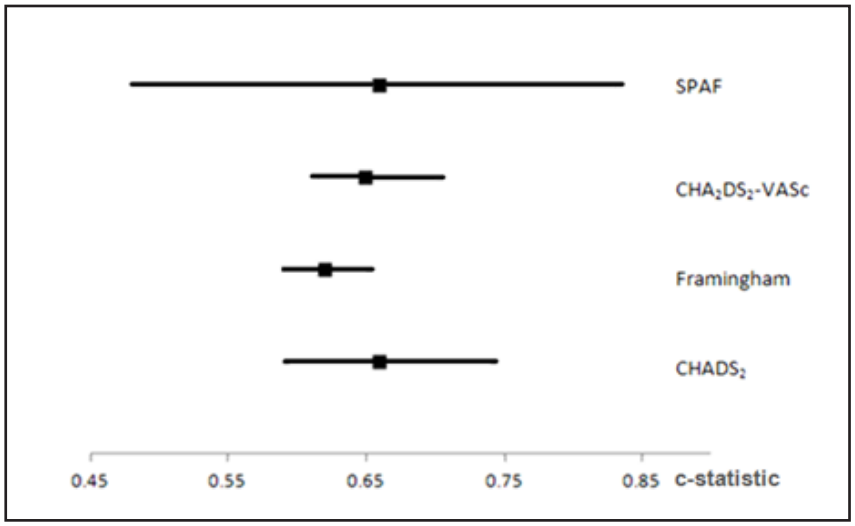


Figure 3: results of the meta-analysis



We are looking for:

Case reports

RAMS is a journal for all (bio)medical students in Nijmegen. Not all students have resources to conduct large scale research. Case reports can be a useful way to publish in this medical journal, since RAMS does not focus on research papers only. The main goal of a case report is to share a valuable clinical lesson, which is perfectly in line with our aim.

For a case report, students are asked to describe symptoms, signs, diagnosis, treatment and follow up of an individual patient. Students are free to choose their own topic. You do not have to discover your own Elephant Man or an entirely new syndrome, you can focus on unexpected associations between diseases or symptoms or unexpected events in the course of observing or treating a patient. Or findings that shed new light on the possible pathogenesis of a disease or an adverse effect, unique or rare features of a disease, unique therapeutic approaches or a positional or quantitative variation of the anatomical structures. Therefore, the topic of a case report does not have to be a rare disease or a rare complication. Consider writing a case report about more common diagnostic or therapeutic challenges that other students might face some day during one of their internships. After all, the most important criteria are that your report is educational and entertaining.

Take permission of your supervisor and an informed consent of the patient into consideration. More detailed information on how to submit a case report can be found at our website. Submissions can be sent to submit.rams@ru.nl.

RAMS

Radboud Annals of Medical Students

www.ramsresearch.nl