Cyclic variation of the common carotid artery structure in relation to prior atherosclerotic burden and physical activity

Inauguraldissertation

zur
Erlangung der Würde eines Dr. sc. med.
vorgelegt der
Medizinischen Fakultät
der Universität Basel

von
Seraina Caviezel
aus Siat, Kanton Graubünden, Schweiz
Basel, 2015

Genehmigt von der Medizinischen Fakultät

auf Antrag von

Fakultätsverantwortlicher	Prof. Dr. med. Arno Schmidt-Trucksäss
Dissertationsleitung	Prof. Dr. med. Arno Schmidt-Trucksäss
Korreferent	Prof. Dr. med. Nino Künzli
Externer Experte	Prof. Dr. med. Giuseppe Schillaci

Basel, den 23.03.2015

Dekan

Prof. Dr. med. Christoph Beglinger

Table of contents

Figures and Tables

FIGURES

TABLES

Acknowledgments

First of all, it is my considerable pleasure to express my deepest gratitude to everyone who contributed to the development and finalisation of this dissertation.

I am very grateful to my supervisor and member of the PhD committee Prof. Dr. med. Arno Schmidt-Trucksäss who gave me the opportunity to work on my PhD thesis at the Department of Sport, Exercise and Health. We had numerous lively discussions resulting in a pleasant collaboration. You gave me room for an individual scientific development and concurrently, you supported me whenever I needed your expertise despite your tight schedule. I really appreciated your support and your trust in me throughout the whole PhD study time.

Prof. Dr. med Arno Schmidt-Trucksäss was not only my supervisor; he was also a member of the PhD committee with Prof. Dr. med. Nino Künzli. Our annual PhD meetings regarding the progress report were very constructive, enabled my process and provided me an optimal working environment. Thank you very much.

I am especially thankful to the members of the SAPALDIA CIMT Working Group for the extensive assistance and feedbacks of all PhD related research projects in many different ways. Your inputs were highly valued: Dr. Julia Dratva, Simon Endes, Dr. Eric de Groot, Prof. Dr. med Nino Künzli, Prof. Dr. Nicole Probst-Hensch, Emmanuel Schaffner, PD Dr. Christian Schindler and Prof. Dr. med. Arno-Schmidt-Trucksäss

A smoothly data transfer with subsequent analysis would not have been possible without the expertise of the biostatistics and computational science team. I wish to express special thanks to Dr. Manfred Baumstark, Emmanuel Schaffner, Michael Tarantino, Dirk Keidel, Prof. Dr. Alexandra Teynor, and PD Dr. Christian Schindler.

Overall, this present PhD thesis was undertaken within the framework of the Department of Sport, Exercise and Health at the University of Basel in cooperation with the Swiss Tropical and Public Health Institute in Basel and the Swiss Cohort Study on Air Pollution and Lung and Heart Diseases In Adults (SAPALDIA). This study could not have been done without the help of the SAPALDIA participants and the whole medical teams, field workers and SAPALDIA researchers. SAPALDIA was supported by the Swiss National Science Foundations (grants no 33CSCO-134276/1, 33CSCO-108796, 3247BO-104283, 3247BO-104288, 3247BO-104284, 3247-065896, 3100-059302, 3200-052720, 3200-042532, 4026-028099, PMPDP3_129021/1, PMPDP3_141671/1), the Federal Office for Forest, Environment and Landscape, the Federal

Office of Public Health, the Federal Office of Roads and Transport, the canton's government of Aargau, Basel-Stadt, Basel-Land, Geneva, Luzern, Ticino, Valais, and Zürich, the Swiss Lung League, the canton's Lung League of Basel Stadt/ Basel Landschaft, Geneva, Ticino, Valais, Graubünden and Zurich, Stiftung ehemals Bündner Heilstätten, SUVA, Freiwillige Akademische Gesellschaft, UBS Wealth Foundation, Talecris Biotherapeutics GmbH, Abbott Diagnostics, European Commission 018996 (GABRIEL), Wellcome Trust WT 084703MA.

During the first two years of my PhD, I had the chance to meet the members of the Reading Center Team namely Livia Grüter, Claudia Mang, Gil Meyer, Phillip Pauli, Yves Schaffner, Mirjam Schneider, Renate Wyss, Dominique Würth and Felix Zimmermann. We analysed together thousands upon thousands of single ultrasound images and were in close collaboration with Prof. Dr. Alexandra Teynor who developed the analysis program. We appreciated the fast improvement in terms of new features and usability of the program. I am sure we will never forget the fancy colours of our analysing system. A very grateful thank goes to PD Dr. Henner Hanssen who trained the SAPALDIA sonographer which was highly important for the quality assurance. I would say out of a regular working relationship of SAPALDIA Reading Center members established a lasting friendship. Thank you very much for this wonderful time and I'm looking forward to see you soon, at latest at the next "Basler Herbstmesse".

Many thanks go to all the former and present colleagues at the Department of Sport, Exercise and Health. Amongst others: My heartfelt thanks go to Dr. Ursina Meyer, Dr. Iris Bänteli-Niederer and Dr. Flavia Bürgi for sharing your PhD experience with me. Furthermore, I would like to thank Monique Nussbaumer, Stefanie Müller, Simon Endes, Yanlei Li, Claudia Mang and Dr. Juliane Schäfer for the various critical discussions in all stages of my PhD. I appreciated it very much that you were completely honest in work- and personal-related questions.

During my PhD I had the opportunity to enhance my statistical and methodological skills by visiting educational courses and presenting our data at different conferences. This would not have been possible without the good organisations and financial support of the Swiss School of Public Health (SSPH+). Thanks to Dr. Sina Henrichs and Prof. Dr. med. Charlotte Braun-Fahrländer who organised and managed this high level academic training program in public health research. Moreover, I gratefully acknowledge the additional financial support for conferences from the "Reisefond für den akademischen Nachwuchs der Universität Basel".

I am also very grateful to Monique Nussbaumer, Anja Rossmeissel, Sofie Hansen, Dr. Inmaculada Aguilera and Dr. Juliane Schäfer for the at least partial proofreading of this dissertation.

Last but not least, my deepest thanks go to my family, my best friends and especially to Simon Fry for their untiring dedication, enormous patience and motivation at all stages of my life.

Summary

Background and aims

Cardiovascular disease (CVD) accounts for the most deaths of non-communicable diseases worldwide. It begins with structural and functional changes of the arterial system commonly known as the atherosclerotic process, starting asymptomatically in early childhood, adapting arterial structure and function with advancing age depending on genetic and environmental exposures and finally resulting in CVD events such as myocardial infarction or stroke. CVD risk prediction today is generally based on risk scores, but substantial disadvantages occur since they account only for specific risk factors at one time point. Carotid structure and function (also called carotid stiffness) parameters measured by ultrasound may overcome this disadvantage, since they can provide information on structural and elastic carotid properties and reflect therefore vascular damage accumulated over time.

Thus, the aims of this thesis were to summarise the state of the arte of ultrasound measurements, to validate the new developed ultrasound analysis system, to assess the variability and reproducibility within the study sample and to investigate the long- and short-term associations of cardiovascular risk factors and carotid stiffness with main focus on physical activity in elderly participants of the SAPALDIA cohort.

Methods

The SAPALDIA cohort study is an ongoing multicentre study with a population-based random sample of adults from eight rural and urban areas started in 1991 (SAPALDIA 1), with a first follow-up in 2001-2003 (SAPALDIA 2) and a second follow-up in 2010-2011 (SAPALDIA 3). In SAPALDIA 3, sequential B-mode ultrasound images of the common carotid artery were examined in 3489 participants (51% women) aged between 50-81 years at the time of examination. Expert readers analysed these ultrasound images with a new analysis system called DYARA (DYnamic ARtery Analysis) according to the state of the art assessed in the review. Thereof, carotid structure parameters were measured and carotid stiffness indices were derived considering blood pressure at time of ultrasound assessment. Validation of the ultrasound analysis program DYARA and reproducibility of carotid parameters were performed in subgroup within the SAPALDIA 3 survey. The presented studies within this thesis comprise cardiovascular risk factor data from the first and second follow-up and therefore, long- and short-term associations with carotid stiffness could be investigated.

Results

The intra- and inter-reader results of the validation study were highly consistent with slightly higher bias for analyses with manual interactions compared to the automatic detection. Among the carotid structure parameters, average values across heart cycle showed lower variability than single images in diastole and systole, whereby the relative difference was smaller in lumen diameter values compared to the carotid intima media thickness (CIMT). Based on different statistical approaches, reproducibility values within SAPALDIA 3 were consistently good to excellent for carotid structure and function indices. Findings additionally revealed that subjects itself were the greatest source of variability between two measurements.

Multivariate regression analyses suggested that most single cardiovascular risk factors in SAPALDIA 2 were long-termly associated with increased carotid stiffness in SAPALDIA 3 except physical activity and high-density lipoprotein cholesterol (HDL-C). HDL-C was the only protective vascular determinant and no relation was observed for physical activity. Most carotid stiffness parameters were similar strong associated within each cardiovascular risk factor (except compliance showed main deviances among several risk factors). Estimating sex-specific associations of atherosclerotic risk factors and carotid stiffness indicated that increased heart rate was more strongly associated with stiffer arteries across all carotid stiffness parameters in men than in women. Low-density lipoprotein cholesterol (LDL-C) was significantly associated with carotid stiffness only in men and triglyceride only in women.

Multifactorial pathway analyses of cardiovascular risk factors in SAPALDIA 3 showed that age was the strongest predictor of carotid stiffness, followed by mean arterial blood pressure and heart rate. Age strongly confounded the association of physical activity and carotid stiffness in multiple regression analyses and therefore, only an univariate association of physical activity and carotid stiffness could be observed.

Conclusion

DYARA tackles the challenge of being able to analyse varying ultrasound image qualities with high precision. The high reproducibility and the feasible application in a large sample size suggest that this program can be recommended for epidemiological research, diagnostics and clinical practice. Long- and short-term cardiovascular exposures have added important information to the overall vascular damage assessed by carotid stiffness for both sexes. Although age was the strongest predictor, sex-differences in long-term associations may indicate a certain differentiated susceptibility to cardiovascular risk factors among men and women, which should be investigated in more detail.

The presented studies within this thesis provide an important basis towards future investigations targeting the early and late consequences of atherosclerosis, its progression and possible implementations of preventive and/or personalised interventions.

Common abbreviations

BMI	Body mass index
BP	Blood pressure
CCA	Common carotid artery
CI	Confidence interval
CIMT	Carotid intima media thickness
CRP	C-reactive protein
CV	Coefficient of variation
CVD	Cardiovascular disease
DAG	Directed acyclic graph
DYARA	DYnamic ARtery Analysis
HDL-C	High-density lipoprotein cholesterol
ICC	Intraclass correlation coefficient
LD	Lumen diameter
LDL-C	Low-density lipoprotein cholesterol
NO	Nitric oxide
PA	Physical activity
SAPALDIA	Swiss Cohort Study on Air Pollution and Lung and Heart Diseases in Adults
SD	Standard deviation

CHAPTER 1

Introduction

INTRODUCTION

Already Thomas Sydenham (1624-1689) – an English Physician and medical doctor – stated: "A man is as old as his arteries" [1]. Four centuries later, arterial research is still of scientific interest, not only due to more refined investigational techniques [2,3], but also due to an increased burden of cardiovascular disease (CVD) [4,5]. CVD is the general term for a systemic disease based on atherosclerotic processes starting asymptomatically in early childhood, adapting arterial structure and function with advancing age depending on genetic and environmental exposures and finally resulting in CVD events such as myocardial infarction or stroke [6,7]. Overall, CVD accounts for the most deaths of non-communicable diseases worldwide [4] and the prevalence increased especially in developing countries during the last few decades while in high-income countries a steady state could be maintained [5,4,7]. However, although primary and secondary prevention may have prolonged a life free of CVD or reduced CVD symptoms, life expectancy is assumed to increase resulting in a population with a higher proportion of elderly people and thus a higher CVD prevalence is expected [4,5,7].

As CVD reduction and cardiovascular health promotion remain a major public health issue [8], it is essential to gain further knowledge on vascular health, which may help to identify people in an early state of the vascular changing process [7]. Moreover, this might provide a solid basis for an early implementation of effective prevention strategies to reverse or at least postpone this process aiming to deliver significant cardiovascular health gains. Thus, this thesis focuses on classical cardiovascular risk factors and their relations to arterial structure and function of the common carotid artery (CCA) in elderly people free of CVD based on a new sophisticated and validated ultrasound analysis technique.

THE DISEASE OF THE ARTERIAL SYSTEM

'Arteriosclerosis' should not be confused with 'atherosclerosis', although the terms are often used interchangeably describing the disease of the arteries [1]. 'Arteriosclerosis' (also called 'senile arteriosclerosis') reflects the generalised stiffening and hardening progression of the arterial system with advancing age while 'atherosclerosis' (also called 'nodular arteriosclerosis') is a specific form of arteriosclerosis defined by a local degenerative process of the artery wall structure in medium to large sized arteries [1,9].

Anatomical background of large arteries

Large arteries are closely located to the heart (e.g. aorta or CCA) and consist of elastic properties to buffer the pulsating blood pressure (BP) from ventricular ejection fraction [1,9,10]. The arterial wall structure is composed of three main zones: the tunica intima, the tunica media and the tunica adventitia [10]. The intima is the inner layer of the artery consisting of a single stratum of vascular endothelium and the internal elastic lamina, which

separates histologically the intima from the media [10]. The media composes smooth muscle cells, elastin and collagen fibres and lies between the intimal and the adventitial boundary [10]. The adventitia is the outer layer of the artery consisting of fibroblasts and fibrocytes surrounded by connective tissue [10].

Basic mechanisms of vascular remodelling

When looking at the basic mechanisms of vascular remodelling, a differentiation between arteriosclerosis and atherosclerosis is needed. Arteriosclerosis is mainly a disease of the medial layer [1]. The degeneration of elastin fibres in the media structure due to tiring effects of cyclic stress is accompanied by an increase in collagenous material and a disorganisation of elastin patterns [10–12]. Overall, investigation of normal vascular ageing are challenging since it is difficult to distinguish between a normal ageing process and other affecting diseases such as atherosclerosis or hypertension [1]. Thus, studies investigating the arterial system are often based on assessments of atherosclerotic changes.

While arteriosclerosis is mainly a systemic disease, atherosclerotic wall alterations are predominantly locally placed and reflect the thickening of the intimal layer [10]. The underlying mechanism are based on accumulation of lipid, inflammatory cells and their waste products, calcium deposits and collagen fibres [10,13,14]. In addition, smooth muscle cells shift from the media to the intima and proliferate resulting in a narrowing of luminal area [10,13,14]. Within the arterial system, wall changes and plaque development are more likely located in arterial segments with low wall shear stress and turbulent blood flow such as the bifurcation of the CCA since the endothelium as first inner boundary is especially exposed to mechanical stress such as BP and other chemical signals [15–20]. As both low wall shear stress and turbulent blood flow affect endothelial cells and their function, this plays also a major role for vascular tone and lumen diameter (LD) regulation [1,6,19]. Intact endothelial cells modulate not only vascular homeostasis by realising vasoconstrictor (endothelin-1, angiotensin II) and vasodilator (nitric oxide (NO), prostacyclin) factors, it also protectively regulates inflammation, cellular and platelet adhesion and proliferation of smooth muscle cells [6,18,19]. If endothelial function is impaired (endothelial dysfunction) anti-atherosclerotic protection is diminished and bioavailability of NO is reduced based on a lower metabolism of endothelial NO synthase (eNOS) [6,18,19].

Overall, vascular remodelling of the intimal and medial layer affects the arterial structure and function [11]. Determination of these changes provides an insight into the cardiovascular system and might improve evaluation of CVD risk assessment [21–23].

ESTIMATION OF CARDIOVASCULAR DISEASE RISK

Understanding the impact of different risk factors on CVD burden is essential to assess the CVD risk [24]. This is even more important for the implementation of preventive treatment aiming to reduce the CVD risk. However, comparisons of general evidence based recommendations of CVD prevention and real applications in clinical practice indicate a persistent gap between the theory and the reality [25]. Risk prevention in daily clinical practice is usually based on treatment of established classical CVD risk factors implemented in middle old to older individuals at high risk [7]. However, the burden of untreated risk factors remained quite constant across decades [26]. For a long-term population strategy, a stricter and earlier treatment allocation of people even at low risk may delay CVD, improve quality of life and prevent CVD events [7,25]. Hence, identifying and classifying individuals at risk and apply the respective treatment may result in substantial population-based health benefit and is a main goal of CVD prevention, CVD risk prediction and also for promotion of cardiovascular health [7,8,27,28].

Classical cardiovascular risk factors

Estimation of CVD risk is commonly based on established risk scores such as Framingham, PROCAM, HEART or ASCVD based on specific combinations of classical CVD risk factors such as age, sex, dyslipidaemia, hypertension, diabetes mellitus and smoking [29–31]. Single CVD risk factors are acknowledged to be associated with CVD morbidity and mortality [32–37]. But it is also recognised that multiple risk factors are able to interact with each other and lead to an increased CVD risk compared to an optimal risk factor profile [26].

Overall, risk score systems are generally well accepted by clinicians and patients due to a cost-effective application and a relatively simple interpretation connected with an applied medication management of classical CVD risk factors [38]. However, risk scores have also substantial disadvantages since they account only for specific risk factors and disregard other important exposures or modifiers such as metabolic syndrome, lifestyle or novel CVD risk factors such as endothelial function [30]. Moreover, the unfavourable load of risk factors can change over time and since risk score systems estimate the risk only at one time point, it does not reflect risk factor burden longitudinally [39]. Vascular biomarkers may overcome this disadvantage since the overall structural and functional vascular changes reflect the accumulated arterial damage of a multifactorial lifetime exposure [31,39,40].

Vascular biomarkers

The terminologies of vascular biomarkers are partially confusing [41]. While structural parameters refer to the arterial anatomy, functional indices describe in general the elasticity or vice versa the stiffness of the arterial structure [23,41]. Arterial stiffness is defined as the reduced ability of the arterial structure to adapt to pressure changes [1], but also other

terms are interchangeably used for arterial stiffness such as distensibility or compliance [23,41]. Depending on the measured arterial segment, arterial stiffness indices give information about the systemic or local arterial stiffness [41].

Among non-invasively determined biomarkers of arterial stiffness, carotid-femoral pulse wave velocity is the gold-standard measurement of systemic arterial stiffness [2]. Systematic reviews and meta-analyses showed that pulse wave velocity is a strong predictor for CVD events and all-cause mortality and improves risk prediction and classification beyond established risk factors [42,43]. However, pulse wave velocity and other systemic measures (e.g. brachial ankle index) only extrapolate the vascular properties over a known distance of the arterial tree although elastic properties vary among different arterial segments [2,44]. Thus, local arterial assessments have the advantage to directly evaluate structural properties and their haemodynamic effects of a particular arterial section [2]. The cylindrical anatomy of the CCA, its size and the superficial location to the skin make high-resolution ultrasound observation of wall and diameter change upon adaptation to pulsating BP feasible [1].

Structural parameters of the common carotid artery

Several studies investigated the CVD risk prediction based on subclinical adaptions of the arterial wall structure and plaque occurrence mostly in the CCA assessed by non-invasive and widely available ultrasound [45–48]. Since ultrasound analyses are not able to measure the single layers, the thickness of the intima-media complex is assessed to measure the atherosclerotic change. Carotid intima-media thickness (CIMT) is defined as the distance between the leading edges of the lumen-intima and the media-adventitia interfaces of the far arterial wall and it is now an established subclinical biomarker for early atherosclerotic progression and prediction of the CVD risk [45–48]. Importantly to mention is, that an increased CIMT has to be distinguished from plaque formation since plaques are always of pathological nature while an increased CIMT addresses the preclinical atherosclerotic stage [49]. A CIMT above 1.5mm or a luminal narrowing of more than 50% from the surrounding tissue is defined as plaque [49]. Hence, for interpretation of preclinical atherosclerotic structure and function of the CCA, plaques were excluded in this thesis.

Besides CIMT, several authors observed an enlargement of the LD with increasing CIMT underlying the mechanism of compensating and preserving the lumen area [50–52]. The outer LD is defined as the distance between the interface of the media-adventitia of the near wall and the media-adventitia interface of the arterial far wall [1]. Although the relationship between both CIMT and LD is dependent, it has been shown that the presence of a higher CIMT and an equivalent enlargement of the LD more precisely classifies an atherosclerotic high-risk phenotype [53]. But also increased LD alone predicts independently CVD events and mortality risk [54,55]. Moreover, it is positively associated with increased BP, left ventricular hypertrophy based on mechanical respond mechanisms [56–58] and several other risk factors such as body mass index (BMI) or smoking [58–60]. As the absolute size of

LD is strongly dependent on body composition (age, sex, height) [52,61] and hemodynamic influences (BP, heart rate or stroke volume) [57], studies focused more on relative dilatations (change of systolic LD and diastolic LD) or dilatation curves (relative change in CIMT and LD across heart cycles in time) together with BP values representing the vascular function (also called functional parameters, carotid stiffness or local arterial stiffness) [62].

Functional parameters of the common carotid artery

While there is evidence based on several researches that carotid-femoral pulse wave velocity is an intermediate outcome and predictor of CVD events and mortality [42,43], carotid stiffness is less often examined and it is still under discussion [55,63–70]. In contrast to pulse wave velocity, some researchers are still struggling with the preconception that a high degree of technical expertise is needed to investigate carotid stiffness using ultrasound, even though structural parameters of the CCA are well established and functional parameters are based on structural indices [2]. In addition, the technical application developed tremendously fast in the last two decades resulting in qualitatively high-resolution ultrasound systems with increased image acquisition speed and integrated automatic image analyses programs, which are now widely available [71–77].

As a basis for high quality and comparability of research, the expert consensus of the general arterial stiffness covered already to some extent the methodological issues for local arterial assessments [2]. However, a specific guideline for carotid stiffness determination is needed. In summary, the most important methodological study criteria for ultrasound assessments [78] and other vascular devices [2] are: the description of the measured arterial segment; the standardisation of examinations, methods and devices; the report of measurement and data validation including description of validation study sample; the exact description of the used arterial stiffness parameter; the use of brachial or central BP for arterial stiffness calculation, the investigated subjects and the outcome or covariate of interest.

Returning to the estimation of CVD risk by carotid stiffness, some studies indicated an association of carotid stiffness and CVD events and/or mortality [63–66] while others did not [55,67–70]. Considering the above mentioned methodological study criterions, all predictability studies measured carotid stiffness near the bifurcation of the CCA and used standardised examination methods and reported the respective reproducibility of carotid stiffness parameters (strain [65–69], distensibility [55,63–70], compliance [55,64,66–69], beta-stiffness index [66–69], Peterson's elastic modulus [66,67], Young's elastic modulus [63,64,66–69], for a detailed description inclusive calculation of these parameters see also chapter 6). However, comparability of results is aggravated since some studies were based on small sample sizes [55,63,65], investigated specified subjects [55,63,65,67] and carotid stiffness parameters were based on brachial BP [55,63–68,70] or central pulse pressure measures [64,69].

Nevertheless, the most recently published study – investigating the predictability of carotid stiffness and CVD events in a population-based study sample – tried to overcome the mentioned concerns and addressed for the first time the issue of carotid stiffness based on different BP measures [64]. They reported independent associations of carotid stiffness and incidence of CVD events and all-cause mortality beyond traditional CVD risk factors and documented that carotid stiffness based on brachial BP (compared to central BP) is an accurate measure in an elderly population [64]. Thus, there is some indication that carotid stiffness might be a predictor of CVD events and mortality in the general population [64].

TRIGGERS OF VASCULAR HEALTH

Vascular remodelling in all sections of the arterial wall is a consequence of adaption due to mechanical- and biochemical-induced stress aiming to preserve a homeostasis (e.g. constant condition of blood supply) as long as possible [11]. Among the triggers of vascular changes, age plays a pivotal role herein [79]. On one hand, age is an element of the normal generalised age-dependent process within the arterial system (arteriosclerosis) [80]. On the other hand, age is determined as a CVD risk factor and reflects a certain possible exposure time to other risk factors [80]. There is clear evidence that carotid stiffness increases with age [11,39,61,62,80,81]. The whole concept of 'vascular ageing' is defined by vascular changes based on a 'normal vascular ageing' process and 'early vascular ageing' (EVA) [39,81]. EVA is the consequence of premature structural and functional changes in the arterial system due to an early exposition of risk factors [39,80,81].

Association of cardiovascular risk factors and carotid stiffness

The sources of EVA are multifactorial [7]. Beside inevitable expositions (such as genetic predisposition, ethnicity, age or sex), the effect of EVA is intensified by an unfavourable lifestyle behaviour and other modifiable risk factors [20,39,82]. The direct pathways of major cardiovascular risk factors and carotid stiffness are presented in figure 1.1. Blood pressure (systolic BP, diastolic BP, mean arterial pressure, hypertension) is a major direct mechanical stress on vascular structure and it is associated with increased carotid stiffness [69,83–89]. However, it is not yet clear whether hypertension or increased stiffness occurs first [85]. Besides BP, previous studies have shown that increased carotid stiffness was partially independently associated with heart rate [90,91], body composition (height, weight, body mass index, waist circumference, skinfold thickness, obesity or adiposity) [69,88,89,92–95], lipids and inflammatory markers (C-reactive protein (CRP), total cholesterol, triglyceride, low-density lipoprotein cholesterol (LDL-C) or low values of high-density lipoprotein cholesterol (HDL-C)) [69,87,89,94–97], diabetes mellitus (insulin, insulin resistance or plasma glucose) [87,94,98–101], smoking [89,94,102] and physical inactivity (or contrarily, physical activity (PA) was associated with reduced carotid stiffness) [103–111].

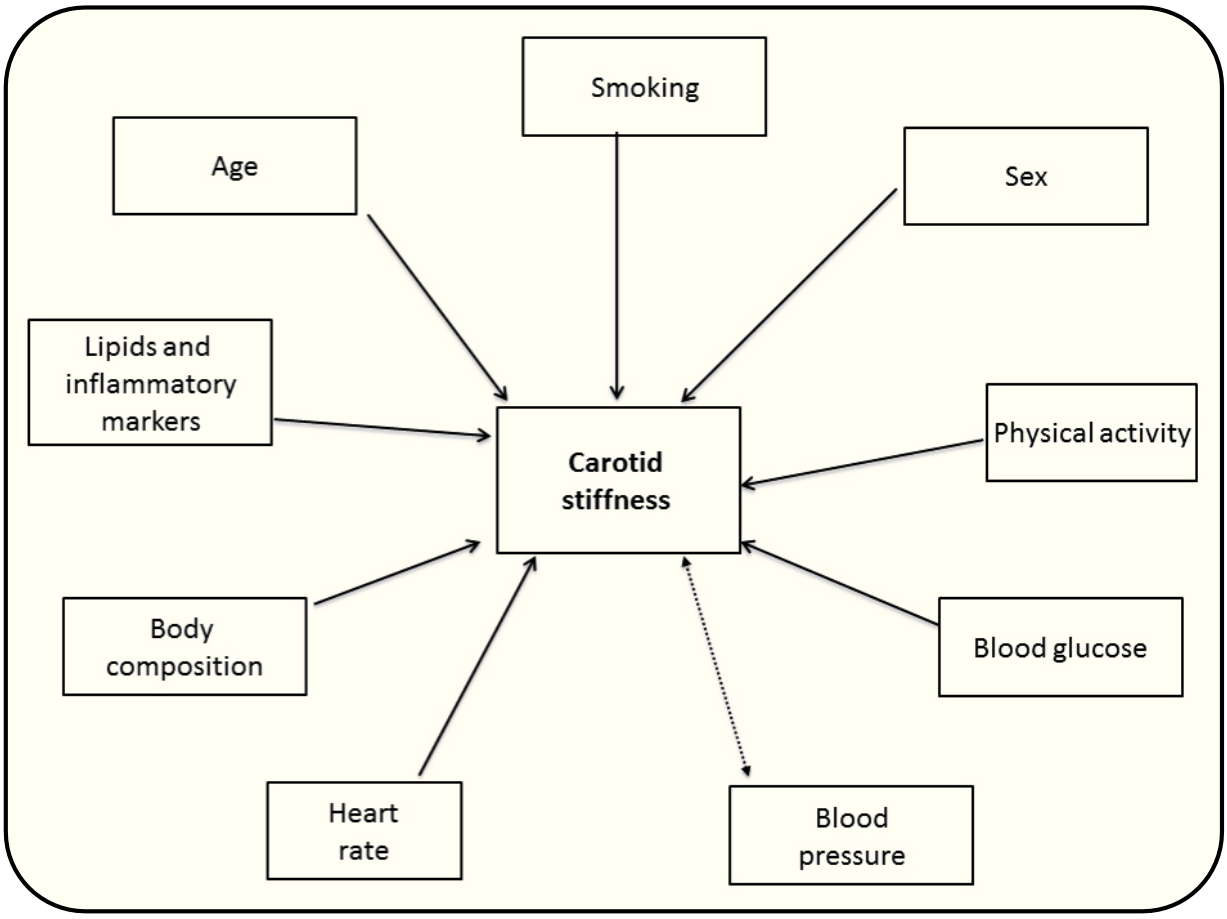

Figure 1.1: Direct pathways of major cardiovascular risk factors and carotid stiffness

Carotid stiffness increased with increasing number of cardiovascular risk factors [40]. A further familiar clustering of cardiovascular risk factors is known as the metabolic syndrome [112]. Metabolic syndrome is diagnosed if three or more of the following five risk factors are fulfilled: elevated waist circumference, triglycerides, BP, fasting glucose and reduced HDL-C [112]. Similar as before, individuals with diagnosed metabolic syndrome [89,95,100,101] and a cluster of at least three metabolic syndrome risk factors [95] were independently associated with stiffer carotid arteries. Although the prevalence of metabolic syndrome did not differ by sex [101], a significant increase of carotid stiffness with increasing number of metabolic syndrome risk factors was observed in women, but not in men [89] suggesting certain sex-specific differences in associations between risk factors and carotid stiffness. Indeed, former studies already reported sex- and age-related cardiovascular morbidity, mortality risk and vascular remodelling [61,113–116]. But so far, only little research has been devoted to the topic of sex-specific associations between different cardiovascular risk factors and carotid stiffness indices, which might be of importance for a better CVD risk stratification.

Risk factor pathways and carotid stiffness

The observed adverse effects in the carotid function based on the burden of cardiovascular risk factors seem to be dose dependent although the respective impact vary between risk factors [40,89,95]. This might suggest that increased carotid stiffness is a consequence of a complex interrelation between different risk factors on vascular remodelling mechanisms [95]. For a deeper understanding of the complex vascular relationships, it is therefore necessary to reflect the different pathways between the major risk factors of carotid stiffness (see figure 1.2).

BP is not only a strong independent carotid stiffness modifier; it acts also as an important mediator since BP is affected by various pathways (age, sex, body composition, heart rate, PA, smoking) [117–123]. In addition, similar known multifactorial influences exist also for heart rate [120–123], body composition [123–126], lipids and inflammatory markers [123,127–129], smoking [130,131] and blood glucose [114,132–134]. Depending on the research question, some of the mentioned risk factors are also confounders. Per definition, a confounder variable affects both, the dependent outcome variable (here carotid stiffness) and the independent exposure (carotid stiffness risk factor) [135]. Thus, major confounders visible in figure 1.1 and figure 1.2 are sex, age and PA, since they possibly affect both, carotid stiffness as well as single risk factors (please see also chapter 8).

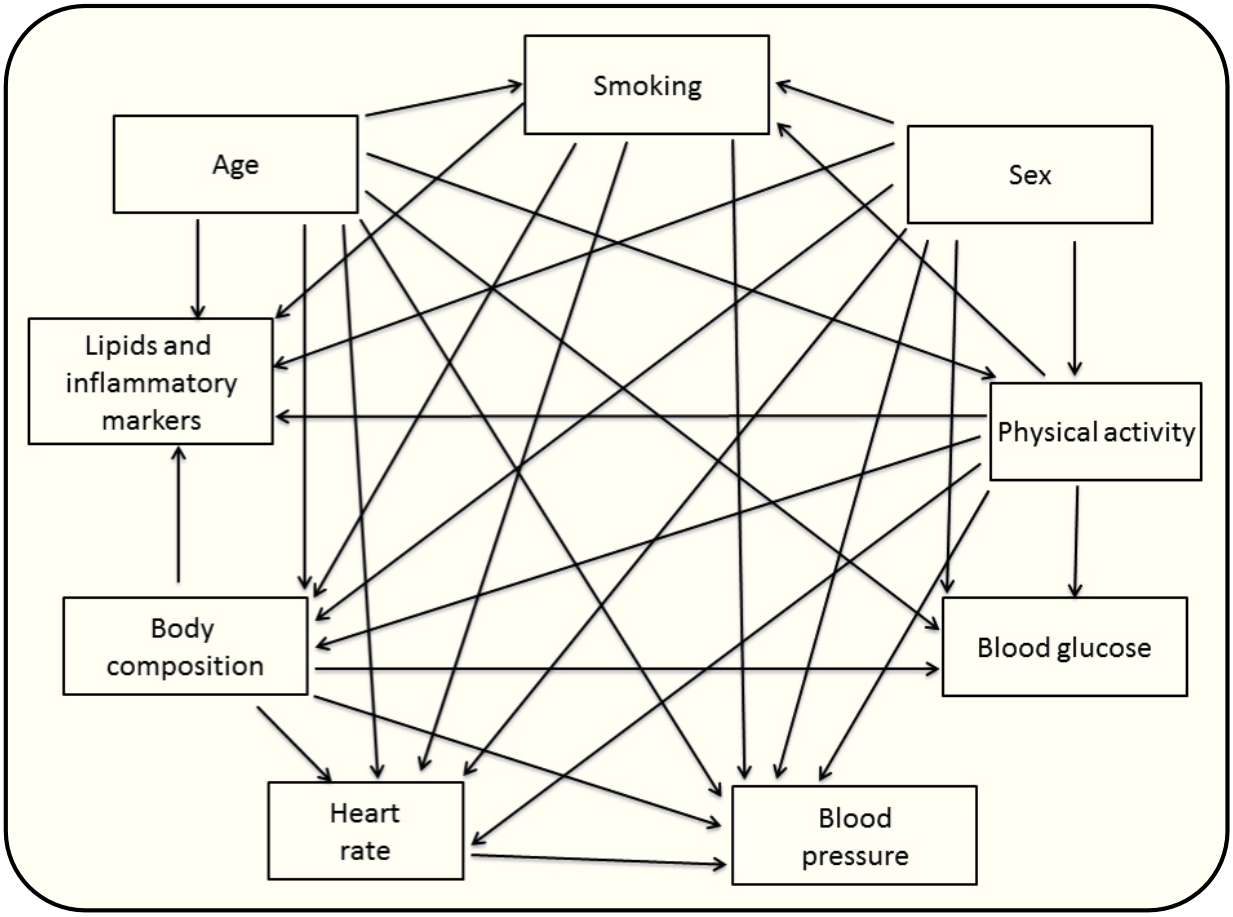

Figure 1.2: Main pathways between major carotid stiffness risk factors

Physical activity and the cardiovascular system

Inactivity or low physical activity PA are nowadays acknowledged cardiovascular risk factors which causes about 6-10% of the non-communicable diseases [136] and contributes majorly to the global burden of disease [24]. Avoiding inactive behaviour and being only 15 min per day physically active can significantly reduce all-cause mortality whereby the health gains were curvilinear [137]. This dose-response effect regarding the amount of PA has also been shown for CVD risk reduction with greatest health gains for those who were previously barely active [138]. Even stronger benefits were yielded after PA or exercise training with higher intensity [139]. Therefore, actual PA guidelines recommend a minimum amount of 150 min per week of moderate intensity or 75 min per week of vigorous PA or a combination of moderate and vigorous intensity (20 min of moderate intensity corresponds to 10 min of vigorous intensity) to improve health outcomes [140,141].

Physical activity plays a special role in the multifarious relationship between the different cardiovascular risk factors since it promotes evidence-based cardiovascular health benefits by several pathways (see figure 1.2). It has been shown that PA favourably modulates BP values, heart rate parameters, body composition, lipid profile and smoking behaviour [123,131,132]. Therefore, mechanisms through which risk factor may influence carotid stiffness are various and PA or exercise training has been discovered and promoted for primary and secondary CVD prevention [142].

Mostly smaller studies investigated the effect of PA on carotid stiffness specified groups considering highly trained versus untrained individuals [103–111]. Their results suggest a certain dose-dependent effect of PA on reduction of carotid stiffness [103–111]. However, the only study including a representative sample indicated only in a sub analysis a weak association between vigorous PA and carotid stiffness [143]. Thus, it is unclear whether these findings can be generalised. In addition, we noticed that divergent associations of different carotid stiffness parameters with different health outcomes within one study have been reported e.g. for CVD risk, mortality or PA [64,66–69,104,107]. It is assumed that all carotid stiffness parameters (strain, distensibility, compliance, beta-stiffness index, Petersons elastic modulus, Young's elastic modulus) yield the same prediction based on the shared pathophysiological mechanisms (e.g. BP and vascular diameter), but up to date, this has never been tested (please see also chapter 7).

Reference

1 Nichols, W. W., O'Rourke, M. F. and Vlachopoulos, C. (2011) Mcdonald's blood flow in arteries: theoretical, experimental and clinical principles 6th ed., Hodder Arnold, London. 1-755

2 Laurent, S., Cockcroft, J., Van Bortel, L., Boutouyrie, P., Giannattasio, C., Hayoz, D., Pannier, B., Vlachopoulos, C., Wilkinson, I. and Struijker-Boudier, H. (2006) Expert consensus document on arterial stiffness: methodological issues and clinical applications. Eur. Heart J. **27**, 2588–2605

3 Faxon, D. P., Creager, M. A., Smith, S. C., Jr, Pasternak, R. C., Olin, J. W., Bettmann, M. A., Criqui, M. H., Milani, R. V., Loscalzo, J., Kaufman, J. A., Jones, D. W. and Pearce, W. H. (2004) Atherosclerotic vascular disease conference: executive summary: atherosclerotic vascular disease conference proceeding for healthcare professionals from a special writing group of the American Heart Association. Circulation. **109**, 2595–2604

4 World Health Organization. (2008) The global burden of disease: 2004 update, World Health Organization, Geneva

5 World Health Organization. (2009) Global health risks: mortality and burden of disease attributable to selected major risks, World Health Organization, Geneva. 1-63

6 Deanfield, J. E., Halcox, J. P. and Rabelink, T. J. (2007) Endothelial function and dysfunction testing and clinical relevance. Circulation. **115**, 1285–1295

7 Perk, J., De Backer, G., Gohlke, H., Graham, I., Reiner, Z., Verschuren, M., Albus, C., Benlian, P., Boysen, G., Cifkova, R., Deaton, C., Ebrahim, S., Fisher, M., Germano, G., Hobbs, R., Hoes, A., Karadeniz, S., Mezzani, A., Prescott, E., Ryden, L., Scherer, M., Syvänne, M., Scholte op Reimer, W. J. M., Vrints, C., Wood, D., Zamorano, J. L. and Zannad, F. (2012) European guidelines on cardiovascular disease prevention in clinical practice (version 2012). The fifth joint task force of the European Society of Cardiology and other societies on cardiovascular disease prevention in clinical practice (constituted by representatives of nine societies and by invited experts). Developed with the special contribution of the European Association for Cardiovascular Prevention & Rehabilitation (EACPR). Eur. Heart J. **33**, 1635–1701

8 Lloyd-Jones, D. M., Hong, Y., Labarthe, D., Mozaffarian, D., Appel, L. J., Van Horn, L., Greenlund, K., Daniels, S., Nichol, G., Tomaselli, G. F., Arnett, D. K., Fonarow, G. C., Ho, P. M., Lauer, M. S., Masoudi, F. A., Robertson, R. M., Roger, V., Schwamm, L. H., Sorlie, P., Yancy, C. W. and Rosamond, W. D. (2010) Defining and setting national goals for cardiovascular health promotion and disease reduction: the American Heart Association's strategic impact goal through 2020 and beyond. Circulation. **121**, 586–613

9 O'Rourke, M. F. and Hashimoto, J. (2007) Mechanical factors in arterial aging: a clinical perspective. J. Am. Coll. Cardiol. **50**, 1–13

10 Gasser, T. C., Ogden, R. W. and Holzapfel, G. A. (2006) Hyperelastic modelling of arterial layers with distributed collagen fibre orientations. J. R. Soc. Interface. **3**, 15–35

11 Greenwald, S. E. (2007) Ageing of the conduit arteries. J. Pathol. **211**, 157–172

12 McEniery, C. M. and Wilkinson, I. B. (2013) The pressures of aging. Hypertension. 1–2

13 Ross, R. (1999) Atherosclerosis - an inflammatory disease. N. Engl. J. Med. **340**, 115–126

14 Weber, C. and Noels, H. (2011) Atherosclerosis: current pathogenesis and therapeutic options. Nat. Med. **17**, 1410–1422

15 Chatzizisis, Y. S., Coskun, A. U., Jonas, M., Edelman, E. R., Feldman, C. L. and Stone, P. H. (2007) Role of endothelial shear stress in the natural history of coronary atherosclerosis and vascular remodeling: molecular, cellular, and vascular behavior. J. Am. Coll. Cardiol. **49**, 2379–2393

16 Shaaban, A. M. and Duerinckx, A. J. (2000) Wall shear stress and early atherosclerosis: a review. Am. J. Roentgenol. **174**, 1657–1665

17 Giannoglou, G. D., Soulis, J. V., Farmakis, T. M., Giannakoulas, G. A., Parcharidis, G. E. and Louridas, G. E. (2005) Wall pressure gradient in normal left coronary artery tree. Med. Eng. Phys. **27**, 455–464

18 Hayoz, D. and Mazzolai, L. (2007) Endothelial function, mechanical stress and atherosclerosis. In Advances in Cardiology (Safar, M. E., and Frohlich, E. D., eds.), KARGER, Basel. 62–75

19 Versari, D., Daghini, E., Virdis, A., Ghiadoni, L. and Taddei, S. (2009) Endothelial dysfunction as a target for prevention of cardiovascular disease. Diabetes Care. **32**, S314–S321

20 Brunner, H., Cockcroft, J. R., Deanfield, J., Donald, A., Ferrannini, E., Halcox, J., Kiowski, W., Luscher, T. F., Mancia, G., Natali, A., Oliver, J. J., Pessina, A. C., Rizzoni, D., Rossi, G. P., Salvetti, A., Spieker, L. E., Taddei, S. and Webb, D. J. (2005) Endothelial function and dysfunction. Part II: association with cardiovascular risk factors and diseases. A statement by the Working Group on Endothelins and Endothelial Factors of the European Society of Hypertension. J. Hypertens. Febr. **23**, 233–246

21 Adji, A., O'Rourke, M. F. and Namasivayam, M. (2011) Arterial stiffness, its assessment, prognostic value, and implications for treatment. Am. J. Hypertens. **24**, 5–17

22 Haluska, B. A., Jeffries, L., Carlier, S. and Marwick, T. H. (2010) Measurement of arterial distensibility and compliance to assess prognosis. Atherosclerosis. **209**, 474–480

23 McVeigh, G. E., Hamilton, P. K. and Morgan, D. R. (2002) Evaluation of mechanical arterial properties: clinical, experimental and therapeutic aspects. Clin. Sci. **102**, 51–67

24 Lim, S. S., Vos, T., Flaxman, A. D., Danaei, G., Shibuya, K., Adair-Rohani, H., AlMazroa, M. A., Amann, M., Anderson, H. R., Andrews, K. G., Aryee, M., Atkinson, C., Bacchus, L. J., Bahalim, A. N., Balakrishnan, K., Balmes, J., Barker-Collo, S., Baxter, A., Bell, M. L., Blore, J. D., Blyth, F., Bonner, C., Borges, G., Bourne, R., Boussinesq, M., Brauer, M., Brooks, P., Bruce, N. G., Brunekreef, B., Bryan-Hancock, C., Bucello, C.,

Buchbinder, R., Bull, F., Burnett, R. T., Byers, T. E., Calabria, B., Carapetis, J., Carnahan, E., Chafe, Z., Charlson, F., Chen, H., Chen, J. S., Cheng, A. T.-A., Child, J. C., Cohen, A., Colson, K. E., Cowie, B. C., Darby, S., Darling, S., Davis, A., et al. (2012) A comparative risk assessment of burden of disease and injury attributable to 67 risk factors and risk factor clusters in 21 regions, 1990–2010: a systematic analysis for the Global Burden of Disease Study 2010. The Lancet. **380**, 2224–2260

25 Kotseva, K., Wood, D., De Backer, G., De Bacquer, D., Pyörälä, K. and Keil, U. (2009) Cardiovascular prevention guidelines in daily practice: a comparison of EUROASPIRE I, II, and III surveys in eight European countries. The Lancet. **373**, 929–940

26 Berry, J. D., Dyer, A., Cai, X., Garside, D. B., Ning, H., Thomas, A., Greenland, P., Van Horn, L., Tracy, R. P. and Lloyd-Jones, D. M. (2012) Lifetime risks of cardiovascular disease. N. Engl. J. Med. **366**, 321–329

27 Blacher, J., Safar, M. E., Pannier, B., Guerin, A. P., Marchais, S. J. and London, G. M. (2002) Prognostic significance of arterial stiffness measurements in end-stage renal disease patients. Curr. Opin. Nephrol. Hypertens. **11**, 629–634

28 Glasser, S. P. and Dudenbostel, T. (2011) The global burden of cardiovascular disease: the role of endothelial function and arterial elasticity in cardiovascular disease as novel and emerging biomarkers. Curr. Cardiovasc. Risk Rep. **5**, 187–195

29 Goff, D. C., Lloyd-Jones, D. M., Bennett, G., Coady, S., D'Agostino, R. B., Gibbons, R., Greenland, P., Lackland, D. T., Levy, D., O'Donnell, C. J., Robinson, J., Schwartz, J. S., Shero, S. T., Smith, S. C., Sorlie, P., Stone, N. J. and Wilson, P. W. F. (2014) 2013 ACC/AHA guideline on the assessment of cardiovascular risk: a report of the American College of Cardiology/American Heart Association task force on practice guidelines. Circulation. S49–73

30 Mozaffarian, D., Wilson, P. W. F. and Kannel, W. B. (2008) Beyond established and novel risk factors: lifestyle risk factors for cardiovascular disease. Circulation. **117**, 3031–3038

31 Erbel, R. and Budoff, M. (2012) Improvement of cardiovascular risk prediction using coronary imaging: subclinical atherosclerosis: the memory of lifetime risk factor exposure. Eur. Heart J. **33**, 1201–1217

32 Ference, B. A., Yoo, W., Alesh, I., Mahajan, N., Mirowska, K. K., Mewada, A., Kahn, J., Afonso, L., Williams Sr, K. A. and Flack, J. M. (2012) Effect of long-term exposure to lower low-density lipoprotein cholesterol beginning early in life on the risk of coronary heart disease: a mendelian randomization analysis. J. Am. Coll. Cardiol. **60**, 2631–2639

33 Gray, L., Lee, I.-M., Sesso, H. D. and Batty, G. D. (2011) Blood pressure in early adulthood, hypertension in middle age, and future cardiovascular disease mortality: HAHS (Harvard Alumni Health Study). J. Am. Coll. Cardiol. **58**, 2396–2403

34 Prospective Studies Collaboration. (2002) Age-specific relevance of usual blood pressure to vascular mortality: a meta-analysis of individual data for one million adults in 61 prospective studies. Lancet. **360**, 1903–1913

35 Lee, C., Joseph, L., Colosimo, A. and Dasgupta, K. (2012) Mortality in diabetes compared with previous cardiovascular disease: a gender-specific meta-analysis. Diabetes Metab. **38**, 420–427

36 Booth, G. L., Kapral, M. K., Fung, K. and Tu, J. V. (2006) Relation between age and cardiovascular disease in men and women with diabetes compared with non-diabetic people: a population-based retrospective cohort study. The Lancet. **368**, 29–36

37 Berger, J. S., Bhatt, D. L., Steinhubl, S. R., Shao, M., Steg, P. G., Montalescot, G., Hacke, W., Fox, K. A., Lincoff, A. M., Topol, E. J. and Berger, P. B. (2009) Smoking, clopidogrel, and mortality in patients with established cardiovascular disease. Circulation. **120**, 2337

38 Wilson PF. (2009) Challenges to improve coronary heart disease risk assessment. JAMA. **302**, 2369–2370

39 Nilsson, P. M., Boutouyrie, P. and Laurent, S. (2009) Vascular aging: a tale of EVA and ADAM in cardiovascular risk assessment and prevention. Hypertension. **54**, 3–10

40 Park, H. E., Cho, G.-Y., Kim, H.-K., Kim, Y.-J. and Sohn, D.-W. (2012) Validation of circumferential carotid artery strain as a screening tool for subclinical atherosclerosis. J. Atheroscler. Thromb. **19**, 349–356

41 Mackenzie, I. S., Wilkinson, I. B. and Cockcroft, J. R. (2002) Assessment of arterial stiffness in clinical practice. QJM. **95**, 67–74

42 Vlachopoulos, C., Aznaouridis, K. and Stefanadis, C. (2010) Prediction of cardiovascular events and all-cause mortality with arterial stiffness: a systematic review and meta-analysis. J. Am. Coll. Cardiol. **55**, 1318–1327

43 Ben-Shlomo, Y., Spears, M., Boustred, C., May, M., Anderson, S. G., Benjamin, E. J., Boutouyrie, P., Cameron, J., Chen, C.-H., Cruickshank, J. K., Hwang, S.-J., Lakatta, E. G., Laurent, S., Maldonado, J., Mitchell, G. F., Najjar, S. S., Newman, A. B., Ohishi, M., Pannier, B., Pereira, T., Vasan, R. S., Shokawa, T., Sutton-Tyrell, K., Verbeke, F., Wang, K.-L., Webb, D. J., Willum Hansen, T., Zoungas, S., McEniery, C. M., Cockcroft, J. R. and Wilkinson, I. B. (2014) Aortic pulse wave velocity improves cardiovascular event prediction: an individual participant meta-analysis of prospective observational data from 17,635 subjects. J. Am. Coll. Cardiol. **63**, 636–646

44 Hughes, S. M. T., Dixon, L. J. and McVeigh, G. E. (2004) Arterial stiffness and pulse wave velocity: problems with terminology. Circulation. **109**, e3

45 Negi, S. I. and Nambi, V. (2012) The role of carotid intimal thickness and plaque imaging in risk stratification for coronary heart disease. Curr. Atheroscler. Rep. 115–123

46 Simon, A., Megnien, J. L. and Chironi, G. (2010) The value of carotid intima-media thickness for predicting cardiovascular risk. Arterioscler. Thromb. Vasc. Biol. **30**, 182–185

47 Nambi, V., Chambless, L., Folsom, A. R., He, M., Hu, Y., Mosley, T., Volcik, K., Boerwinkle, E. and Ballantyne, C. M. (2010) Carotid intima-media thickness and presence or absence of plaque improves prediction of coronary heart disease risk: the ARIC (Atherosclerosis Risk In Communities) Study. J. Am. Coll. Cardiol. **55**, 1600–1607

48 Lorenz, M. W., Markus, H. S., Bots, M. L., Rosvall, M. and Sitzer, M. (2007) Prediction of clinical cardiovascular events with carotid intima-media thickness: a systematic review and meta-analysis. Circulation. **115**, 459–467

49 Touboul, P.-J., Hennerici, M. G., Meairs, S., Adams, H., Amarenco, P., Bornstein, N., Csiba, L., Desvarieux, M., Ebrahim, S., Fatar, M., Hernandez Hernandez, R., Jaff, M., Kownator, S., Prati, P., Rundek, T., Sitzer, M., Schminke, U., Tardif, J.-C., Taylor, A., Vicaut, E., Woo, K. S., Zannad, F. and Zureik, M. (2007) Mannheim carotid intima-media thickness consensus (2004-2006): an update on behalf of the advisory board of the 3rd and 4th Watching the Risk Symposium 13th and 15th European Stroke Conferences, Mannheim, Germany, 2004, and Brussels, Belgium, 2006. Cerebrovasc. Dis. **23**, 75–80

50 Glagov, S., Weisenberg, E., Zarins, C. K., Stankunavicius, R. and Kolettis, G. J. (1987) Compensatory enlargement of human atherosclerotic coronary arteries. N. Engl. J. Med. **316**, 1371–1375

51 Crouse, J. R., Goldbourt, U., Evans, G., Pinsky, J., Sharrett, A. R., Sorlie, P., Riley, W. and Heiss, G. (1994) Arterial enlargement in the atherosclerosis risk in communities (ARIC) cohort. In vivo quantification of carotid arterial enlargement. The ARIC Investigators. Stroke J. Cereb. Circ. **25**, 1354–1359

52 Polak, J. F., Kronmal, R. A., Tell, G. S., O'Leary, D. H., Savage, P. J., Gardin, J. M., Rutan, G. H. and Borhani, N. O. (1996) Compensatory increase in common carotid artery diameter: relation to blood pressure and artery intima-media thickness in older adults. Stroke. **27**, 2012–2015

53 Eigenbrodt, M. L., Bursac, Z., Tracy, R. E., Mehta, J. L., Rose, K. M. and Couper, D. J. (2008) B-mode ultrasound common carotid artery intima-media thickness and external diameter: cross-sectional and longitudinal associations with carotid atherosclerosis in a large population sample. Cardiovasc. Ultrasound. **6**, 10

54 Eigenbrodt, M. L., Sukhija, R., Rose, K. M., Tracy, R. E., Couper, D. J., Evans, G. W., Bursac, Z. and Mehta, J. L. (2007) Common carotid artery wall thickness and external diameter as predictors of prevalent and incident cardiac events in a large population study. Cardiovasc. Ultrasound. **5**, 11

55 Van Dijk, R. A., Dekker, J. M., Nijpels, G., Heine, R. J., Bouter, L. M. and Stehouwer, C. D. (2001) Brachial artery pulse pressure and common carotid artery diameter: mutually independent associations with mortality in subjects with a recent history of impaired glucose tolerance. Eur. J. Clin. Invest. **31**, 756–763

56 Kronmal, R. A., Smith, V.-E., O'Leary, D. H., Polak, J. F., Gardin, J. M. and Manolio, T. A. (1996) Carotid artery measures are strongly associated with left ventricular mass in older adults (A report from the Cardiovascular Health Study). Am. J. Cardiol. **77**, 628–633

57 Polak, J. F., Wong, Q., Johnson, W. C., Bluemke, D. A., Harrington, A., O'Leary, D. H. and Yanez, N. D. (2011) Associations of cardiovascular risk factors, carotid intima-media thickness and left ventricular mass with inter-adventitial diameters of the common carotid artery: the Multi-Ethnic Study of Atherosclerosis (MESA). Atherosclerosis. **218**, 344–349

58 Kawamoto, R., Tomita, H., Oka, Y. and Ohtsuka, N. (2006) Association between risk factors and carotid enlargement. Intern. Med. **45**, 503–509

59 Lloyd, K. D., Barinas-Mitchell, E., Kuller, L. H., Mackey, R. H., Wong, E. A. and Sutton-Tyrrell, K. (2012) Common carotid artery diameter and cardiovascular risk factors in overweight or obese postmenopausal women. Int. J. Vasc. Med. **2012**

60 Jensen-Urstad, K., Jensen-Urstad, M. and Johansson, J. (1999) Carotid artery diameter correlates with risk factors for cardiovascular disease in a population of 55-year-old subjects. Stroke. **30**, 1572–1576

61 Vaidya, D., Heckbert, S. R., Wasserman, B. A. and Ouyang, P. (2012) Sex-specific association of age with carotid artery distensibility: Multi-Ethnic Study of Atherosclerosis. J. Womens Health. **21**, 516–520

62 Schmidt-Trucksäss, A., Grathwohl, D., Schmid, A., Boragk, R., Upmeier, C., Keul, J. and Huonker, M. (1999) Structural, functional, and hemodynamic changes of the common carotid artery with age in male subjects. Arterioscler. Thromb. Vasc. Biol. **19**, 1091–1097

63 Blacher, J., Pannier, B., Guerin, A. P., Marchais, S. J., Safar, M. E. and London, G. M. (1998) Carotid arterial stiffness as a predictor of cardiovascular and all-cause mortality in end-stage renal disease. Hypertension. **32**, 570–574

64 Van Sloten, T. T., Schram, M. T., van den Hurk, K., Dekker, J. M., Nijpels, G., Henry, R. M. A. and Stehouwer, C. D. A. (2014) Local stiffness of the carotid and femoral artery is associated with incident cardiovascular events and all-cause mortality: the Hoorn Study. J. Am. Coll. Cardiol. **63**, 1739–1747

65 Barenbrock, M., Kosch, M., Jöster, E., Kisters, K., Rahn, K.-H. and Hausberg, M. (2002) Reduced arterial distensibility is a predictor of cardiovascular disease in patients after renal transplantation. J. Hypertens. **20**, 79–84

66 Yang, E. Y., Chambless, L., Sharrett, A. R., Virani, S. S., Liu, X., Tang, Z., Boerwinkle, E., Ballantyne, C. M. and Nambi, V. (2012) Carotid arterial wall characteristics are associated with incident ischemic stroke but not coronary heart disease in the Atherosclerosis Risk in Communities (ARIC) Study. Stroke. **43**, 103–108

67 Dijk, J. M., Algra, A., van der Graaf, Y., Grobbee, D. E. and Bots, M. L. (2005) Carotid stiffness and the risk of new vascular events in patients with manifest cardiovascular disease. The SMART study. Eur. Heart J. **26**, 1213–1220

68 Störk, S., van den Beld, A. W., von Schacky, C., Angermann, C. E., Lamberts, S. W. J., Grobbee, D. E. and Bots, M. L. (2004) Carotid artery plaque burden, stiffness, and mortality risk in elderly men: a prospective, population-based cohort study. Circulation. **110**, 344–348

69 Leone, N., Ducimetière, P., Gariépy, J., Courbon, D., Tzourio, C., Dartigues, J.-F., Ritchie, K., Alpérovitch, A., Amouyel, P., Safar, M. E. and Zureik, M. (2008) Distension of the carotid artery and risk of coronary events: the Three-City Study. Arterioscler. Thromb. Vasc. Biol. **28**, 1392–1397

70 Mattace-Raso, F. U. S., van der Cammen, T. J. M., Hofman, A., van Popele, N. M., Bos, M. L., Schalekamp, M. A. D. H., Asmar, R., Reneman, R. S., Hoeks, A. P. G., Breteler, M. M. B. and Witteman, J. C. M. (2006) Arterial stiffness and risk of coronary heart disease and stroke: the Rotterdam Study. Circulation. **113**, 657–663

71 Pignoli, P., Tremoli, E., Poli, A., Oreste, P. and Paoletti, R. (1986) Intimal plus medial thickness of the arterial wall: a direct measurement with ultrasound imaging. Circulation. **74**, 1399 –1406

72 Wendelhag, I., Gustavsson, T., Suurküla, M., Berglund, G. and Wikstrand, J. (1991) Ultrasound measurement of wall thickness in the carotid artery: fundamental principles and description of a computerized analysing system. Clin. Physiol. **11**, 565–577

73 Gustavsson, T., Liang, Q., Wendelhag, I. and Wikstrand, J. (1994) A dynamic programming procedure for automated ultrasonic measurement of the carotid artery. Comput. Cardiol. 297–300

74 Schmidt-Trucksäss, A., Cheng, D., Sandrock, M., Schulte-Mönting, J., Rauramaa, R., Huonker, M. and Burkhardt, H. (2001) Computerized analysing system using the active contour in ultrasound measurement of carotid artery intima-media thickness. Clin. Physiol. **21**, 561–569

75 Faita, F., Gemignani, V., Bianchini, E., Giannarelli, C., Ghiadoni, L. and Demi, M. (2008) Real-time measurement system for evaluation of the carotid intima-media thickness with a robust edge operator. J Ultrasound Med. **27**, 1353–1361

76 Molinari, F., Zeng, G. and Suri, J. S. (2010) A state of the art review on intima-media thickness (IMT) measurement and wall segmentation techniques for carotid ultrasound. Comput. Methods Programs Biomed. **100**, 201–221

77 Teynor, A., Caviezel, S., Dratva, J., Künzli, N. and Schmidt-Trucksäss, A. (2012) An automated, interactive analysis system for ultrasound sequences of the common carotid artery. Ultrasound Med. Biol. **38**, 1440–1450

78 Wikstrand, J. (2007) Methodological considerations of ultrasound measurement of carotid artery intima–media thickness and lumen diameter. Clin. Physiol. Funct. Imaging. **27**, 341–345

79 Safar, M. E., Levy, B. I. and Struijker-Boudier, H. (2003) Current perspectives on arterial stiffness and pulse pressure in hypertension and cardiovascular diseases. Circulation. **107**, 2864–2869

80 Najjar, S. S., Scuteri, A. and Lakatta, E. G. (2005) Arterial aging: is it an immutable cardiovascular risk factor? Hypertension. **46**, 454–462

81 Nilsson, P. M., Lurbe, E. and Laurent, S. (2008) The early life origins of vascular ageing and cardiovascular risk: the EVA syndrome. J. Hypertens. **26**, 1049–1057

82 Nilsson, P. M. (2008) Early vascular aging (EVA): consequences and prevention. Vasc. Health Risk Manag. **4**, 547–552

83 Benetos, A., Laurent, S., Hoeks, A. P., Boutouyrie, P. H. and Safar, M. E. (1993) Arterial alterations with aging and high blood pressure. A noninvasive study of carotid and femoral arteries. Arterioscler. Thromb. Vasc. Biol. **13**, 90–97

84 Liao, D., Arnett, D. K., Tyroler, H. A., Riley, W. A., Chambless, L. E., Szklo, M. and Heiss, G. (1999) Arterial stiffness and the development of hypertension: the ARIC Study. Hypertension. **34**, 201–206

85 Glasser, S. P. and Arnett, D. K. (2008) Vascular stiffness and the "Chicken-or-the-Egg" question. Hypertension. **51**, 177–178

86 Arnett, D. K., Glasser, S. P., McVeigh, G., Prineas, R., Finklestein, S., Donahue, R., Cohn, J. N. and Sinaiko, A. (2001) Blood pressure and arterial compliance in young adults: the Minnesota Children's Blood Pressure Study. Am. J. Hypertens. **14**, 200–205

87 Miao, D.-M., Ye, P., Xiao, W.-K., Gao, P., Zhang, J.-Y. and Wu, H.-M. (2011) Influence of low high-density lipoprotein cholesterol on arterial stiffening and left ventricular diastolic dysfunction in essential hypertension. J. Clin. Hypertens. **13**, 710–715

88 Ferreira, I., van de Laar, R. J., Prins, M. H., Twisk, J. W. and Stehouwer, C. D. (2012) Carotid stiffness in young adults: a life-course analysis of its early determinants: the Amsterdam Growth and Health Longitudinal Study. Hypertension. **59**, 54–61

89 Koskinen, J., Magnussen, C. G., Viikari, J. S. A., Kähönen, M., Laitinen, T., Hutri-Kähönen, N., Lehtimäki, T., Jokinen, E., Raitakari, O. T. and Juonala, M. (2012) Effect of age, gender and cardiovascular risk factors on carotid distensibility during 6-year follow-up. The cardiovascular risk in Young Finns Study. Atherosclerosis. **224**, 474–479

90 Cunha, R. S., Pannier, B., Benetos, A., Siche, J.-P., London, G. M., Mallion, J. M. and Safar, M. E. (1997) Association between high heart rate and high arterial rigidity in normotensive and hypertensive subjects. J Hypertens. **15**, 1423–1430

91 Whelton, S. P., Blankstein, R., Al-Mallah, M. H., Lima, J. A. C., Bluemke, D. A., Hundley, W. G., Polak, J. F., Blumenthal, R. S., Nasir, K. and Blaha, M. J. (2013) Association of resting heart rate with carotid and aortic arterial stiffness: Multi-Ethnic Study of Atherosclerosis. Hypertension. **62**, 477–484

92 Redheuil, A., Yu, W.-C., Wu, C. O., Mousseaux, E., de Cesare, A., Yan, R., Kachenoura, N., Bluemke, D. and Lima, J. A. C. (2010) Reduced ascending aortic strain and distensibility: earliest manifestations of vascular aging in humans. Hypertension. **55**, 319–326

93 Brinkley, T. E., Hsu, F.-C., Carr, J. J., Hundley, W. G., Bluemke, D. A., Polak, J. F. and Ding, J. (2011) Pericardial fat is associated with carotid stiffness in the Multi-Ethnic Study of Atherosclerosis. Nutr. Metab. Cardiovasc. Dis. **21**, 332–338

94 Sharrett, A. R., Ding, J., Criqui, M. H., Saad, M. F., Liu, K., Polak, J. F., Folsom, A. R., Tsai, M. Y., Burke, G. L. and Szklo, M. (2006) Smoking, diabetes, and blood cholesterol differ in their associations with subclinical atherosclerosis: the Multiethnic Study of Atherosclerosis (MESA). Atherosclerosis. **186**, 441–447

95 Scuteri, A., Najjar, S. S., Muller, D. C., Andres, R., Hougaku, H., Metter, E. J. and Lakatta, E. G. (2004) Metabolic syndrome amplifies the age-associated increases in vascular thickness and stiffness. J. Am. Coll. Cardiol. **43**, 1388–1395

96 Aggoun, Y., Bonnet, D., Sidi, D., Girardet, J. P., Brucker, E., Polak, M., Safar, M. E. and Levy, B. I. (2000) Arterial mechanical changes in children with familial hypercholesterolemia. Arterioscler. Thromb. Vasc. Biol. **20**, 2070–2075

97 Roman, M. J., Devereux, R. B., Schwartz, J. E., Lockshin, M. D., Paget, S. A., Davis, A., Crow, M. K., Sammaritano, L., Levine, D. M., Shankar, B.-A., Moeller, E. and Salmon, J. E. (2005) Arterial stiffness in chronic inflammatory diseases. Hypertension. **46**, 194–199

98 Emoto, M., Nishizawa, Y., Kawagishi, T., Maekawa, K., Hiura, Y., Kanda, H., Izumotani, K., Shoji, T., Ishimura, E., Inaba, M., Okuno, Y. and Morii, H. (1998) Stiffness indexes β of the common carotid and femoral arteries are associated with insulin resistance in NIDDM. Diabetes Care. **21**, 1178–1182

99 Salomaa, V., Riley, W., Kark, J. D., Nardo, C. and Folsom, A. R. (1995) Non–insulin-dependent diabetes mellitus and fasting glucose and insulin concentrations are associated with arterial stiffness indexes: the ARIC Study. Circulation. **91**, 1432–1443

100 Stehouwer, C. D. A., Henry, R. M. A. and Ferreira, I. (2008) Arterial stiffness in diabetes and the metabolic syndrome: a pathway to cardiovascular disease. Diabetologia. **51**, 527–539

101 Della-Morte, D., Gardener, H., Denaro, F., Boden-Albala, B., Elkind, M. S. V., Paik, M. C., Sacco, R. L. and Rundek, T. (2010) Metabolic syndrome increases carotid artery stiffness: the Northern Manhattan Study. Int. J. Stroke. **5**, 138–144

102 Liang, Y.-L., Shiel, L. M., Teede, H., Kotsopoulos, D., McNeil, J., Cameron, J. D. and McGrath, B. P. (2001) Effects of blood pressure, smoking, and their interaction on carotid artery structure and function. Hypertension. **37**, 6–11

103 Van de Laar, R. J., Ferreira, I., van Mechelen, W., Prins, M. H., Twisk, J. W. and Stehouwer, C. D. (2010) Lifetime vigorous but not light-to-moderate habitual physical activity impacts favorably on carotid stiffness in young adults: the Amsterdam Growth and Health Longitudinal Study. Hypertension. **55**, 33–39

104 Cook, J. N., DeVan, A. E., Schleifer, J. L., Anton, M. M., Cortez-Cooper, M. Y. and Tanaka, H. (2006) Arterial compliance of rowers: implications for combined aerobic and strength training on arterial elasticity. Am. J. Physiol. Heart Circ. Physiol. **290**, H1596–1600

105 DeSouza, C. A., Shapiro, L. F., Clevenger, C. M., Dinenno, F. A., Monahan, K. D., Tanaka, H. and Seals, D. R. (2000) Regular aerobic exercise prevents and restores age-related declines in endothelium-dependent vasodilation in healthy men. Circulation. **102**, 1351–1357

106 Nualnim, N., Barnes, J. N., Tarumi, T., Renzi, C. P. and Tanaka, H. (2011) Comparison of central artery elasticity in swimmers, runners, and the sedentary. Am. J. Cardiol. **107**, 783–787

107 Tanaka, H., Dinenno, F. A., Monahan, K. D., Clevenger, C. M., DeSouza, C. A. and Seals, D. R. (2000) Aging, habitual exercise, and dynamic arterial compliance. Circulation. **102**, 1270–1275

108 Sugawara, J., Otsuki, T., Tanabe, T., Hayashi, K., Maeda, S. and Matsuda, M. (2006) Physical activity duration, intensity, and arterial stiffening in postmenopausal women. Am. J. Hypertens. **19**, 1032–1036

109 Kozakova, M., Palombo, C., Mhamdi, L., Konrad, T., Nilsson, P., Staehr, P. B., Paterni, M. and Balkau, B. (2007) Habitual physical activity and vascular aging in a young to middle-age population at low cardiovascular risk. Stroke. **38**, 2549–2555

110 Schmidt-Trucksäss, A., Grathwohl, D., Frey, I., Schmid, A., Boragk, R., Upmeier, C., Keul, J. and Huonker, M. (1999) Relation of leisure-time physical activity to structural and functional arterial properties of the common carotid artery in male subjects. Atherosclerosis. **145**, 107–114

111 Schmidt-Trucksäss, A., Schmid, A., Brunner, C., Scherer, N., Zäch, G., Keul, J. and Huonker, M. (2000) Arterial properties of the carotid and femoral artery in endurance-trained and paraplegic subjects. J. Appl. Physiol. **89**, 1956–1963

112 Alberti, K. G. M. M., Eckel, R. H., Grundy, S. M., Zimmet, P. Z., Cleeman, J. I., Donato, K. A., Fruchart, J.-C., James, W. P. T., Loria, C. M. and Smith, S. C. (2009) Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. Circulation. **120**, 1640–1645

113 Lerner, D. J. and Kannel, W. B. (1986) Patterns of coronary heart disease morbidity and mortality in the sexes: a 26-year follow-up of the Framingham population. Am. Heart J. **111**, 383–390

114 Juutilainen, A., Kortelainen, S., Lehto, S., Rönnemaa, T., Pyörälä, K. and Laakso, M. (2004) Gender difference in the impact of type 2 diabetes on coronary heart disease risk. Diabetes Care. **27**, 2898–2904

115 Marlatt, K. L., Kelly, A. S., Steinberger, J. and Dengel, D. R. (2013) The influence of gender on carotid artery compliance and distensibility in children and adults. J. Clin. Ultrasound. **41**, 340–346

116 Vermeersch, S. J., Rietzschel, E. R., De Buyzere, M. L., De Bacquer, D., De Backer, G., Van Bortel, L. M., Gillebert, T. C., Verdonck, P. R. and Segers, P. (2008) Age and gender related patterns in carotid-femoral PWV and carotid and femoral stiffness in a large healthy, middle-aged population. J. Hypertens. **26**, 1411–1419

117 Sandberg, K. and Ji, H. (2012) Sex differences in primary hypertension. Biol. Sex Differ. **3**, 7

118 Reckelhoff, J. F. (2001) Gender differences in the regulation of blood pressure. Hypertension. **37**, 1199–1208

119 Foulds, H. J. A., Bredin, S. S. D. and Warburton, D. E. R. (2012) The relationship between hypertension and obesity across different ethnicities. J. Hypertens. Febr. 2012. **30**, 359–367

120 Santos, M. A. A., Sousa, A. C. S., Reis, F. P., Santos, T. R., Lima, S. O. and Barreto-Filho, J. A. (2013) Does the aging process significantly modify the mean heart rate? Arq. Bras. Cardiol. **101**, 388–398

121 Brinkworth, G. D., Noakes, M., Buckley, J. D. and Clifton, P. M. (2006) Weight loss improves heart rate

recovery in overweight and obese men with features of the metabolic syndrome. Am. Heart J. **152**, 693.e1–693.e6

122 Dietrich, D. F., Schwartz, J., Schindler, C., Gaspoz, J.-M., Barthélémy, J.-C., Tschopp, J.-M., Roche, F., Eckardstein, A. von, Brändli, O., Leuenberger, P., Gold, D. R. and Ackermann-Liebrich, U. (2007) Effects of passive smoking on heart rate variability, heart rate and blood pressure: an observational study. Int. J. Epidemiol. **36**, 834–840

123 Kujala, U. M. (2009) Evidence on the effects of exercise therapy in the treatment of chronic disease. Br. J. Sports Med. **43**, 550–555

124 Koster, A., Visser, M., Simonsick, E. M., Yu, B., Allison, D. B., Newman, A. B., van Eijk, J. T. M., Schwartz, A. V., Satterfield, S., Harris, T. B. and for the Health, A. and B. C. S. (2010) Association between fitness and changes in body composition and muscle strength. J. Am. Geriatr. Soc. **58**, 219–226

125 Kleppinger, A., Litt, M. D., Kenny, A. M. and Oncken, C. A. (2010) Effects of smoking cessation on body composition in postmenopausal women. J. Womens Health. **19**, 1651–1657

126 White, U. A. and Tchoukalova, Y. D. (2014) Sex dimorphism and depot differences in adipose tissue function. Biochim. Biophys. Acta. **1842**, 377–92

127 Gepner, A. D., Piper, M. E., Johnson, H. M., Fiore, M. C., Baker, T. B. and Stein, J. H. (2011) Effects of smoking and smoking cessation on lipids and lipoproteins: outcomes from a randomized clinical trial. Am. Heart J. **161**, 145–151

128 Wilsgaard, T. and Arnesen, E. (2004) Change in serum lipids and body mass index by age, sex, and smoking status: the Tromsø Study 1986–1995. Ann. Epidemiol. **14**, 265–273

129 Wang, X., Magkos, F. and Mittendorfer, B. (2011) Sex differences in lipid and lipoprotein metabolism: it's not just about sex hormones. J. Clin. Endocrinol. Metab. **96**, 885–893

130 Dedobbeleer, N., Béland, F., Contandriopoulos, A.-P. and Adrian, M. (2004) Gender and the social context of smoking behaviour. Soc. Sci. Med. **58**, 1–12

131 Kaczynski, A. T., Manske, S. R., Mannell, R. C. and Grewal, K. (2008) Smoking and physical activity: a systematic review. Am. J. Health Behav. **32**, 93–110

132 Herman, W. H., Hoerger, T. J., Brandle, M., Hicks, K., Sorensen, S., Zhang, P., Hamman, R. F., Ackermann, R. T., Engelgau, M. M., Ratner, R. E. and Diabetes Prevention Program Research Group. (2005) The cost-effectiveness of lifestyle modification or metformin in preventing type 2 diabetes in adults with impaired glucose tolerance. Ann. Intern. Med. **142**, 323–332

133 Bano, G. (2013) Glucose homeostasis, obesity and diabetes. Best Pract. Res. Clin. Obstet. Gynaecol. **27**, 715–726

134 Pani, L. N., Korenda, L., Meigs, J. B., Driver, C., Chamany, S., Fox, C. S., Sullivan, L., D'Agostino, R. B. and Nathan, D. M. (2008) Effect of aging on a1c levels in individuals without diabetes evidence from the Framingham Offspring Study and the National Health and Nutrition Examination Survey 2001–2004. Diabetes Care. **31**, 1991–1996

135 Hernán, M. A., Hernández-Díaz, S., Werler, M. M. and Mitchell, A. A. (2002) Causal knowledge as a prerequisite for confounding evaluation: an application to birth defects epidemiology. Am. J. Epidemiol. **155**, 176–184

136 Lee, I.-M., Shiroma, E. J., Lobelo, F., Puska, P., Blair, S. N. and Katzmarzyk, P. T. (2012) Effect of physical inactivity on major non-communicable diseases worldwide: an analysis of burden of disease and life expectancy. Lancet. **380**, 219–229

137 Wen, C. P., Wai, J. P. M., Tsai, M. K., Yang, Y. C., Cheng, T. Y. D., Lee, M.-C., Chan, H. T., Tsao, C. K., Tsai, S. P. and Wu, X. (2011) Minimum amount of physical activity for reduced mortality and extended life expectancy: a prospective cohort study. Lancet. **378**, 1244–1253

138 Sattelmair, J., Pertman, J., Ding, E. L., Kohl, H. W., Haskell, W. and Lee, I.-M. (2011) Dose response between physical activity and risk of coronary heart disease: a meta-analysis. Circulation. **124**, 789–795

139 Swain, D. P. and Franklin, B. A. (2006) Comparison of cardioprotective benefits of vigorous versus moderate intensity aerobic exercise. Am. J. Cardiol. **97**, 141–147

140 World Health Organization. (2010) Global recommendations on physical activity for health, World Health Organization, Geneva, Switzerland, 1-58

141 Federal Office of Sport FOSPO, Federal Office of Public Health FOPH, Health Promotion Switzerland, bfu – Swiss Council for Accident Prevention, Swiss Accident Insurance Fund (Suva), Health and Physical Activity and Network Switzerland. (2013) Health - enhancing physical activity core document for Switzerland, Maggingen: FOSPO, 1-28.

142 Swift, D. L., Lavie, C. J., Johannsen, N. M., Arena, R., Earnest, C. P., O'Keefe, J. H., Milani, R. V., Blair, S. N. and Church, T. S. (2013) Physical activity, cardiorespiratory fitness, and exercise training in primary and secondary coronary prevention. Circ. J. **77**, 281–292

143 Schmitz, K. H., Arnett, D. K., Bank, A., Liao, D., Evans, G. W., Evenson, K. R., Stevens, J., Sorlie, P. and Folsom, A. R. (2001) Arterial distensibility and physical activity in the ARIC study. Med. Sci. Sports Exerc. **33**, 2065–2071

CHAPTER 2

PhD research objectives

PHD RESEARCH OBJECTIVES

Summarising the previously mentioned gaps of knowledge on which research objectives of the publications were based, the aims of this thesis were:

Background of study aim 1: Manual measurements of carotid intima media thickness (CIMT) are nowadays widely replaced by sophisticated ultrasound analyses programs, which are able to analyse carotid structure across heart cycle. This allows the investigation of carotid function, also known as functional parameters or carotid stiffness. However, since carotid stiffness is based on structural parameters, a review about carotid structure assessments, validations results and possible predictability of cardiovascular disease (CVD) risk assessment provides a solid basis for further research (Chapter 4, Swiss Med Wkly. 2012;142:w13705).

Aim 1: To describe the anatomical and histological background of artery wall structure; to summarise standardisations of ultrasound examinations and analysis using different measurement methods; to report measurement-variability; to describe normal values of CIMT and link it to CIMT progression based on cardiovascular risk factors; to review whether a supplemental implementation of CIMT measurements, compared to validated cardiovascular risk assessments, would enhance the power of discrimination between healthy and pathological values; and how the latter one could be implemented in clinical practice.

Background of study aim 2: As described in our review, automatic ultrasound programs showed the highest reproducibility. However, there is no gold standard for analysing ultrasound images. A major point of criticism on automatic programs is that misdetection by the program due to insufficient ultrasound data had to be discarded from the evaluation. The development of a novel analysis program called DYnamic ARtery Analysis (DYARA) solved this deficit but it had to be validated (Chapter 5, Ultrasound Med Biol. 2012 Aug;38(8):1440-50).

Aim 2: To describe the detection algorithm of DYARA; to assess the inter-reader and intra-reader variability for sequential images based on completely automatic detection and combined manual and automatic detection; to make the complete validation dataset freely available upon request for an easy comparison of further validation studies.

Background of study aim 3: In order to investigate and validate not only the ultrasound program but also the SAPALDIA ultrasound data quality, a validation study considering different structural and functional carotid parameters had to be executed within a subsample of SAPALDIA study population and within the same study setting (Chapter 6, Atherosclerosis. 2013 Dec;231(2):448–55).

Aim 3: To investigate the SAPALDIA ultrasound data quality; to describe the SAPALDIA ultrasound study sample, the ultrasound examination and the respective sequential image analysis, definitions of carotid structural (CIMT, lumen diameter) and the carotid functional indices (strain, distensibility, compliance, β-stiffness index, Peterson's elastic modulus and Young's elastic modulus); to investigate the variability and reproducibility of carotid structural and functional parameters by means of repeated ultrasound scans using different statistical approaches; and to compare our findings of structural and functional reproducibility with other different population based studies.

Background of study aim 4: Even though it has been reported that incidence of cardiovascular morbidity and mortality is sex- and age-related, it has never been investigated whether carotid stiffness parameters (a discussed biomarker for CVD) are sex-specifically associated with cardiovascular risk factors. In addition, it is also unknown whether all carotid stiffness parameters provide similar association patterns (Chapter 7, Atherosclerosis 2014 Jun;235(2):576-84) .

Aim 4: To examine the associations of cardiovascular risk factors with six different carotid stiffness parameters separately for men and women, using multivariate regression analyses to deal with multiple comparison issues; to investigate whether sex differences in these associations exist; and to assess the heterogeneity of these association patterns among all six carotid stiffness parameters.

Background of study aim 5: Although regular physical activity (PA) has been shown to reduce CVD risk in the general population, reduction in carotid stiffness was only found comparing extreme groups of highly trained vs. untrained subjects. However, it is unclear whether these associations remain if considering a representative sample (Publication 5, submitted to PLOS ONE).

Aim 5: To determine whether moderate, vigorous or total PA are associated with reduced carotid stiffness adjusting for main cardiovascular risk factors; to describe pathway associations of PA, cardiovascular risk factors and carotid stiffness based on prior assumed effect directions.

CHAPTER 3

SAPALDIA – The Swiss Cohort Study on Air Pollution and Lung and Heart Diseases in Adults

SAPALDIA – The Swiss Cohort Study on Air Pollution and Lung and Heart Diseases in Adults

This thesis is implemented in the 'Swiss Cohort Study on Air Pollution and Lung and Heart Diseases in Adults' (SAPALDIA) and focuses on the cyclic variation of the common carotid structure in relation to prior atherosclerotic burden and physical activity (PA). To better understand the study development and cohort participants, a short summary of the Swiss demography and the main SAPALDIA aims, results and methodology are given in the following pages.

Swiss demography - background

In the last century, the Swiss population has more than doubled and the age composition fundamentally changed [1]. While in 1900, 40.7% of the overall population were under 20 years, 5.8% older than 64 years and 0.5% older than 80 years the actual distributions in 2009 changed radically to 21.0%, 16.9% and 4.8%, respectively [1]. The ageing population is a consequence of the industrial and medical development combined with a certain economic wealth, which raises life expectancy by a simultaneous decrease in birth rate [1]. Thus, it is not surprising that, within an ageing population, chronic diseases based on age-related changes occur more frequently with advancing age, although these diseases may manifest in earlier life stages due to unfavourable lifestyle [2]. In Switzerland, cardiovascular diseases (CVD) are the leading cause of death since more than 20 years, followed closely by cancer and at some distance by respiratory diseases, which is still a major public health issue [3].

SAPALDIA - background

Given the described demographical background, the first SAPALDIA examination (SAPALDIA 1) was initiated in 1991 with a primary focus on respiratory health outcomes, allergies and air pollution exposure [4]. SAPALDIA is a multicentre cohort study based on a random sample of 9651 adults (18-60 years at first examination) from eight different Swiss communities (Aarau, Basel, Davos, Genève, Lugano, Montana, Payerne, Wald) representing a wide range of cultural and environmental conditions across Switzerland [4]. Based on the scientific findings obtained in SAPALDIA 1, the Swiss Federal Council implemented air pollution emission limits in their air pollution control guidelines [5], which contributed to the scientific evidence revised by the World Health Organization (WHO) for the publication of the Air Quality Guidelines in 2006 [6].

In the first follow-up survey (SAPALDIA 2) in 2001-2003, 8047 participants (83% of participants in SAPALDIA 1) underwent a new examination. The research focus was extended to cardiovascular health, with special focuses (besides air pollution) on sex and gender-related risk factors, second-hand tobacco smoke exposure and genetic and molecular issues

[7]. Methodological details of cross-sectional and longitudinal investigations in SAPALDIA 1 and SAPALDIA 2 are described elsewhere [4,7]. Main results of SAPALDIA 2 attracted worldwide attention since they showed for the first time that reducing exposure to air pollutants, even from low levels, could attenuate the normal age-related decline in lung function in the general adulthood [5,8]. Among other results, exposure to nitrogen dioxide was associated with reduced heart rate variability in elderly women and adults with CVD, suggesting a relation between air pollutant and cardiac dysfunction [9,10]. A list of all SAPALDIA publications can be found on the SAPALDIA website [11].

SAPALDIA 3

SAPALDIA 3 was the second follow-up survey performed in 2010-2011 focusing again on air pollution, cardiovascular health with special attention to systemic inflammation and chronic diseases in particular to asthma, COPD (chronic obstructive pulmonary disease) and atherosclerosis [5]. The new generated SAPALDIA database, combining three examinations over 20 years in a uniform format, is an optimal basis for current and future investigations. In addition, the follow-up rate between SAPALDIA 1 and SAPALDIA 3 among survivors was about 68%, which shows a high level of commitment to the study.

SAPALDIA 3 – ultrasound data

Within SAPALDIA 3, new measurements of arterial vessels health state were performed. Among all vascular measurements, arterial structure and function of the common carotid artery was of particular interest for this dissertation. Therefore, ultrasound examinations were performed in 3489 SAPALDIA participants aged 50 years and older within the same eight Swiss regions using standardised ultrasound instruments (UF-870 machine LA385-16 MHz array transducer, Fukuda Denshi, Japan) [12,13]. Medical staff was trained and certificated prior to the study [12,13]. Participants were bilaterally examined (ear to ear and horizontal scans) in supine position after a rest of at least 10 minutes with rotated neck standardised by a 45° foam wedge [12,13]. Ultrasound scans were transferred to the Swiss Tropical and Public Health Institute mirrored to the Department of Sport, Exercise and Health and offline analysed by the full automatic ultrasound analysis program called **DY**namic **AR**tery **A**nalysis (DYARA) [12,13]. Therefore, expert readers analysed carotid structure directly proximal of the carotid bifurcation over exactly on centimetre across at least one heart cycle and intimal and adventitial layer of for wall and adventitia layer of the near wall [12,13]. Results were automatically saved in a local database and at the end of the study uploaded to the official SAPALDIA database. The detailed description of ultrasound assessment and analyses, DYARA validation and SAPALDIA ultrasound data validation can be found in chapter 5 and chapter 6 [12,13].

References

1 Bundesamt für Statistik. (2013) Switzerland's population 2012. Retrieved May 16, 2014, from http://www.bfs.admin.ch/bfs/portal/en/index/news/publikationen.html?publicationID=5381

2 World Health Organization. (2009) Global health risks: mortality and burden of disease attributable to selected major risks, World Health Organization, Geneva

3 Bundesamt für Statistik. (2012) Todesfälle: Anzahl, Entwicklung und Ursachen. Retrieved December 3, 2012, from http://www.bfs.admin.ch/bfs/portal/de/index/themen/14/02/04/key/01.html

4 Martin, B. W., Ackermann-Liebrich, U., Leuenberger, P., Künzli, N., Zemp Stutz, E., Keller, R., Zellweger, J. P., Wüthrich, B., Monn, C., Blaser, K., Bolognini, G., Bongard, J. P., Brändli, O., Braun, P., Defila, C., Domenighetti, G., Grize, L., Karrer, W., Keller-Wossidlo, H., Medici, T. C., Peeters, A., Perruchoud, A. P., Schindler, C., Schoeni, M. H., Schwartz, J., Solari, G., Tschopp, J.-M. and Villiger, B. (1997) SAPALDIA: methods and participation in the cross-sectional part of the Swiss Study on Air Pollution and Lung Diseases in Adults. Soz.- Präventivmedizin. **42**, 67–84

5 Eidgenössisches Departement für Umwelt, Verkehr, Energie und Kommunikation UVEK and Bundesamt für Umwelt BAFU. (2013) Faktenblatt - SAPALDIA - Swiss Cohort Study on Air Pollution and Lung and Heart Diseases in Adults, 1-10

6 World Health Organization. (2006) Air quality guidelines - global update 2005. WHO. 1-22. Retrieved January 12, 2015, from http://www.who.int/phe/health_topics/outdoorair/outdoorair_aqg/en/

7 Ackermann-Liebrich, U., Kuna-Dibbert, B., Probst-Hensch, N. M., Schindler, C., Felber Dietrich, D., Zemp Stutz, E., Bayer-Oglesby, L., Baum, F., Brändli, O., Brutsche, M., Downs, S. H., Keidel, D., Gerbase, M. W., Imboden, M., Keller, R., Knöpfli, B., Künzli, N., Nicod, L., Pons, M., Staedele, P., Tschopp, J.-M., Zellweger, J.-P. and Leuenberger, P. (2005) Follow-up of the Swiss Cohort Study on Air Pollution and Lung Diseases in Adults (SAPALDIA 2) 1991-2003: methods and characterization of participants. Soz.-Präventivmedizin. **50**, 245–263

8 Downs, S. H., Schindler, C., Liu, L.-J. S., Keidel, D., Bayer-Oglesby, L., Brutsche, M. H., Gerbase, M. W., Keller, R., Künzli, N., Leuenberger, P., Probst-Hensch, N. M., Tschopp, J.-M., Zellweger, J.-P., Rochat, T., Schwartz, J. and Ackermann-Liebrich, U. (2007) Reduced Exposure to PM10 and Attenuated Age-Related Decline in Lung Function. N. Engl. J. Med. **357**, 2338–2347

9 Dietrich, D. F., Schindler, C., Schwartz, J., Barthélémy, J.-C., Tschopp, J.-M., Roche, F., Eckardstein, A. von, Brändli, O., Leuenberger, P., Gold, D. R., Gaspoz, J.-M. and Ackermann-Liebrich, U. (2006) Heart rate variability in an ageing population and its association with lifestyle and cardiovascular risk factors: results of the SAPALDIA study. Europace. **8**, 521–529

10 Dietrich, D. F., Gemperli, A., Gaspoz, J.-M., Schindler, C., Liu, L.-J. S., Gold, D. R., Schwartz, J., Rochat, T., Barthelemy, J.-C., Pons, M., Roche, F., Probst Hensch, N. M., Bridevaux, P.-O., Gerbase, M. W., Neu, U. and Ackermann-Liebrich, U. (2008) Differences in Heart Rate Variability Associated with Long-Term Exposure to NO2. Environ. Health Perspect. **116**, 1357–1361

11 SAPALDIA website - List of SAPALDIA publications in medical journals. Retrieved May 19, 2014, from http://www.sapaldia.net/en/content/blogcategory/52/287/

12 Teynor, A., Caviezel, S., Dratva, J., Künzli, N. and Schmidt-Trucksäss, A. (2012) An automated, interactive analysis system for ultrasound sequences of the common carotid artery. Ultrasound Med. Biol. **38**, 1440–1450

13 Caviezel, S., Dratva, J., Schaffner, E., Teynor, A., Baumstark, M. W., Schindler, C., de Groot, E., Burdet, L., Rothe, T., Pons, M., Gaspoz, J.-M., Rochat, T., Künzli, N., Probst-Hensch, N. and Schmidt-Trucksäss, A. (2013) Variability and reproducibility of carotid structural and functional parameters assessed with transcutaneous ultrasound – results from the SAPALDIA cohort study. Atherosclerosis. **231**, 448–45

CHAPTER 4

Carotid intima-media thickness as a biomarker of subclinical atherosclerosis

Authors
Marcus Bauer
Seraina Caviezel
Alexandra Teynor
Raimund Erbel
Amir A. Mahabadi
Arno Schmidt-Trucksäss

Accepted version*, published in:
Swiss Med Wkly. 2012;142:w13705
Doi: 10.4414/smw.2012.13705

*Minor editorial modifications possible due to harmonisation of the thesis

CAROTID INTIMA-MEDIA THICKNESS AS A BIOMARKER OF SUBCLINICAL ATHEROSCLEROSIS

Abstract

Intima-media thickness of the carotid artery (CIMT) and its increase is associated with several cardiovascular risk factors and manifest cardiovascular diseases (CVD). CIMT is suggested to be an important biomarker of subclinical atherosclerosis. CIMT is measured in B-mode ultrasound images of the carotid tree as a typical double line of the arterial wall. CIMT is best visible in the measurement segment of the distal common carotid artery (CCA) with lowest measurement variability. The measurement is most reliable over a one centimeter-segment with automatic or semiautomatic reading methods, which minimizes reading errors. Further structured training of sonographer and reader is important for valid and reproducible results. CIMT is an accepted predictor for future cardiovascular events independent of age, gender and cardiovascular risk factors. Measurement seems to be best applicable in patients with intermediate risk in order to readjust cardiovascular risk. Plaques in the carotid tree and thickening of the CIMT are different atherosclerotic processes. From childhood to early adulthood CIMT is the only atherosclerotic marker of the carotid tree; plaques occur later in life. Both parameters contribute independently to risk assessment for future cardio-vascular events.

Aims of this review are to outline measurement procedures, reproducibility, prognostic value and ability to discriminate healthy subject and patients with manifest disease in a practical and scientifically contemporary manner.

Key words: carotid intima-media thickness; B-mode sonography; subclinical atherosclerosis; cardiovascular prevention

Introduction

Carotid intima-media thickness (CIMT), recorded with B-mode sonography, is an important marker to quantify atherosclerotic burden in the common carotid artery (CCA). The last twenty years, the value of CIMT-measurement for risk estimation of atherosclerotic events (for e.g. myocardial infarction, stroke, sudden cardiac death) increased more than ever.

Aim of the present review article is to 1) present anatomical and histological background of the vessel wall structure, 2) introduce the history of CIMT-imaging, to demonstrate 3) aspects of reproducibility, 4) prospective value in regard to cardiovascular endpoints and medical treatment, 5) the power of discrimination between healthy and pathological values, 6) the additional benefit of CIMT-measurement compared to established cardiovascular risk stratification algorithms, and 7) its implementation in the daily clinical practice.

Anatomy and Histology

The right CCA has its source in the Truncus brachiocephalicus, while the left CCA originates from the aortic arch. In its cervical course the CCA is medial located, slightly behind the internal jugular vein [1]. The carotid bifurcation (CB) into the internal (ICA) and external carotid artery (ECA) is located at the level of the 4th cervical vertebra [1]. The carotid artery has a superficial course, so that ultrasound examinations usually can be performed without bigger problems. In some cases the variability of the location of the carotid bulb makes the identification of the ICA and ECA somewhat difficult.

The histological mural structure of the CCA is composed of three layers:

1) Intima (Tunica intima): inner layer; monolayer of endothelial cells; between Tunica intima and Tunica media: internal elastic lamina
2) Media (Tunica media): mainly composed of longitudinal smooth muscle cells, surrounded by connective tissue; containing elastic lamella that provides elastic property of vessels
3) Adventitia (Tunica externa / adventitia): outer layer; generally embedded in the circumjacent tissue

Histopathology

Atherosclerosis is a systemic and chronic inflammatory disease, which may cause CVD, the most frequent cause of death in the world [2]. A long term thesis in the development of atherosclerosis was the “response-to-injury” theory [3], in which a physical injury of endothelium was considered to be responsible for atherosclerotic changes of vessel walls. This view was completed the last three decades, since endothelial dysfunction has been considered to be a functional trigger [4,5]. Briefly, the infiltration of low-density lipoprotein cholesterol (LDL-C) through the endothelium, LDL-deposition in the intima and the following oxidative and enzymatic processes has been described [6]. Hence, the intima-media complex of arterial walls plays an essential role in the pathogenesis of atherosclerosis and may reflect different stages in the development of the disease: a hypertensive hypertrophic response of medial cells can be observed in early phases of atherosclerosis (quantified by CIMT-measurement), while carotid plaque formation are often seen in later stages of atherosclerosis, which may be caused by inflammation, oxidation, endothelial dysfunction, and/or smooth muscle cell proliferation [7]. An increased CIMT is typically seen at the CCA, while carotid plaque formations are more frequent at the carotid bulb (CB) or internal carotid artery (ICA). Plaque formation at the CB or ICA are more associated with hyperlipidaemia and MI, while an increased CIMT at the CCA shows a stronger relationship to hypertension and stroke [8].

History of CIMT-measurements

Already in the 1980s Pignoli et al. could demonstrate a highly significant association between histological findings of the CCA and respective ultrasound examinations [9]. Since then, associations between CIMT and a) traditional and non-traditional cardiovascular risk factors, b) the extent and severity of atherosclerosis c) as well as cardio- and cerebrovascular events (outcome) had been examined and described in different studies ([4-6]; table 4.1). Regarding cardiovascular risk factors, an increased age has the most impact on an increased CIMT: depending on different studies, age may explain 50-80% of the variability for an increased CIMT. It has been described an annual increase of CIMT in the CCA about 0,007mm [10], with a nearby 5 years delay in women compared to men of the same age.

Methods exposure

B-mode sonography of the carotid artery is a safe, cheap, quick and painless examination, free of radiation exposure to the patient and enables a detailed evaluation of different regions of the carotid artery. Currently, B-mode sonography of the carotid artery mainly allows a noninvasive visualization and assessment of arterial wall changes via measurement and quantification of a) CIMT and b) carotid plaque formation:

a) CIMT is defined as the viewable distance between the lumen-intima- and the media-adventitia interface (see figure 4.1)

b) Carotid plaque formation differ from CIMT (as defined) by an increase of CIMT about at least 0,5 mm or an increase about 50% compared the adjacent CIMT as well as an increase of thickness about more than 1,5 mm [17].

   Another possibility is to regard CIMT as a continuous distance that also integrates those vessel regions in CIMT-measurements that are defined as “plaque formation”. In those cases CIMT is defined as mean CIMT in CCA or the mean maximum CIMT in the CCA, the CB and / or the ICA [18].

Table 4.1: Prospective studies about the prediction of cardiovascular events via CIMT-measurement in subjects without manifest cardiovascular atherosclerotic disease Hazard Ratio, RR = Relative Risk, CCA = common carotid artery, CB = carotid bifurcation, ICA = internal carotid artery), * = follow-up [11].

Author	Study	Partici-pants	Age	Follow-up	Endpoints	Region of interest	Risk estimation	Comment
Chambless et al. 1997 [11]	ARIC	12841	45 - 64 years	4-7 years	fatal and non-fatal cardiovascular events	CCA, CB,ICA	for CIMT > 1 vs. < 1 mm, HR adjusted for Diabetes, HDL-, LDL-cholesterol, hypertension, smoking status, study center, age and race 1.18 (1.06–1.32) in men and 1.42 (1.24-1.64) in women	CIMT = mean CIMT from 6 different segments
O'Leary et al. 1999 [12]	CHS	4.476	72.5 years (mean age at beginning of the study)	6.2 years (median)	myocardial infarction, stroke, combination	CCA, ICA	RR-combined endpoint (adjusted for age, gender, blood pressure (sys, dia), smoking (pack years), Diabetes, atrial fibrillation) for max. CIMT CCA: 2.22 (1.58-3.13) for >1.18 mm (highest) vs. <0.87 mm (lowest CIMT-quintile); 2.47 (1.59–3.85) for >1.81 mm (highest) vs. <0.90 mm (lowest CIMT-quintile)	Primary measurement value: max. CIMT
Iglesias del Sol et al. 2002 [13]	Rotterdam	1.721	> 55 years	4.6 years (mean age)	cardio- or cerebro-vascular disease	CCA, CB, ICA	RR 1.41 (95% CI, 1.25-1.82) for stroke and 1.43 (95% CI, 1.16-1.78) for myocardial infarction	Primary measurement value: max. CIMT; separated for different segments
Lorenz et al. 2006 [14]	CAPS	5.056	19–90 years; mean age 50.1 years	4.2 years (mean age)	myocardial infarction, stroke, combined endpoint	CCA, CB, ICA	HR (adjusted for risk factors) for CIMT-CCA > 0.79mm vs. < 0.63 mm 1.85 (1.09–3.15), for CIMT-CB > 0.79mm vs. < 0.63 mm 1.27 (0.80–1.99), for CIMT-ICA > 0.79mm vs. < 0.63 mm 1.25 (0.84–1.86) for combined endpoint	mean CIMT; far wall
***Nambi et al. 2010 [15]**	ARIC	13.145	45 - 64 years	15.1 years (mean age)	fatal and non-fatal cardiovascular events, revascularisation	CCA, CB, ICA	men: CIMT increased AUC from 0.674 (only risk factors) up to 0.690 (95% CI for difference of the adjusted AUC: 0.009 - 0.022) women: CIMT increased AUC from 0.759 (only risk factors) up to 0.762 (95% CI for difference of the adjusted AUC: - 0.002 - 0.006)	CIMT = mean CIMT from 6 different segments
Plichart et al. 2011 [16]	Three-City	5.895	65-85 years; mean age 73.3 years	5.4 years (mean age)	new diagnosed coronary heart disease, fatal cardiovascular event	CCA	HR 5. vs. 1. quintile = 0.8; 95% CI = 0.5–1.2; p for trend < 0.48) (adjusted for risk factors)	mean CIMT; far wall; plaque-free segment

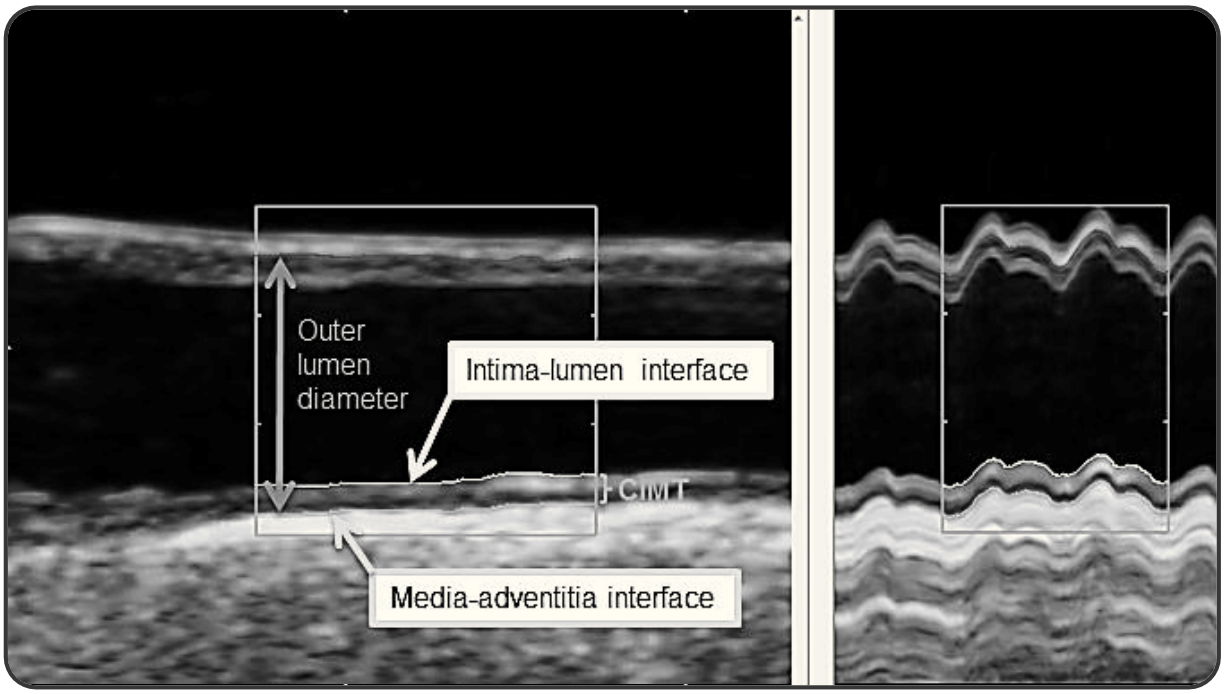

Figure 4.1: Ultrasound image
Ultrasound image of the common carotid (longitudinal axis) with tracing lines of automatic contour-detection at the lumen-intima and the media-adventitia interface. Outer lumen diameter in the common carotid artery in B-mode (left) over two heart cycles and with M-mode (right) generated by 180 single images of a this clip. The mean CIMT at the far wall was 0.87 mm.

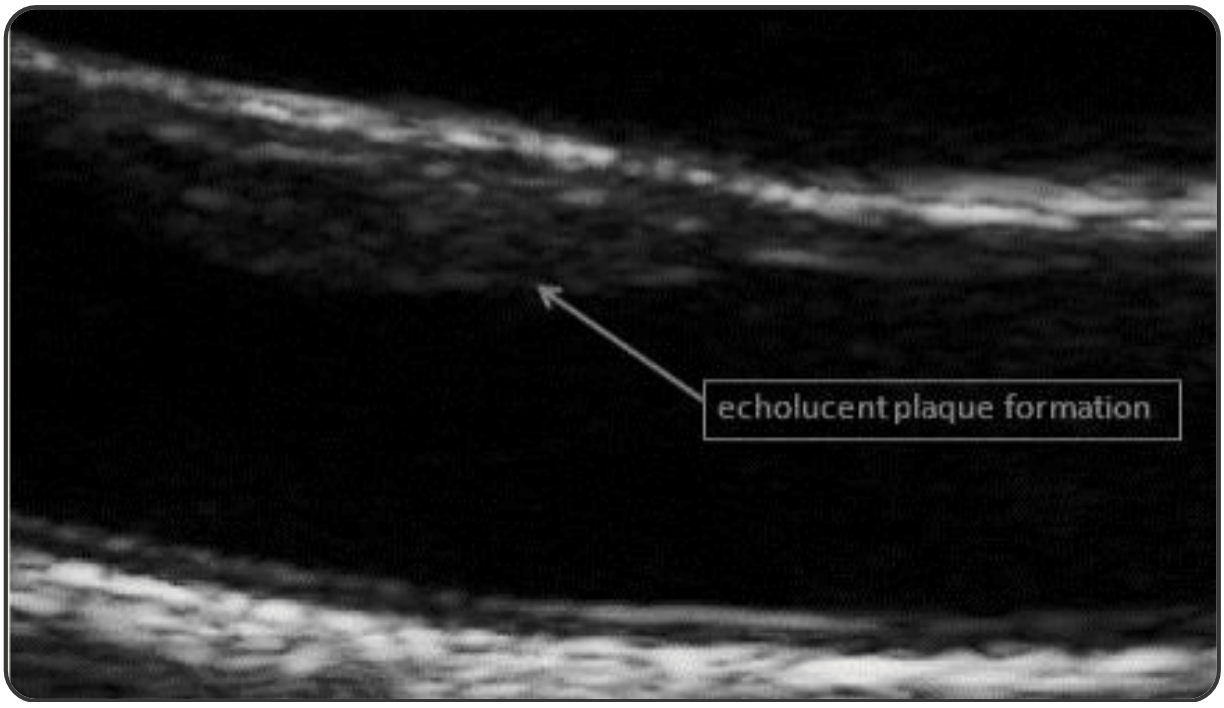

Figure 4.2: Carotid plaque formation
Echolucent carotid plaque formation at the near wall of the right common carotid artery (male study participant of the population-based Heinz Nixdorf Recall study) [19].

Proposals for a standardized preparation and performance of carotid ultrasound examinations

a) Preparation

1. Dimly lit room
2. Room temperature 22-25°C
3. Lying position
4. Examination position: end-of-heading; with neck extended
5. ECG-electrodes (control of heart cycles) [20]
6. Standardized head-position (45°-50°-position to the right / left)
7. Minimum requirements transducer: ultrasound frequencies 5-15 MHz (better: 10 MHz linear ultrasound transducer); appropriate depth of focus
8. Refresh rate: ≥25 Hz (minimal compression)
9. Gain: ~ 60 dB

b) Performance

1. Sequential-based records over 3-4 heart cycles (longitudinal axis)
2. optimal image-adjustment: visualizing the double line pattern of the carotid artery (near and far wall of the CCA)
3. Record of at least three fixed images (longitudinal axis)
   → Optimal: vertical dipping ultrasound-rays (artery in horizontal focus)
4. end-diastolic records of images /sequences (see “Preparation”)
5. Image-acquisition: ICA and ECA
   - Using Pulse waved Doppler to distinguish between ICA and ECA
   - ICA: greater caliber than ECA
   - ICA: no extracranial vessel branches (ECA: supine thyroid artery)
6. Sequential-based record of the vessel diameter (detection plaque formation)
   → Record of carotid plaque formation (longitudinal and transversal)

An ultrasound arc (see “Meijer-Arc”) can be used for longitudinal-records in anterior, middle and posterior position.

Different methods of CIMT-measurements

Originally, CIMT-measurements were performed via a manual method that could be integrated in ultrasound systems or per additional acquired software. However, the last few years it could be demonstrated that this method is associated with a higher reader-subjectivity compared to automatic or semiautomatic (automatic + manual correction) measurement software [21].

These can be implemented in the ultrasound system or can base a) on an image-analysis (contour-detection) (Figure 4.1) or b) on the analysis of radiofrequency signals. The radiofrequency - analysis is performed with single images or continuously over more heart cycles. It has been shown that sequentially-based CIMT-measurement contributes to an improved differentiation between subjects with and without coronary heart disease, compared to the analysis of a single image (AUC for mean CIMT 0.82 [95% CI 0.68 – 0.94] versus 0.64 [95% CI 0.55 – 0.80]) [22].

CIMT-measurement is possible in different segments of the carotid artery: in the CCA, CIMT is measured automatically over a distance about 1 cm [23]. Further vessel segments, like the ICA and ECA or CB are commonly only depictable over a limited distance. Therefore, maximum CIMT is measured in these segments. Up to now, the different measurement-localizations and –methods resulted in inconsistent CIMT-protocols. The limited standardization is the vulnerable weakness of this non-imaging method. Per electrocardiogram it is possible to standardize the CIMT-measurement during a complete heart cycle (R-wave) which is especially important in single images, because CIMT may vary between 5-10% during a heartbeat. Regarding the R-wave in the electrocardiogram, the wave represents the end-diastolic moment – the moment of the thickest CIMT [20,24]. Utilization of Meijer`s carotid arc is a further instrument to optimize reproducibility of CIMT-measurements over specific carotid segments and time. This semicircle is divided into 30° angles one after another (30°, 60°, 90°, 120°, …) and was used in different studies (e.g. METEOR study) to optimize data acquisition [25].

Reproducibility

Measurement-variability of CIMT depends on the carotid segment that is examined. The intraobserver variability of maximum CIMT in the CCA was found to be 0.14±0.16 mm and 0.13±011 mm for mean CIMT. The interobserver variability is reported to be slightly higher (0.20±0.26 mm and 0.18±0.24 mm, respectively) [26]. In repetitive CIMT-measurements of the CCA the absolute mean difference in the CCA was only 20-25% of ICA or the CB (0.11±0.08 mm vs. 0.60 up to 0.66 mm) [27]. Due to the speckle pattern caused by the differences of acoustic impedance between wall components the near wall of the CCA and CB have a slightly higher CIMT-variability compared to the far wall [28].

Compared to CB (76 up to 96%) and ICA (54 up to 81%) the CCA can be depicted in nearby all patients (94 up to 99%). This applies also for the far wall compared to near wall (CCA 97% vs. 88%, CB 87% vs. 80% and ICA 76 vs. 49%) [29,30]. Additionally, anatomic conditions and especially the experience of the reader influence the visibility considerably.

Normal CIMT-values

Classification of CIMT-values in healthy and pathological values varies considerable, depending on the CIMT measurement protocol. Therefore, conclusions from measured CIMT must be drawn, by consideration of the underlying protocol. Every reader should pay attention that the same measurement value in two different studies may be considered as normal on one and as pathologic on the other hand.

Nowadays, there are two main possibilities for the evaluation of CIMT-values: the utilization of a) fixed cut-off values or b) percentile-distribution. For fixed cut-off values, a prognostic value for prediction of future cardiovascular events has been established [11,16,31].

Fixed CIMT-values ≥1mm were described in the literature, however, such high values are not achieved in different studies which can be explained by in- or exclusion of plaque formation (see also page 3) [10,32,33]. The differences in observations have resulted in the utilization of percentile-distributions [34]. Both, fixed cut-off- and percentile values have been previously and base on different measurement protocols. [35]. Like other markers of subclinical atherosclerosis (e.g. coronary artery calcium, ankle brachial index), CIMT increases with an increased age [10]. In contrast to other well-known cardiovascular risk factors, normal and pathological values differ over the years. Hence, it is reasonable to provide gender- and age-depending CIMT-values in consideration of the CIMT-protocol. Generally, Stein et al. provided very detailed information about gender- and aged-stratified CIMT-values in different international studies and the respective measured carotid segments [35]. In order to simplify these values, Jäger et al. developed an equation for calculating an individual CIMT threshold value (mm) from calculations of average CIMT as a function of age: Decade of life/10+ 0.2mm [36]. CIMT-based percentile-values are supplied on the homepage of the Heinz Nixdorf Recall study for a general population between 45- and 75 years [19].

CIMT-Progression

CIMT-progression has been examined in different studies and depends on included regions of CIMT-measurement [10,37]. Recently, Lorenz et al. included n=36.984 participants from 16 different studies in a meta-analysis to investigate CIMT-progression as a predictor of cardiovascular events: the association remained unproven [38].However, due to well-known histopathological mechanisms in the intima-media complex (see above) and calculated cardiovascular risks being associated with an increased CIMT, the question of CIMT-interference via medical treatment raised up the last decade. Therefore, in many studies CIMT-regression and –progression, induced by medical treatment of lipid metabolism, were conducted. It could be demonstrated that 40-mg dose of rosuvastatin significantly decreased maximum CIMT-progression over 12 carotid segments (common carotid, carotid bulb, internal carotid) in middle-aged individuals [37]. Based on these METEOR-results, the predictive value of CIMT-measurement as a detection and monitoring tool in subjects with low Framingham risk score was shown [37]. The effect of other different LDL-lowering

therapies on the regression of CIMT could be proven in different studies [39–41]. Furthermore, in some studies the association between different statins and CIMT-regression were examined [42,43]. CIMT-measurement, used as a monitoring tool in lipid-lowering studies, suffered a practical test and passed it, when Kastelein et al. examined the "null-effect" of LDL-C: a combined therapy with ezetimibe and simvastatin did not result in a significant difference in changes in intima-media thickness, as compared with simvastatin alone [44]. Despite many comments in public, Brown et al. pointed out, that different preconditions led to the observed results [45].

Benefit of CIMT-measurements in addition to traditional and established cardiovascular risk stratification algorithms

Actual cardiovascular risk stratification relies on risk calculations that base on the inclusion of different traditional and established risk factors (e.g. Framingham Risk Score, European HEART Score). However, the solely utilization of these risk scores is afflicted with different problems. There is a variation in risk estimation in different populations [46], different endpoints in risk algorithms are evaluated (e.g. coronary morbidity, CV-mortality), many important lifestyle and other risk factors are not included in stratification algorithms [47], and cross-sectional risk assessment does not account for variation in risk factor exposure.

Up today, risk factors are used to classify cardiovascular risk in low, intermediate and high risk. Subjects with a low risk are recommended to modify risk factors, while high-risk subjects receive a medical therapy. The management for subjects with an intermediate risk is unsettled. These persons are recommended for further risk assessment including new markers of a subclinical atherosclerosis (for example CIMT-measurement). However, the value of CIMT-measurement in the cardiovascular risk stratification is still under debate.

For reclassification of cardiovascular risk via CIMT-measurement (according the Framingham Risk Score), the investigators of the Atherosclerosis Risk in Communities *Study* (ARIC-Study) provided data about 13.145 subjects (mean observation: 15.1 years) that were observed with regard to the onset of acute myocardial infarction, coronary death and cardiovascular revascularization [15]. A total of 16.7% subjects with intermediate risk (5-20% 10-year risk) could be reclassified.

Using CIMT- and carotid plaque-measurement in all carotid segments, a total of 9.9% subjects could be reclassified. In subjects with an intermediate risk (5-20%), 12.4% persons were reclassified in the low risk group and 10.8% in the high risk group. The number of reclassification was considerably lower in the Carotid Atherosclerosis Progression *Study* (CAPS-Study) (5.3%) [48]. However, in this study cardiovascular risk has been evaluated according the European Heart Score that calculate fatal cardiovascular events as the outcome [48]. So the data are not comparable with each other.

For subjects with a 10-year risk for cardiovascular events of 6-20%, the American Heart Association (AHA) finds CIMT-measurements reasonable. Hence, CIMT-measurement is one of the few biomarker that is attributed to the class IIa / level of evidence B. However, a suitable technical equipment and profound course of instruction and experience of the reader are conditions to keep up a high quality of the measurement method [49].

Whether an increased CIMT is able to support correct clinical decision making and lead to specified anti-atherosclerotic therapy has not been investigated in meaningful endpoint-studies until today. Additionally, appropriate clinical guidelines, with inclusion of CIMT-measurements in the treatment of patients as well as proof of cost-effectiveness are still missing.

Possible implementation of CIMT-measurements in the daily clinical practice, based on a combination of ASE-, AHA- and SHAPE-Task Force-recommendations

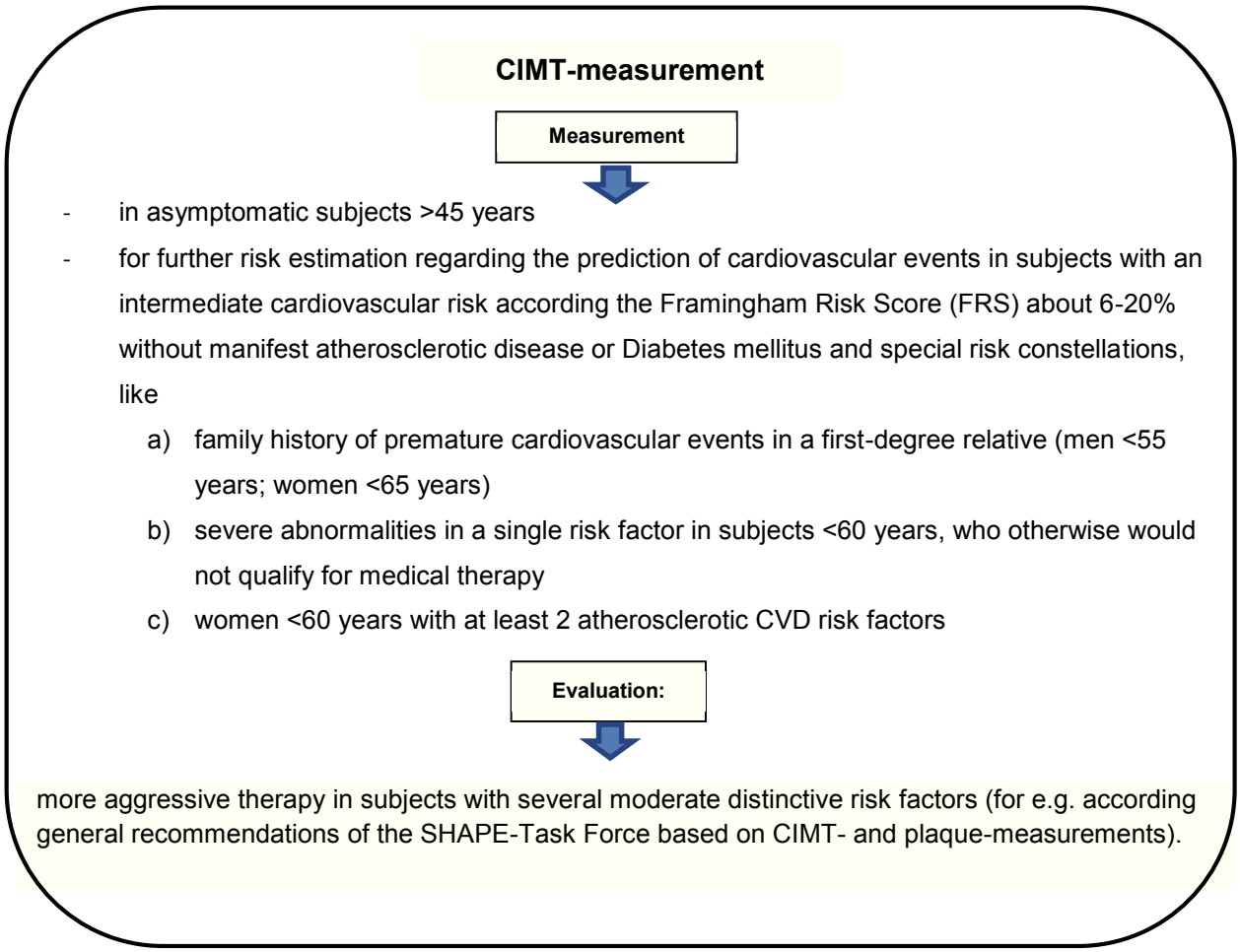

Figure 4.3: Potential implementation of CIMT-measurements in the daily clinical practice
General recommendation of SHAPE-Task Force [8]

Table 4.2: Potential clinical implementation of CIMT-measurements with respective suggestions of therapy

	CIMT	Carotid plaque formation	Therapy	LDL-C
moderate high risk	<1mm and 50.-75. Pctl.	no plaque	lifestyle modification	<130mg/dl (<3.37 mmol/l)
high risk	≥1mm or >75. Pctl.	<50% stenosis	agressive lifestyle modification	<100mg/dl (<2.59 mmol/l)
very high risk	≥1mm or >75. Pctl.	≥50% stenosis	agressive lifestyle modification	<70mg/dl (<1.82 mmol/l) myocardial ischemic test

Lifestyle modification = lifestyle modifications and a LDL-C target of <130 mg/dl (<3.37 mmol/l); targeting to <100 mg/dl (<2.59 mmol/l) is optional.
Aggressive Lifestyle modification = lifestyle modifications and a LDL-C target of <100 mg/dl (<2.59 mmol/l); targeting to <70 mg/dl (<1.82 mmol/l) is optional.

Quantification of carotid plaque formation or CIMT-measurement

Embedding carotid plaque formation in CIMT-measurements is debatable, since they are reported to be different biological and genetic atherosclerotic phenotypes [50]. It is certain that plaque formation is always pathologic. In a meta-analysis about 11 population-based studies (n=54.336 subjects) carotid plaque formation had a significantly higher diagnostic precision in the prediction of myocardial infarction compared to an increased CIMT (AUC 0.64 vs. 0.61) [51]. The additive inclusion of plaque formation in CIMT-measurements may improve risk prediction of coronary heart disease [15].

The last three decades, quantification and evaluation of carotid plaque formation changed remarkable. In addition to cursory assessments like degree of stenosis and echogenicity, other distinctive features were used to investigate carotid plaques. The most common used criteria for plaque investigation are echogenicity (echolucent, echogenic, mixed echogenicity), echogenic distribution pattern (homogeneous versus inhomogeneous) and evaluation of surface structure (regular versus irregular). Furthermore, measurement of two dimensions (2D) and three-dimensions (3D) are used to quantify total plaque area and total plaque volume [52,53]. Latterly, plaque vascularization on contrast-enhanced ultrasound are developed to optimize cardiovascular risk prediction [54,55]. Because the prevalence of carotid plaques in a population at 60 years is 60-90% [56] it seems to be of additional benefit for risk prediction to take this different ultrasound derived pattern into consideration at least in an elderly population. Finally both, quantification of CIMT and carotid plaque formation provide different information of the atherosclerotic status and burden in the carotid artery. Taking together these two parameters has been shown to result in a superior risk prediction for coronary heart disease than with one of the parameters alone [15].

Conclusion

CIMT-measurement and plaque detection are already established measurement methods for detection of subclinical atherosclerosis in many studies. However, solid and efficient trainings of sonographer and reader are required for sufficient CIMT-quantification in studies as well as daily clinical practice. Among different CIMT-measurement methods, automatically based methods show the highest reproducibility. To differ between normal and pathological CIMT values an exact consideration of the underlying measurement protocol is obligatory. CIMT-measurement is a suitable method for an improvement of risk stratification in subjects with an intermediate risk factor profile above traditional atherosclerotic risk factors. The combination of CIMT-measurement and quantification of carotid plaque formation further increases the predictive value for first cardiovascular events.

References

1 Maier, H. (1987) Präparierkurs Präparieranweisungen und Theorie 2., erw. Aufl., Deutscher Ärzte-Verlag, Köln. 1-475

2 Faxon, D. P., Creager, M. A., Smith, S. C., Jr, Pasternak, R. C., Olin, J. W., Bettmann, M. A., Criqui, M. H., Milani, R. V., Loscalzo, J., Kaufman, J. A., Jones, D. W. and Pearce, W. H. (2004) Atherosclerotic Vascular Disease Conference: Executive summary: Atherosclerotic Vascular Disease Conference proceeding for healthcare professionals from a special writing group of the American Heart Association. Circulation. **109**, 2595–2604

3 Ross, R., Faggiotto, A., Bowen-Pope, D. and Raines, E. (1984) The role of endothelial injury and platelet and macrophage interactions in atherosclerosis. Circulation. **70**, III77–82

4 Gossl, M., Lerman, L. O. and Lerman, A. (2007) Frontiers in nephrology: early atherosclerosis--a view beyond the lumen. J. Am. Soc. Nephrol. JASN. **18**, 2836–2842

5 Weber, C. and Noels, H. (2011) Atherosclerosis: current pathogenesis and therapeutic options. Nat. Med. **17**, 1410–1422

6 Hansson, G. K. (2005) Inflammation, atherosclerosis, and coronary artery disease. N. Engl. J. Med. **352**, 1685–1695

7 Hegele, R. A. (1996) The pathogenesis of atherosclerosis. Clin. Chim. Acta Int. J. Clin. Chem. **246**, 21–38

8 Johnsen, S. H. and Mathiesen, E. B. (2009) Carotid plaque compared with intima-media thickness as a predictor of coronary and cerebrovascular disease. Curr. Cardiol. Rep. **11**, 21–27

9 Pignoli, P., Tremoli, E., Poli, A., Oreste, P. and Paoletti, R. (1986) Intimal plus medial thickness of the arterial wall: a direct measurement with ultrasound imaging. Circulation. **74**, 1399 –1406

10 Bauer, M., Möhlenkamp, S., Lehmann, N., Schmermund, A., Roggenbuck, U., Moebus, S., Stang, A., Mann, K., Jöckel, K.-H. and Erbel, R. (2009) The effect of age and risk factors on coronary and carotid artery atherosclerotic burden in males—Results of the Heinz Nixdorf Recall Study. Atherosclerosis. **205**, 595–602

11 Chambless, L. E., Heiss, G., Folsom, A. R., Rosamond, W., Szklo, M., Sharrett, A. R. and Clegg, L. X. (1997) Association of Coronary Heart Disease Incidence with Carotid Arterial Wall Thickness and Major Risk Factors: The Atherosclerosis Risk in Communities (ARIC) Study, 1987–1993. Am. J. Epidemiol. **146**, 483 –494

12 O'Leary, D. H., Polak, J. F., Kronmal, R. A., Manolio, T. A., Burke, G. L. and Wolfson, S. K. (1999) Carotid-artery intima and media thickness as a risk factor for myocardial infarction and stroke in older adults. Cardiovascular Health Study Collaborative Research Group. N. Engl. J. Med. **340**, 14–22

13 Iglesias del Sol, A., Bots, M. L., Grobbee, D. E., Hofman, A. and Witteman, J. C. M. (2002) Carotid intima-media thickness at different sites: relation to incident myocardial infarction. The Rotterdam Study. Eur. Heart J. **23**, 934 –940

14 Lorenz, M. W., von Kegler, S., Steinmetz, H., Markus, H. S. and Sitzer, M. (2006) Carotid intima-media thickening indicates a higher vascular risk across a wide age range: prospective data from the Carotid Atherosclerosis Progression Study (CAPS). Stroke. **37**, 87 –92

15 Nambi, V., Chambless, L., Folsom, A. R., He, M., Hu, Y., Mosley, T., Volcik, K., Boerwinkle, E. and Ballantyne, C. M. (2010) Carotid intima-media thickness and presence or absence of plaque improves prediction of coronary heart disease risk: the ARIC (Atherosclerosis Risk In Communities) study. J. Am. Coll. Cardiol. **55**, 1600–1607

16 Plichart, M., Celermajer, D. S., Zureik, M., Helmer, C., Jouven, X., Ritchie, K., Tzourio, C., Ducimetière, P. and Empana, J.-P. (2011) Carotid intima-media thickness in plaque-free site, carotid plaques and coronary heart disease risk prediction in older adults. The Three-City Study. Atherosclerosis. **219**, 917–924

17 Touboul, P.-J., Hennerici, M. G., Meairs, S., Adams, H., Amarenco, P., Bornstein, N., Csiba, L., Desvarieux, M., Ebrahim, S., Fatar, M., Hernandez Hernandez, R., Jaff, M., Kownator, S., Prati, P., Rundek, T., Sitzer, M., Schminke, U., Tardif, J.-C., Taylor, A., Vicaut, E., Woo, K. S., Zannad, F. and Zureik, M. (2007) Mannheim carotid intima-media thickness consensus (2004-2006). An update on behalf of the Advisory Board of the 3rd and 4th Watching the Risk Symposium, 13th and 15th European Stroke Conferences, Mannheim, Germany, 2004, and Brussels, Belgium, 2006. Cerebrovasc. Dis. Basel Switz. **23**, 75–80

18 Dogan, S., Kastelein, J. J. P., Grobbee, D. E. and Bots, M. L. (2011) Mean common or mean maximum carotid intima-media thickness as primary outcome in lipid-modifying intervention studies. J. Atheroscler. Thromb. **18**, 946–957

19 Heinz Nixdorf Recall Studie. Retrieved January 10, 2012, from https://www.uni-due.de/recall-studie/

20 Oren, A., Vos, L. E., Uiterwaal, C. S. P. M., Grobbee, D. E. and Bots, M. L. (2003) Cardiovascular risk factors and increased carotid intima-media thickness in healthy young adults: the Atherosclerosis Risk in Young Adults (ARYA) Study. Arch. Intern. Med. **163**, 1787–1792

21 Kanters, S. D., Algra, A., van Leeuwen, M. S. and Banga, J. D. (1997) Reproducibility of in vivo carotid intima-media thickness measurements: a review. Stroke J. Cereb. Circ. **28**, 665–671

22 Haller, C., Schulz, J., Schmidt-Trucksäss, A., Burkardt, H., Schmitz, D., Dickhuth, H.-H. and Sandrock, M. (2007) Sequential based analysis of Intima-Media Thickness (IMT) in common carotid artery studies. Atherosclerosis. **195**, e203–e209

23 Cheng, D., Schmidt-Trucksäss, A., Cheng, K. and Burkhardt, H. (2002) Using snakes to detect the intimal and adventitial layers of the common carotid artery wall in sonographic images. Comput. Methods Programs Biomed. **67**, 27–37

24 Jourdan, C., Wühl, E., Litwin, M., Fahr, K., Trelewicz, J., Jobs, K., Schenk, J.-P., Grenda, R., Mehls, O., Tröger, J. and Schaefer, F. (2005) Normative values for intima-media thickness and distensibility of large

arteries in healthy adolescents. J. Hypertens. **23**, 1707–1715

25 Bots, M. L., Evans, G. W., Riley, W. A. and Grobbee, D. E. (2003) Carotid intima-media thickness measurements in intervention studies: design options, progression rates, and sample size considerations: a point of view. Stroke. **34**, 2985

26 O'Leary, D. H., Polak, J. F., Wolfson, S. K., Bond, M. G., Bommer, W., Sheth, S., Psaty, B. M., Sharrett, A. R. and Manolio, T. A. (1991) Use of sonography to evaluate carotid atherosclerosis in the elderly. The Cardiovascular Health Study. CHS Collaborative Research Group. Stroke J. Cereb. Circ. **22**, 1155–1163

27 Lorenz, M. W., Markus, H. S., Bots, M. L., Rosvall, M. and Sitzer, M. (2007) Prediction of Clinical Cardiovascular Events With Carotid Intima-Media Thickness. Circulation. **115**, 459 –467

28 Stensland-Bugge, E., Bønaa, K. H. and Joakimsen, O. (1997) Reproducibility of ultrasonographically determined intima-media thickness is dependent on arterial wall thickness. The Tromsø Study. Stroke J. Cereb. Circ. **28**, 1972–1980

29 Montauban van Swijndregt, A. D., De Lange, E. E., De Groot, E. and Ackerstaff, R. G. . (1999) An in vivo evaluation of the reproducibility of intima-media thickness measurements of the carotid artery segments using B-mode ultrasound. Ultrasound Med. Biol. **25**, 323–330

30 Espeland, M. A., Craven, T. E., Riley, W. A., Corson, J., Romont, A. and Furberg, C. D. (1996) Reliability of Longitudinal Ultrasonographic Measurements of Carotid Intimal-Medial Thicknesses. Stroke. **27**, 480 –485

31 Bots, M. L., Hoes, A. W., Koudstaal, P. J., Hofman, A. and Grobbee, D. E. (1997) Common Carotid Intima-Media Thickness and Risk of Stroke and Myocardial Infarction : The Rotterdam Study. Circulation. **96**, 1432 –1437

32 Salonen, J. and Salonen, R. (1991) Ultrasonographically assessed carotid morphology and the risk of coronary heart disease. Arterioscler. Thromb. Vasc. Biol. **11**, 1245 –1249

33 Chambless, L. E., Folsom, A. R., Clegg, L. X., Sharrett, A. R., Shahar, E., Nieto, F. J., Rosamond, W. D. and Evans, G. (2000) Carotid Wall Thickness is Predictive of Incident Clinical Stroke: The Atherosclerosis Risk in Communities (ARIC) Study. Am. J. Epidemiol. **151**, 478 –487

34 Naghavi, M., Falk, E., Hecht, H. S., Jamieson, M. J., Kaul, S., Berman, D., Fayad, Z., Budoff, M. J., Rumberger, J., Naqvi, T. Z., Shaw, L. J., Faergeman, O., Cohn, J., Bahr, R., Koenig, W., Demirovic, J., Arking, D., Herrera, V. L. M., Badimon, J., Goldstein, J. A., Rudy, Y., Airaksinen, J., Schwartz, R. S., Riley, W. A., Mendes, R. A., Douglas, P. and Shah, P. K. (2006) From Vulnerable Plaque to Vulnerable Patient—Part III: Executive Summary of the Screening for Heart Attack Prevention and Education (SHAPE) Task Force Report. Am. J. Cardiol. **98**, 2–15

35 Stein, J. H., Korcarz, C. E., Hurst, R. T., Lonn, E., Kendall, C. B., Mohler, E. R., Najjar, S. S., Rembold, C. M. and Post, W. S. (2008) Use of carotid ultrasound to identify subclinical vascular disease and evaluate cardiovascular disease risk: a consensus statement from the American Society of Echocardiography Carotid Intima-Media Thickness Task Force endorsed by the Society for Vascular Medicine. J. Am. Soc. Echocardiogr. **21**, 93–111

36 Jäger, K. A. and Staub, D. (2009) Did you measure the intima-media thickness? Ultraschall Med. Stuttg. Ger. 1980. **30**, 434–437

37 Crouse, J. R., 3rd, Raichlen, J. S., Riley, W. A., Evans, G. W., Palmer, M. K., O'Leary, D. H., Grobbee, D. E. and Bots, M. L. (2007) Effect of rosuvastatin on progression of carotid intima-media thickness in low-risk individuals with subclinical atherosclerosis: the METEOR Trial. JAMA J. Am. Med. Assoc. **297**, 1344–1353

38 Lorenz, M. W., Polak, J. F., Kavousi, M., Mathiesen, E. B., Völzke, H., Tuomainen, T.-P., Sander, D., Plichart, M., Catapano, A. L., Robertson, C. M., Kiechl, S., Rundek, T., Desvarieux, M., Lind, L., Schmid, C., DasMahapatra, P., Gao, L., Ziegelbauer, K., Bots, M. L. and Thompson, S. G. (2012) Carotid intima-media thickness progression to predict cardiovascular events in the general population (the PROG-IMT collaborative project): a meta-analysis of individual participant data. Lancet. **379**, 2053–2062

39 Salonen, R., Nyyssönen, K., Porkkala, E., Rummukainen, J., Belder, R., Park, J. S. and Salonen, J. T. (1995) Kuopio Atherosclerosis Prevention Study (KAPS). A population-based primary preventive trial of the effect of LDL lowering on atherosclerotic progression in carotid and femoral arteries. Circulation. **92**, 1758–1764

40 Hodis, H. N., Mack, W. J., LaBree, L., Selzer, R. H., Liu, C., Liu, C., Alaupovic, P., Kwong-Fu, H. and Azen, S. P. (1996) Reduction in carotid arterial wall thickness using lovastatin and dietary therapy: a randomized controlled clinical trial. Ann. Intern. Med. **124**, 548–556

41 De Groot, E., Jukema, J. W., van Boven, A. J., Reiber, J. H., Zwinderman, A. H., Lie, K. I., Ackerstaff, R. A. and Bruschke, A. V. (1995) Effect of pravastatin on progression and regression of coronary atherosclerosis and vessel wall changes in carotid and femoral arteries: a report from the Regression Growth Evaluation Statin Study. Am. J. Cardiol. **76**, 40C–46C

42 Taylor, A. J., Kent, S. M., Flaherty, P. J., Coyle, L. C., Markwood, T. T. and Vernalis, M. N. (2002) ARBITER: Arterial Biology for the Investigation of the Treatment Effects of Reducing Cholesterol: a randomized trial comparing the effects of atorvastatin and pravastatin on carotid intima medial thickness. Circulation. **106**, 2055–2060

43 Smilde, T. J., van Wissen, S., Wollersheim, H., Trip, M. D., Kastelein, J. J. and Stalenhoef, A. F. (2001) Effect of aggressive versus conventional lipid lowering on atherosclerosis progression in familial hypercholesterolaemia (ASAP): a prospective, randomised, double-blind trial. Lancet. **357**, 577–581

44 Kastelein, J. J. P., Akdim, F., Stroes, E. S. G., Zwinderman, A. H., Bots, M. L., Stalenhoef, A. F. H., Visseren, F. L. J., Sijbrands, E. J. G., Trip, M. D., Stein, E. A., Gaudet, D., Duivenvoorden, R., Veltri, E. P., Marais, A. D. and de Groot, E. (2008) Simvastatin with or without ezetimibe in familial

hypercholesterolemia. N. Engl. J. Med. **358**, 1431–1443

45 Brown, B. G. and Taylor, A. J. (2008) Does ENHANCE diminish confidence in lowering LDL or in ezetimibe? N. Engl. J. Med. **358**, 1504–1507

46 Hense, H.-W., Schulte, H., Löwel, H., Assmann, G. and Keil, U. (2003) Framingham risk function overestimates risk of coronary heart disease in men and women from Germany--results from the MONICA Augsburg and the PROCAM cohorts. Eur. Heart J. **24**, 937–945

47 Mozaffarian, D., Wilson, P. W. F. and Kannel, W. B. (2008) Beyond Established and Novel Risk Factors Lifestyle Risk Factors for Cardiovascular Disease. Circulation. **117**, 3031–3038

48 Lorenz, M. W., Schaefer, C., Steinmetz, H. and Sitzer, M. (2010) Is carotid intima media thickness useful for individual prediction of cardiovascular risk? Ten-year results from the Carotid Atherosclerosis Progression Study (CAPS). Eur. Heart J. **31**, 2041 –2048

49 Greenland, P., Lauer, M. S., Beller, G. A., Benjamin, E. J., Budoff, M. J., Fayad, Z. A., Foster, E., Hlatky, M. A., Hodgson, J. M., Kushner, F. G., Lauer, M. S., Shaw, L. J., Smith, S. C., Taylor, A. J., Weintraub, W. S. and Wenger, N. K. (2010) 2010 ACCF/AHA Guideline for Assessment of Cardiovascular Risk in Asymptomatic Adults. Circulation. **122**, e584 –e636

50 Spence, J. (2008) The importance of distinguishing between diffuse carotid intima-media thickening and focal plaque. Can. J. Cardiol. **24**, 61C–64C

51 Inaba, Y., Chen, J. A. and Bergmann, S. R. (2012) Carotid plaque, compared with carotid intima-media thickness, more accurately predicts coronary artery disease events: A meta-analysis. Atherosclerosis. **220**, 128–133

52 Spence, J. D. and Hegele, R. A. (2004) Non-invasive assessment of atherosclerosis risk. Curr. Drug Targets Cardiovasc. Haematol. Disord. **4**, 125–128

53 Landry, A., Spence, J. D. and Fenster, A. (2005) Quantification of carotid plaque volume measurements using 3D ultrasound imaging. Ultrasound Med. Biol. **31**, 751–762

54 Staub, D., Partovi, S., Schinkel, A. F. L., Coll, B., Uthoff, H., Aschwanden, M., Jaeger, K. A. and Feinstein, S. B. (2011) Correlation of carotid artery atherosclerotic lesion echogenicity and severity at standard US with intraplaque neovascularization detected at contrast-enhanced US. Radiology. **258**, 618–626

55 Staub, D., Patel, M. B., Tibrewala, A., Ludden, D., Johnson, M., Espinosa, P., Coll, B., Jaeger, K. A. and Feinstein, S. B. (2010) Vasa vasorum and plaque neovascularization on contrast-enhanced carotid ultrasound imaging correlates with cardiovascular disease and past cardiovascular events. Stroke J. Cereb. Circ. **41**, 41–47

56 Joakimsen, O., Bonaa, K. H., Stensland-Bugge, E. and Jacobsen, B. K. (1999) Age and sex differences in the distribution and ultrasound morphology of carotid atherosclerosis: the Tromsø Study. Arterioscler. Thromb. Vasc. Biol. **19**, 3007–3013

CHAPTER 5

An automated, interactive analysis system for ultrasound sequences of the common carotid artery

Authors
Alexandra Teynor
Seraina Caviezel
Julia Dratva
Nino Künzli
Arno Schmidt-Trucksäss

Accepted version*, published in:
Ultrasound in Med. & Biol., Vol. 38, No. 8, pp. 1440–1450, 2012
Doi: 10.1016/j.ultrasmedbio.2012.03.015

*Minor editorial modifications possible due to harmonisation of the thesis

AN AUTOMATED, INTERACTIVE ANALYSIS SYSTEM FOR ULTRASOUND SEQUENCES OF THE COMMON CAROTID ARTERY

Abstract:

Structural parameters of the Common Carotid Artery (CCA) have shown to correlate with the risk of cardiovascular disease (CVD), but their precise measurement is challenging. We developed an automatic detection system with manual interaction capabilities that can reliably analyze B-Mode ultrasound sequences of the CCA over several heart cycles.

We evaluated 3824 frames from 40 sequences in two data qualities. Two readers measured the intima media thickness (CIMT) and the lumen diameter at two evaluation times (T1/T2).

A Bland-Altman analysis of the average CIMT showed a bias ± SD of 0.002 ± 0.010 mm (T1), -0.004 ± 0.008 mm (T2) for completely automatic detections, and -0.004 ± 0.010 mm (T1), -0.003 ± 0.010 mm (T2) for clips with manual corrections.

The combination of automated analysis and manual intervention provides precise parameters as biomarkers for the atherosclerotic process and makes the system suitable for large scale epidemiological research, diagnostic and clinical practice.

Key Words: Common carotid artery, Intima media thickness, Lumen diameter, Automatic ultrasound analysis, Dual dynamic programming, Interactive system.

Introduction

Structural parameters of the common carotid artery (CCA) as the carotid intima media thickness (CIMT) have shown to correlate with the risk of cardiovascular diseases (CVD) [1–3]. It is confirmed by many studies that a change in wall structure is a reaction to numerous physiological and pathological factors as age or hypertension [4–6]. They showed that the CIMT is not only a surrogate marker, but a direct consequence of the atherosclerotic process.

CIMT and lumen diameter (LD) measurements can be achieved using B-Mode ultrasound images (e.g. Gustavsson et al. [7], Touboul et al. [8] an Wendelhaag et al. [9]) or directly by radio frequency based measurement methods (e.g. Hoeks et al. [10] or Rossi et al. [11]). While the latter ones are known for a higher resolution [12] and avoid image processing artifacts [13], B-Mode images are still the prevalent output of conventional US-machines, and thus the focus of this article.

Reliable CIMT measurements in B-Mode US images are challenging in practice due to the varying quality of ultrasound images dependent on the training of the sonographer, the imaging protocols, the ultrasound settings, and the patient's anatomy. Speckle noise, echo

dropouts and motion artifacts are resulting issues. Thus, completely automated analysis systems will fail in some cases. Completely manual reading systems are not ideal either, since they are very time consuming and have shown higher variability than automatic systems [14].

The measurement of relevant parameters from ultrasound images of the CCA has been attempted already since 1991 [9]. Soon it became evident that a manual, at worst single point measurement of the CIMT is insufficient as a reliable description of the condition of a cardiovascular system. (Semi-) automated methods were soon presented, the earliest by Touboul et al. [8], where intensity gradients in the ultrasound image were used as features. Cheng et al. [15] presented a snake based algorithm that has shown low intra- and inter-reader variability [14], however the exact initialization of the algorithm has shown to be difficult. Loizou et al. [16] improved on this by extended preprocessing and an improved initialization procedure. Gustavsson et al. [7] used a dynamic programming approach and more complex features as edge strength, echo intensity and continuity, together with learned weight factors. Cheng et al. [17] extended this concept again to detect double contours, which lead to more stable detection results.

Because of increased computational power and more sophisticated analysis algorithms, it became possible to analyze ultrasound sequences over several heart cycles. This revealed a variation of the CIMT of around 10% during a heart cycle [18]. Haller et al. [19] showed that a sequential analysis can offer a more extensive examination of the carotid wall than when using single images.

The most recent systems for automated CIMT analysis were presented by Faita et al. [20], Lee et al. [21] and Molinari et al. [22]. Faita et al. [20] developed an automatic real-time analysis system with a heuristic search for measuring the CIMT. The system has shown very low inter- and intra- reader variability, but is only able to process single images. Misdetections had to be discarded from their evaluations. Lee et al. [21] used directional features for their dynamic programming approach in single images. This has shown to be an advantage for skewed or bended arteries. Molinari et al. [22] presented two image analysis systems, CULEXsa and CALEXia. The first one is based on signal analysis; the second one is based on an integrated approach using feature extraction, line fitting and classification. They deliberately disclaim the use of manual correction, which leads to about 15% data that cannot be evaluated by CULEXsa.

The aim of this article is to present and evaluate a novel analysis program, **DY**namic **AR**tery **A**nalysis (DYARA), with superior detection and analysis capabilities. It automatically measures the CIMT and the arterial LD in every time frame in ultrasound sequences and offers the automatic detection of the minimum and maximum distention of the artery during a heart cycle. Whenever an automatic detection step fails due to a difficult imaging situation, the user can correct this instead of simply discarding the data. The system can reliably analyze B-Mode sequences of different quality over several heart cycles, thus making it suitable for large scale clinical research.

Materials and Methods

Study design

For the evaluation of the system, we use data of participants of the SAPALDIA study (**S**wiss Cohort Study on **A**ir **P**ollution **a**nd **L**ung and Heart **D**iseases **I**n **A**dults. SAPALDIA is a multi-center cohort study in eight Swiss urban and rural regions, representing a broad range of environmental conditions (Aarau, Basel, Davos, Genève, Lugano, Montana, Payerne, Wald) [23,24]. The cohort population was recruited in 1991 as a population–based, random sample of adults, 18–60 years) to study the impact of air pollution on respiratory health. The second survey in 2001/02 extended the original study question to CVD. The third survey held in 2010/11 repeated the measures and data collection of the earlier survey and included an ultrasound examination of the carotid artery in participants > 50 years of age (N ≈ 4000). The Institute of Exercise and Health Sciences is responsible for all readings of the recorded ultrasound sequences using the DYARA analysis system. The study protocol was approved by the ethical committees of the respective cantons, in which the study centers are situated. Participants gave written informed consent prior to participating in the study.

The analyzed ultrasound images for this article belong to 40 study subjects with a mean age (SD) of 65.6 (8.7) years, range 52-81 years, including 19 females and 21 males. For each participant, ultrasound sequences of the CCA were acquired with Fukuda Denshi UF-870 scanners equipped with a 10 MHz transducer. We used 20 consecutive clips where all readers agreed that the automatic detection was flawless (CA = completely automatic) and 20 consecutive clips were some corrections of the automatic tracing result were necessary (MA = manual and automatic). The temporal resolution of the US system is 10.7 ms per frame, i.e. 93 Hz. The datasets were analyzed over one entire heart cycle, each containing 68 to 131 single images depending on the heart rate. Altogether 3824 frames were analyzed by two experienced readers (R1 and R2) at two different points in time (T1 and T2), at least one day apart. Since CIMT is dependent on the location in the carotid tree, one reader marked the proximal start of the 1cm measurement region according to the Mannheim Consensus [25]. Then both readers were free to set the beginning of the evaluation box (region of interest) in an interval of 30 pixels around this marker. The analyzed clips started and ended in end-diastole. Typically, every research group evaluates their image analysis system with a different dataset, which makes a comparison of the results very difficult. To facilitate direct comparability, we make the complete dataset and the respective analysis of each patient, which was used for the evaluation of the program freely available upon request.

Ultrasound examination

The ultrasound clips for SAPALDIA were acquired in a standardized way. Each subject rested in supine position on an examination couch prior to the examination by a trained and certified sonographer. The head of the patient was rotated by 45° to either the left or the right side. For each patient, four locations were scanned: the left and right CCA, each from

ear to ear as well as horizontally. All clips had to follow certain criteria: the carotid bulb had to be visible on the left side of the image, the artery positioned horizontally and the intimal and adventitial boundaries of the arterial wall had to be visible over at least a 1cm segment proximal to the bulb for at least three heart cycles. In this study, one scan position per subject was used for the analysis.

Ultrasound analysis

After the ultrasound examination, the datasets were sent to the SAPALDIA data management, checked and then sent to the reading center (Department of Sports Medicine, University Basel). There the clips were analyzed with the novel analysis program. The readers set a 1cm bounding box (266 pixels) in the x-y view (B-Mode view) of the program to indicate where the measurements should be performed. Then the number of heart cycles to be analyzed was marked in the x-z view (virtual M-Mode). The lumen-intima (LIF) and the media-adventitia interfaces of the far wall (MAF), as well as the media-adventitia interface of the near wall (MAN) were then automatically detected and the results could be verified in both views. A schematic diagram of the interfaces is shown in Figure 5.1. Examples of original images with detected lines are shown in Figure 5.2.

Whenever a misdetection took place by the automatic analysis due to difficult data quality, the reader could supersede the result by editing the respective contour as much as necessary. When the reader has controlled all tracing results, CIMT and LD were calculated for the selected region. Markers of the arterial minimum (end-diastole) and the maximum distention (systole) were displayed automatically. Additionally, the readers traced 5 consecutive frames of each dataset completely manual (CM), resulting in 5 x 40 = 200 manually traced images per reader and time point. The temporal location of the 5 frames was distributed evenly between systole and diastole over the datasets. From these tracings, a manual ground truth was created by averaging the contours for both readers and evaluation times for each clip.

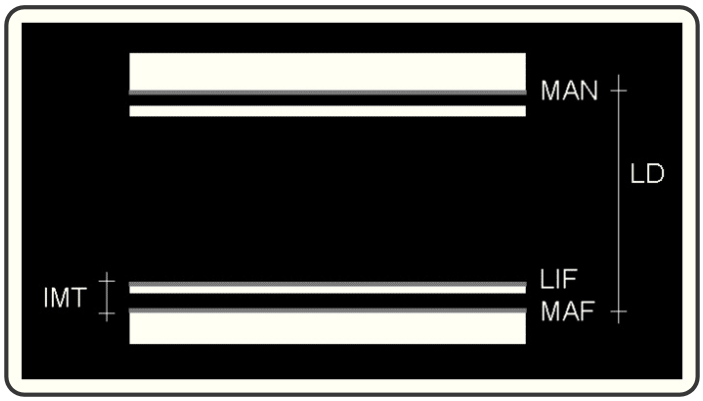

Figure 5.1: Artery interfaces
Media adventitia oft the near wall (MAN), lumen intima (LIF) and media adventitia (MAF) interface of the far wall. The lumen diameter (LD) and the carotid intima media thickness (CIMT) are also marked.

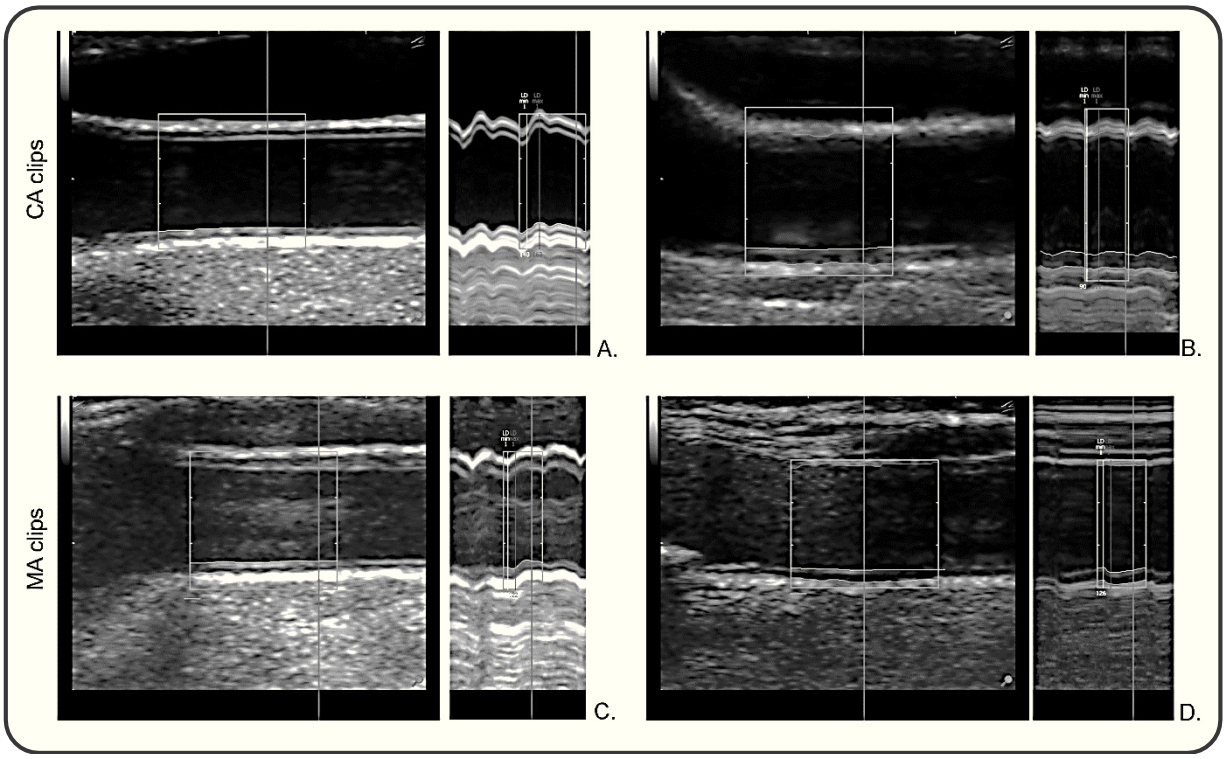

Figure 5.2: Examples of original common carotid artery sequences with bounding boxes and sample detection

A.& B.: Clips that could be analyzed completely automatically (CA). C.& D.: Clips were manual corrections had to be performed (MA). For each sequence, an x-y view (current B-mode frame, left side) and an x-z view (virtual M-mode, right side) are shown. Vertical red lines in either view indicate the location of the other view, respectively. The white vertical line shows the minimum lumen diameter in end-diastole and the blue line shows the maximum lumen diameter in systole.

Detection Algorithm

To achieve a system with low measurement variability, a key ingredient is a robust, repeatable contour detection algorithm that minimizes manual correction steps to the absolute minimum. In this work, we use a dual dynamic programming approach based on the work of Cheng et al. [17]. However, instead of using the original image features presented in their work, we use features inspired by Gustavsson et al. [7], which have proven to be more robust in our experiments.

The idea of dynamic programming is to build a solution to a problem from (simpler) sub-problems, instead of trying to solve everything at once. In our case, we try to calculate optimal contours based on evolving them pixel by pixel, from left to right in each frame of the clip. Optimality in this case is defined by a cost function that describes how well our solution fits to a model that we have defined. We look at a $M \times N$ pixel grid, with $(0,0)$ being in the upper left corner, representing the ultrasound image area under consideration. Let $f(x,y)$ be a normalized image feature with $0 \leq f(x,y) \leq 1$. We are simultaneously looking for a contour $B_1 = \{(x_1, y1_1), (x_2, y1_2), \dots, (x_N, y1_N)\}$ representing the LIF boundary together with a contour $B_2 = \{(x_1, y2_1), (x_2, y2_2), \dots, (x_N, y2_N)\}$, representing the MAF boundary.

In order to obtain an anatomically plausible solution, we enforce certain constraints. The first one is that $1 \leq y1 \leq y2 \leq M$. This prevents the boundaries from crossing each other. A second one is that the contours must have a minimal and maximal distance (d_{min}, d_{max}), where $0 < d_{min} < d_{max} \ll M$ and $d_{min} < y2 - y1 < d_{max}$. To ensure a certain degree of smoothness of each contour, we use a parameter $d_r > 0$ which indicates in which range a contour might deviate from the previous stage. Small values of d_r will lead to smooth contours. Since we consider two contours simultaneously, we can also restrict the change in distance between the curves. This parameter is referred to as dual curve smoothness and is defined as $|w_x - w_{x-1}|$ where $w_x = y2_x - y1_x$.

For the calculation of the cost function, we use two different established feature terms. The main role plays the gradient magnitude of the derivation of the ultrasound image in y direction. Since we are going to minimize a cost function later on, we use 1 minus the gradient magnitude and denote it as $f_G(x, y)$. Using only this term would force a contour to lie exactly at the point with the highest degree of intensity change. In order to tweak the detected contours towards a boundary that would be manually chosen, we also use the intensity image itself as proposed by Gustavsson et al. [7]: we calculate the average "brightness below" a contour as well as the average "darkness above" inside a predefined area and combine this to a feature $f_I(x, y)$.

The cost function used in this work then looks like:

$$\begin{aligned} C(x, y1, y2) = \min_{\{j1,j2\} \in \{-d_r,\cdots,d_r\}} \{ & C(x-1, y1+j1, y2+j2) \\ & + \lambda_G \left(f_G(x, y1) + f_G(x, y2)\right) \\ & + \lambda_I \left(f_I(x, y1) + f_I(x, y2)\right) \\ & + \lambda_{scc}(|j1| + |j2|) \\ & + \lambda_{dcs} |w_x - w_{x-1}|\} \end{aligned}$$

As initialization for the previous cost of the first location, the gradient values of the first position are used. The weight factors λ define the relative contribution of the different terms. We use 0.8 for λ_G (gradient), 0.15 for λ_I (intensity), 0.025 for λ_{scc} (single curve smoothness) and 0.025 for λ_{dcs} (dual curve smoothness). These values were determined prior to the study on different, independent datasets: several detection results were presented to expert readers who selected the visually best parameter combination. The optimal values $j1^*$ and $j2^*$ (i.e. the j1 and j2 where $C(x, y1, y2)$ is minimal) can be stored and traced backwards once the cost function for the entire double contour has been evaluated.

The far wall interfaces are always detected by dual dynamic programming. In some ultrasound sequences, the near wall does not show a double contour, though. Then the algorithm is likely to miss the MAN because it searches for a "best solution" to a double contour. In these cases, the operator might choose to select a dynamic programming algorithm that searches for a single contour following Gustavson et al. [7].

From the three interfaces, we calculate several parameters for further evaluation. Let $\overline{LD_t} = \sum_{x=a}^{b} (MAF_x - MAN_x) / w$ be the averaged LD in frame *t*, where *a* is the x-coordinate of the beginning of the bounding box, *b* the end and *w* the width ($w = b - a + 1$). Then we define the avg. LD to be $\sum_{t=c}^{d} \overline{LD_t} / n$, where *c* is the first frame of the heart cycle, *d* the last one and *n* the number of frames ($n = d - c + 1$). For the systolic/diastolic measurements we use the frame where $\overline{LD}$ is maximal/minimal, i.e. sys. LD = $\overline{LD}_{\arg\max(\overline{LD_t})}$ and dias. LD = $\overline{LD}_{\arg\min(\overline{LD_t})}$. The CIMT values are calculated in a similar fashion: $\overline{CIMT_t} = \sum_{x=a}^{b} (MAF_x - LIF_x) / w$, and the avg. CIMT is $\sum_{t=c}^{d} \overline{CIMT_t} / n$. The sys. CIMT = $\overline{CIMT}_{\arg\max(\overline{LD_t})}$ and the dias. CIMT = $\overline{CIMT}_{\arg\min(\overline{LD_t})}$. When we consider more than one heart cycle, the respective values are averaged.

Statistical Analysis

In order to show the general characteristics of the data, we calculated descriptive statistics in form of mean, SD and range of the measurements of the 40 datasets. To assess inter- and intra-reader variability, we computed bias and SD with the Bland Altman method, as well as Bland Altman plots of selected results. The interval of agreement (IoA = ±1.96*SD) is shown in the plots as dashed lines. We also computed the relative scales of bias and SD in percent of the respective mean values. As a cross check of the method, we analyzed scatter plots of the percental change in LD versus the percentual change in CIMT relative to the respective diastolic values for each measurement value of a patient. All analyses were performed in with the R statistical software version 2.12.0 [26] on an Ubuntu Linux system.

Results

Descriptive statistics for average, diastolic and systolic measurements for CIMT and LD for both readers are shown in Table 5.1. The results for T2 are very similar and are thus omitted due to space reasons. Table 5.2 shows results for the inter-reader analysis.

Bland-Altman plots for average CIMT and average LD are shown in Figure 5.3. The bias in CIMT measurements for CA clips was 0.002 mm (T1) and -0.004 mm (T2), for MA clips -0.004 mm (T1) and -0.003 mm (T2). The relative scales of these values (in percent of the respective mean values) are 0.3% (T1) and 0.5% (T2) for CA clips, 0.5% (T1) and 0.4% (T2) for MA clips. The LD bias in good quality clips was -0.003 mm (T1) and -0.016 mm (T2), for MA clips it was higher: -0.021 mm (T1) and -0.015 mm (T2). The relative values for the LD bias are 0.04% (T1) and 0.2% (T2) for CA, 0.3% (T1) and 0.2% (T2) for MA clips. Low inter-reader variability could be achieved for average CIMT in CA clips. The SD between R1 and R2 was 0.010 mm for T1 and 0.008 mm for T2. Here we obtain relative values of 1.2% (T1) and 1.0% (T2). The SD of MA clips are about in the same range at 0.010 mm, relative value 1.3%, for T1 and 1.4% for T2. In general, LD variability was higher than CIMT variability, and measurements of medium clips had a higher variability than of good clips.

Table 5.1: Descriptive statistics for for completely automatic (CA) and manual and automatic (MA) clips at evaluation time T1

Mean values together with standard deviation (SD) and range of the data in mm.

	R1		R2	
	mean (SD)	**range**	**mean (SD)**	**range**
CA clips T1				
Avg. CIMT	0.78 (0.15)	0.56-1.09	0.78 (0.15)	0.56-1.09
Sys. CIMT	0.77 (0.15)	0.54-1.05	0.77 (0.14)	0.54-1.04
Dias. CIMT	0.80 (0.16)	0.58-1.16	0.80 (0.15)	0.58-1.14
Avg. LD	7.33 (1.03)	5.63-10.05	7.33 (1.04)	5.62-10.09
Sys. LD	7.54 (1.05)	5.78-10.28	7.53 (1.06)	5.77-10.31
Dias. LD	7.14 (1.01)	5.50-9.86	7.14 (1.02)	5.50-9.90
MA clips T1				
Avg. CIMT	0.74 (0.12)	0.47-0.94	0.73 (0.12)	0.46-0.94
Sys. CIMT	0.72 (0.12)	0.48-0.97	0.71 (0.11)	0.46-0.95
Dias. CIMT	0.75 (0.12)	0.48-0.96	0.75 (0.12)	0.48-0.97
Avg. LD	7.65 (0.73)	6.09-8.99	7.63 (0.73)	6.07-8.95
Sys. LD	7.88 (0.75)	6.32-9.25	7.85 (0.73)	6.31-9.22
Dias. LD	7.45 (0.73)	5.89-8.73	7.43 (0.73)	5.87-8.70

Table 5.2: Bland-Altman statistics for inter-reader variability for completely automatic (CA) and manual and automatic (MA) quality clips for observation times T1 and T2

Inter-reader variability (R1 vs. R2)				
	Bias in mm (% of mean value)		**SD in mm (% of mean value)**	
	T1	**T2**	**T1**	**T2**
CA clips				
Avg. CIMT	0.002 (0.3 %)	-0.004 (-0.5 %)	0.010 (1.2 %)	0.008 (1.0 %)
Sys. CIMT	0.000 (0.1 %)	-0.003 (-0.4 %)	0.006 (0.8 %)	0.013 (1.6 %)
Dias. CIMT	0.006 (0.7 %)	-0.007 (-0.9 %)	0.019 (2.4 %)	0.012 (1.5 %)
Avg. LD	-0.003 (-0.0 %)	-0.016 (-0.2 %)	0.025 (0.3 %)	0.019 (0.3 %)
Sys. LD	-0.005 (-0.1 %)	-0.016 (-0.2 %)	0.022 (0.3 %)	0.026 (0.3 %)
Dias. LD	-0.001 (-0.0 %)	-0.019 (-0.3 %)	0.034 (0.5 %)	0.025 (0.4 %)
Ma clips				
Avg. CIMT	-0.004 (-0.5 %)	-0.003 (-0.4 %)	0.010 (1.3 %)	0.010 (1.4 %)
Sys. CIMT	-0.010 (-1.3 %)	-0.017 (-2.3 %)	0.016 (2.2 %)	0.023 (3.2 %)
Dias. CIMT	0.000 (0.0 %)	-0.005 (0.7 %)	0.026 (3.5 %)	0.037 (5.0 %)
Avg. LD	-0.021 (-0.3 %)	-0.015 (-0.2 %)	0.027 (0.4 %)	0.032 (0.4 %)
Sys. LD	-0.030 (-0.4 %)	-0.027 (-0.3 %)	0.039 (0.5 %)	0.041 (0.5 %)
Dias. LD	-0.017 (-0.2 %)	-0.011 (-0.1 %)	0.045 (0.6 %)	0.051 (0.7 %)

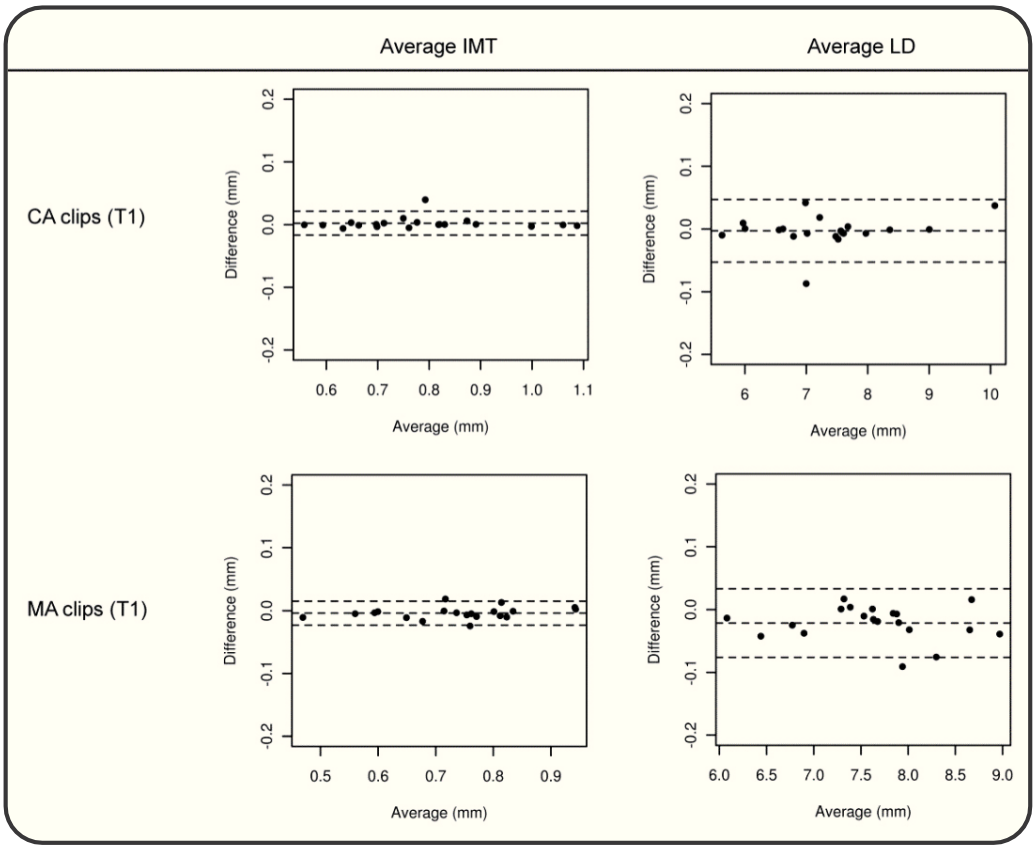

Figure 5.3: Bland-Altman plots for inter-reader variability for for completely automatic (CA) and manual and automatic (MA) datasets at evaluation time T1 (reader R1 vs. reader R2)

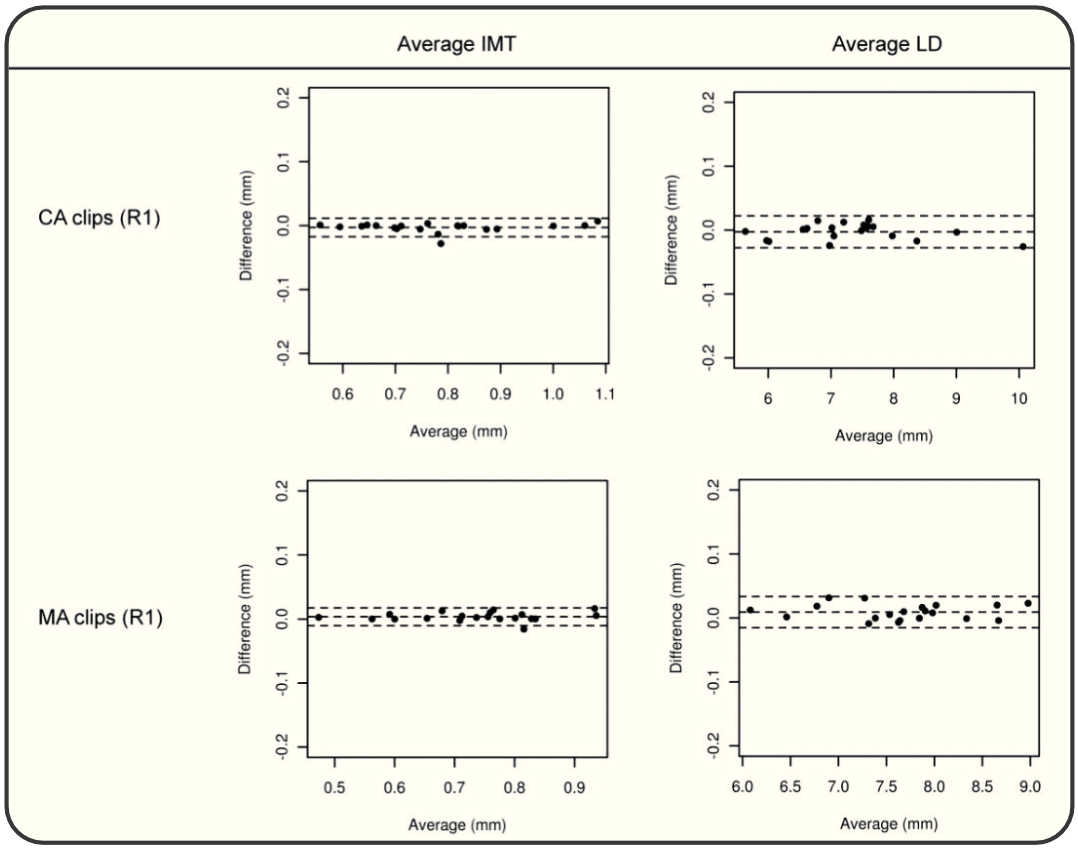

Figure 5.4: Bland-Altman plots for intra-reader variability for completely automatic (CA) and manual and automatic (MA) data of reader R1 (time T1 vs. time T2)

The values for intra-reader variability are shown in Table 5.3 and Figure 5.4. The bias of R1 for average CIMT measurements of CA clips was -0.003 mm and for MA clips 0.004 mm. Results for R2 were very similar. Regarding the SD for the average CIMT, identical results were observed for CA and MA clips: 0.007 mm (R1) and 0.008 mm (R2). Again, variability for the LD measurements was higher than the respective CIMT measurements.

Table 5.4 lists inter-reader results for completely manual measurements. We can observe a bias of 0.048 mm (MA dataset, T2) to 0.061 mm (MA dataset, T1) for CIMT and 0.016 mm (MA dataset, T2) to 0.73 mm (CA dataset, T1) for LD measurements. The SD is in the range of 0.028 mm (CA dataset, T2) to 0.042mm (CA dataset, T1) for CIMT and 0.038 mm (MA dataset, T2) to 0.068 mm (CA dataset, T1) for LD.

Table 5.3: Bland-Altman statistics for intra-reader variability (R1 vs. R2) for completely automatic (CA) and manual and automatic (MA) clips

Intra-reader variability (R1 vs. R2)				
	Bias in mm (% of mean value)		SD in mm (% of mean value)	
	R1	R2	R1	R2
CA clips				
Avg. CIMT	-0.003 (-0.4 %)	0.003 (0.4 %)	0.007 (0.9 %)	0.008 (1.0 %)
Sys. CIMT	0.000 (0.1 %)	0.004 (0.6 %)	0.008 (1.1 %)	0.013 (1.7 %)
Dias. CIMT	-0.008 (-0.9 %)	0.005 (0.6 %)	0.017 (2.2 %)	0.012 (1.5 %)
Avg. LD	-0.003 (-0.0 %)	0.011 (0.1 %)	0.012 (0.2 %)	0.017 (0.2 %)
Sys. LD	0.001 (0.0 %)	0.012 (0.2 %)	0.017 (0.2 %)	0.017 (0.2 %)
Dias. LD	-0.006 (-0.1 %)	0.013 (0.2 %)	0.019 (0.3 %)	0.020 (0.3 %)
MA Clips				
Avg. CIMT	0.004 (0.5 %)	0.002 (0.3 %)	0.007 (0.9 %)	0.008 (1.1 %)
Sys. CIMT	-0.007 (-0.9 %)	0.000 (0.1 %)	0.027 (3.7 %)	0.017 (2.4 %)
Dias. CIMT	0.009 (1.2 %)	0.004 (0.5 %)	0.019 (2.6 %)	0.011 (1.5 %)
Avg. LD	0.009 (0.1 %)	-0.003 (0.0 %)	0.012 (0.2 %)	0.014 (0.2 %)
Sys. LD	0.009 (0.1 %)	0.005 (0.1 %)	0.027 (0.3 %)	0.019 (0.2 %)
Dias. LD	0.009 (0.1 %)	-0.003 (0.0 %)	0.038 (0.5 %)	0.012 (0.2 %)

Table 5.4: Bland-Altman statistics for inter-reader variability for completely manually (CM) evaluated data for evaluation times T1 and T2

Inter-reader variability (T1 vs. T2) for completely manually (CM) evaluated data				
	Bias in mm (% of mean value)		SD in mm (% of mean value)	
	T1	T2	T1	T2
CA dataset – CM				
Avg. CIMT	0.055 (7.7 %)	0.053 (7.3 %)	0.042 (5.8 %)	0.028 (3.9 %)
Avg. LD	0.073 (1.0 %)	0.057 (0.8 %)	0.068 (0.9 %)	0.066 (0.9 %)
MA dataset – CM				
Avg. CIMT	0.061 (9.2 %)	0.048 (7.1 %)	0.036 (5.5 %)	0.033 (4.9 %)
Avg. LD	0.056 (0.7 %)	0.016 (0.2 %)	0.052 (0.7 %)	0.038 (0.5 %)

5 frames of each clip were either evaluated completely automatic (CA) or manual and automatic (MA).

Results for the inter-method analysis are given in Table 5.5. The bias / SD of CA to CM measurements are 0.057/0.031 mm (CIMT) and 0.077/0.061 mm (LD), respectively; for MA vs. CM measurements we obtain 0.064/0.027 mm (CIMT) and 0.049/0.048 mm (LD).

Figure 5.5 shows a scatter plot of the percent change in LD versus the percent change in CIMT for each patient at each time. The patient-to-patient variation of strain measurements is approximately fivefold higher than the actual intra-reader variability (T1 vs. T2).

Figure 5.6 shows the comparison of the virtual M-mode of the automatic tracing across one complete heart cycle (left side) and a completely manual tracing by an expert reader (right side). The more irregular line of the manual tracing is a clear indicator of the higher variability compared to the automatic tracing.

Table 5.5: Bland-Altman statistics for inter-method variability (CA/MA vs. CM)

	Inter-method variability (CA/MA vs. CM)			
	CA vs. CM		**MA vs. CM**	
	Bias in mm (% of mean)	**SD in mm (% of mean)**	**Bias in mm (% of mean)**	**SD in mm (% of mean)**
Avg. CIMT	0.057 (7.6 %)	0.031 (4.1 %)	0.064 (9.1 %)	0.027 (3.8 %)
Avg. LD	0.077 (1.1 %)	0.061 (0.8 %)	0.049 (0.6 %)	0.048 (0.6 %)

Bland-Altman statistics for completely automatic (CA) or manual and automatic (MA) measurements against a completely manual (CM) ground truth (average of manual tracings of R1 and R2 for times T1 and T2).

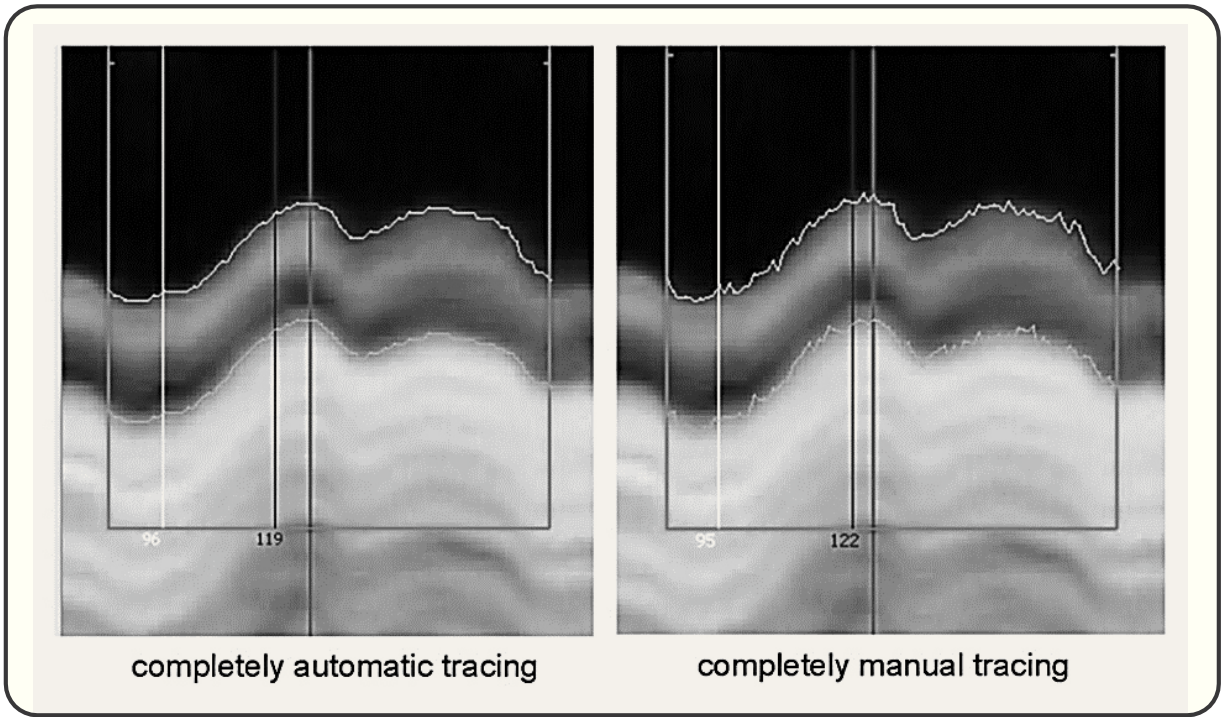

Figure 5.5: Virtual M-mode showing the results of automatic vs. completely manual tracing

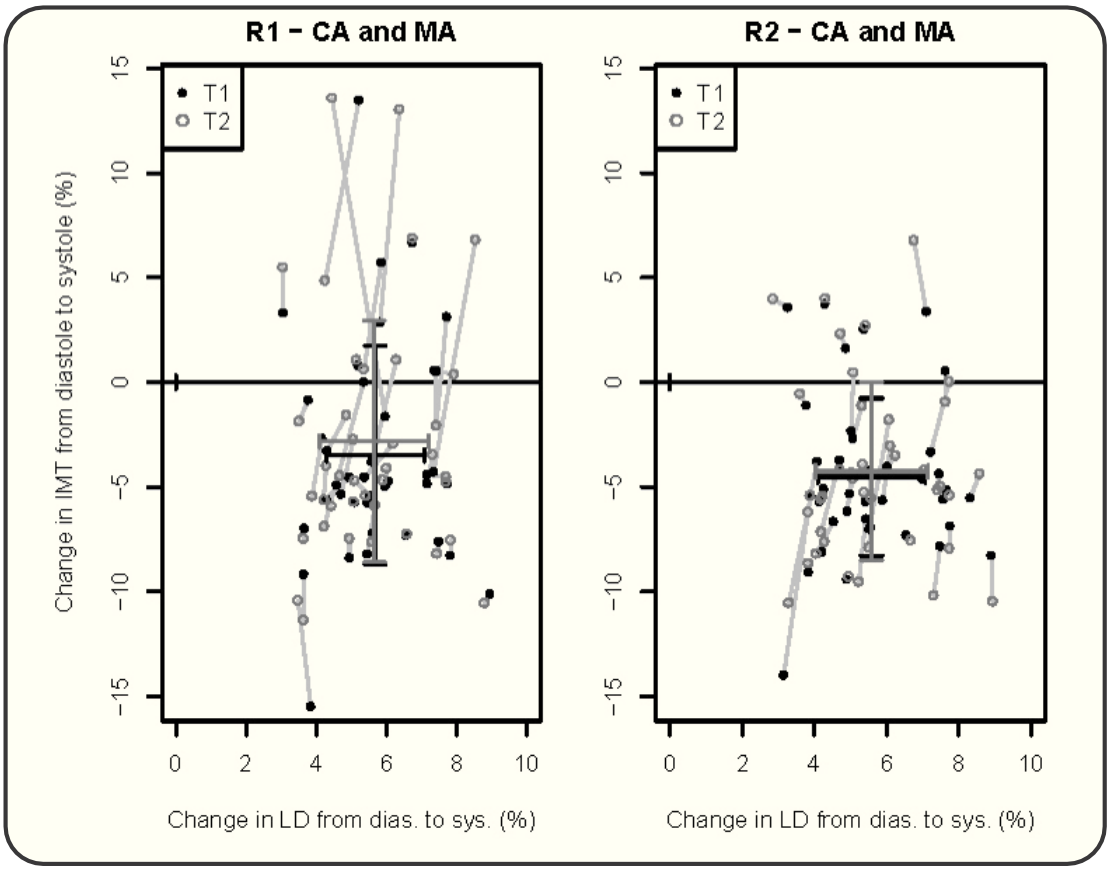

Figure 5.6: Examples for lumen diameter (LD) and carotid intima media thickness values (CIMT) for both readers and evaluation times over a heart cycle
Vertical lines show the position of the diastole as determined by the respective readers. In the third row, the percentual changes of CIMT vs. LD are shown, together with the hypothetical relation when assuming conservation of vessel wall volume (black line).

Discussion

Changes of the CCA, especially an increase of the CIMT, enlargement of the LD and the reduction of the local compliance are accepted biomarkers for the atherosclerotic process of the arterial system and especially the CIMT is increasingly used in clinical trials, clinical practice and prediction of cardio-vascular events [1,27–29]. Automatic analyzing systems are attractive due to lower measurement variability compared to manual tracing of the CIMT [14,20,22]. However, two issues are not sufficiently addressed so far. These are the measurement of sectional CIMT and LD over the complete heart cycle in B-mode images and the handling of images with lower quality impeding the automatic measurement procedure.

The presented novel analysis software DYARA targets to solve both issues. First, it is able to analyze the named structural and functional parameters of the CCA with high inter- and intra-reader consistency automatically across the complete heart cycle. In fact, the SD for the average CIMT are as good or better as the currently best system we are aware of: Faita et al. [20], SD for average CIMT, Operator 1 vs. Operator 2: ±0.010 mm. The implemented algorithm based on dual dynamic programming and enhanced features has shown highly consistent detection results.

Second, the software enables the reader to interact with the automatically traced lines if the result is not sufficient. Frames with echo dropouts or much noise do not have to be discarded in our system because of the possibility for manual editing of the traced lines. The resulting SD of the CIMT in MA clips is comparable to the CA results. Corrections could be executed in a highly consistent fashion by quickly comparing the results to other parts of the detected contours or neighboring frames. This is a clear advantage in large scale research studies with different centers. If large amounts of data are collected from several locations and examiners, it is likely that some frames are flawed. The combination of automatic and manual detection allows us to fully utilize the available data.

The system is capable of analyzing the CIMT and the LD over the complete heart cycle, allowing studies that analyze the dynamic properties of the artery like Paini et al. [30] or Bianchini et al. [31]. This is an important issue since CIMT and LD changes across the heart cycle [18]. In our system, the end-systolic as well as end-diastolic frames are detected automatically by determination of the lowest and highest LD. Hence, the use of ECG markers for control of the heart cycle is dispensable. However, the values of the systolic and the diastolic LD and CIMT (in single frames) have a higher variability compared to the average CIMT and average LD. This is plausible since the averaged values contain all frames of the entire heart cycle, where as for the systolic/diastolic results only one single measurement is taken.

The inter-reader analysis of the completely manual measurements showed a higher bias and SD than within the automatic method and thus the superiority of the (semi-)automatic system. When we compared the CA/MA measurements against a manual ground truth, we could show that the automatic methods detected on average the CIMT about 0.06 mm wider than human observers. A certain bias will most likely exist against other automatic systems and must be taken into consideration when comparing absolute CIMT and LD values. An automatic unbiasing procedure as proposed by Rossi et al. [11] could be a solution for this issue. The high variability of the manual tracings becomes visible when looking at a virtual M-mode in Figure 5.5. For human observers, it is difficult to meet exactly the same location in successive frames. The automatic tracing procedure provides more consistent results.

The scatter of the percent changes in LD and CIMT between the patients is higher than the mean differences within repeated measurements of a reader (Figure 5.6). This offers the opportunity to analyze the strain relation of LD to CIMT based on our methodology. When assuming a conservation of vascular wall volume, the percent change for IMT should be nearly equal to the percent change in LD. This does not hold for all cases. The positive changes in CIMT are mainly due to measurement inaccuracies, and can thus serve as a quality check. The remaining values still do not all show this expected ratio. This has also been reported by others (e.g., Selzer et al. 2001 [18]) and might be investigated in future research.

Our system can offer reliable measurements from B-Mode ultrasound images, although the resolution of the underlying images (266 pixel per cm, i.e. 37.6 µm per measurement) is lower than in RF based methods, that are usually in the order of 21-23 µm per measurement [11,31]. The temporal resolution of both approaches is the same and dependant of the underlying US-equipment. Our data has a very high temporal resolution of 93 Hz, which is necessary if the CIMT and the LD should be measured accurately in the rapid transition from diastole to systole.

The data for our study was acquired according to a standardized protocol and evaluated by expert readers. The results show the full capabilities of the system. The CIMT and LD measurements are affected by the location of the 1cm measurement range (see Mannheim Consensus). So an important factor for reproducible results is a good training of the readers. Excellent usability of the system and sophisticated visual control features help the users to place the bounding box at the right location. Further investigations will deal with an arbitrary setting of the bounding box and the effect of readers with different skill levels.

In conclusion, DYARA is a highly precise detection system providing a combination of automated analysis together with manual interaction capabilities avoiding to discard images with poorer contour quality. Thus, it comes closer to cope with the varying quality of ultrasound images and clips in clinical practice as well as in research settings.

Acknowledgments

We would like to thank Prof. Dr.-Ing. Hans Burkhardt, University of Freiburg, who supported the development of an early version of the analysis system. Janina Schulz played a vital role in this phase. Dr. Manfred Baumstark helped on all server-side issues of the project. The system was developed in close collaboration with the SAPALDIA readers, which constantly gave feedback on features and the usability of the program.

References

1 Lorenz, M. W., Markus, H. S., Bots, M. L., Rosvall, M. and Sitzer, M. (2007) Prediction of clinical cardiovascular events with carotid intima-media thickness: a systematic review and meta-analysis. Circulation. **115**, 459–467

2 O'Leary, D. H., Polak, J. F., Kronmal, R. A., Manolio, T. A., Burke, G. L. and Wolfson, S. K. (1999) Carotid-artery intima and media thickness as a risk factor for myocardial infarction and stroke in older adults. Cardiovascular Health Study Collaborative Research Group. N. Engl. J. Med. **340**, 14–22

3 Simon, A., Megnien, J. L. and Chironi, G. (2010) The value of carotid intima-media thickness for predicting cardiovascular risk. Arterioscler. Thromb. Vasc. Biol. **30**, 182–185

4 Espeland, M. A., Tang, R., Terry, J. G., Davis, D. H., Mercuri, M. and Crouse, J. R., 3rd. (1999) Associations of risk factors with segment-specific intimal-medial thickness of the extracranial carotid artery. Stroke. **30**, 1047–1055

5 Homma, S., Hirose, N., Ishida, H., Ishii, T. and Araki, G. (2001) Carotid plaque and intima-media thickness assessed by b-mode ultrasonography in subjects ranging from young adults to centenarians. Stroke. **32**, 830–835

6 Zielinski, T., Dzielinska, Z., Januszewicz, A., Rynkun, D., Makowiecka Ciesla, M., Tyczynski, P., Prejbisz, A., Demkow, M., Kadziela, J., Naruszewicz, M., Januszewicz, M., Juraszynski, Z., Korewicki, J. and Ruzyllo, W. (2007) Carotid intima-media thickness as a marker of cardiovascular risk in hypertensive patients with coronary artery disease. Am. J. Hypertens. **20**, 1058–1064

7 Gustavsson, T., Liang, Q., Wendelhag, I. and Wikstrand, J. (1994) A dynamic programming procedure for automated ultrasonic measurement of the carotid artery. Comput. Cardiol. 297–300

8 Touboul, P. J., Prati, P., Scarabin, P. Y., Adrai, V., Thibout, E. and Ducimetière, P. (1992) Use of monitoring software to improve the measurement of carotid wall thickness by B-mode imaging. J. Hypertens. Suppl. **10**, S37–41

9 Wendelhag, I., Gustavsson, T., Suurküla, M., Berglund, G. and Wikstrand, J. (1991) Ultrasound measurement of wall thickness in the carotid artery: fundamental principles and description of a computerized analysing system. Clin. Physiol. **11**, 565–577

10 Hoeks, A. P. G., Willekes, C., Boutouyrie, P., Brands, P. J., Willigers, J. M. and Reneman, R. S. (1997) Automated detection of local artery wall thickness based on M-line signal processing. Ultrasound Med. Biol. **23**, 1017–1023

11 Rossi, A. C., Brands, P. J. and Hoeks, A. P. G. (2010) Automatic localization of intimal and adventitial carotid artery layers with noninvasive ultrasound: a novel algorithm providing scan quality control. Ultrasound Med. Biol. **36**, 467–479

12 Laurent, S., Cockcroft, J., Van Bortel, L., Boutouyrie, P., Giannattasio, C., Hayoz, D., Pannier, B., Vlachopoulos, C., Wilkinson, I. and Struijker-Boudier, H. (2006) Expert consensus document on arterial stiffness: methodological issues and clinical applications. Eur. Heart J. **27**, 2588 –2605

13 Rossi, A. C., Brands, P. J. and Hoeks, A. P. G. (2009) Nonlinear processing in B-mode ultrasound affects carotid diameter assessment. Ultrasound Med. Biol. **35**, 736–747

14 Schmidt-Trucksäss, A., Cheng, D., Sandrock, M., Schulte-Mönting, J., Rauramaa, R., Huonker, M. and Burkhardt, H. (2001) Computerized analysing system using the active contour in ultrasound measurement of carotid artery intima-media thickness. Clin. Physiol. **21**, 561–569

15 Cheng, D.-C., Cheng, K.-S., Schmidt-Trucksäss, A., Sandrock, M., Pu, Q. and Burkhardt, H. (1999) Automatic detection of the intimal and the adventitial layers of the common carotid artery wall in ultrasound B-mode images using snakes. Werner B Ed Proc. 10th Int. Conf. Image Anal. Process. Los Alamitos CA IEEE Comput. Soc. 452–457

16 Loizou, C. P., Pattichis, C. S., Pantziaris, M., Tyllis, T. and Nicolaides, A. (2007) Snakes based segmentation of the common carotid artery intima media. Med. Biol. Eng. Comput. **45**, 35–49

17 Cheng, D.-C., Jiang, X., Schmidt-Trucksäss, A. and Cheng, K.-S. (2006) Automatic intima-media thickness measurement of carotid artery wall in B-mode sonographic images. Proc. 3rd IEEE Int. Symp. Biomed. Imaging Nano Macro Arlingt. VA IEEE. **30**, 912–915

18 Selzer, R. H., Mack, W. J., Lee, P. L., Kwong-Fu, H. and Hodis, H. N. (2001) Improved common carotid elasticity and intima-media thickness measurements from computer analysis of sequential ultrasound frames. Atherosclerosis. **154**, 185–193

19 Haller, C., Schulz, J., Schmidt-Trucksäss, A., Burkardt, H., Schmitz, D., Dickhuth, H.-H. and Sandrock, M. (2007) Sequential based analysis of Intima-Media Thickness (IMT) in common carotid artery studies. Atherosclerosis. **195**, e203–e209

20 Faita, F., Gemignani, V., Bianchini, E., Giannarelli, C., Ghiadoni, L. and Demi, M. (2008) Real-time measurement system for evaluation of the carotid intima-media thickness with a robust edge operator. J Ultrasound Med. **27**, 1353

21 Lee, Y.-B., Choi, Y.-J. and Kim, M.-H. (2010) Boundary detection in carotid ultrasound images using dynamic programming and a directional Haar-like filter. Comput. Biol. Med. **40**, 687–697

22 Molinari, F., Zeng, G. and Suri, J. S. (2010) Intima-media thickness: setting a standard for a completely automated method of ultrasound measurement. IEEE Trans Ultrason Ferroelectr Freq Control. **57**, 1112–1124

23 Ackermann-Liebrich, U., Kuna-Dibbert, B., Probst-Hensch, N. M., Schindler, C., Felber Dietrich, D., Zemp Stutz, E., Bayer-Oglesby, L., Baum, F., Brändli, O., Brutsche, M., Downs, S. H., Keidel, D., Gerbase, M. W., Imboden, M., Keller, R., Knöpfli, B., Künzli, N., Nicod, L., Pons, M., Staedele, P., Tschopp, J.-M., Zellweger, J.-P. and Leuenberger, P. (2005) Follow-up of the Swiss Cohort Study on Air Pollution and Lung Diseases in Adults (SAPALDIA 2) 1991-2003: methods

and characterization of participants. Soz.- Präventivmedizin. **50**, 245–263

24 Martin, B. W., Ackermann-Liebrich, U., Leuenberger, P., Künzli, N., Zemp Stutz, E., Keller, R., Zellweger, J. P., Wüthrich, B., Monn, C., Blaser, K., Bolognini, G., Bongard, J. P., Brändli, O., Braun, P., Defila, C., Domenighetti, G., Grize, L., Karrer, W., Keller-Wossidlo, H., Medici, T. C., Peeters, A., Perruchoud, A. P., Schindler, C., Schoeni, M. H., Schwartz, J., Solari, G., Tschopp, J.-M. and Villiger, B. (1997) SAPALDIA: methods and participation in the cross-sectional part of the Swiss Study on Air Pollution and Lung Diseases in Adults. Soz.- Präventivmedizin. **42**, 67–84

25 Touboul, P. J., Hennerici, M. G., Meairs, S., Adams, H., Amarenco, P., Desvarieux, M., Ebrahim, S., Fatar, M., Hernandez Hernandez, R., Kownator, S., Prati, P., Rundek, T., Taylor, A., Bornstein, N., Csiba, L., Vicaut, E., Woo, K. S. and Zannad, F. (2004) Mannheim intima-media thickness consensus. Cerebrovasc. Dis. **18**, 346–349

26 R Development Core Team. (2011) R: a language and environment for statistical computing, Vienna, Austria. Retrieved December 23, 2011, from http://www.r-project.org/

27 Eigenbrodt, M. L., Bursac, Z., Tracy, R. E., Mehta, J. L., Rose, K. M. and Couper, D. J. (2008) B-mode ultrasound common carotid artery intima-media thickness and external diameter: cross-sectional and longitudinal associations with carotid atherosclerosis in a large population sample. Cardiovasc. Ultrasound. **6**, 10

28 Haluska, B. A., Jeffries, L., Carlier, S. and Marwick, T. H. (2010) Measurement of arterial distensibility and compliance to assess prognosis. Atherosclerosis. **209**, 474–480

29 Nambi, V., Chambless, L., Folsom, A. R., He, M., Hu, Y., Mosley, T., Volcik, K., Boerwinkle, E. and Ballantyne, C. M. (2010) Carotid intima-media thickness and presence or absence of plaque improves prediction of coronary heart disease risk: the ARIC (Atherosclerosis Risk In Communities) study. J. Am. Coll. Cardiol. **55**, 1600–1607

30 Paini, A., Boutouyrie, P., Calvet, D., Zidi, M., Agabiti-Rosei, E. and Laurent, S. (2007) Multiaxial mechanical characteristics of carotid plaque: analysis by multiarray echotracking system. Stroke. **38**, 117–123

31 Bianchini, E., Bozec, E., Gemignani, V., Faita, F., Giannarelli, C., Ghiadoni, L., Demi, M., Boutouyrie, P. and Laurent, S. (2010) Assessment of carotid stiffness and intima-media thickness from ultrasound data. J. Ultrasound Med. **29**, 1169 –1175

CHAPTER 6

Variability and reproducibility of carotid structural and functional parameters assessed with transcutaneous ultrasound – results from the SAPALDIA Cohort Study

Authors

Seraina Caviezel
Julia Dratva
Emmanuel Schaffner
Alexandra Teynor
Manfred W. Baumstark
Christian Schindler
Eric de Groot
Luc Burdet
Thomas Rothe
Marco Pons
Jean-Michel Gaspoz
Thierry Rochat
Nino Künzli
Nicole Probst-Hensch
Arno Schmidt-Trucksäss

Accepted version*, published in:

Atherosclerosis. 2013 Dec;231(2):448–55.
Doi: 10.1016/j.atherosclerosis.2013.10.010.

*Minor editorial modifications possible due to harmonisation of the thesis

VARIABILITY AND REPRODUCIBILITY OF CAROTID STRUCTURAL AND FUNCTIONAL PARAMETERS ASSESSED WITH TRANSCUTANEOUS ULTRASOUND – RESULTS FROM THE SAPALDIA COHORT STUDY

Abstract

Objective: Carotid intima media thickness (CIMT) and local stiffness are vascular biomarkers of atherosclerotic burden. We investigated the variability and reproducibility of clinically relevant structural (CIMT, lumen diameter (LD)) and functional parameters (strain, distensibility, compliance, β-stiffness index, Peterson's elastic modulus and Young's elastic modulus) measured in B-mode ultrasound sequences of the common carotid artery (CCA) in the second follow-up of the **S**wiss Cohort Study on **A**ir **P**ollution **a**nd **L**ung and Heart **D**iseases **I**n **A**dults (SAPALDIA 3).

Methods: Ultrasound sequential images were examined twice over a 1 cm segment across at least one heart cycle in 165 SAPALDIA 3 participants. To assess variability and reproducibility of structural and functional parameters, individual coefficients of variation (CV), intraclass correlation (ICC), Bland-Altman plots and mixed effect regressions were used.

Results: ICCs of repeated examinations ranged between 0.67 and 0.77 for blood pressure indices, between 0.87 and 0.97 for structural properties and between 0.75 and 0.79 for functional parameters. CV was lowest in structural parameters (1.6-4.6%), followed by blood pressure (5.1-7.9%) and functional indices (11.0-13.1%). Variations in all parameters were predominantly explained by subjects (>74% in functional, >82% in structural properties). Bland-Altman plots for functional indices showed mean and standard deviation of the respective mean value of 4.2(19.6)% for strain, 1.9(24.4)% for distensibility, 2.4(22.2)% for compliance, 3.0(24.4)% for β-stiffness index, 0.9(25.7)% for Peterson's elastic modulus and 1.2(27.9)% for Young's elastic modulus.

Conclusion: The results show that SAPALDIA 3 measurements of transcutaneous ultrasound examinations have an excellent reproducibility of structural parameters and a good reproducibility of functional indices.

Key words: Common carotid artery; Ultrasound; Arterial stiffness; Intima media thickness; Diameter; Reproducibility

Introduction

CVD is a major cause of death [1]. The development and application of validated surrogate endpoints to identify risk and evaluate therapeutic intervention have become increasingly available in the last decades [2–5]. Based on numerous clinical studies non-invasive structural or functional endpoints as carotid intima media thickness (CIMT) and lumen

diameter (LD) assessed by B-mode ultrasound or aortic stiffness assessed by pulse wave velocity are now widely accepted as identifiers of the atherosclerotic status and predictors of atherosclerotic events, respectively [6–9]. Carotid functional indices such as distensibility are under discussion regarding their validity and sensitivity to predict cardiovascular events in the general population [9–12].

The widespread availability of non-invasive high resolution ultrasound in combination with advanced automated reading software [13] allow the in-vivo evaluation and modelling of the haemodynamic effects and properties of the arterial wall in humans in cross-sectional and follow-up studies [14]. CIMT and LD describe the anatomic and structural properties of the CCA segment, whereas vascular function can be described by changes in CIMT and LD across the heart cycle.

Accuracy and repeatability of CIMT and carotid LD measurements have a decisive impact on the sample sizes needed for cross-sectional and longitudinal risk identification and assessment of treatment efficacy. This implies that the evaluation of data quality needs a thorough methodical validation of all parameters used for future analyses within the same study setting. We therefore investigated the variability and reproducibility of carotid structural wall parameters (CIMT, LD) and the carotid functional indices strain, distensibility, compliance, β-stiffness index, Peterson's elastic modulus and Young's elastic modulus by means of repeated ultrasound scans. For our purposes a randomly selected sample of participants of the SAPALDIA 3 cohort, a general population study (second follow-up of the **S**wiss Cohort Study on **A**ir **P**ollution **a**nd **L**ung and Heart **D**iseases **I**n **A**dults), was selected and scanned a second time on top of the study visit. To better understand the significance of our reproducibility study, we additionally compared our results with different population based studies [9,15–22].

Methods

Study design and subjects

The SAPALDIA multicentre cohort study recruited in 1991 is a population-based random sample of adults (18-60 years) from eight areas of Switzerland representing rural and urban environmental conditions (Aarau, Basel, Davos, Genève, Lugano, Montana, Payerne, Wald) [23]. SAPALDIA 3 was executed in 2010/2011. It is the second follow-up of the SAPALDIA cohort after the previous survey's in 2001/2003 and 1991 [23,24]. In SAPALDIA 3 carotid B-mode ultrasound scans were performed in 3489 participants (49% males, 51% females) turning 50 years within the same year or older, mean age 63.9 (SD 8.1, range 50-81) years. A health screening questionnaire was applied before health examinations and participants were excluded partially or fully if a health risk for a clinical examination existed. Ethical approval had been granted by the respective Swiss cantonal ethical committees. Participants gave written informed consent according to their preferences either globally for all examinations or separately for single assessments.

To assess variability and reproducibility of carotid structural and functional parameters repeated scans were performed in a representative random sample of 165 SAPALDIA 3 participants (see figure 6.1). Participants were examined on two different occasions, at least one night in between scans and within a maximum of three months. Sequential image analyses were consecutively performed within SAPALDIA 3 and all readers were blinded in terms of the initial and the repeat ultrasound examination date (intersession validation).

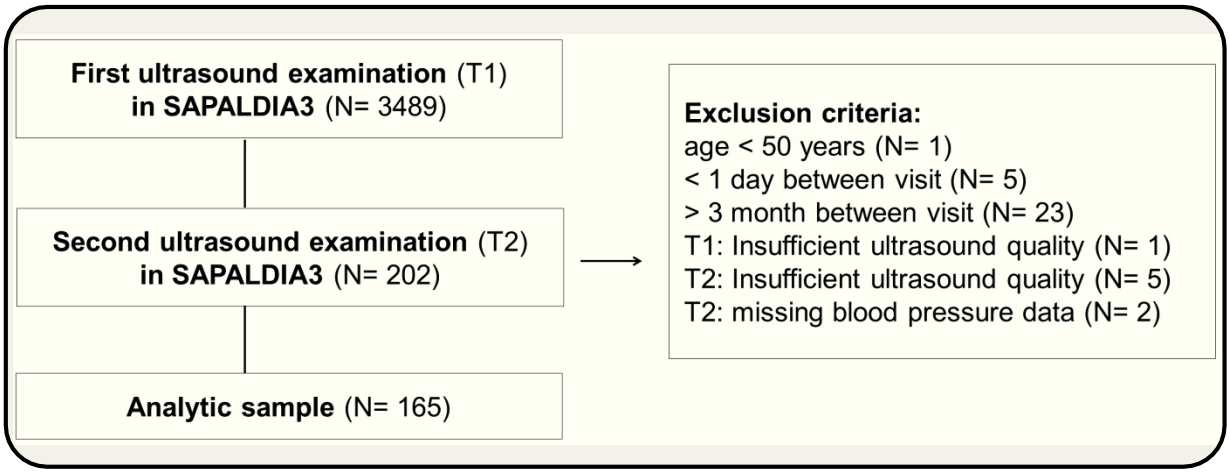

Figure 6.1: Participation and feasibility flow chart

Ultrasound examination

Examinations were performed following a standardised ultrasound scan protocol. Eight standardised ultrasound instruments (Fukuda Denshi UF-870) equipped with an LA38 5-16 MHz linear array transducers. The ultrasound temporal resolution was 10.47ms per frame. All sonographers were trained, certified prior to study entry and supervised by two collaborating vascular labs: the Department of Vascular Medicine at the Academic Medical Center/Imagelab, University of Amsterdam and Erichem, The Netherlands and the Division Sport and Exercise Medicine at the Institute of Exercise and Health Science, University of Basel, Switzerland. To guide sonographers through the scan procedures, all ultrasound instrumentation was equipped with the SAPALDIA 3 application protocol, jointly developed by Vascular Imaging/Imagelab, ISSW and Fukuda Denshi.

Before start of the ultrasound examination, each individual rested in supine position for at least 10 minutes in a 22-25°C dimly lit room. During the bilateral carotid scans the head rested comfortably against a 45° foam wedge to standardise the horizontal and ear-to-ear angles of insolation of the four right and left distal CCA locations: In all CCA B-mode ultrasound scans the sonographer aimed to visualise the following standardised and predefined anatomic arterial wall structures: the lumen-intima to media-adventitia layer (CIMT) of the far wall and the media-adventitia interface of the far wall to the media-adventitia interface of the near wall (outer LD) were assessed longitudinally in the CCA along the 1 cm arterial wall segment proximal to the carotid bifurcation for a duration of at least

three heart cycles (see figure 6.2) [25]. Furthermore, left and right brachial systolic (sBP) and diastolic blood pressure (dBP) were measured at the upper arm with a standard oscillometric device (OMRON 705IT, OMRON HEALTHCARE, Kyoto, Japan) immediately upon measuring CIMT on the respective side.

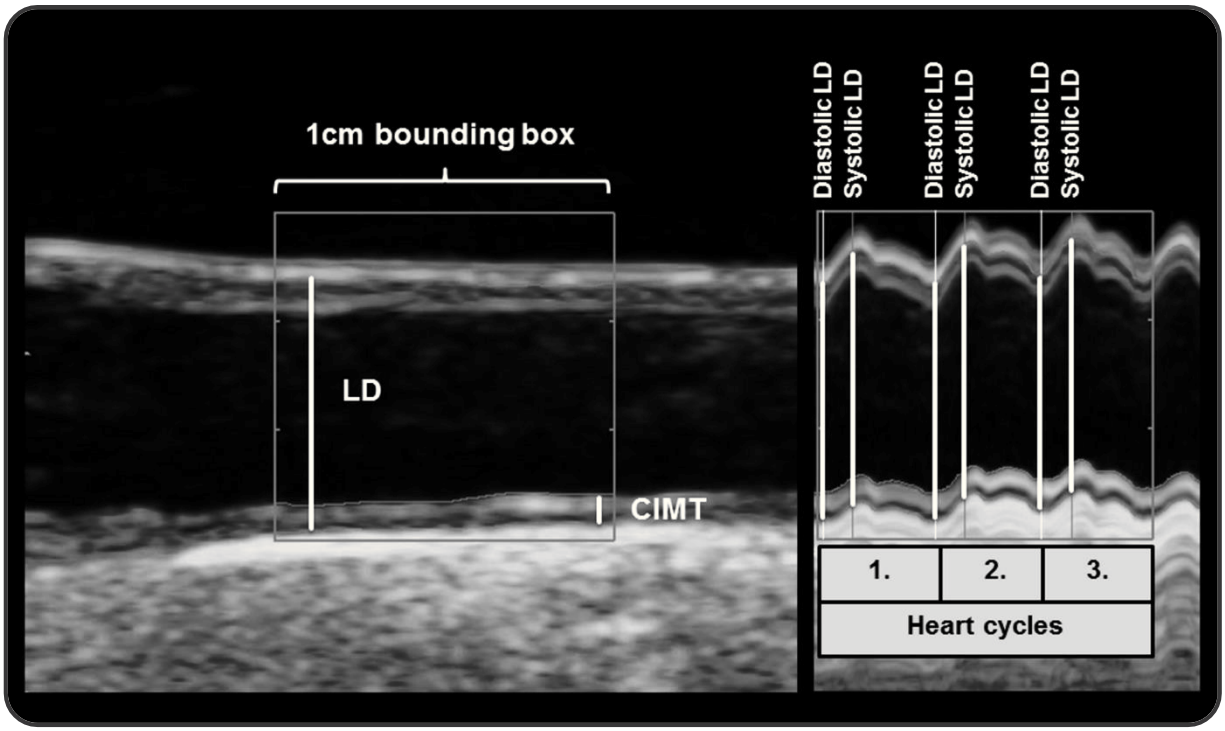

Figure 6.2: Ultrasound images of the common carotid artery
B-mode (left) and M-mode (right) ultrasound image of the common carotid artery with automatic contour detection lines (LD between adventitia far wall and adventitia near wall and CIMT as intima-media thickness of the far wall) over three heart cycles.

All ultrasound examination data were saved as native format pooled per examination and transferred to the Swiss Tropical and Public Health Institute, Basel, Switzerland, which centrally administered the examined data. The native files were converted by a Fukuda Denshi extraction file program to a sequence of B-mode images in Portable Network Graphics (PNG) format. Afterwards, the image sequences were loaded into the B-mode image analysis program **DY**namic **AR**tery **A**nalysis (DYARA). The program interface shows the B-mode image sequences as x-y view on the left side and a virtual M-mode as y-z view on the right side (see figure 6.2) [13]. Validation for intra- and interreader variability is described in detail elsewhere [13].

Sequential images analyses

Data acquisition was conducted according to standardised SAPALDIA 3 protocols referring to Mannheim Consensus [25], thus plaque in the measurement region of the CCA and images with insufficient quality were excluded from analysis. In order to ensure reproducibility, expert ultrasound readers tagged the start of measurement systematically directly proximal to the opening of the carotid bifurcation in the B-mode DYARA user interface. Then, a bounding box of exactly 1 cm was generated by DYARA according to image resolution (266 pixels per cm). The bounding box indicated the region of interest for automatic detection of the intimal and adventitial layer of the far wall and the media – adventitia interface of the near wall. The analysed sequential images started and ended in end-diastole containing one to three heart cycles (see figure 6.2).

DYARA is advantageously reader independent, but it allows manual interaction as much as necessary whenever the measurement lacked information due to difficult ultrasound data quality. When the reading was finished (approximately 10 to 15 minutes per ultrasound scan), the raw data, contour detection results and calculated parameters were automatically saved in Hierarchical Data Format (HDF5). Simultaneously, a database was generated containing structural data (average, systolic and diastolic CIMT and LD, respectively). These were all based on the average of four location analyses (left and right CCA, each in ear to ear and horizontal view) of a 1 cm segment across at least one heart cycle (see table 6.1).

Functional parameters

In general, local functional indices reflect the local elasticity of the CCA. They are defined as the relation of blood pressure (BP) change (stress) to cyclic variation of structural indices. Hence, functional parameters are based on structural indices.

Table 6.1 presents the structural definitions and functional parameter formulas used in SAPALDIA 3. Strain describes the relative carotid distension without considering any BP changes. It only considers the relative change of LD across heart cycles [14]. The distensibility coefficient (DC) represents the relative change of LD for a given pulse pressure and reflects the carotid elasticity [2,14]. The compliance coefficient (CC) measures the absolute change of the cross-sectional LD area for a given pressure change which displays the buffer capacity [2,14]. Peterson's elastic modulus (EP) measures the needed pressure change for a given relative LD change [14]. Young's elastic modulus (EINC) incorporates the wall thickness and therefore expresses a wall-dependent elasticity [8]. The β-stiffness index (Bstiff) describes the ratio between the logarithmized quotient of systolic and diastolic blood pressure to strain and therefore, it is dimensionless [26]. Lower values of strain, DC and CC and higher values of EP, EINC, and Bstiff correspond to a higher arterial stiffness.

Table 6.1: Structural and functional parameters based on a 1 cm detection segment

Parameter	unit	Definition / formula	
aLD	mm	average of LD in all analysed ultrasound frames	
dLD	mm	average of the minimal LD in all analysed heart cycles	
sLD	mm	average of the maximum LD in all analysed heart cycles	
aCIMT	mm	average of CIMT in all analysed images across the heart cycle	
dCIMT	mm	average of CIMT measured in all analysed heart cycles at diastolic (minimal) LD	
sCIMT	mm	average of CIMT measured in all analysed heart cycles at systolic (maximal) LD	
deltaLD	mm	sLD-dLD	
Strain	%	deltaLD / dLD	[14]
DC	1/kPa	$((2 \times deltaLD \times dLD)+(deltaLD)^2)/(PP\times dLD^2)$	[2]
CC	mm^2/kPa	$\pi\times((2\times deltaLD \times dLD)+(deltaLD)^2)/(4\times PP)$	[2]
Bstiff	no unit	(ln(sBP/dBP))/(deltaLD/dLD)	[26]
EP	kPa	dLD×PP/deltaLD	[14]
EINC	kPa	dLD/(aCIMT × DC)	[8]

a = average, d = diastolic, s = systolic, BP = blood pressure, PP = pulse pressure (sBP – dBP), deltaLD = delta lumen diameter, CIMT = carotid intima media thickness, LD = lumen diameter, DC = distensibility coefficient, CC = compliance coefficient, Bstiff = β-stiffness index, EP = Peterson's elastic modulus, EINC = Young's elastic modulus, ln = natural logarithm.

Statistical analyses

To assess differences in structural and functional carotid parameters between two ultrasound examinations (T1, T2), mean differences, 95% confidence interval (CI) and p-values were calculated using paired t-tests. Functional parameters were log-transformed; p-values <0.05 were considered as statistically significant.

Furthermore, for each parameter, individual between-visit coefficients of variation (CV) defined as the ratio of the standard deviation to the mean of the two measurements of the respective subject, were determined and intraclass correlation coefficients (ICC) were computed using one-way analysis of variance models (according to McGraw and Wong ICC(1) [27] and Shrout and Fleiss ICC(1,1) [28]). In addition, Bland-Altman plots were generated showing mean differences and estimated limits of agreement of the examined carotid functional parameters [29].

Finally, mixed linear models were used to quantify the different sources of total variation. Therefore, the model included a fixed factor distinguishing between the first and the second measurement and random intercepts were assigned to variables subject, sonographer, and

reader. Then, the contributions to the total variance of these random intercept factors and of uncontrolled influences at the level of individual measurements were estimated.

All analyses were performed using the statistical software STATA (StataCorp, Release 12. Statistical Software, College Station, TX: StataCorp LPTexas, USA).

Results

Descriptive statistics of study population characteristics for both examinations (T1, T2) and the respective p-values of paired t-tests are shown in table 6.2. The analytic sample size included 165 SAPALDIA 3 subjects. 40.6% were men, with a mean age of 63.6(7.5) years, an age range of 50-80 years and a mean body mass index of 26.0(3.4) kg/m^2. 59.4% were women, with a mean age of 61.0(6.9) years, an age range of 50-79 years and a mean body mass index of 23.7(3.5) kg/m^2. On average, 19.3(18.1) days were between the two ultrasound examinations.

Table 6.2: Descriptive characteristics of study population with number (N), mean value and standard deviation (SD) and the p-value for the difference of the two examinations (T1, T2)

Parameter	unit	T1		T2		p-value
		N	mean(SD)	N	mean(SD)	
sBP	mmHg	165	131.93(17.63)	165	127.03(15.20)	<0.001
dBP	mmHg	165	77.15(9.71)	165	74.23(8.76)	<0.001
PP	mmHg	165	54.78(12.34)	165	52.8(10.91)	0.001
aCIMT	mm	165	0.71(0.11)	165	0.71(0.12)	0.81
sCIMT	mm	165	0.68(0.11)	165	0.68(0.12)	0.72
dCIMT	mm	165	0.73(0.12)	165	0.74(0.12)	0.46
aLD	mm	165	7.34(0.78)	165	7.29(0.80)	0.001
sLD	mm	165	7.54(0.78)	165	7.48(0.82)	<0.001
dLD	mm	165	7.14(0.78)	165	7.10(0.80)	0.002
deltaLD	mm	165	0.40(0.11)	165	0.38(0.11)	0.002
strain	%	165	5.63(1.67)	165	5.40(1.59)	0.016
DC	10^{-3}/kPa	165	16.64(6.31)	165	16.33(5.63)	0.73
CC	mm^2/kPa	165	0.65(0.21)	165	0.63(0.20)	0.25
Bstiff	no unit	165	10.43(3.81)	165	10.74(3.51)	0.016
EP	kPa	165	143.07(59.51)	165	141.84(53.37)	0.78
EINC	kPa	165	708.33(306.64)	165	699.80(270.06)	0.95

a = average, d = diastolic, s = systolic, BP = blood pressure, PP = pulse pressure, CIMT = carotid intima media thickness, LD = lumen diameter, DC = distensibility coefficient, CC = compliance coefficient, Bstiff = β-stiffness index, EP = Peterson's elastic modulus, EINC = Young's elastic modulus.

Table 6.3 shows means of ICCs and means of individual CVs with 95% CI. Additionally, table 6.3 expresses how much of the variance of different examination variables is explained by the factors study subject, sonographer or reader and how much of the variance is unexplained. The ICCs were 0.71 for systolic BP, 0.67 for diastolic BP and 0.77 for PP between T1 and T2. ICCs for structural properties ranged between 0.87 and 0.91 for CIMT and between 0.96 and 0.97 for LD. Finally, ICCs of functional parameters ranged between of 0.75 and 0.79. Measurement variability was lowest in LD (CV = 1.6-1.7%), followed by CIMT (CV = 3.7-4.6) and BP (CV = 5.08-7.87). Greater measurement variability was observed for functional indices (CV = 11.0-13.1%). For all parameters, most of the variance was explained by the factor subject (>71.1%), while the factors sonographer and reader accounted for less than 2.9% and 1.6%, respectively, of the total variance.

Table 6.3: Mean ICC and mean CV with 95% confidence interval (CI) and percent variation explained by the factors study subject, sonographer, reader and residuals

Para-meter	Mean ICC (95% CI)	Mean CV (95% CI) [%]	Variance explained by subject [%]	Variance explained by sonographer [%]	Variance explained by reader [%]	Unexplained variance [%]
sBP	0.71 (0.63-0.78)	5.08 (4.39-5.77)	74.44	0.00		25.56
dBP	0.67 (0.59-0.75)	5.21 (4.48-5.94)	71.09	0.00		28.91
PP	0.77 (0.70-0.83)	7.87 (6.87-8.86)	77.82	0.00		22.18
aCIMT	0.89 (0.87-0.93)	3.98 (3.52-4.44)	86.28	2.66	1.25	9.81
sCIMT	0.87 (0.84-0.91)	4.57 (4.00-5.14)	82.38	2.89	1.60	13.13
dCIMT	0.91 (0.88-0.94)	3.70 (3.25-4.15)	89.42	1.51	0.81	8.25
aLD	0.97 (0.96-0.98)	1.60 (1.40-1.80)	96.32	0.00	0.32	3.36
sLD	0.96 (0.95-0.97)	1.67 (1.46-1.87)	96.12	0.00	0.32	3.56
dLD	0.96 (0.96-0.98)	1.59 (1.39-1.80)	96.48	0.00	0.26	3.26
deltaLD	0.77 (0.71-0.83)	11.16 (9.74-12.59)	75.04	0.00	0.21	24.75
strain	0.77 (0.71-0.83)	10.99 (9.53-12.45)	77.71	0.00	0.00	22.29
DC	0.77 (0.71-0.84)	12.14 (10.58-13.71)	80.73	0.00	0.00	19.27
CC	0.75 (0.69-0.82)	12.11 (10.56-13.65)	74.27	0.00	0.01	25.72
Bstiff	0.75 (0.68-0.82)	11.43 (9.81-13.06)	75.98	0.00	0.00	24.02
EP	0.79 (0.73-0.85)	11.95 (10.42-13.49)	80.74	0.00	0.00	19.26
EINC	0.77 (0.71-0.83)	13.07 (11.37-14.78)	78.47	0.00	0.00	21.53

a = average, d = diastolic, s = systolic, BP = blood pressure, PP = pulse pressure, CIMT = carotid intima media thickness, LD = lumen diameter, DC = distensibility coefficient, CC = compliance coefficient, Bstiff = β-stiffness index, EP = Peterson's elastic modulus, EINC = Young's elastic modulus.

Bland-Altman plots for carotid function parameters with mean(SD) of the differences and the respective values in percentage of the mean were for strain 0.2(1.1) % and 4.2(19.6) %, for DC 0.3(4) 10^{-3}/kPa and 1.9(24.4) %, for CC 0.0(0.1) mm^2/kPa and 2.4(22.2) %, for Bstiff -0.3(2.6) and 3.0(24.4) %, for EP 1.2(36.6) kPa and 0.9(25.7) %, and for EINC 8.5(196.2) kPa and 1.2(27.9) % (see figure 6.3). In none of the parameters the Bland-Altman plot revealed a drift with increasing values thus indicating stable analysis across the complete range of analysed parameters within the cohort.

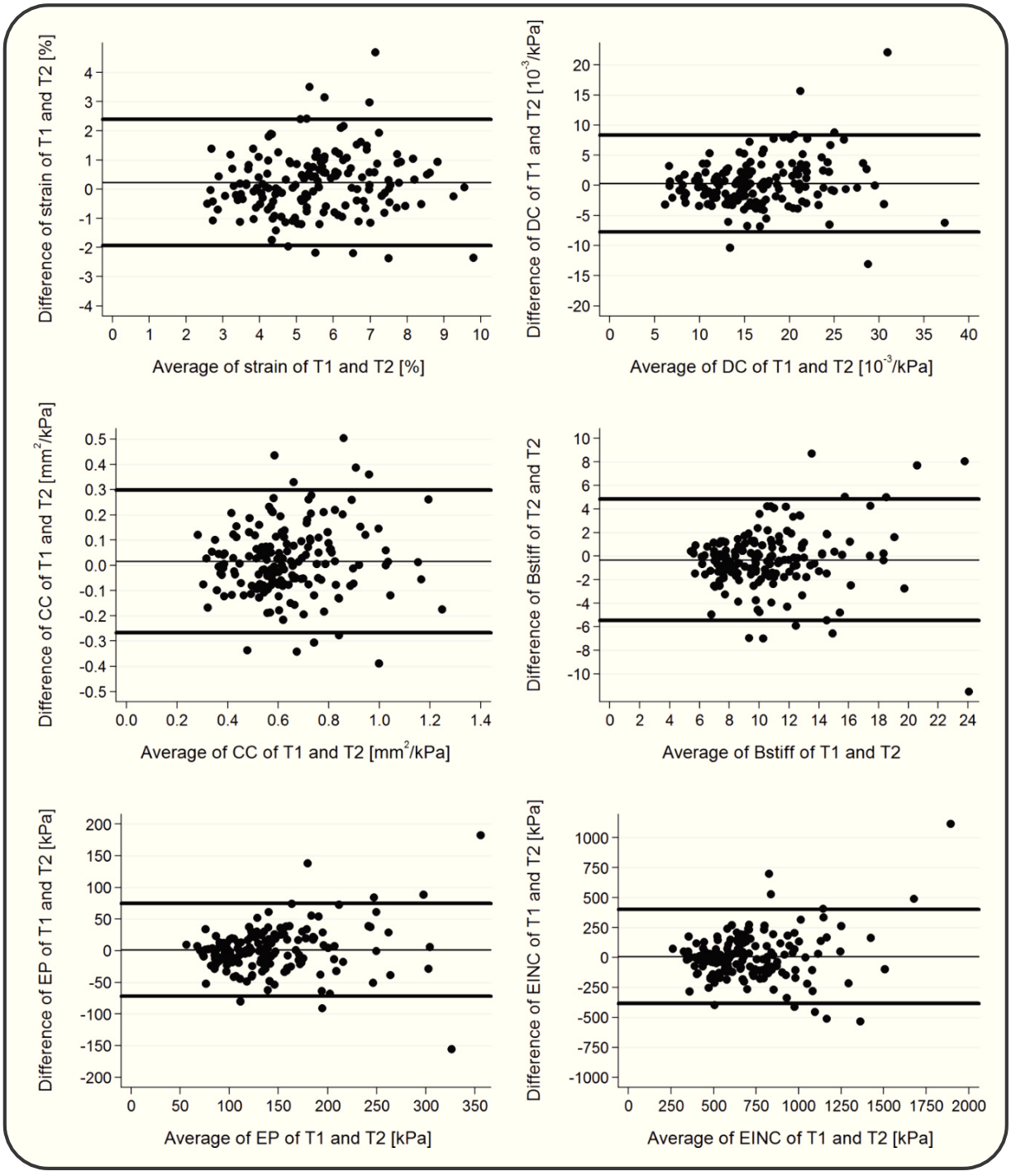

Figure 6.3: Bland-Altman plots of functional parameters at two ultrasound examinations (T1, T2) with mean difference as thin black line and limits of agreement as bold black lines

Legend: DC = distensibility coefficient, CC = compliance coefficient, stiffness = β-stiffness index, EP = Peterson's elastic modulus, EINC = Young's elastic modulus.

Discussion

The main results of this validation study show that the structural parameters obtained from SAPALDIA 3 study have an excellent reproducibility and functional indices a good reproducibility. Measurement variability was lowest in structural parameters followed by functional indices and BP variables. To our knowledge this is the first epidemiological study which analysed reproducibility of all generally used parameters of carotid structure and function to describe risk for cardiovascular events within a representative sample of the total study cohort.

To better understand the significance of our reproducibility study, we compared results of structural and functional reproducibility analyses of different population based studies with our data (see table 6.4). We chose these studies, because at least one structural and functional carotid parameter was used as a covariate or outcome. In general, reproducibility of structural parameters was higher than in the Rotterdam [22], Northern Manhattan Prospective Cohort Study (NOMAS) [20] and Hoorn Study [18] and in line with the Multi Ethnic Study of Atherosclerosis Study (MESA) [19]. Functional parameters showed higher ICCs than in the Atherosclerosis Risk In Communities Study (ARIC) [15] or slightly lower reproducibility compared to the Rotterdam [9,22], Hoorn [18] or MESA Study [19]. Overall, structural, functional and BP parameters were in good agreement with results reported in literature [9,15,18–22] (see table 6.4). However, comparisons of reproducibility results for structural and functional parameters within population based studies remain challenging due to three main aspects, namely differences in vascular measurement regions (e.g. near or far wall of CCA), in reproducibility aspects of study design (e.g. intra- and interreader, intra- and interobserver, intra- and intersession) and in statistical reproducibility analyses (e.g. Pearson and Spearman correlation coefficient, CV, ICC).

From a methodological point of view, measurement variability depends on anatomical location [16,30,31]. Therefore, it is essential that only measurements relating to the same wall segments can be directly compared. Even the selected studies differ in calculating the CIMT (i.e. mean maximum or near plus far wall etc.) and thus are not one to one comparable according to reproducibility with our study and among each other.

Further, a study design considering rereading a single ultrasound examination (inter- and intrareader) only reflects reading reproducibility but not the complete process including the sonographer’s contribution and the reading process. In order to investigate study data quality in a way relevant for the epidemiologic research, replicate ultrasound examinations and readings must be performed by the same technicians and their protocols and systems within the same study population to guarantee that data quality depends on population characteristics of the examined cohort such as age, sex, morbidities and other factors.

From a statistical point of view, the use of different statistical methods for reproducibility assessment and interpretation has been recommended [32]. Reproducibility analysis by Pearson or Spearman correlation coefficient is still very common [16,17], but these

correlation measures cannot identify systematic errors [29,33]. CV is dimensionless, usually expressed in percent, and can directly be compared across different scales. However, these scales must be limited by zero since CV expresses the standard deviation as percentage of the mean [32]. ICC can assess reproducibility of one or more repeated measurements by expressing the relation of explained variance by subject to total variance [33]. But since there are several possibilities to calculate ICC, exact description of ICC formula is required [27,28,33]. Mixed model analysis has the advantage of determining the explained variance due to different factors and one can express each factor as percentage of explained variance. As a consequence, we examined reproducibility data by specified mixed linear models, ICC, CV, and Bland-Altman to give mean differences and estimated limits of agreement. This multi-parametric approach gives a thorough view into the quality of the reproducibility of the SAPALDIA 3 data being unique so far on the cohort level.

In general, the total variability is the sum of the different components or sources of variability within a study and it should be as low as possible. In theory, the unexplained variance ideally should be zero and the remaining variability should be attributed to the subject. In real clinical practice, however, no measurement system is perfect and this is also true for our carotid ultrasound measurements within the SAPALDIA 3 study. Thus, the total variability of measurements can be explained by the population and its subjects, temporal effects, sonographers and readers. The mixed model analyses showed that less than 2.9% of the measurement variation was explained by sonographer or reader and the remaining variability of more than 74-81% for functional and 82-96% for structural parameters were explained by the subject. Within the presented population based studies only the ARIC study reported random effects analysis of variance [15], which best describes the distribution of different variability sources. Comparing ARIC and SAPALDIA 3, SAPALDIA 3 explained more variance by the factor subject for dLD, strain, EP and DC, while a slightly lower variance explained by subject was found for deltaLD and CC [15]. Thus, sample sizes needed for future SAPALDIA cross-sectional and longitudinal risk identification will be comparable or slightly lower than in ARIC providing a solid basis for the upcoming analysis with carotid structural and functional parameters as predictors or outcome.

Table 6.4: Overview of structural and functional reproducibility within population based studies

Cohort and Reference	Cohort		Reproducibility study							
	N	**age [years]**	**N**	**age [years]**	**ICC**		**CV [%]**		**Remarks**	
ARIC [15]	15800	Range: 45-64	36	Range: 45-64	Interobserver dBP PP dLD deltaLD strain DC CC EINC	 0.57 0.69 0.65 0.76 0.67 0.67 0.77 0.66	Interobserver dBP PP dLD deltaLD strain DC CC EINC	 7# 18# 8# 29# 26# 32# 38# 35#	„No history of hypertension, angina pectoris or coronary heart disease“ [15]. #CV of between-person calculated by random effects analysis of variance [15].	
CHS [16]	5201	> 65	N = 22 for aCIMT N = 24 for dLD	Mean age: 68.6 SD: 1.2					Interreader aCIMT dLD	**Spearman** 0.91 0.85
FOSC [17]	5124 Ultrasound 3377	Mean age: 57.9 SD: 9.6	37	n.p.					Interreader dCIMT sCIMT	**Pearson** 0.94 0.93
Hoorn Study [18]	2484 Ultrasound 822	Range: 50-74	10	Mean age: 58.2 SD: 9.5			Intraobserver aCIMT aLD deltaLD DC CC EINC	 10.9 2.9 5.3 7.0 6.0 11.6		
MESA [19]	6814	Range: 45-84	Intraobserver N=31 for CIMT N=211 for DC and EINC Interobserver N=10 for DC and EINC intrareader N=71 for CIMT N=204 for DC and EINC Interreader N=77 for CIMT	Not published	Intraobserver CIMT DC EINC Interobserver DC EINC Intrareader CIMT DC EINC Interreader CIMT	 0.95 0.71 0.69 0.85 0.84 0.98 0.68 0.80 0.87			CIMT is the average of maximal CIMT in near and far wall [19].	

Table 6.4 continued

Cohort and Reference	Cohort		Reproducibility study						
	N	**age [years]**	**N**	**age [years]**	**ICC**		**CV [%]**		**Remarks**
NOMAS **[20,21]**	3298 Ultrasound 1133	Mean age : 65.4 SD : 8.8	88	n.p.			Intrareader CIMT Interreader CIMT	5.4 7.5	**Correlation coefficient** Intrareader CIMT 0.94 Interreader CIMT 0.77 CIMT is the average of the near and the far wall of the maximal CCA CIMT, the maximal bifurcation CIMT, and the maximal ICA CIMT [21].
Rotterdam **[9,22]**	7983	> 50	47	n.p.	CIMT DC	0.74 0.8			CIMT is the average of the near and far wall CIMT [23].
SAPALDIA (current article)	3489	Range: 50-81 Mean age: 63.90 SD: 8.07	**165**	Range: 50-80 Mean age: 62.1 SD:7.3	intersession dBP sBP PP aCIMT dCIMT sCIMT aLD dLD sLD deltaLD strain DC CC Bstiff EP EINC	 0.67 0.71 0.77 0.89 0.91 0.87 0.97 0.96 0.96 0.77 0.77 0.75 0.75 0.75 0.79 0.77	intersession dBP sBP PP aCIMT dCIMT sCIMT aLD dLD sLD deltaLD strain DC CC Bstiff EP EINC	 5.21 5.08 7.87 3.98 3.70 4.57 1.60 1.59 1.67 11.16 10.99 12.14 12.11 11.43 11.95 13.07	

a = average, d = diastolic, s = systolic, BP = blood pressure, PP = pulse pressure, CIMT = carotid intima media thickness, LD = lumen diameter, DC = distensibility coefficient, CC = compliance coefficient, Bstiff = β-stiffness index, EP = Peterson's elastic modulus, EINC = Young's elastic modulus, ARIC = Atherosclerosis Risk in Communities, CHS = the Cardiovascular Health Study, FOSC = Framingham offspring study, Hoorn = Hoorn Study, MESA = Multi Ethnic Study of Atherosclerosis, NOMAS = Northern Manhattan Prospective Cohort Study, Rotterdam = Rotterdam study, SAPALDIA = Swiss Cohort Study on Air Pollution and Lung and Heart Diseases In Adults, n.p. = not published, Interobserver = two ultrasound visits and minimal two different readers, Intraobserver = two ultrasound visits and the same reader, Interreader = one ultrasound visit and minimal two different readers, Interreader = one ultrasound visit and the same reader, intersession = two ultrasound visits with randomised reading procedure.

Conclusion and perspectives

In summary, in order to investigate study data quality for further epidemiologic research, we evaluated the reproducibility and sources of variability of structural and functional distal CCA wall and lumen ultrasound measurements in a representative random sample of participants in the multicentre SAPALDIA 3 cohort. Structural parameters have an excellent reproducibility and functional indices a good reproducibility. This will provide a solid basis for the exploratory SAPALDIA 3 ultrasound data analyses to assess the early and late consequences of atherosclerosis progression and to evaluate the associations of preventive or prognostic determinants and carotid ultrasound structural and functional parameters which may help to improve cardiovascular risk stratification by using carotid ultrasound in clinical practice.

Acknowledgments

The authors thank the members of the Reading Center of the Institute of Exercise and Health Science, the SAPALDIA Team, the SAPALDIA participants and sonographers for their excellent work.

Funding

No conflict of interest stated for all authors.

Paper abbreviations

- BP = blood pressure
    - dBP = diastolic blood pressure
    - sBP = systolic blood pressure
    - PP = pulse pressure = systolic blood pressure – diastolic blood pressure

- CCA = common carotid artery
- DYARA = **DY**namic **AR**tery **A**nalysis

- Functional parameters
    - DC = distensibility coefficient
    - CC = compliance coefficient
    - Bstiff = β-stiffness index
    - EP = Peterson's elastic modulus
    - EINC = Young's elastic modulus

- Structural parameters
    - CIMT = carotid intima media thickness
        - aCIMT = average carotid intima media thickness
        - dCIMT = diastolic intima media thickness
        - sCIMT = systolic intima media thickness
    - LD = lumen diameter
        - aLD = average lumen diameter
        - dLD = diastolic lumen diameter
        - sLD = systolic lumen diameter
        - deltaLD = systolic lumen diameter – diastolic lumen diameter

- Statistical terms
    - CI = confidence interval
    - CV = coefficient of variation
    - ICC = intraclass coefficient
    - SD = standard deviation
    - ln = natural logarithm

- Studies acronyms
    - ARIC = Atherosclerosis Risk in Communities
    - CHS = the Cardiovascular Health Study
    - FOSC = Framingham offspring study
    - Hoorn = Hoorn Study
    - MESA = Multi Ethnic Study of Atherosclerosis
    - NOMAS = Northern Manhattan Prospective Cohort Study
    - Rotterdam = Rotterdam study
    - SAPALDIA = Swiss Cohort Study on Air Pollution and Lung and Heart Diseases In Adults

References

1 World Health Organization. (2008) The global burden of disease: 2004 update, World Health Organization, Geneva. 1-146

2 Van Bortel, L. M., Duprez, D., Starmans-Kool, M. J., Safar, M. E., Giannattasio, C., Cockcroft, J., Kaiser, D. R. and Thuillez, C. (2002) Clinical applications of arterial stiffness, Task Force III: recommendations for user procedures. Am. J. Hypertens. **15**, 445–452

3 Perk, J., De Backer, G., Gohlke, H., Graham, I., Reiner, Z., Verschuren, M., Albus, C., Benlian, P., Boysen, G., Cifkova, R., Deaton, C., Ebrahim, S., Fisher, M., Germano, G., Hobbs, R., Hoes, A., Karadeniz, S., Mezzani, A., Prescott, E., Ryden, L., Scherer, M., Syvänne, M., Scholte op Reimer, W. J. M., Vrints, C., Wood, D., Zamorano, J. L. and Zannad, F. (2012) European Guidelines on cardiovascular disease prevention in clinical practice (version 2012). The Fifth Joint Task Force of the European Society of Cardiology and Other Societies on Cardiovascular Disease Prevention in Clinical Practice (constituted by representatives of nine societies and by invited experts). Developed with the special contribution of the European Association for Cardiovascular Prevention & Rehabilitation (EACPR). Eur. Heart J. **33**, 1635–1701

4 Roman, M. J., Naqvi, T. Z., Gardin, J. M., Gerhard-Herman, M., Jaff, M. and Mohler, E. (2006) Clinical Application of Noninvasive Vascular Ultrasound in Cardiovascular Risk Stratification: A Report from the American Society of Echocardiography and the Society of Vascular Medicine and Biology. J. Am. Soc. Echocardiogr. **19**, 943–954

5 Laurent, S., Cockcroft, J., Van Bortel, L., Boutouyrie, P., Giannattasio, C., Hayoz, D., Pannier, B., Vlachopoulos, C., Wilkinson, I. and Struijker-Boudier, H. (2006) Expert consensus document on arterial stiffness: methodological issues and clinical applications. Eur. Heart J. **27**, 2588–2605

6 Lorenz, M. W., Markus, H. S., Bots, M. L., Rosvall, M. and Sitzer, M. (2007) Prediction of clinical cardiovascular events with carotid intima-media thickness: a systematic review and meta-analysis. Circulation. **115**, 459–467

7 Simon, A., Megnien, J. L. and Chironi, G. (2010) The value of carotid intima-media thickness for predicting cardiovascular risk. Arterioscler. Thromb. Vasc. Biol. **30**, 182–185

8 Leone, N., Ducimetière, P., Gariépy, J., Courbon, D., Tzourio, C., Dartigues, J.-F., Ritchie, K., Alpérovitch, A., Amouyel, P., Safar, M. E. and Zureik, M. (2008) Distension of the Carotid Artery and Risk of Coronary Events: The Three-City Study. Arterioscler. Thromb. Vasc. Biol. **28**, 1392–1397

9 Mattace-Raso, F. U. S., van der Cammen, T. J. M., Hofman, A., van Popele, N. M., Bos, M. L., Schalekamp, M. A. D. H., Asmar, R., Reneman, R. S., Hoeks, A. P. G., Breteler, M. M. B. and Witteman, J. C. M. (2006) Arterial Stiffness and Risk of Coronary Heart Disease and Stroke: The Rotterdam Study. Circulation. **113**, 657–663

10 Blacher, J., Safar, M. E., Pannier, B., Guerin, A. P., Marchais, S. J. and London, G. M. (2002) Prognostic significance of arterial stiffness measurements in end-stage renal disease patients. Curr. Opin. Nephrol. Hypertens. **11**, 629–634

11 Odink, A. E., Mattace-Raso, F. U. S., van der Lugt, A., Hofman, A., Hunink, M. G. M., Breteler, M. M. B., Krestin, G. P. and Witteman, J. C. M. (2008) The association of arterial stiffness and arterial calcification: the Rotterdam Study. J. Hum. Hypertens. **22**, 205–207

12 Barenbrock, M., Kosch, M., Jöster, E., Kisters, K., Rahn, K.-H. and Hausberg, M. (2002) Reduced arterial distensibility is a predictor of cardiovascular disease in patients after renal transplantation. J. Hypertens. **20**, 79–84

13 Teynor, A., Caviezel, S., Dratva, J., Künzli, N. and Schmidt-Trucksäss, A. (2012) An automated, interactive analysis system for ultrasound sequences of the common carotid artery. Ultrasound Med. Biol. **38**, 1440–1450

14 Nichols, W. W., O'Rourke, M. F. and Vlachopoulos, C. (2011) McDonald's Blood Flow in Arteries: Theoretical, Experimental and Clinical Principles 6th ed., Hodder Arnold, London. 1-768

15 Arnett, D. K., Chambless, L. E., Kim, H., Evans, G. W. and Riley, W. (1999) Variability in ultrasonic measurements of arterial stiffness in the atherosclerosis risk in communities study. Ultrasound Med. Biol. **25**, 175–180

16 O'Leary, D. H., Polak, J. F., Wolfson, S. K., Bond, M. G., Bommer, W., Sheth, S., Psaty, B. M., Sharrett, A. R. and Manolio, T. A. (1991) Use of sonography to evaluate carotid atherosclerosis in the elderly. The Cardiovascular Health Study. CHS Collaborative Research Group. Stroke. **22**, 1155–1163

17 Polak, J. F., Meisner, A., Pencina, M. J., Wolf, P. A. and D'Agostino, R. B. (2012) Variations in Common Carotid Artery Intima-Media Thickness during the Cardiac Cycle: Implications for Cardiovascular Risk Assessment. J. Am. Soc. Echocardiogr. **25**, 1023–1028

18 Henry, R. M. A., Kostense, P. J., Spijkerman, A. M. W., Dekker, J. M., Nijpels, G., Heine, R. J., Kamp, O., Westerhof, N., Bouter, L. M. and Stehouwer, C. D. A. (2003) Arterial Stiffness Increases With Deteriorating Glucose Tolerance Status The Hoorn Study. Circulation. **107**, 2089–2095

19 Fernandes, V. R. S., Polak, J. F., Cheng, S., Rosen, B. D., Carvalho, B., Nasir, K., McClelland, R., Hundley, G., Pearson, G., O'Leary, D. H., Bluemke, D. A. and Lima, J. A. C. (2008) Arterial Stiffness Is Associated With Regional Ventricular Systolic and Diastolic Dysfunction: The Multi-Ethnic Study of Atherosclerosis. Arterioscler. Thromb. Vasc. Biol. **28**, 194–201

20 Della-Morte, D., Gardener, H., Denaro, F., Boden-Albala, B., Elkind, M. S. V., Paik, M. C., Sacco, R. L. and Rundek, T. (2010) Metabolic syndrome increases carotid artery stiffness: The Northern Manhattan Study. Int. J. Stroke. **5**, 138–144

21 Rundek, T., Elkind, M. S., Pittman, J., Boden-Albala, B., Martin, S., Humphries, S. E., Juo, S. H. and Sacco, R. L. (2002) Carotid Intima-Media Thickness Is Associated With Allelic Variants of Stromelysin-1, Interleukin-6,

and Hepatic Lipase Genes The Northern Manhattan Prospective Cohort Study. Stroke. **33**, 1420–1423

22 Van Popele, N. M., Grobbee, D. E., Bots, M. L., Asmar, R., Topouchian, J., Reneman, R. S., Hoeks, A. P. G., van der Kuip, D. A. M., Hofman, A. and Witteman, J. C. M. (2001) Association between arterial stiffness and atherosclerosis: the Rotterdam Study. Stroke. **32**, 454–460

23 Martin, B. W., Ackermann-Liebrich, U., Leuenberger, P., Künzli, N., Zemp Sutzt, E., Keller, R., Zellweger, J. P., Wüthrich, B., Monn, C., Blaser, K., Bolognini, G., Bongard, J. P., Brändli, O., Braun, P., Defila, C., Domenighetti, G., Grize, L., Karrer, W., Keller-Wossidlo, H., Medici, T. C., Peeters, A., Perruchoud, A. P., Schindler, C., Schoeni, M. H., Schwartz, J., Solari, G., Tschopp, J. M. and Villiger, B. (1997) SAPALDIA: methods and participation in the cross-sectional part of the Swiss Study on Air Pollution and Lung Diseases in Adults. Soz.- Präventivmedizin. **42**, 67–84

24 Ackermann-Liebrich, U., Kuna-Dibbert, B., Probst-Hensch, N. M., Schindler, C., Felber Dietrich, D., Zemp Stutz, E., Bayer-Oglesby, L., Baum, F., Brändli, O., Brutsche, M., Downs, S. H., Keidel, D., Gerbase, M. W., Imboden, M., Keller, R., Knöpfli, B., Künzli, N., Nicod, L., Pons, M., Staedele, P., Tschopp, J.-M., Zellweger, J.-P. and Leuenberger, P. (2005) Follow-up of the Swiss Cohort Study on Air Pollution and Lung Diseases in Adults (SAPALDIA 2) 1991-2003: methods and characterization of participants. Soz.-Präventivmedizin. **50**, 245–263

25 Touboul, P.-J., Hennerici, M. G., Meairs, S., Adams, H., Amarenco, P., Bornstein, N., Csiba, L., Desvarieux, M., Ebrahim, S., Fatar, M., Hernandez Hernandez, R., Jaff, M., Kownator, S., Prati, P., Rundek, T., Sitzer, M., Schminke, U., Tardif, J.-C., Taylor, A., Vicaut, E., Woo, K. S., Zannad, F. and Zureik, M. (2007) Mannheim carotid intima-media thickness consensus (2004-2006). An update on behalf of the Advisory Board of the 3rd and 4th Watching the Risk Symposium, 13th and 15th European Stroke Conferences, Mannheim, Germany, 2004, and Brussels, Belgium, 2006. Cerebrovasc. Dis. **23**, 75–80

26 O'Rourke, M. F., Staessen, J. A., Vlachopoulos, C., Duprez, D. and Plante, G. E. (2002) Clinical applications of arterial stiffness; definitions and reference values. Am J Hypertens. **15**, 426–444

27 McGraw, K. O. and Wong, S. P. (1996) Forming inferences about some intraclass correlation coefficients. Psychol. Methods. **1**, 30–46

28 Shrout, P. E. and Fleiss, J. L. (1979) Intraclass correlations: uses in assessing rater reliability. Psychol. Bull. **86**, 420–428

29 Bland, J. M. and Altman, D. G. (1986) Statistical methods for assessing agreement between two methods of clinical measurement. The Lancet. **327**, 307–310

30 Stensland-Bugge, E., Bønaa, K. H. and Joakimsen, O. (1997) Reproducibility of ultrasonographically determined intima-media thickness is dependent on arterial wall thickness. The Tromsø Study. Stroke. **28**, 1972–1980

31 Bauer, M., Caviezel, S., Teynor, A., Erbel, R., Mahabadi, A. A. and Schmidt-Trucksäss, A. (2012) Carotid intima-media thickness as a biomarker of subclinical atherosclerosis. Swiss Med. Wkly. **142**, 1-9

32 Atkinson, G. and Nevill, A. M. (1998) Statistical methods for assessing measurement error (reliability) in variables relevant to sports medicine. Sports Med. **26**, 217–238

33 Weir, J. P. (2005) Quantifying test-retest reliability using the intraclass correlation coefficient and the SEM. J. Strength Cond. Res. **19**, 231–240

CHAPTER 7

Sex-specific associations of cardiovascular risk factors with carotid stiffness – results from the SAPALDIA Cohort Study

Authors

Seraina Caviezel
Julia Dratva
Emmanuel Schaffner
Christian Schindler
Elisabeth Zemp Stutz
Eric de Groot
Luc Burdet
Thomas Rothe
Marco Pons
Jean-Michel Gaspoz
Thierry Rochat
Nino Künzli
Nicole Probst-Hensch
Arno Schmidt-Trucksäss

Accepted version*, published in:

Atherosclerosis 2014 Jun;235(2):576-84.
Doi: 10.1016/j.atherosclerosis.2014.05.963.

*Minor editorial modifications possible due to harmonisation of the thesis

SEX-SPECIFIC ASSOCIATIONS OF CARDIOVASCULAR RISK FACTORS WITH CAROTID STIFFNESS – RESULTS FROM THE SAPALDIA COHORT STUDY

Abstract

Objective: Manifestation of cardiovascular disease (CVD) occurs with clear sex differences. Carotid stiffness parameters are increasingly used for CVD risk assessment but the sex specific association with CVD risk factors as well as association patterns between carotid stiffness parameters are largely unknown, which we investigated in SAPALDIA population-based cohort participants.

Methods: Risk factors of 2545 participants without clinically manifest disease were evaluated in 2001 2003 and different carotid stiffness parameters were assessed in carotid ultrasound scans in 2010-2011. Stratified and non-stratified mixed linear models and multivariate regression analyses were used to examine sex specific associations, differences and association patterns of single risk factors and carotid stiffness parameters.

Results: High-density lipoprotein cholesterol (HDL-C) was the only significant protective determinant of reduced carotid stiffness for both sexes (ranges of carotid stiffness parameters: -3.7; -0.8% of changes in geometric mean per 1SD of the risk factor on an inverted scale) and significant adverse risk factors were body mass index (BMI) (-0.5; 4.7%), systolic (-1.23; 4.7%) and diastolic blood pressure (1.4; 4.4%), heart rate (2.7; 7.9%), C-reactive protein (0.6; 3.3%) and smoking (2.82; 1%), all p-values of multivariate analyses were < 0.01. Sex differences with stiffer carotid stiffness parameters in men were observed for increased heart rate (p=0.001) and low-density lipoprotein cholesterol (LDL-C) (p<0.001) and in women for triglyceride (p<0.003). Similar association patterns were found for most carotid stiffness parameters.

Conclusion: Sex-specific associations of cardiovascular risk factors may reflect a sex-specific burden of atherosclerotic risk factors and similar association patterns across different carotid stiffness parameters within men and women may allow the use of carotid stiffness parameters in an exchangeable manner.

Introduction

Six major cardiovascular risk factors, hypertension, smoking, elevated blood glucose and cholesterol, physical inactivity and obesity cause 42.1% of total mortality worldwide and 60.2% in high-income countries [1]. An early assessment of cardiovascular risk might therefore support the implementation of preventive or therapeutic interventions in asymptomatic but at risk subjects to reduce the economic burden of cardiovascular disease (CVD) [2]. Risk prediction today is generally based on risk scores like Framingham, PROCAM, HEART or ASCVD [3,4]. Substantial disadvantages of the score systems are that they account

only for specific risk factors at one time point, not reflecting risk factor burden longitudinally [3]. Vascular imaging biomarkers may overcome this disadvantage since the vasculature may indicate accumulated risk at an initial state, expressed by the deterioration of vascular function, and later on by structural changes in shape of arteriosclerosis and/or atherosclerosis [5,6].

Among non-invasive imaging biomarkers carotid stiffness has been increasingly examined in recent years. Novel software applications facilitate the analysis of single ultrasound images and of sequential images over several heart cycles [7–13]. Carotid stiffness parameters derived from such analyses are local measures of arterial stiffness [14] and encompass strain, distensibility, compliance, β-stiffness index, Peterson's elastic modulus and Young's elastic modulus [15–19]. Shared pathophysiological mechanisms (e.g. blood pressure (BP) or vascular diameter) among carotid stiffness parameters should theoretically result in similar associations with risk factors. However, this has not been tested neither in both sexes nor separately within men and women [18–26]. Further, we noticed that some studies used more than one carotid stiffness parameter, and reported more or less divergent associations of risk factors and carotid stiffness parameters in their results [18–22]. In addition, some investigators did not use the same statistical approach consistently for all carotid stiffness parameters, which impairs the comparability of the results [20,21]. The gained impressions indicate that differences in associations between these six carotid stiffness measurements may exist and should be tested. Moreover, sex differences in subclinical CVD measured with carotid ultrasound were not yet investigated.

However, sex- and age-related cardiovascular morbidity and mortality risk and vascular remodeling, respectively, have been reported [22–25]. Women have a lower incidence of cardiovascular morbidity and mortality in middle age with an approximation after age 45 years [22] and on the vascular level, carotid stiffness parameters have been shown to stiffen with age in both sexes, but with greater impact in older women compared to men [23,24]. Studies among measurements of regional or systemic arterial stiffness such as carotid-femoral or aortic pulse wave velocity have shown to predict cardiovascular events and mortality [14,26]. Aortic stiffness was independently associated with different cardiovascular risk factors in men [27]. Moreover, a large prospective study reported sex-specific different associations of risk factors on aortic stiffness [28]. In contrast, negligible sex differences were shown for hemodynamic parameters and age investigating reference values [29]. However, conclusions have been shown to depend on sex and arterial segment [30]. On this background and focusing on local measurement of arterial stiffness, it is not clear yet whether cardiovascular risk factors and their cardiovascular burden result in identical vascular changes for men and women in the common carotid artery (CCA).

This study intends to deepen the understanding of the long-term associations and patterns between several classical CVD risk factors and different carotid stiffness parameters, separately for men and women initially free of CVD diagnosis based on the SAPALDIA cohort study (Swiss Cohort Study on Air Pollution And Lung and Heart Diseases In Adults).

Methods

Study design and subjects

SAPALDIA baseline assessments were conducted in 1991 in a random population sample of adults aged 18-60 years (N=9651) from eight rural and urban areas of Switzerland (Aarau, Basel, Davos, Genève, Lugano, Montana, Payerne, Wald) [31]. The original focus of the study was on air pollution and respiratory health. In the follow-up surveys in 2001-2003 (SAPALDIA 2, N=8047) and in 2010-2011 (SAPALDIA 3, N=6087) the focus was extended to cardiovascular health [32]. In SAPALDIA 2, cardiovascular risk factors were assessed in 6190 subjects (49% male, 51% females) and medical treatment was collected by means of self-report, asking for intake of antihypertensive drugs, lipid lowering agents, glucose lowering agents and beta-blocker within the last 30 days. In SAPALDIA 3, sequential B-mode ultrasound images of the CCA were obtained from 3489 participants (49% male, 51% females) with an age range between 50-80 years at the time of the examination.

A total of 3410 subjects participated in both follow-up examinations. After excluding subjects with missing data on anthropometric parameters, classical risk factors, heart rate and doctor diagnosed diabetes, stroke or myocardial infarction, 2545 participants were available for analysis (see figure 1). Excluded SAPALDIA participants were more likely to be male, smokers and less physically active, had slightly higher weight, blood pressure, lipids and inflammatory markers compared to the study sample. The respective Swiss cantonal ethical committees granted the ethical approval and participants gave written informed consent according to their preferences either globally for all examinations or separately for single assessments.

Measurements of risk factors at SAPALDIA 2

The following CVD risk factors of SAPALDIA 2 were considered in the analyses: height, weight, body mass index (BMI), systolic BP, diastolic BP, pulse pressure, heart rate, creatinine, C-reactive protein (CRP), total cholesterol, triglyceride, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), pack years of smoking and physical activity (PA). Methods of SAPALDIA 2 are described elswhere [32]. In short, weight was measured without heavy clothes with a calibrated digital electronic scale (TERRAILLON, Bradford, MA, USA). Height was assessed by a permanently fixed telescopic scale (SECA, Hamburg, Germany). All BP measurements were performed in sitting position twice after a rest of at least 10 minutes with a standard oscillometric device (OMRON 705 CP, Tokyo, Japan) at the left upper arm. The second measurement was executed within three minutes. Venous blood samples of the antecubital vein were drawn in sitting position, centrifuged and the serum fractioned within four hours, stored at -80° Centigrade. Pack years of smoking and PA state were self-reported using questionnaires. Moderate PA was defined as activity causing breathlessness or sweating and totalling at least two hours per week.

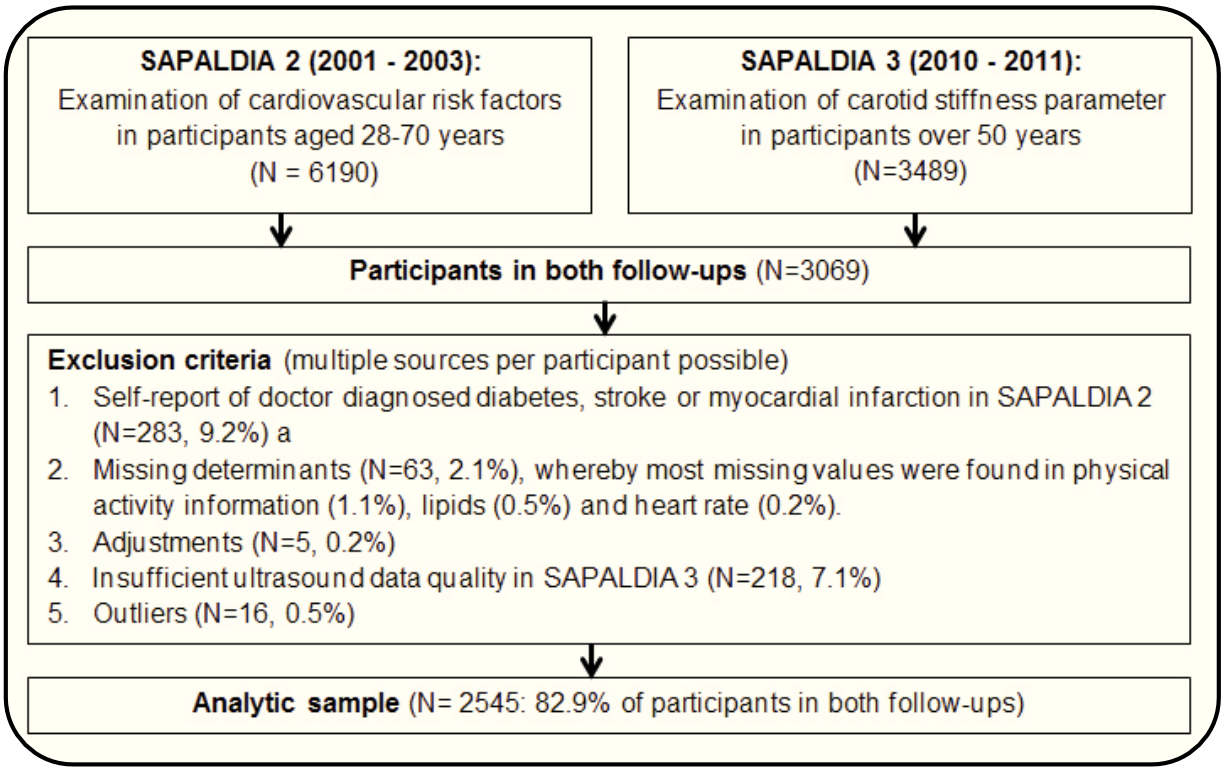

Figure 7.1: Analytic sample - flow chart of subject inclusion
Exclusion criteria are presented as numbers and as percentage of participants in both follow-ups.

Measurements of carotid stiffness parameters at SAPALDIA 3

Centrally trained and certified sonographers performed ultrasound examinations in all eight centres using the same standardised ultrasound instruments (UF-870 machine LA38 5-16 MHz array transducer, Fukuda Denshi, Japan) and scan protocol. Bilateral sequential B-mode ultrasound scans of predefined anatomic CCA structures (distal intimal and adventitial layer of the far wall and adventitial layer of the near wall) were accomplished in supine position after at least 10 minutes rest with the neck rotated to either the right or left side standardised by a 45° foam wedge. Immediately after ultrasound examination BP was measured with a standard oscillometric device (OMRON 705IT, OMRON HEALTHCARE, Kyoto, Japan) at the left and right upper arm. Mean arterial pressure was calculated as one-third of the pulse pressure added to the diastolic BP [15].

For ultrasound data analyses, a full automatic and validated B-mode image analysis program was used [13,33]. Expert readers traced intimal and adventitial layer of the far wall and media-adventitia interface of the near wall directly proximal of the carotid bifurcation over exactly one centimetre across at least one heart cycle in each predefined ultrasound angle. Whenever detection of wall layers lacked information due to insufficient ultrasound image quality, manual correction was done as much as necessary. Detection results and raw data were automatically saved and a database with single results of carotid intima media thickness (CIMT) in maximum systole and end-diastole and the average over heart cycles was generated. Outer lumen diameter was assessed simultaneously. From this data, carotid

stiffness parameters namely strain, distensibility, compliance, β-stiffness index, Peterson's elastic modulus and Young's elastic modulus were calculated (see supplement table 7.4). Lower values of strain, distensibility and compliance and higher values of Peterson's elastic modulus, Young's elastic modulus and β-stiffness index, respectively, correspond to an increased carotid stiffness.

The variability and reproducibility of all carotid stiffness parameters were investigated previously in 165 SAPALDIA 3 participants [33]. Intraclass correlation coefficients (ICC) of replicate ultrasound measurements ranged between 0.75-0.79 for carotid stiffness indices. Coefficients of variation (CV) ranged between 11.0-13.1% [33].

Statistical analyses

Sex-specific descriptive analyses of the study population characteristics and cardiovascular risk factors at SAPALDIA 2 and hemodynamic parameters at SAPALDIA 3 were performed. Sex differences were assessed by the two sample t-test. Carotid stiffness parameters got close to normally distributed after log transformation. Statistical significance was defined as $p<0.05$. The Bonferroni-Holm method was used to assess statistical significance in multiple comparisons.

To investigate sex-specific associations between SAPALDIA 2 cardiovascular risk factors and SAPALDIA 3 carotid stiffness parameters and to appropriately deal with multiple comparison issues we performed stratified multivariate regression analyses considering all carotid stiffness parameters as six-dimensional outcome and included each CVD risk factor separately along with age, education level (obligatory school education, higher education and college education) and medication information (antihypertensive, lipid lowering, glucose lowering treatment) at SAPALDIA 2, mean arterial BP and study centres at SAPALDIA 3. The raw p-value of each risk factor was calculated using an F-test with 6 numerator degrees of freedom. The statistically significant risk factors were determined using the Bonferroni-Holm method. For the graphical illustration we calculated sex-stratified mixed linear models considering the same covariates as above but study centres were now treated as random intercepts to account for regional clustering.

To investigate sex differences, we performed multivariate regression analyses for each CVD risk factor separately including interaction terms of sex with the respective risk factor and all covariates. The raw p-value of the sex-risk factor interaction was calculated using an F-test with 6 numerator degrees of freedom, and the statistically significant sex-risk factor interactions were determined using the Bonferroni-Holm method.

To investigate sex-specific association patterns and to assess potential heterogeneity of the effects of a given risk factor on the different log-transformed carotid stiffness parameters, all risk factors were first z-standardised separately for men and women, enabling each effect estimate to be interpreted as percentage change in the geometric mean of the respective

carotid stiffness parameter associated with an increase in the respective risk factor by one standard deviation [34]. To have higher values of carotid stiffness parameters correspond to a stiffer artery, strain, compliance and distensibility were multiplied by minus one (labelled as inverted scale). Sex-stratified multivariate regressions and mixed linear models and were conducted considering standardised risk factors and the same covariates as in the sex-specific association analyses. To quantify the degree of heterogeneity in the associations of a given risk factor with the 6 carotid stiffness parameters, we performed F-tests with 5 numerator degrees of freedom testing the null hypothesis of equal regression coefficients. Based on these analyses and to explore heterogeneity, five different multivariate carotid stiffness models were defined. All analyses were performed using the statistical software STATA (StataCorp 12, Statistical Software, College Station, TX: StataCorp LPTexas, USA).

Results

Sex-specific characteristics of the study population with respect to cardiovascular risk factors assessed in SAPALDIA 2 and carotid stiffness and hemodynamic parameters assessed in SAPALDIA 3 are shown in table 7.1.

Sex-specific associations and sex differences

Detailed sex-specific adjusted association estimates between different carotid stiffness parameters and non-standardised cardiovascular risk factors are reported in the supplement table 7.5. According to the multivariate analyses with subsequent Bonferroni-Holm correction of p-values, significant associations for both sexes were found for height, weight, body mass index, systolic and diastolic BP, pulse pressure, heart rate, CRP, HDL-C and smoking, as well as for total cholesterol and LDL-C in men and for creatinine and triglyceride in women (table 7.2). Multivariate analyses with interaction terms and subsequent Bonferroni-Holm correction of p-values showed significant sex differences for LDL-C, heart rate and triglyceride (table 7.2). Adjusted associations of log transformed carotid stiffness parameters with z-standardised risk factors are displayed in figure 7.2 and detailed estimates are presented in the supplement table 7.6.

Table 7.1: Characteristics of study population with respect to cardiovascular risk factors assessed in SAPALDIA 2 and carotid stiffness and hemodynamic parameters assessed in SAPALDIA 3, for men and women

	Characteristics	Units	Men (N=1211)	Women (N=1334)	p-value*
			Mean(SD)	Mean(SD)	
SAPALDIA 2	Age	years	55.08(7.99)	55.22(8.00)	0.66
	Height	cm	175.37(6.31)	162.62(6.11)	<0.001
	Weight	kg	81.36(11.33)	65.50(11.56)	<0.001
	Body mass index	kg/m^2	26.45(3.34)	24.77(4.22)	<0.001
	Systolic BP	mmHg	132.93(17.10)	121.49(18.37)	<0.001
	Diastolic BP	mmHg	83.44(10.67)	77.44(10.27)	<0.001
	Pulse pressure	mmHg	49.49(10.88)	44.05(11.89)	<0.001
	Heart rate	bpm	69.45(10.70)	70.67(9.50)	0.002
	Creatinine	µmol/l	94.43(10.55)	81.81(9.20)	<0.001
	CRP	mg/l	1.99(3.34)	1.95(2.61)	0.75
	Total cholesterol	mmol/l	6.16(1.08)	6.16(1.12)	0.96
	Triglyceride	mmol/l	2.10(1.24)	1.55(0.90)	<0.001
	HDL-C	mmol/l	1.35(0.37)	1.72(0.43)	<0.001
	LDL-C	mmol/l	3.85(0.99)	3.74(1.04)	0.005
	Smoking	pack years	12.98(20.19)	7.56(13.84)	<0.001
	Physical activity	%	38.1	23.1	<0.001
	Medication intake	%	18.7	13.4	<0.001
	Anti-hypertensive	%	13.8	10.9	0.03
	Lipid-lowering	%	8.1	3.4	<0.001
	Glucose-lowering	%	0.0	0.1†	0.3
SAPALDIA 3	Systolic BP	mmHg	137.88(16.94)	132.42(18.92)	<0.001
	Diastolic BP	mmHg	79.94(9.46)	76.53(9.18)	<0.001
	Pulse pressure	mmHg	57.94(12.04)	55.88(14.22)	<0.001
	Mean arterial BP	mmHg	99.25(11.09)	95.16(11.42)	<0.001
	Stiffness parameters	**Units**	**Geometric mean (95% CI)**	**Geometric mean (95% CI)**	**p-value**
	Strain	%	5.13 (5.04; 5.23)	5.25 (5.16; 5.34)	<0.001
	Compliance	mm^2/kPa	0.65 (0.64; 0.66)	0.57 (0.56; 0.58)	<0.001
	Distensibility	$10^{-3}/kPa$	0.0139 (0.0136; 0.0142)	0.0149 (0.0146; 0.0152)	<0.001
	β-stiffness index	no unit	10.48 (10.28; 10.69)	10.23 (10.03; 10.43)	<0.001
	Peterson's EM	kPa	147.5 (144.4; 150.7)	137.9 (134.9; 141.0)	<0.001
	Young's EM	kPa	744.3 (728.0; 761.0)	660.4 (645.8; 675.4)	<0.001

CI = confidence interval, N = number, SD = standard deviation, BP = Blood pressure, CRP = C-reactive protein, EM = elastic modulus HDL-C = high-density lipoprotein cholesterol, LDL-C = low-density lipoprotein cholesterol, Medication intake = anti-hypertensive or lipid- or glucose-lowering medication, *p-value for differences between men and women, † self-reported glucose lowering medication but no self-reported doctor diagnosed diabetes.

Table 7.2: Significance of the associations of single cardiovascular risk factors (per unit) across all six carotid stiffness parameters (log-transformed) separately for men, women and for the respective sex differences

Risk factors	Men p-value	Women p-value	Sex difference p-value
Height	<0.001 †	<0.001 †	0.74
Weight	<0.001 †	<0.001 †	0.20
Body mass index	<0.001 †	<0.001 †	0.02
Systolic blood pressure	<0.001 †	<0.001 †	0.41
Diastolic blood pressure	0.01 †	<0.001 †	0.04
Pulse pressure	<0.001 †	<0.001 †	0.04
Heart rate	<0.001 †	<0.001 †	0.001 †
Creatinine	0.05	0.007 †	0.67
C-reactive protein	0.006 †	<0.001 †	0.06
Total cholesterol	<0.001 †	0.03	0.016
Triglyceride	0.03	<0.001 †	0.003 †
HDL-C	<0.001 †	<0.001 †	0.58
LDL-C	<0.001 †	0.04	<0.001 †
Smoking	<0.001 †	0.001 †	0.51
Physical activity	0.79	0.57	0.84

P-values were based on multivariate regression analyses with a subsequent F-test performed.
† = Significant after Bonferroni-Holm correction, HDL-C = high-density lipoprotein cholesterol, LDL-C = low-density lipoprotein cholesterol.

Sex-specific association patterns

Association patterns of carotid stiffness parameters with standardised risk factors are visualised in the figure 7.3, separately for men and women. Overall, strong mutual correlations were found for Young's elastic modulus, Peterson's elastic modulus, β-stiffness index and distensibility (|r| = 0.89-0.99) while compliance and strain were less strongly correlated among themselves (with r = 0.76) and with the other carotid stiffness parameters (|r| = 0.74-0.88).

Multivariate analyses showed significant heterogeneity in association patterns within different standardised risk factors (supplement table 7.7). Homogeneous association patterns across all carotid stiffness parameters were found for PA in both sexes, for diastolic BP, creatinine and triglyceride in men and for cholesterol and LDL-C in women. Pairwise comparisons showed that associations of compliance with height, weight, body mass index, pulse pressure, creatinine, HDL-C and smoking strongly differed from those of other carotid stiffness parameters for both sexes and with systolic BP and heart rate in women. Moreover, associations of strain with systolic and pulse pressure differed from those of the other carotid stiffness parameters in both sexes and for diastolic BP and heart rate in women. Further sub analyses showed that heterogeneity in LDL-C and total cholesterol was removed in a model without Young's elastic modulus in men (data not shown). The ranges of estimated effects of standardised risk factors on carotid stiffness parameters are presented in the table 7.3. Association patterns are shown in figure 7.2 and figure 7.3 and detailed information are presented in supplement table 7.6-7.7.

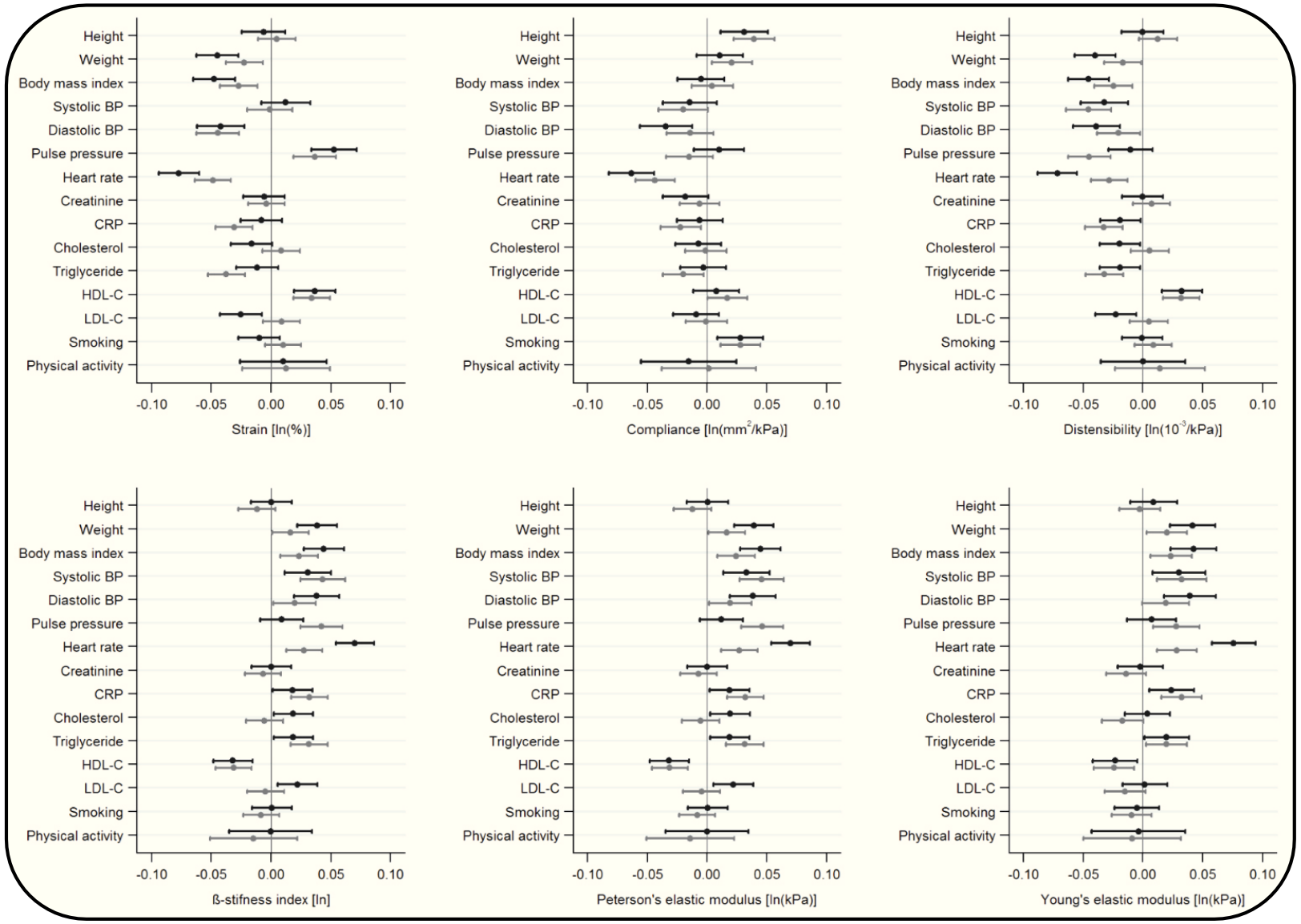

Figure 7.2: Sex-specific associations of single cardiovascular risk factors (per 1 SD) for each carotid stiffness parameter (log-transformed). Women = grey line, men = black line
Association estimates were based on mixed linear regression analyses adjusted for age, education level, medication, mean arterial pressure and study centre. Physical activity is a binary variable. BP = blood pressure, Cholesterol = total cholesterol, CRP = C-reactive protein, HDL-C = high density lipoprotein cholesterol, LDL-C = low density lipoprotein cholesterol, SD = standard deviation.

Discussion

Our analyses provide estimates of long-term adverse effects of cardiovascular risk factors assessed in SAPALDIA 2 on carotid stiffness parameters assessed ten years later in SAPALDIA 3. To our knowledge, this is the first population-based study in elderly Caucasians investigating the sex-specific associations, sex differences and carotid stiffness association patterns of established CVD risk factors with several carotid stiffness parameters. While we find a high agreement for the effects of most CVD risk factors in men and women (strongest for anthropometrics and hemodynamic parameters), the analyses reveal effects of different size in men and women for heart rate, LDL-C and triglyceride. The only long-term determinant showing consistent protective associations with all carotid stiffness parameters in both sexes was HDL-C. The hypothesis of similar association patterns between carotid stiffness parameters and risk factors was partially rejected. Despite the high to medium mutual correlations of carotid stiffness parameters, their associations with some of the risk factors showed partially significant heterogeneity. Associations of compliance with anthropometric variables were different from those of the other carotid stiffness parameters and strain showed a distinct association pattern with the BP variables.

Sex differences in associations of risk factors and carotid stiffness parameters

Increased heart rate was more strongly associated with stiffer arteries across all carotid stiffness parameters in men as compared to women. This association is pathophysiologically important since an enhanced mechanical load induces vascular smooth muscle cell growth and collagen deposition, processes potentially associated with arterial stiffening [15]. An association between increased heart rate and increased carotid stiffness has been reported in a cross-sectional analysis in normotensive as well as in hypertensive subjects in a clinical setting [35,36]. Further, increase of heart rate by ventricular pacing caused reduced distensibility of the carotid artery [37]. Moreover, it has been shown that an increased heart rate is related to increased incidence of CVD and mortality [38].

Further sex differences in association estimates were observed for LDL-C and triglyceride. Since the basic lipid profile also includes HDL-C and total cholesterol beside LDL-C and triglyceride [39], a discussion of the whole panel is needed. HDL-C was the only determinant significantly associated with less carotid stiffness in both sexes. Triglyceride was significantly associated with increased carotid stiffness in women, but not in men. The exact opposite was found for LDL-C and total cholesterol, although the sex-difference for total cholesterol lost its statistical significance after Bonferroni-Holm correction. A recent study concluded that lower non-HDL-C concentration in women was related to lower cholesterol synthesis and absorption [40]. Values of total cholesterol in SAPALDIA 2 did not differ between men and women. Nevertheless, the meta-analysis of ‘prospective Studies Collaboration’ has shown that a reduction of 1 mmol/l of total cholesterol was associated with a reduction of ischaemic heart disease by one sixth to one third in participants aged 50 years or older [41].

Similarly, in our study, a 1 mmol/l increase of total cholesterol was associated with significant increases carotid stiffness of |1.5 to 1.8%| in the geometric means of strain, distensibility, β-stiffness index and Peterson's elastic modulus in men, but not in women (supplement table 7.5).

Overall, the observed sex differences in the associations of carotid stiffness-parameters with heart rate, triglyceride and LDL-C were statistically significant. This is potentially relevant to clinical treatment and preventive strategies.

Carotid stiffness parameter patterns

Multivariate analyses using z-standardized risk factors showed very similar association patterns across risk factors for Peterson's elastic modulus, β-stiffness index and distensibility and this suggests that they could be used in an exchangeable manner. With the exception of few risk factors, this extends to strain and Young's elastic modulus. The question arises whether all carotid stiffness parameters need to be measured in future clinical studies.

Strain differed from other stiffness parameters solely in its positive association with BP values. At a first glance, this positive association seems implausible, since we would expect an inverse association. Lower values of strain corresponding to a stiffer artery and, on the opposite, high strain values are an expression of an improved vascular function as shown for HDL-C. However, BP and especially pulse pressure have a direct mechanical impact on diameter change resulting in increased strain values since strain is only the relative change of the diameter without accounting for BP values (supplement table 7.4). Investigation of multiple risk factors may result in divergent associations with strain and therefore we recommend using and interpreting the respective results with caution. In case of Young's elastic modulus, different associations were observed for LDL-C and total cholesterol. One must keep in mind that its formula includes CIMT and therefore reflects the atherosclerotic process in a slightly different way.

Compliance showed deviating association patterns with a range of risk factors. This might be due to the fact that the mathematical formula of compliance is the only one containing the cross-sectional area, instead of the diameter of the CCA (supplement table 7.4). Since most of the risk factor are associated with increased carotid diameter, as shown also by Polak et al. [42], their impact might be stronger for compliance than for the other carotid stiffness parameters. Based on our results we question whether compliance is a suitable parameter for cardiovascular risk assessment.

Table 7.3: Ranges of association estimates of cardiovascular risk factors on carotid stiffness parameters for different models

	Risk factors	Model 1: All CS parameters (min; max)	Model 2: All CS parameters except compliance (min; max)	Model 3: All CS parameters except strain (min; max)	Model 4: All CS parameters except compliance and strain (min; max)	Model 5: All CS parameters except compliance, strain and Young's elastic modulus (min; max)
Men	Height	(-3.16; 0.89)	(0.03; 0.89)	(-3.16; 0.89)	(0.03; 0.89)	(0.03; 0.04)
	Weight	(-1.07; 4.39)	(3.93; 4.39)	(-1.07; 4.23)	(3.93; 4.23)	(3.93; 3.99)
	Body mass index	(0.51; 4.67)	(4.31; 4.67)	(0.51; 4.56)	(4.31; 4.56)	(4.46; 4.56)
	Systolic blood pressure	(-1.23; 3.33)	(-1.23; 3.33)	(1.44; 3.33)	(3.05; 3.33)	(3.10; 3.33)
	Diastolic blood pressure	(3.38; 4.12)	(3.83; 4.12)	(3.38; 4.00)	(3.83; 4.00)	(3.83; 3.89)
	Pulse pressure	(-5.41; 1.20)	(-5.41; 1.20)	(-1.00; 1.20)	(0.72; 1.20)	(0.91; 1.20)
	Heart rate	(6.14; 7.88)	(6.92; 7.88)	(6.14; 7.88)	(6.92; 7.88)	(6.92; 7.26)
	Creatinine	(-0.23; 1.79)	(-0.23; 0.58)	(-0.23; 1.79)	(-0.23; 0.04)	(0.02; 0.04)
	C-reactive protein	(0.61; 2.42)	(0.81; 2.42)	(0.61; 2.42)	(1.81; 2.42)	(1.81; 1.90)
	Total cholesterol	(0.37; 1.93)	(0.37; 1.93)	(0.37; 1.93)	(0.37; 1.93)	(1.88; 1.93)
	Trigyceride	(0.32; 2.00)	(1.15; 2.00)	(0.32; 2.00)	(1.88; 2.00)	(1.88; 1.92)
	HDL-C	(-3.71; -0.78)	(-3.71; -2.30)	(-3.30; -0.78)	(-3.30; -2.30)	(-3.30; -3.12)
	LDL-C	(0.16; 2.50)	(0.16; 2.50)	(0.16; 2.24)	(0.16; 2.24)	(2.22; 2.24)
	Smoking	(-2.82; 1.00)	(-0.51; 1.00)	(-2.82; 0.08)	(-0.51; 0.08)	(0.07; 0.08)
	Physical activity	(-1.04; 1.53)	(-1.04; 0.01)	(-0.37; 1.53)	(-0.37; 0.01)	(-0.05; 0.01)
Women	Height	(-4.01; -0.26)	(-1.26; -0.26)	(-4.01; -0.26)	(-1.26; -0.26)	(-1.26; -1.17)
	Weight	(-2.10; 2.22)	(1.64; 2.22)	(-2.10; 2.02)	(1.64; 2.02)	(1.64; 1.69)
	Body mass index	(-0.43; 2.67)	(2.36; 2.67)	(-0.43; 2.45)	(2.36; 2.45)	(2.39; 2.45)
	Systolic blood pressure	(0.13; 4.68)	(0.13; 4.68)	(1.96; 4.68)	(3.27; 4.68)	(4.41; 4.68)
	Diastolic blood pressure	(1.41; 4.37)	(1.92; 4.37)	(1.41; 2.03)	(1.92; 2.03)	(1.96; 2.03)
	Pulse pressure	(-3.71; 4.71)	(-3.71; 4.71)	(1.48; 4.71)	(2.82; 4.71)	(4.32; 4.71)
	Heart rate	(2.74; 4.77)	(2.74; 4.77)	(2.74; 4.26)	(2.74; 2.86)	(2.74; 2.81)
	Creatinine	(-1.40; 0.64)	(-1.40; 0.37)	(-1.40; 0.64)	(-1.40; -0.67)	(-0.73; -0.67)
	C-reactive protein	(2.18; 3.27)	(3.05; 3.27)	(2.18; 3.27)	(3.22; 3.27)	(3.22; 3.26)
	Total cholesterol	(-1.70; 0.11)	(-1.70; -0.55)	(-1.70; 0.11)	(-1.70; -0.55)	(-0.57; -0.55)
	Trigyceride	(1.97; 3.69)	(1.97; 3.69)	(1.97; 3.23)	(1.97; 3.23)	(3.19; 3.23)
	HDL-C	(-3.47; -1.71)	(-3.47; -2.40)	(-3.25; -1.71)	(-3.25; -2.40)	(-3.25; -3.07)
	LDL-C	(-1.51; 0.06)	(-1.51; -0.47)	(-1.51; 0.06)	(-1.51; -0.47)	(-0.48; -0.47)
	Smoking	(-2.82; -0.82)	(-1.01; -0.82)	(-2.82; -0.82)	(-0.96; -0.82)	(-0.86; -0.82)
	Physical activity	(-1.46; -0.14)	(-1.46; -0.91)	(-1.46; -0.14)	(-1.46; -0.91)	(-1.46; -1.39)

Values are expressed as percent change in geometric mean per 1 SD increment. Association estimates were based on mixed linear regression models adjusted for age, education level, medication, mean arterial pressure and study centre. Scales for strain, compliance and distensibility were inverted. Physical activity is a binary variable. CRP = C-reactive protein, HDL-C = high-density lipoprotein cholesterol, LDL-C = low-density lipoprotein cholesterol, SD = standard deviation.

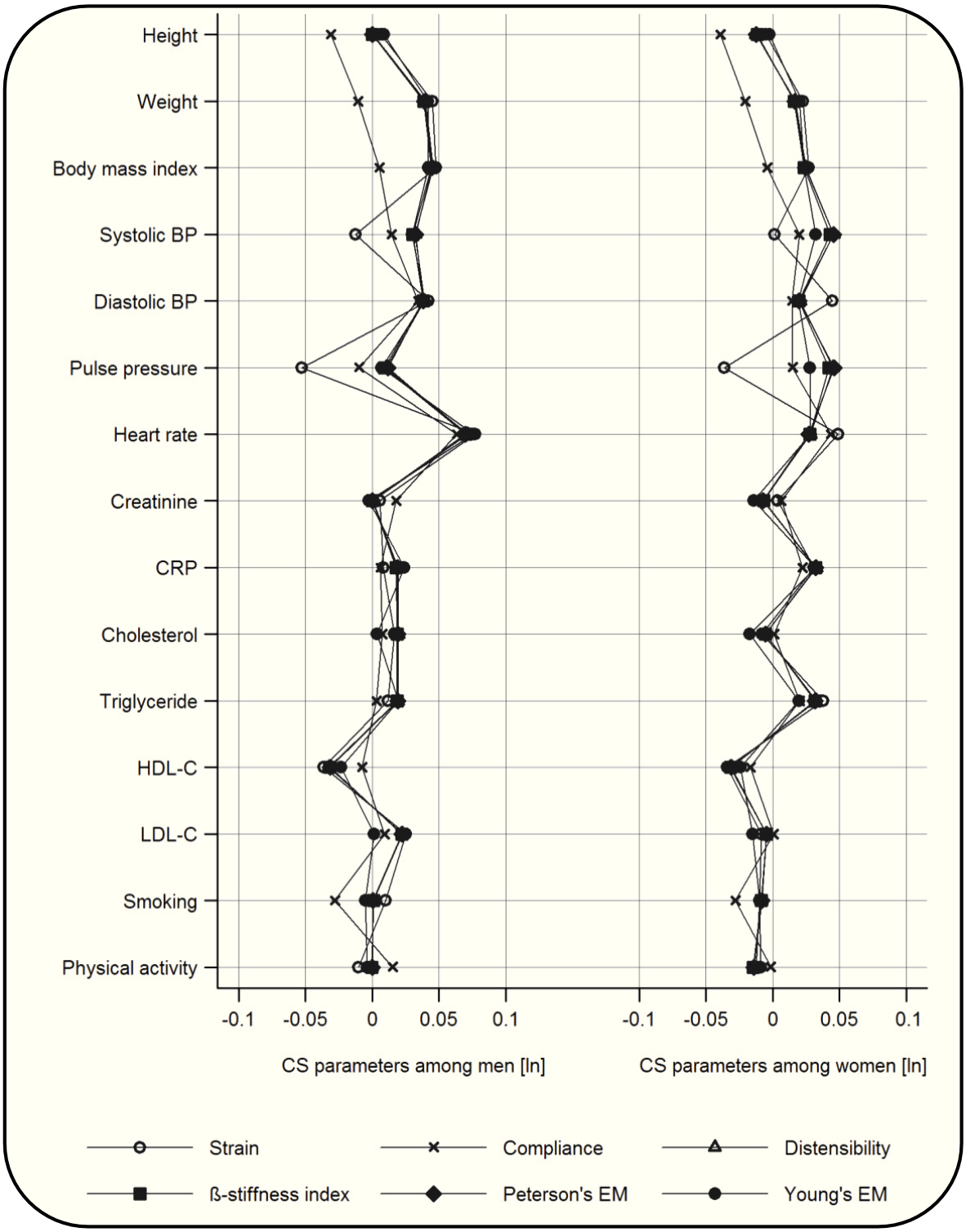

Figure 7.3: Association patterns of cardiovascular risk factors (per 1 SD) and six carotid stiffness parameters (log-transformed) separately for men and women

Association estimates were based on mixed linear regression analyses adjusted for age, education level, medication, mean arterial pressure and study centre. Scales for strain, compliance and distensibility were inverted. Physical activity is a binary variable. BP = blood pressure, Cholesterol = total cholesterol, CRP = C-reactive protein, EM = elastic modulus, HDL-C = high-density lipoprotein cholesterol, LDL-C = low-density lipoprotein cholesterol, SD = standard deviation.

Strengths and limitations

The highly standardised procedures of biomarker and ultrasound assessment, image analyses and validation combined in a large cohort are clear strengths of this study. It allowed us to investigate the longitudinal associations of CVD risk factors assessed in SAPALDIA 2 with carotid stiffness parameters obtained in SAPALDIA 3 for the first time. However, the study population is primarily Caucasian and the age range is limited to 50-80 years at the last examination we measured brachial instead of central BP which may have overestimated carotid stiffness [14]. Although the difference of brachial and central pulse pressure decreases with advancing age [15], it's not clear how this variability - which is also dependent on heart rate - affects the association estimates, especially if the differences vary in males and females [43]. Furthermore, some cardiovascular diagnoses are based on self-report and we could not investigate the longitudinal change in carotid stiffness itself since carotid stiffness was measured only in SAPALDIA 3. Therefore, it is not possible to infer a causal relationship between risk factors and carotid stiffness parameters. Prior studies have documented the predictive power of single carotid stiffness parameters [18,44,45], but did not compare the sensitivity and predictability of carotid stiffness parameters among each other. Although we found very similar association pattern for most of the carotid stiffness parameters, we recommend to investigate all these carotid stiffness parameters in correlation with hard cardiovascular endpoints in order to confirm our results and to precisely figure out whether similarities justify exchangeability of different carotid stiffness parameters.

Conclusion and perspectives

This first investigation of long-term associations of cardiovascular risk factors assessed in 2001-2003 with several common carotid stiffness parameters measured ten years later in an elderly population without diagnosed CVD shows sex differences in association estimates for heart rate, LDL-C and triglyceride. Increased heart rate was more strongly associated with stiffer arteries across all carotid stiffness parameters in men than in women. LDL-C was significantly associated with carotid stiffness only in men and triglyceride only in women. These results emphasize the importance of clarifying and estimating sex-specific associations of atherosclerotic risk factors. We were able to show comparable CVD risk association patterns for distensibility, β-stiffness index, Peterson's elastic modulus and partially Young's elastic modulus in men and women, suggesting that these carotid stiffness parameters might be used in an exchangeable manner. Since transcutaneous ultrasound measurement and their reproducible automated image analyses become increasingly available, a simplification of the use and interpretation of carotid stiffness parameters might facilitate implementation of these instruments in clinical practice.

Acknowledgments

This study could not have been done without the help of the study participants, technical and administrative support, the medical teams and field workers at the local study sites, the SAPALDIA team and the members of the Department of Sport, Exercise and Health. The authors thank for their excellent work.

Disclosures

No conflict of interest stated for all authors.

Sources of Funding

The Swiss Study on Air Pollution and Lung and Heart Diseases In Adults (SAPALDIA) was supported by the Swiss National Science Foundations (grants no 33CSCO-134276/1, 33CSCO-108796, 3247BO-104283, 3247BO-104288, 3247BO-104284, 3247-065896, 3100-059302, 3200-052720, 3200-042532, 4026-028099, PMPDP3_129021/1, PMPDP3_141671/1), the Federal Office for Forest, Environment and Landscape, the Federal Office of Public Health, the Federal Office of Roads and Transport, the canton's government of Aargau, Basel-Stadt, Basel-Land, Geneva, Luzern, Ticino, Valais, and Zürich, the Swiss Lung League, the canton's Lung League of Basel Stadt/ Basel Landschaft, Geneva, Ticino, Valais, Graubünden and Zurich, Stiftung ehemals Bündner Heilstätten, SUVA, Freiwillige Akademische Gesellschaft, UBS Wealth Foundation, Talecris Biotherapeutics GmbH, Abbott Diagnostics, European Commission 018996 (GABRIEL), Wellcome Trust WT 084703MA.

References

1 World Health Organisation. (2009) Global health risks: mortality and burden of disease attributable to selected major risks, World Health Organization, Geneva, 1-63

2 Gibbons, R. J., Jones, D. W., Gardner, T. J., Goldstein, L. B., Moller, J. H. and Yancy, C. W. (2008) The American Heart Association's 2008 Statement of Principles for Healthcare Reform. Circulation. **118**, 2209–2218

3 Erbel, R. and Budoff, M. (2012) Improvement of cardiovascular risk prediction using coronary imaging: subclinical atherosclerosis: the memory of lifetime risk factor exposure. Eur. Heart J. **33**, 1201–1217

4 Goff, D. C., Lloyd-Jones, D. M., Bennett, G., Coady, S., D'Agostino, R. B., Gibbons, R., Greenland, P., Lackland, D. T., Levy, D., O'Donnell, C. J., Robinson, J., Schwartz, J. S., Shero, S. T., Smith, S. C., Sorlie, P., Stone, N. J. and Wilson, P. W. F. (2013) 2013 ACC/AHA Guideline on the Assessment of Cardiovascular Risk A Report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. Circulation. 01.cir.0000437741.48606.98

5 Nilsson, P. M., Boutouyrie, P. and Laurent, S. (2009) Vascular aging: a tale of EVA and ADAM in cardiovascular risk assessment and prevention. Hypertension. **54**, 3–10

6 Ross, R. (1999) Atherosclerosis - an inflammatory disease. N. Engl. J. Med. **340**, 115–126

7 Wendelhag, I., Gustavsson, T., Suurküla, M., Berglund, G. and Wikstrand, J. (1991) Ultrasound measurement of wall thickness in the carotid artery: fundamental principles and description of a computerized analysing system. Clin. Physiol. **11**, 565–577

8 Cheng, D.-C., Jiang, X., Schmidt-Trucksäss, A. and Cheng, K.-S. (2006) Automatic intima-media thickness measurement of carotid artery wall in B-mode sonographic images. Proc. 3rd IEEE Int. Symp. Biomed. Imaging Nano Macro Arlingt. VA IEEE. **30**, 912–915

9 Haller, C., Schulz, J., Schmidt-Trucksäss, A., Burkardt, H., Schmitz, D., Dickhuth, H.-H. and Sandrock, M. (2007) Sequential based analysis of Intima-Media Thickness (IMT) in common carotid artery studies. Atherosclerosis. **195**, e203–e209

10 Faita, F., Gemignani, V., Bianchini, E., Giannarelli, C., Ghiadoni, L. and Demi, M. (2008) Real-time measurement system for evaluation of the carotid intima-media thickness with a robust edge operator. J Ultrasound Med. **27**, 1353–1361

11 Lee, Y.-B., Choi, Y.-J. and Kim, M.-H. (2010) Boundary detection in carotid ultrasound images using dynamic programming and a directional Haar-like filter. Comput. Biol. Med. **40**, 687–697

12 Molinari, F., Zeng, G. and Suri, J. S. (2010) Intima-media thickness: setting a standard for a completely automated method of ultrasound measurement. IEEE Trans Ultrason Ferroelectr Freq Control. **57**, 1112–1124

13 Teynor, A., Caviezel, S., Dratva, J., Künzli, N. and Schmidt-Trucksäss, A. (2012) An automated, interactive analysis system for ultrasound sequences of the common carotid artery. Ultrasound Med. Biol. **38**, 1440–1450

14 Laurent, S., Cockcroft, J., Van Bortel, L., Boutouyrie, P., Giannattasio, C., Hayoz, D., Pannier, B., Vlachopoulos, C., Wilkinson, I. and Struijker-Boudier, H. (2006) Expert consensus document on arterial stiffness: methodological issues and clinical applications. Eur. Heart J. **27**, 2588 –2605

15 Nichols, W. W., O'Rourke, M. F. and Vlachopoulos, C. (2011) McDonald's Blood Flow in Arteries: Theoretical, Experimental and Clinical Principles 6th ed., Hodder Arnold, London, 1-755

16 O'Rourke, M. F., Staessen, J. A., Vlachopoulos, C., Duprez, D. and Plante, G. E. (2002) Clinical applications of arterial stiffness; definitions and reference values. Am J Hypertens. **15**, 426–444

17 Van Bortel, L. M., Duprez, D., Starmans-Kool, M. J., Safar, M. E., Giannattasio, C., Cockcroft, J., Kaiser, D. R. and Thuillez, C. (2002) Clinical applications of arterial stiffness, Task Force III: recommendations for user procedures. Am. J. Hypertens. **15**, 445–452

18 Leone, N., Ducimetière, P., Gariépy, J., Courbon, D., Tzourio, C., Dartigues, J.-F., Ritchie, K., Alpérovitch, A., Amouyel, P., Safar, M. E. and Zureik, M. (2008) Distension of the carotid artery and risk of coronary events: The Three-City Study. Arterioscler. Thromb. Vasc. Biol. **28**, 1392–1397

19 Tanaka, H., Dinenno, F. A., Monahan, K. D., Clevenger, C. M., DeSouza, C. A. and Seals, D. R. (2000) Aging, Habitual Exercise, and Dynamic Arterial Compliance. Circulation. **102**, 1270–1275

20 Tseke, P., Grapsa, E., Stamatelopoulos, K., Samouilidou, E., Protogerou, A., Papamichael, C. and Laggouranis, A. (2006) Atherosclerotic risk factors and carotid stiffness in elderly asymptomatic HD patients. Int. Urol. Nephrol. **38**, 801–809

21 Roman, M. J., Devereux, R. B., Schwartz, J. E., Lockshin, M. D., Paget, S. A., Davis, A., Crow, M. K., Sammaritano, L., Levine, D. M., Shankar, B.-A., Moeller, E. and Salmon, J. E. (2005) Arterial stiffness in chronic inflammatory diseases. Hypertension. **46**, 194–199

22 Lerner, D. J. and Kannel, W. B. (1986) Patterns of coronary heart disease morbidity and mortality in the sexes: a 26-year follow-up of the Framingham population. Am. Heart J. **111**, 383–390

23 Marlatt, K. L., Kelly, A. S., Steinberger, J. and Dengel, D. R. (2013) The influence of gender on carotid artery compliance and distensibility in children and adults. J. Clin. Ultrasound. **41**, 340–346

24 Vaidya, D., Heckbert, S. R., Wasserman, B. A. and Ouyang, P. (2012) Sex-specific association of age with carotid artery distensibility: Multi-Ethnic Study of Atherosclerosis. J. Womens Health. **21**, 516–520

25 Coutinho, T., Borlaug, B. A., Pellikka, P. A., Turner, S. T. and Kullo, I. J. (2013) Sex differences in arterial stiffness and ventricular-arterial interactions. J. Am. Coll. Cardiol. **61**, 96–103

26 Vlachopoulos, C., Aznaouridis, K. and Stefanadis, C. (2010) Prediction of cardiovascular events and all-cause mortality with arterial stiffness: a systematic review and meta-analysis. J. Am. Coll. Cardiol. **55**, 1318–1327

27 McEniery, C. M., Spratt, M., Munnery, M., Yarnell, J., Lowe, G. D., Rumley, A., Gallacher, J., Ben-Shlomo, Y., Cockcroft, J. R. and Wilkinson, I. B. (2010) An analysis of prospective risk factors for aortic stiffness in men: 20-year follow-up from the Caerphilly prospective study. Hypertension. **56**, 36–43

28 Johansen, N. B., Vistisen, D., Brunner, E. J., Tabák, A. G., Shipley, M. J., Wilkinson, I. B., McEniery, C. M., Roden, M., Herder, C., Kivimäki, M. and Witte, D. R. (2012) Determinants of aortic stiffness: 16-year follow-up of the Whitehall II Study. PLOS ONE. **7**, e37165

29 Arterial Stiffness Collaboration. (2010) Determinants of pulse wave velocity in healthy people and in the presence of cardiovascular risk factors: “establishing normal and reference values.” Eur. Heart J. **31**, 2338–2350

30 Van Der Heijden-Spek, J. J., Staessen, J. A., Fagard, R. H., Hoeks, A. P., Boudier, H. A. S. and Van Bortel, L. M. (2000) Effect of age on brachial artery wall properties differs from the aorta and is gender dependent: a population study. Hypertension. **35**, 637–642

31 Martin, B. W., Ackermann-Liebrich, U., Leuenberger, P., Künzli, N., Zemp Stutz, E., Keller, R., Zellweger, J. P., Wüthrich, B., Monn, C., Blaser, K., Bolognini, G., Bongard, J. P., Brändli, O., Braun, P., Defila, C., Domenighetti, G., Grize, L., Karrer, W., Keller-Wossidlo, H., Medici, T. C., Peeters, A., Perruchoud, A. P., Schindler, C., Schoeni, M. H., Schwartz, J., Solari, G., Tschopp, J.-M. and Villiger, B. (1997) SAPALDIA: methods and participation in the cross-sectional part of the Swiss Study on Air Pollution and Lung Diseases in Adults. Soz.- Präventivmedizin. **42**, 67–84

32 Ackermann-Liebrich, U., Kuna-Dibbert, B., Probst-Hensch, N. M., Schindler, C., Felber Dietrich, D., Zemp Stutz, E., Bayer-Oglesby, L., Baum, F., Brändli, O., Brutsche, M., Downs, S. H., Keidel, D., Gerbase, M. W., Imboden, M., Keller, R., Knöpfli, B., Künzli, N., Nicod, L., Pons, M., Staedele, P., Tschopp, J.-M., Zellweger, J.-P. and Leuenberger, P. (2005) Follow-up of the Swiss Cohort Study on Air Pollution and Lung Diseases in Adults (SAPALDIA 2) 1991-2003: methods and characterization of participants. Soz.- Präventivmedizin. **50**, 245–263

33 Caviezel, S., Dratva, J., Schaffner, E., Teynor, A., Baumstark, M. W., Schindler, C., de Groot, E., Burdet, L., Rothe, T., Pons, M., Gaspoz, J.-M., Rochat, T., Künzli, N., Probst-Hensch, N. and Schmidt-Trucksäss, A. (2013) Variability and reproducibility of carotid structural and functional parameters assessed with transcutaneous ultrasound – results from the SAPALDIA cohort study. Atherosclerosis. **231**, 448–455

34 Kirkwood, B. and Sterne, J. (2003) Essentials of medical statistics 2nd ed., Blackwell Publishing company, Massachusetts, 1-501

35 Cunha, R. S., Pannier, B., Benetos, A., Siche, J.-P., London, G. M., Mallion, J. M. and Safar, M. E. (1997) Association between high heart rate and high arterial rigidity in normotensive and hypertensive subjects. J Hypertens. **15**, 1423–1430

36 Whelton, S. P., Blankstein, R., Al-Mallah, M. H., Lima, J. A. C., Bluemke, D. A., Hundley, W. G., Polak, J. F., Blumenthal, R. S., Nasir, K. and Blaha, M. J. (2013) Association of resting heart rate with carotid and aortic arterial stiffness: Multi-Ethnic Study of Atherosclerosis. Hypertension. **62**, 477–484

37 Giannattasio, C., Vincenti, A., Failla, M., Capra, A., Cirò, A., De Ceglia, S., Gentile, G., Brambilla, R. and Mancia, G. (2003) Effects of heart rate changes on arterial distensibility in humans. Hypertension. **42**, 253–256

38 Lang, C. C., Gupta, S., Kalra, P., Keavney, B., Menown, I., Morley, C. and Padmanabhan, S. (2010) Elevated heart rate and cardiovascular outcomes in patients with coronary artery disease: clinical evidence and pathophysiological mechanisms. Atherosclerosis. **212**, 1–8

39 Perk, J., De Backer, G., Gohlke, H., Graham, I., Reiner, Z., Verschuren, M., Albus, C., Benlian, P., Boysen, G., Cifkova, R., Deaton, C., Ebrahim, S., Fisher, M., Germano, G., Hobbs, R., Hoes, A., Karadeniz, S., Mezzani, A., Prescott, E., Ryden, L., Scherer, M., Syvänne, M., Scholte op Reimer, W. J. M., Vrints, C., Wood, D., Zamorano, J. L. and Zannad, F. (2012) European Guidelines on cardiovascular disease prevention in clinical practice (version 2012). The Fifth Joint Task Force of the European Society of Cardiology and Other Societies on Cardiovascular Disease Prevention in Clinical Practice (constituted by representatives of nine societies and by invited experts). Developed with the special contribution of the European Association for Cardiovascular Prevention & Rehabilitation (EACPR). Eur. Heart J. **33**, 1635–1701

40 Matthan, N. R., Zhu, L., Pencina, M., D’Agostino, R. B., Schaefer, E. J. and Lichtenstein, A. H. (2013) Sex-specific differences in the predictive value of cholesterol homeostasis markers and 10-year cardiovascular disease event rate in framingham offspring study participants. J. Am. Heart Assoc. **2**, e005066

41 Prospective Studies Collaboration, Lewington, S., Whitlock, G., Clarke, R., Sherliker, P., Emberson, J., Halsey, J., Qizilbash, N., Peto, R. and Collins, R. (2007) Blood cholesterol and vascular mortality by age, sex, and blood pressure: a meta-analysis of individual data from 61 prospective studies with 55000 vascular deaths. Lancet. **370**, 1829–1839

42 Polak, J. F., Wong, Q., Johnson, W. C., Bluemke, D. A., Harrington, A., O’Leary, D. H. and Yanez, N. D. (2011) Associations of cardiovascular risk factors, carotid intima-media thickness and left ventricular mass with inter-adventitial diameters of the common carotid artery: The Multi-Ethnic Study of Atherosclerosis (MESA). Atherosclerosis. **218**, 344–349

43 McEniery, C. M., Yasmin, McDonnell, B., Munnery, M., Wallace, S. M., Rowe, C. V., Cockcroft, J. R. and Wilkinson, I. B. (2008) Central Pressure: Variability and Impact of Cardiovascular Risk Factors The Anglo-Cardiff Collaborative Trial II. Hypertension. **51**, 1476–1482

44 Barenbrock, M., Kosch, M., Jöster, E., Kisters, K., Rahn, K.-H. and Hausberg, M. (2002) Reduced arterial distensibility is a predictor of cardiovascular disease in patients after renal transplantation. J. Hypertens. **20**, 79–84

45 Blacher, J., Safar, M. E., Pannier, B., Guerin, A. P., Marchais, S. J. and London, G. M. (2002) Prognostic significance of arterial stiffness measurements in end-stage renal disease patients. Curr. Opin. Nephrol. Hypertens. **11**, 629–634

Supplement Table 7.4: Formulas of carotid stiffness parameters based on a one centimetre detection segment

Parameter	Unit	Formula	
Strain	%	deltaLD / dLD	[15]
Distensibility	1/kPa	$((2 \times deltaLD \times dLD)+(deltaLD)^2)/(PP\times dLD^2)$	[17]
Compliance	mm^2/kPa	$\pi\times((2\times deltaLD \times dLD)+(deltaLD)^2)/(4\times PP)$	[17]
β-stiffness index	no unit	(ln(sBP/dBP))/(deltaLD/dLD)	[16]
Peterson's elastic modulus	kPa	dLD×PP/deltaLD	[15]
Young's elastic modulus	kPa	dLD/(aCIMT × distensibility)	[18]

aCIMT = average carotid intima media thickness across heart cycle, deltaLD = delta lumen diameter, dLD = diastolic lumen diameter, dBP= diastolic blood pressure, sBP = systolic blood pressure, PP = pulse pressure (sBP – dBP), ln = natural logarithm.

Supplement Table 7.5: Sex-specific associations of cardiovascular risk factors (per unit) with six CS parameters for men, women and for the respective sex differences

	risk factors	units	men			women			sex differences p-value
			Change in geometric mean (CI) [%]		p-value	Change in geometric mean (CI) [%]		p-value	
Strain [%]	**height**	10 cm	-1.01	(-3.81 ; 1.87)	0.49	0.81	(-1.75 ; 3.44)	0.54	0.35
	weight	10 kg	-3.89	(-5.35 ; -2.40)	0.00	-1.93	(-3.22 ; -0.61)	0.00	0.05
	BMI	5 kg/m^2	-6.91	(-9.34 ; -4.42)	0.00	-3.16	(-4.96 ; -1.33)	0.00	0.02
	systolic BP	10 mmHg	0.72	(-0.48 ; 1.93)	0.24	-0.07	(-1.09 ; 0.96)	0.89	0.33
	diastolic BP	10 mmHg	-3.87	(-5.64 ; -2.07)	0.00	-4.26	(-5.89 ; -2.59)	0.00	0.76
	pulse pressure	10 mmHg	4.96	(3.16 ; 6.78)	0.00	3.11	(1.59 ; 4.66)	0.00	0.13
	heart rate	10 bpm	-6.96	(-8.41 ; -5.49)	0.00	-5.01	(-6.50 ; -3.50)	0.00	0.07
	creatinine	1 µmol/l	-0.06	(-0.22 ; 0.11)	0.51	-0.04	(-0.21 ; 0.13)	0.63	0.90
	CRP	1 mg/l	-0.24	(-0.76 ; 0.27)	0.36	-1.18	(-1.76 ; -0.59)	0.00	0.02
	cholesterol	1 mmol/l	-1.51	(-3.09 ; 0.09)	0.07	0.75	(-0.66 ; 2.18)	0.30	0.04
	triglyceride	1 mmol/l	-0.93	(-2.32 ; 0.47)	0.19	-4.10	(-5.76 ; -2.42)	0.00	0.00
	HDL-C	1 mmol/l	10.33	(5.32 ; 15.59)	0.00	8.24	(4.47 ; 12.13)	0.00	0.52
	LDL-C	1 mmol/l	-2.53	(-4.24 ; -0.79)	0.00	0.84	(-0.66 ; 2.37)	0.27	0.00
	smoking	pack years	-0.05	(-0.14 ; 0.04)	0.26	0.07	(-0.04 ; 0.18)	0.20	0.09
	moderate PA	yes/no	1.04	(-2.56 ; 4.77)	0.57	1.26	(-2.40 ; 5.06)	0.50	0.93

Supplement Table 7.5 continued

	risk factors	units	men			women			sex differences p-value
			Change in geometric mean (CI) [%]		p-value	Change in geometric mean (CI) [%]		p-value	
Compliance [mm2/kPa]	**height**	10 cm	5.05	(1.79 ; 8.41)	0.00	6.65	(3.70 ; 9.69)	0.00	0.48
	weight	10 kg	0.95	(-0.77 ; 2.69)	0.28	1.82	(0.34 ; 3.32)	0.02	0.46
	BMI	5 kg/m²	-0.77	(-3.65 ; 2.20)	0.61	0.51	(-1.54 ; 2.60)	0.63	0.49
	systolic BP	10 mmHg	-0.85	(-2.14 ; 0.47)	0.21	-1.07	(-2.18 ; 0.05)	0.06	0.80
	diastolic BP	10 mmHg	-3.17	(-5.15 ; -1.15)	0.00	-1.38	(-3.22 ; 0.51)	0.15	0.20
	pulse pressure	10 mmHg	0.92	(-1.01 ; 2.88)	0.35	-1.24	(-2.85 ; 0.39)	0.14	0.09
	heart rate	10 bpm	-5.75	(-7.39 ; -4.08)	0.00	-4.48	(-6.13 ; -2.81)	0.00	0.29
	creatinine	1 µmol/l	-0.17	(-0.35 ; 0.01)	0.07	-0.07	(-0.25 ; 0.11)	0.45	0.44
	CRP	1 mg/l	-0.18	(-0.75 ; 0.39)	0.53	-0.84	(-1.49 ; -0.19)	0.01	0.14
	cholesterol	1 mmol/l	-0.68	(-2.44 ; 1.11)	0.45	-0.10	(-1.63 ; 1.46)	0.90	0.63
	triglyceride	1 mmol/l	-0.26	(-1.79 ; 1.30)	0.74	-2.21	(-4.06 ; -0.31)	0.02	0.12
	HDL-C	1 mmol/l	2.12	(-3.03 ; 7.54)	0.43	4.02	(0.03 ; 8.16)	0.05	0.58
	LDL-C	1 mmol/l	-0.93	(-2.85 ; 1.03)	0.35	-0.06	(-1.70 ; 1.61)	0.94	0.51
	smoking	pack years	0.14	(0.04 ; 0.23)	0.00	0.20	(0.08 ; 0.32)	0.00	0.41
	moderate PA	yes/no	-1.53	(-5.39 ; 2.50)	0.45	0.14	(-3.75 ; 4.19)	0.94	0.56
Distensibility [10-3/kPa]	**height**	10 cm	-0.07	(-2.82 ; 2.76)	0.96	2.06	(-0.57 ; 4.76)	0.12	0.28
	weight	10 kg	-3.47	(-4.91 ; -2.01)	0.00	-1.47	(-2.79 ; -0.12)	0.03	0.05
	BMI	5 kg/m²	-6.61	(-8.98 ; -4.17)	0.00	-2.90	(-4.73 ; -1.03)	0.00	0.02
	systolic BP	10 mmHg	-1.87	(-3.00 ; -0.73)	0.00	-2.45	(-3.46 ; -1.44)	0.00	0.46
	diastolic BP	10 mmHg	-3.60	(-5.34 ; -1.82)	0.00	-1.98	(-3.70 ; -0.23)	0.03	0.20
	pulse pressure	10 mmHg	-0.97	(-2.64 ; 0.73)	0.26	-3.71	(-5.15 ; -2.25)	0.00	0.02
	heart rate	10 bpm	-6.48	(-7.90 ; -5.04)	0.00	-2.93	(-4.49 ; -1.35)	0.00	0.00
	creatinine	1 µmol/l	0.00	(-0.16 ; 0.16)	0.97	0.08	(-0.09 ; 0.25)	0.36	0.49
	CRP	1 mg/l	-0.57	(-1.07 ; -0.07)	0.03	-1.24	(-1.84 ; -0.65)	0.00	0.09
	cholesterol	1 mmol/l	-1.79	(-3.32 ; -0.23)	0.02	0.51	(-0.92 ; 1.96)	0.49	0.03
	triglyceride	1 mmol/l	-1.54	(-2.88 ; -0.19)	0.03	-3.55	(-5.24 ; -1.82)	0.00	0.07
	HDL-C	1 mmol/l	9.16	(4.32 ; 14.23)	0.00	7.70	(3.90 ; 11.65)	0.00	0.65
	LDL-C	1 mmol/l	-2.27	(-3.94 ; -0.57)	0.01	0.47	(-1.06 ; 2.01)	0.55	0.02
	smoking	pack years	0.00	(-0.09 ; 0.08)	0.92	0.06	(-0.05 ; 0.17)	0.28	0.35
	moderate PA	yes/no	0.01	(-3.47 ; 3.62)	0.99	1.42	(-2.30 ; 5.29)	0.46	0.59

Supplement Table 7.5 continued

	risk factors	units	men			women			sex differences p-value
			Change in geometric mean (CI) [%]		p-value	Change in geometric mean (CI) [%]		p-value	
β-stiffness index [no unit]	**height**	10 cm	0.04	(-2.65 ; 2.81)	0.98	-1.91	(-4.38 ; 0.62)	0.14	0.30
	weight	10 kg	3.47	(1.96 ; 4.99)	0.00	1.42	(0.09 ; 2.77)	0.04	0.05
	BMI	5 kg/m²	6.83	(4.18 ; 9.55)	0.00	2.84	(0.94 ; 4.77)	0.00	0.02
	systolic BP	10 mmHg	1.80	(0.65 ; 2.96)	0.00	2.38	(1.35 ; 3.42)	0.00	0.47
	diastolic BP	10 mmHg	3.63	(1.80 ; 5.50)	0.00	1.93	(0.19 ; 3.71)	0.03	0.19
	pulse pressure	10 mmHg	0.83	(-0.83 ; 2.52)	0.33	3.62	(2.11 ; 5.16)	0.00	0.02
	heart rate	10 bpm	6.77	(5.19 ; 8.38)	0.00	2.96	(1.35 ; 4.60)	0.00	0.00
	creatinine	1 µmol/l	0.00	(-0.16 ; 0.16)	0.98	-0.07	(-0.24 ; 0.09)	0.38	0.52
	CRP	1 mg/l	0.54	(0.05 ; 1.04)	0.03	1.24	(0.64 ; 1.83)	0.00	0.08
	cholesterol	1 mmol/l	1.74	(0.19 ; 3.31)	0.03	-0.49	(-1.88 ; 0.91)	0.49	0.04
	triglyceride	1 mmol/l	1.51	(0.17 ; 2.88)	0.03	3.61	(1.84 ; 5.42)	0.00	0.07
	HDL-C	1 mmol/l	-8.26	(-12.24 ; -4.11)	0.00	-7.02	(-10.23 ; -3.70)	0.00	0.64
	LDL-C	1 mmol/l	2.25	(0.55 ; 3.98)	0.01	-0.45	(-1.93 ; 1.04)	0.55	0.02
	smoking	pack years	0.00	(-0.08 ; 0.09)	0.93	-0.06	(-0.17 ; 0.05)	0.27	0.35
	moderate PA	yes/no	-0.05	(-3.45 ; 3.47)	0.98	-1.46	(-5.00 ; 2.20)	0.43	0.58
Peterson's elastic modulus [kPa]	**height**	10 cm	0.05	(-2.65 ; 2.82)	0.97	-1.99	(-4.46 ; 0.56)	0.12	0.28
	weight	10 kg	3.51	(2.00 ; 5.04)	0.00	1.45	(0.11 ; 2.80)	0.03	0.05
	BMI	5 kg/m²	6.90	(4.24 ; 9.63)	0.00	2.90	(1.00 ; 4.84)	0.00	0.02
	systolic BP	10 mmHg	1.94	(0.78 ; 3.10)	0.00	2.52	(1.48 ; 3.57)	0.00	0.46
	diastolic BP	10 mmHg	3.64	(1.80 ; 5.51)	0.00	1.91	(0.16 ; 3.69)	0.03	0.18
	pulse pressure	10 mmHg	1.11	(-0.56 ; 2.80)	0.20	3.94	(2.42 ; 5.49)	0.00	0.01
	heart rate	10 bpm	6.75	(5.16 ; 8.36)	0.00	2.89	(1.27 ; 4.53)	0.00	0.00
	creatinine	1 µmol/l	0.00	(-0.16 ; 0.16)	0.98	-0.08	(-0.24 ; 0.09)	0.34	0.48
	CRP	1 mg/l	0.57	(0.07 ; 1.06)	0.02	1.24	(0.64 ; 1.84)	0.00	0.09
	cholesterol	1 mmol/l	1.78	(0.23 ; 3.36)	0.02	-0.49	(-1.88 ; 0.91)	0.49	0.03
	triglyceride	1 mmol/l	1.55	(0.20 ; 2.92)	0.02	3.58	(1.80 ; 5.40)	0.00	0.08
	HDL-C	1 mmol/l	-8.20	(-12.19 ; -4.03)	0.00	-6.99	(-10.21 ; -3.65)	0.00	0.65
	LDL-C	1 mmol/l	2.27	(0.56 ; 4.00)	0.01	-0.45	(-1.93 ; 1.05)	0.56	0.02
	smoking	pack years	0.00	(-0.08 ; 0.09)	0.93	-0.06	(-0.17 ; 0.05)	0.28	0.37
	moderate PA	yes/no	0.01	(-3.40 ; 3.54)	1.00	-1.39	(-4.95 ; 2.30)	0.46	0.58

Supplement Table 7.5 continued

	risk factors	units	men			women			sex differences p-value
			Change in geometric mean (CI) [%]		p-value	Change in geometric mean (CI) [%]		p-value	
Young's elastic modulus [kPa]	**height**	10 cm	1.42	(-1.67 ; 4.59)	0.37	-0.43	(-3.20 ; 2.42)	0.77	0.39
	weight	10 kg	3.72	(2.02 ; 5.46)	0.00	1.75	(0.27 ; 3.25)	0.02	0.09
	BMI	5 kg/m^2	6.53	(3.53 ; 9.62)	0.00	2.81	(0.71 ; 4.95)	0.01	0.05
	systolic BP	10 mmHg	1.77	(0.47 ; 3.09)	0.01	1.77	(0.63 ; 2.92)	0.00	1.00
	diastolic BP	10 mmHg	3.75	(1.66 ; 5.87)	0.00	1.87	(-0.06 ; 3.84)	0.06	0.20
	pulse pressure	10 mmHg	0.67	(-1.21 ; 2.58)	0.49	2.37	(0.70 ; 4.06)	0.01	0.19
	heart rate	10 bpm	7.35	(5.54 ; 9.18)	0.00	3.01	(1.23 ; 4.83)	0.00	0.00
	creatinine	1 µmol/l	-0.02	(-0.20 ; 0.16)	0.81	-0.15	(-0.34 ; 0.03)	0.10	0.31
	CRP	1 mg/l	0.72	(0.16 ; 1.28)	0.01	1.24	(0.59 ; 1.90)	0.00	0.24
	cholesterol	1 mmol/l	0.34	(-1.39 ; 2.10)	0.70	-1.52	(-3.03 ; 0.01)	0.05	0.11
	triglyceride	1 mmol/l	1.61	(0.09 ; 3.16)	0.04	2.20	(0.26 ; 4.19)	0.03	0.64
	HDL-C	1 mmol/l	-6.09	(-10.70 ; -1.24)	0.01	-5.49	(-9.11 ; -1.73)	0.00	0.85
	LDL-C	1 mmol/l	0.16	(-1.73 ; 2.09)	0.87	-1.45	(-3.07 ; 0.18)	0.08	0.21
	smoking	pack years	-0.03	(-0.12 ; 0.07)	0.59	-0.07	(-0.19 ; 0.05)	0.25	0.57
	moderate PA	yes/no	-0.37	(-4.20 ; 3.61)	0.85	-0.91	(-4.86 ; 3.20)	0.66	0.85

Values are expressed as percentage change in the geometric mean per unit increment. Association estimates were based on mixed linear regression models adjusted for age, education level, medication, mean arterial pressure and study centre. Physical activity is a binary variable. CI = confidence interval, p = p value, BMI = body mass index, BP = blood pressure, cholesterol = total cholesterol, CRP = C-reactive protein, HDL-C = high density lipoprotein cholesterol, LDL C = low density lipoprotein cholesterol, PA = physical activity, SD = standard deviation.

*Sex-specific associations in effect estimates per unit increment between men and women for single risk factors and CS parameters were calculated based on sex-stratified mixed linear models adjusted for age, education level, medication, mean arterial pressure and study centre. Sex-difference p-values were calculated based on sex-stratified log-estimates using the following formulas:

Sex- difference with 95% CI: $(\text{coefficient}_{\text{women}} - \text{coefficient}_{\text{men}}) \pm 1.96 \times \sqrt{SE(women)^2 + SE(men)^2}$

p-value: $1 - F[(\text{coefficient}_{\text{women}} - \text{coefficient}_{\text{men}})^2 / ((\text{standard error}_{\text{women}})^2 + (\text{standard error}_{\text{men}})^2)]$, where F = Chi2-distribution function with 1 degree of freedom.

Supplement Table 7.6: Sex-specific associations of cardiovascular risk factors (per 1 SD) with six CS parameters for men, women and for the respective sex differences

	Risk factors	Men			Women		
		Change in geometric mean (CI) per 1SD [%]		p	Change in geometric mean (CI) per 1SD [%]		p
Strain [%]	Height	-0.64	(-2.42; 1.17)	0.49	0.50	(-1.07; 2.09)	0.54
	Weight	-4.39	(-6.04; -2.72)	0.00	-2.22	(-3.72; -0.70)	0.00
	Body mass index	-4.67	(-6.34; -2.97)	0.00	-2.67	(-4.20; -1.12)	0.00
	Systolic blood pressure	1.23	(-0.81; 3.32)	0.24	-0.13	(-2.00; 1.78)	0.89
	Diastolic blood pressure	-4.12	(-6.01; -2.20)	0.00	-4.37	(-6.05; -2.66)	0.00
	Pulse pressure	5.41	(3.45; 7.40)	0.00	3.71	(1.89; 5.56)	0.00
	Heart rate	-7.43	(-8.97; -5.87)	0.00	-4.77	(-6.18; -3.33)	0.00
	Creatinine	-0.58	(-2.30; 1.17)	0.51	-0.37	(-1.88; 1.16)	0.63
	CRP	-0.81	(-2.52; 0.92)	0.36	-3.05	(-4.54; -1.54)	0.00
	Total Cholesterol	-1.63	(-3.33; 0.10)	0.07	0.84	(-0.74; 2.44)	0.30
	Triglyceride	-1.15	(-2.86; 0.58)	0.19	-3.69	(-5.18; -2.17)	0.00
	HDL-C	3.71	(1.94; 5.52)	0.00	3.47	(1.90; 5.05)	0.00
	LDL-C	-2.50	(-4.18; -0.78)	0.00	0.88	(-0.69; 2.46)	0.27
	Smoking	-1.00	(-2.71; 0.74)	0.26	1.01	(-0.51; 2.55)	0.20
	Physical activity	1.04	(-2.56; 4.77)	0.57	1.26	(-2.40; 5.06)	0.50
Compliance [mm2/kPa]	Height	3.16	(1.12; 5.23)	0.00	4.01	(2.24; 5.81)	0.00
	Weight	1.07	(-0.87; 3.06)	0.28	2.10	(0.39; 3.85)	0.02
	Body mass index	-0.51	(-2.45; 1.47)	0.61	0.43	(-1.30; 2.19)	0.63
	Systolic blood pressure	-1.44	(-3.64; 0.80)	0.21	-1.96	(-3.97; 0.09)	0.06
	Diastolic blood pressure	-3.38	(-5.49; -1.22)	0.00	-1.41	(-3.31; 0.52)	0.15
	Pulse pressure	1.00	(-1.10; 3.14)	0.35	-1.48	(-3.38; 0.47)	0.14
	Heart rate	-6.14	(-7.88; -4.36)	0.00	-4.26	(-5.83; -2.67)	0.00
	Creatinine	-1.79	(-3.66; 0.12)	0.07	-0.64	(-2.28; 1.04)	0.45
	CRP	-0.61	(-2.49; 1.31)	0.53	-2.18	(-3.84; -0.50)	0.01
	Total Cholesterol	-0.73	(-2.63; 1.20)	0.45	-0.11	(-1.83; 1.64)	0.90
	Triglyceride	-0.32	(-2.22; 1.62)	0.74	-1.98	(-3.65; -0.28)	0.02
	HDL-C	0.78	(-1.14; 2.73)	0.43	1.71	(0.01; 3.44)	0.05
	LDL-C	-0.92	(-2.81; 1.02)	0.35	-0.06	(-1.77; 1.67)	0.94
	Smoking	2.82	(0.87; 4.81)	0.00	2.82	(1.14; 4.54)	0.00
	Physical activity	-1.53	(-5.39; 2.50)	0.45	0.14	(-3.75; 4.19)	0.94

Supplement table 7.6 continued

	Risk factors	Men			Women		
		Change in geometric mean (CI) per 1SD [%]		p	Change in geometric mean (CI) per 1SD [%]		p
Distensibility [10-3/kPa]	Height	-0.04	(-1.79; 1.73)	0.96	1.26	(-0.35; 2.88)	0.12
	Weight	-3.93	(-5.54; -2.28)	0.00	-1.69	(-3.22; -0.14)	0.03
	Body mass index	-4.46	(-6.09; -2.80)	0.00	-2.45	(-4.00; -0.87)	0.00
	Systolic blood pressure	-3.18	(-5.08; -1.24)	0.00	-4.46	(-6.26; -2.62)	0.00
	Diastolic blood pressure	-3.83	(-5.69; -1.94)	0.00	-2.03	(-3.79; -0.24)	0.03
	Pulse pressure	-1.05	(-2.87; 0.79)	0.26	-4.39	(-6.09; -2.66)	0.00
	Heart rate	-6.92	(-8.43; -5.39)	0.00	-2.79	(-4.27; -1.28)	0.00
	Creatinine	-0.04	(-1.72; 1.67)	0.97	0.73	(-0.82; 2.30)	0.36
	CRP	-1.89	(-3.53; -0.23)	0.03	-3.22	(-4.72; -1.68)	0.00
	Total Cholesterol	-1.93	(-3.58; -0.25)	0.02	0.57	(-1.03; 2.20)	0.49
	Triglyceride	-1.91	(-3.56; -0.24)	0.03	-3.19	(-4.71; -1.64)	0.00
	HDL-C	3.30	(1.58; 5.06)	0.00	3.25	(1.66; 4.86)	0.00
	LDL-C	-2.24	(-3.89; -0.57)	0.01	0.48	(-1.10; 2.09)	0.55
	Smoking	-0.08	(-1.76; 1.63)	0.92	0.86	(-0.68; 2.42)	0.28
	Physical activity	0.01	(-3.47; 3.62)	0.99	1.42	(-2.30; 5.29)	0.46
β-stiffness index [no unit]	Height	0.03	(-1.68; 1.76)	0.98	-1.17	(-2.69; 0.38)	0.14
	Weight	3.94	(2.23; 5.68)	0.00	1.64	(0.10; 3.21)	0.04
	Body mass index	4.51	(2.77; 6.28)	0.00	2.39	(0.80; 4.01)	0.00
	Systolic blood pressure	3.10	(1.12; 5.12)	0.00	4.41	(2.49; 6.37)	0.00
	Diastolic blood pressure	3.88	(1.92; 5.88)	0.00	1.99	(0.20; 3.81)	0.03
	Pulse pressure	0.91	(-0.90; 2.75)	0.33	4.32	(2.51; 6.16)	0.00
	Heart rate	7.26	(5.56; 8.99)	0.00	2.81	(1.28; 4.36)	0.00
	Creatinine	0.02	(-1.63; 1.69)	0.98	-0.67	(-2.16; 0.84)	0.38
	CRP	1.81	(0.15; 3.50)	0.03	3.25	(1.69; 4.85)	0.00
	Total Cholesterol	1.88	(0.20; 3.58)	0.03	-0.55	(-2.10; 1.01)	0.49
	Triglyceride	1.88	(0.21; 3.57)	0.03	3.23	(1.65; 4.84)	0.00
	HDL-C	-3.15	(-4.73; -1.54)	0.00	-3.09	(-4.54; -1.61)	0.00
	LDL-C	2.22	(0.54; 3.93)	0.01	-0.47	(-2.00; 1.08)	0.55
	Smoking	0.08	(-1.57; 1.75)	0.93	-0.84	(-2.31; 0.66)	0.27
	Physical activity	-0.05	(-3.45; 3.47)	0.98	-1.46	(-5.00; 2.20)	0.43

Supplement table 7.6 continued

	Risk factors	Men			Women		
		Change in geometric mean (CI) per 1SD [%]		p	Change in geometric mean (CI) per 1SD [%]		p
Peterson's elastic modulus [kPa]	**Height**	0.03	(-1.68; 1.77)	0.97	-1.22	(-2.75; 0.34)	0.12
	Weight	3.99	(2.27; 5.73)	0.00	1.67	(0.12; 3.25)	0.03
	Body mass index	4.56	(2.81; 6.33)	0.00	2.45	(0.84; 4.07)	0.00
	Systolic blood pressure	3.33	(1.35; 5.36)	0.00	4.68	(2.74; 6.65)	0.00
	Diastolic blood pressure	3.89	(1.92; 5.89)	0.00	1.96	(0.16; 3.79)	0.03
	Pulse pressure	1.20	(-0.61; 3.05)	0.20	4.71	(2.88; 6.56)	0.00
	Heart rate	7.24	(5.53; 8.97)	0.00	2.74	(1.20; 4.30)	0.00
	Creatinine	0.02	(-1.63; 1.70)	0.98	-0.73	(-2.23; 0.79)	0.34
	CRP	1.90	(0.24; 3.60)	0.02	3.26	(1.68; 4.86)	0.00
	Total Cholesterol	1.93	(0.25; 3.64)	0.02	-0.55	(-2.10; 1.02)	0.49
	Triglyceride	1.92	(0.25; 3.62)	0.02	3.21	(1.61; 4.83)	0.00
	HDL-C	-3.12	(-4.70; -1.51)	0.00	-3.07	(-4.53; -1.59)	0.00
	LDL-C	2.24	(0.55; 3.95)	0.01	-0.47	(-2.00; 1.09)	0.56
	Smoking	0.07	(-1.58; 1.75)	0.93	-0.82	(-2.31; 0.69)	0.28
	Physical activity	0.01	(-3.40; 3.54)	1.00	-1.39	(-4.95; 2.30)	0.46
Young's elastic modulus [kPa]	**Height**	0.89	(-1.05; 2.87)	0.37	-0.26	(-1.97; 1.47)	0.77
	Weight	4.23	(2.29; 6.21)	0.00	2.02	(0.31; 3.76)	0.02
	Body mass index	4.31	(2.34; 6.32)	0.00	2.36	(0.60; 4.16)	0.01
	Systolic blood pressure	3.05	(0.81; 5.34)	0.01	3.27	(1.16; 5.43)	0.00
	Diastolic blood pressure	4.00	(1.77; 6.28)	0.00	1.92	(-0.06; 3.95)	0.06
	Pulse pressure	0.72	(-1.32; 2.81)	0.49	2.82	(0.84; 4.85)	0.01
	Heart rate	7.88	(5.94; 9.86)	0.00	2.86	(1.16; 4.58)	0.00
	Creatinine	-0.23	(-2.09; 1.66)	0.81	-1.40	(-3.04; 0.26)	0.10
	CRP	2.42	(0.53; 4.34)	0.01	3.27	(1.53; 5.04)	0.00
	Total Cholesterol	0.37	(-1.50; 2.27)	0.70	-1.70	(-3.38; 0.02)	0.05
	Triglyceride	2.00	(0.11; 3.92)	0.04	1.97	(0.23; 3.75)	0.03
	HDL-C	-2.30	(-4.11; -0.46)	0.01	-2.40	(-4.03; -0.75)	0.00
	LDL-C	0.16	(-1.71; 2.06)	0.87	-1.51	(-3.18; 0.19)	0.08
	Smoking	-0.51	(-2.36; 1.37)	0.59	-0.96	(-2.60; 0.70)	0.25
	Physical activity	-0.37	(-4.20; 3.61)	0.85	-0.91	(-4.86; 3.20)	0.66

Values are expressed as percentage change in the geometric mean per 1 SD increment. Association estimates were based on mixed linear regression models adjusted for age, education level, medication, mean arterial pressure and study centre. Continuous risk factors were standardised. CI = confidence interval, p = p value, BMI = body mass index, BP = blood pressure, cholesterol = total cholesterol, CRP = C-reactive protein, HDL-C = high density lipoprotein cholesterol, LDL C = low density lipoprotein cholesterol, PA = physical activity, SD = standard deviation.

Supplement Table 7.7: Significance of heterogeneity in the associations of single cardiovascular risk factors (per 1 SD) across all six CS parameters (log-transformed) separately for men, women

	Risk factors	Hetero-geneity	Strain vs Comp	Strain vs Dist	Strain vs Bstiff	Strain vs EP	Strain vs Youngs	Comp vs Dist	Comp vs Bstiff	Comp vs EP	Comp vs Youngs	Dist vs Bstiff	Dist vs EP	Dist vs Youngs	Bstiff vs EP	Bstiff vs Youngs	EP vs Youngs
Men	**Height**	<0.001†	<0.001†	0.14	0.13	0.15	0.75	<0.001†	<0.001†	<0.001†	<0.001†	0.82	0.73	0.05	0.99	0.05	0.05
	Weight	<0.001†	<0.001†	0.27	0.15	0.19	0.54	<0.001†	<0.001†	<0.001†	<0.001†	<0.001†	<0.001†	0.80	0.18	0.56	0.63
	Body mass index	<0.001†	<0.001†	0.68	0.44	0.53	0.36	<0.001†	<0.001†	<0.001†	<0.001†	<0.001†	<0.001†	0.38	0.14	0.60	0.53
	Systolic BP	<0.001†	<0.001†	<0.001†	<0.001†	<0.001†	<0.001†	0.01	0.02	0.01	0.10	<0.001†	0.07	0.67	<0.001†	0.94	0.60
	Diastolic BP	0.02	0.38	0.55	0.42	0.45	0.71	0.57	0.67	0.67	0.65	0.02†	<0.001†	0.93	0.87	0.78	0.79
	Pulse pressure	<0.001†	<0.001†	<0.001†	<0.001†	<0.001†	<0.001†	0.00†	0.00†	<0.001†	0.05	<0.001†	<0.001†	0.48	<0.001†	0.72	0.32
	Heart rate	<0.001†	0.04	0.21	0.10	0.10	0.87	0.13	0.22	0.24	0.09	<0.001†	<0.001†	0.29	0.42	0.15	0.14
	Creatinine	0.03	0.08	0.16	0.14	0.16	0.15	0.00†	0.00†	0.00†	0.01†	0.63	0.37	0.53	0.93	0.56	0.57
	CRP	0.00†	0.75	0.01†	0.02	0.02	0.01†	0.02	0.03	0.02	0.02	<0.001†	0.29	0.25	0.00†	0.16	0.23
	Total Cholesterol	<0.001†	0.18	0.47	0.59	0.53	0.04	0.03	0.05	0.04	0.62	0.01	0.10	<0.001†	0.13	<0.001†	<0.001†
	Triglyceride	0.02	0.20	0.09	0.12	0.11	0.18	0.00†	0.01†	0.01†	0.03	0.04	0.18	0.89	0.18	0.75	0.83
	HDL-C	<0.001†	<0.001†	0.39	0.32	0.31	0.03	<0.001†	<0.001†	<0.001†	0.04	0.13	<0.001†	0.02	0.41	0.03	0.04
	LDL-C	<0.001†	0.02	0.61	0.49	0.54	<0.001†	0.02	0.02	0.02	0.32	0.04	0.02	<0.001†	0.58	<0.001†	<0.001†
	Smoking	<0.001†	<0.001†	0.02	0.02	0.02	0.01	<0.001†	<0.001†	<0.001†	0.00†	0.86	0.47	0.16	0.76	0.17	0.18
	Physical activity	0.69	0.09	0.28	0.29	0.28	0.68	0.24	0.22	0.24	0.25	0.63	0.62	0.60	0.39	0.63	0.58
Women	**Height**	<0.001†	<0.001†	0.10	0.13	0.12	0.68	<0.001†	<0.001†	<0.001†	<0.001†	0.04	0.23	0.00†	0.14	0.01	0.01†
	Weight	<0.001†	<0.001†	0.24	0.17	0.21	0.66	<0.001†	<0.001†	<0.001†	<0.001†	0.02	0.02	0.37	0.34	0.26	0.31
	Body mass index	<0.001†	<0.001†	0.63	0.45	0.54	0.52	<0.001†	<0.001†	<0.001†	<0.001†	<0.001†	0.00†	0.71	0.09	0.97	0.84
	Systolic BP	<0.001†	0.01	<0.001†	<0.001†	<0.001†	<0.001†	<0.001†	<0.001†	<0.001†	0.14	<0.001†	0.70	0.00†	<0.001†	0.01	0.00†
	Diastolic BP	<0.001†	<0.001†	<0.001†	<0.001†	<0.001†	<0.001†	0.41	0.50	0.54	0.65	0.04	<0.001†	0.78	0.42	0.94	1.00
	Pulse pressure	<0.001†	<0.001†	<0.001†	<0.001†	<0.001†	<0.001†	<0.001†	<0.001†	<0.001†	0.08	<0.001†	<0.001†	<0.001†	<0.001†	<0.001†	<0.001†
	Heart rate	<0.001†	0.33	<0.001†	<0.001†	<0.001†	<0.001†	<0.001†	<0.001†	<0.001†	0.02	0.12	<0.001†	0.95	0.03	0.82	0.67
	Creatinine	0.00†	0.61	0.02	0.02	0.02	<0.001†	0.00†	0.00†	0.00†	<0.001†	0.13	0.62	0.03	0.05	0.02	0.03
	CRP	0.01†	0.11	0.72	0.82	0.82	0.83	0.02†	0.02	0.02	0.09	0.05	0.00†	0.88	0.88	0.97	0.98
	Total Cholesterol	0.03	0.09	0.55	0.52	0.53	0.12	0.12	0.13	0.13	0.00†	0.69	0.40	<0.001†	0.92	<0.001†	<0.001†
	Triglyceride	<0.001†	0.00†	0.29	0.23	0.23	<0.001†	0.01	0.01	0.02	0.88	0.10	<0.001†	<0.001†	0.49	<0.001†	<0.001†
	HDL-C	<0.001†	0.00†	0.66	0.55	0.55	0.07	<0.001†	0.00†	0.00†	0.22	0.08	<0.001†	0.02	0.61	0.04	0.05
	LDL-C	0.06	0.10	0.38	0.36	0.37	0.25	0.21	0.22	0.22	0.01†	0.74	0.42	0.00†	0.88	0.00†	0.00†
	Smoking	<0.001†	0.00†	0.74	0.72	0.70	0.97	<0.001†	<0.001†	<0.001†	0.00†	0.74	0.18	0.70	0.62	0.68	0.64
	Physical activity	0.75	0.76	0.79	0.74	0.80	0.92	0.51	0.48	0.52	0.84	0.47	0.78	0.60	0.35	0.55	0.62

P-values for heterogeneity were tested for each risk factor based on the null hypothesis of equal regression coefficients across all six CS parameters using an F-test with 5 numerator degrees of freedom. Pairwise comparisons of effect estimates of CS parameters were performed using F-tests with 1 numerator degree of freedom. †= significant after Bonferroni-Holm correction, Bstiff = β-stiffness index, Comp = compliance, Dist = distensibility, Youngs = Young's elastic modulus, EP = Peterson's elastic modulus, BP = blood pressure, CRP = C-reactive protein, HDL-C = high density lipoprotein cholesterol, LDL C = low density lipoprotein cholesterol.

CHAPTER 8

Carotid stiffness and physical activity in elderly – results from the SAPALDIA 3 Cohort Study

Authors

Seraina Caviezel
Julia Dratva
Emmanuel Schaffner
Christian Schindler
Simon Endes
Christine Autenrieth
Miriam Wanner
Brian Martin
Eric de Groot
Jean-Michel Gaspoz
Nino Künzli
Nicole Probst-Hensch
Arno Schmidt-Trucksäss

Submitted version*:

Submitted to PLOS ONE

Submission date: 09th January 2015

*For the accepted version published in Plos One please see "Carotid Stiffness and Physical Activity in Elderly-A Short Report of the SAPALDIA 3 Cohort Study", PLoS One. 2015 Jun 2;10(6):e0128991 Doi: 10.1371/journal.pone.0128991

CAROTID STIFFNESS AND PHYSICAL ACTIVITY IN ELDERLY – RESULTS FROM THE SAPALDIA 3 COHORT STUDY

Abstract

Introduction: Regular physical activity has been shown to reduce cardiovascular disease risk in the general population. While smaller studies in specified groups (highly trained versus untrained individuals) indicate a certain dose-dependent effect of physical activity on reduction of carotid stiffness - which is an indicator of subclinical vascular disease - it is unclear whether this association is present in a representative sample. Thus, we investigated this question cross-sectionally in participants from the population-based SAPALDIA cohort study.

Methods: Self-reported total, moderate and vigorous physical activity and distensibility as a measure of local arterial stiffness among 1636 participants aged 50 to 81 years without clinically manifest diseases were evaluated. Mixed regression models and pathway analyses were used to examine associations of physical activity intensity with distensibility.

Results: Vigorous physical activity, but not total nor moderate physical activity, was significantly associated with increased distensibility (= reduced carotid stiffness) in univariate analyses (percent change in the geometric mean and 95% confidence interval per 10min/week increase in vigorous physical activity = 0.11 (0.03-0.19), p<0.01; in total physical activity = 0.03 (0.00-0.06), p=0.08; in moderate physical activity = 0.02 (-0.02-0.06), p=0.45). These associations disappeared when we additionally adjusted for age. Pathway analyses showed that age (standardised beta -0.48) was the strongest predictor of distensibility followed by mean arterial blood pressure (standardised beta -0.35) and heart rate (standardised beta -0.18). All pathways of vigorous physical activity (direct or intermediate) were marginal and statistically non-significant.

Conclusion: We found no evidence for an association of physical activity with carotid stiffness in the general middle-age to elderly population adjusted for most important confounders and risk factors.

Introduction

Physical inactivity is a generally accepted cardiovascular risk factor which causes cardiovascular disease (CVD) and a high percentage of global mortality [1–3]. Therefore, being regularly physically active can improve health at all ages by different pathways (e.g. enhancing cardiorespiratory, metabolic or mental health) resulting in a reduced CVD risk and mortality [3–8]. CVD begins with structural and functional changes of the arterial system commonly known as the atherosclerotic process [9,10]. The vascular damage accumulated

over time can be assessed as arterial stiffness, which can be directly measured in the common carotid artery (CCA) [11].

While regular physical activity (PA) has been shown to reduce CVD risk in the general population, results of inverse associations between an increased PA level and a reduced carotid stiffness were mostly limited to two or three healthy groups with specific group characteristics such as highly trained versus untrained subjects [12–19]. Although these reports suggest a certain dose-dependent effect of PA on carotid stiffness [12–19], the specific group distinctions constrict a more generalizable conclusion. In addition, there is no consent on how different types and intensities of PA influence different parameters of arterial stiffness across the lifespan, since arterial stiffness varies in different arterial segments along the arterial tree with age.

Thus, the aim of this study was to assess whether an increasing amount of self-reported total, moderate and vigorous PA is associated with reduced carotid stiffness in a general middle-aged and elderly population without a diagnosis of CVD from the SAPALDIA cohort (Swiss Cohort Study on Air Pollution And Lung and Heart Diseases In Adults).

Methods

Study design and subjects

The SAPALDIA cohort study is an ongoing multicentre cohort study with a population-based random sample of adults from eight rural and urban areas of Switzerland (Aarau, Basel, Davos, Genève, Lugano, Montana, Payerne, Wald) with baseline assessment in 1991 and first follow-up assessment in 2001-2003 [20,21]. This study is based on the second follow-up assessment (SAPALDIA 3) which was conducted in 2010-2011. Sequential B-mode ultrasound images of the CCA were assessed in 3489 participants (51% female) aged between 50-81 years at the time of examination. PA was assessed using the long form of the International Physical Activity Questionnaire (IPAQ). Data from 1636 participants were available for analyses after exclusion of individuals with doctor diagnosed diabetes, stroke, myocardial infarction, angina pectoris, heart failure at time of ultrasound examination or missing ultrasound, IPAQ or additional covariate data (figure 8.1).

The study complies with the declaration of Helsinki and ethical clearance was obtained and approved from the respective cantonal ethical committees (Aargau, Basel, Geneva, Grisons, Ticino, Valais, Vaud and Zurich) and participants gave written informed consent according to their preferences either globally for all examinations or separately for single assessments.

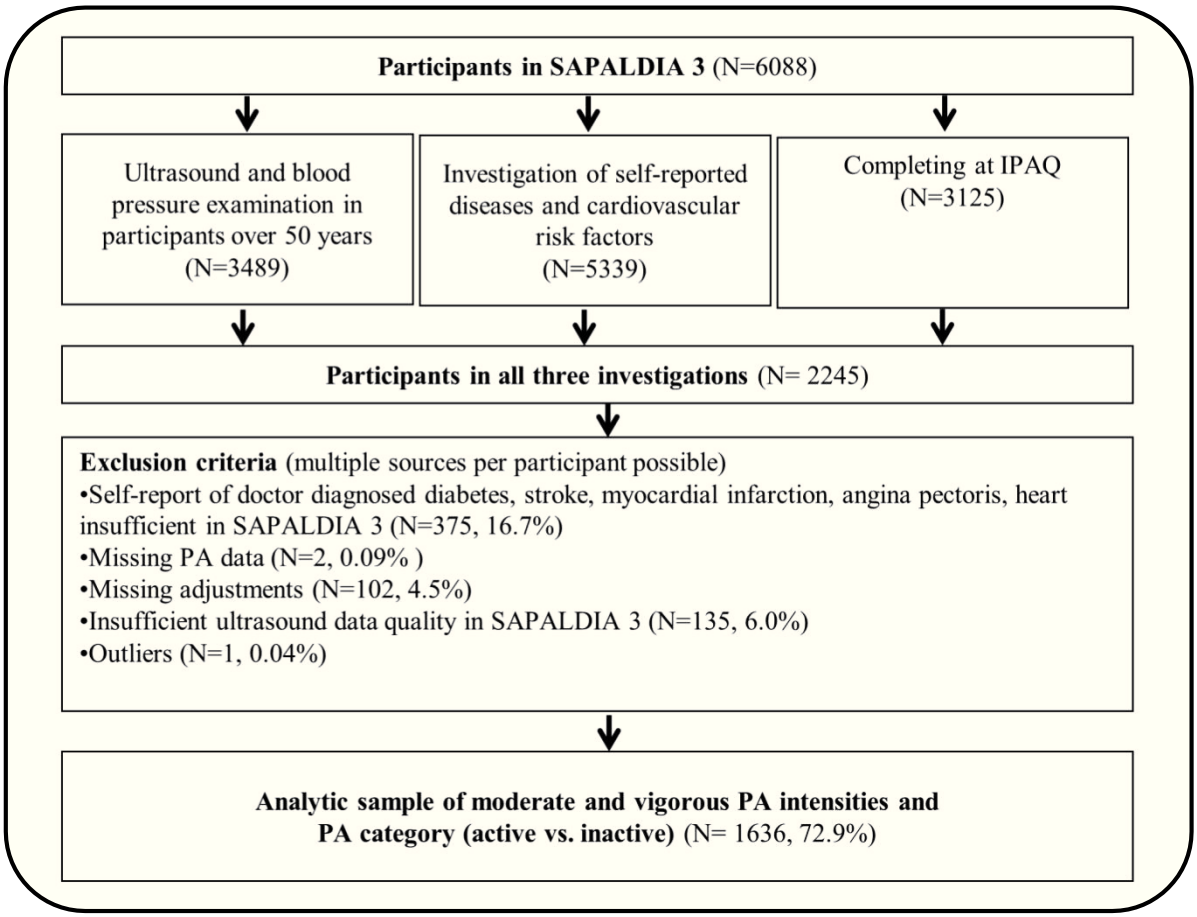

Figure 8.1: Flow chart of subject inclusion
IPAQ = International Physical Activity Questionnaire, PA = physical activity, affected adjustments = body mass index, mean arterial pressure, heart rate, smoking.

Carotid stiffness – distensibility

Ultrasound B-mode scans of the CCA were performed by centrally trained and certified sonographers in all eight centres. The same standardised ultrasound instruments (UF-870 machine LA385-16 MHz array transducer, Fukuda Denshi, Japan) and scan protocol were used. Participants' common carotid arteries were bilaterally examined in supine position after a rest of at least 10 minutes with the neck rotated by 45° to either right or left side standardized with a foam wedge. Blood pressure (BP) was measured immediately after ultrasound examination with a standard oscillometric device (OMRON 705IT, OMRON HEALTHCARE, Kyoto, Japan) at the left and right upper arm. Mean arterial pressure was defined as diastolic BP added to one-third of the pulse pressure [22].

Ultrasound scans were analysed offline by the fully automatic ultrasound program DYARA (DYnamic ARtery Analysis program) [23,24]. Expert readers tagged the carotid structure directly proximal of the carotid bifurcation over exactly one centimetre and analysed the intimal and adventitial layer of the carotid wall and adventitia layer of the near wall for a duration of at least one heart cycle [23,24]. Detailed information about the reading process and DYARA algorithm are described elsewhere [23,24]. Distensibility was calculated as

([1/kPa] = ((2 × deltaLD × dLD)+(deltaLD)^2)/(PP× dLD^2)) [25], where deltaLD is the systolic-diastolic lumen diameter difference, dLD the diastolic lumen diameter and PP the pulse pressure. Lower values of distensibility correspond to an increased carotid stiffness.

The variability and reproducibility of carotid stiffness parameters were evaluated in 165 participants of the SAPALDIA 3 population within the identical study setting [24]. The coefficient of variation of replicate ultrasound measurements was 12.14% for distensibility with an intraclass correlation coefficient (ICC) of 0.77 [24].

Physical activity parameters

Self-reported PA was assessed using the validated IPAQ long form [26,27]. IPAQ data cleaning and item analysis were conducted according to the IPAQ protocol for data processing and analysis [28]. In short, moderate and vigorous continuous values expressed as min/week were derived from 27 items and four domains (work, transport, domestic/garden, leisure time) [28]. Values of moderate or vigorous intensity were only considered if at least 10 minute bouts were obtained. Values above 1260 min/week (3hours/day) were truncated [28]. The sum of moderate and vigorous PA intensity was calculated and defined as the total PA.

Table 8.1: Characteristics of the study population by sex described by numbers (N) and percentages (%), mean value and standard deviation (SD) or median and interquartile range (p25, p75)

Characteristics	Units	Men	Women
N participants	N (%)	712 (43.5)	924 (56.5)
No medication intake	N (%)	463 (39.9)	696 (60.1)
Medication intake	N (%)	249 (52.2)	228 (47.8)
Age	Mean (SD) years	63.0 (7.6)	63.0 (7.8)
Height	Mean (SD) cm	175.1 (6.3)	162.0 (6.3)
Weight	Mean (SD) kg	81.8 (11.9)	65.7 (12.3)
Body mass index	Mean (SD) kg/m2	26.7 (3.5)	25.1 (4.6)
Diastolic BP	Mean (SD) mmHg	80.1 (9.4)	76.5 (9.1)
Systolic BP	Mean (SD) mmHg	137.5 (17.3)	131.5 (17.9)
Mean BP	Mean (SD) mmHg	99.2 (11.2)	94.8 (11.1)
Pulse pressure	Mean (SD) mmHg	57.4 (12.1)	55.1 (13.1)
Heart rate	Mean (SD) bpm	68.1 (10.6)	69.1 (9.2)
Smoking	Median (p25, p75) pack years	2.5 (0, 24)	0 (0, 12)
Distensibility	Median (p25, p75) 10^{-3}/kPa	14.5 (11.4, 18.1)	15.8 (12.0, 20.1)
Total PA	Median (p25, p75) min/week	510 (180, 1105)	590 (240, 1195)
Moderate PA	Median (p25, p75) min/week	360 (120, 00)	510 (193, 1080)
Vigorous PA	Median (p25, p75) min/week	25 (0, 225)	0 (0, 113)

BP = blood pressure, PA = physical activity

Statistical analysis

Descriptive analyses of the study population characteristics and hemodynamic parameters were performed. Box plots of distensibility values and moderate and vigorous PA stratified by potential confounders (sex and age) were generated (figure 8.2). Distensibility was skewed on the original scale and was normalised by log-transformation. Statistical significance was defined for p-values <0.05.

To examine the associations of distensibility with PA, an a priori directed acyclic graph (DAG) was generated considering potential confounder variables and referring to the indirect effects of PA via intermediate endpoints. Based on the defined variables in the DAG, a stepwise forward and backward selection using the likelihood ratio test was used to define the final set of variables for analyses. The same procedure was repeated to check for potential interaction and non-linearity terms of covariates. Education level (obligatory school education, higher education and college education) did not reach the inclusion significance level of 0.2. The final DAG pathways for vigorous PA are shown in figure 8.3.

Considering the final different pathways of the DAG (figure 8.3), the univariate, the total and the direct effect estimates of moderate and vigorous PA on distensibility were calculated using different multiple mixed linear regression models. Model 1 was an univariate model of distensibility and PA determinants; model 2 additionally adjusted for potential confounders sex and age; model 3 included possible mediators like heart rate, body mass index (BMI), mean arterial BP and smoking and was further adjusted for medication (yes/no: antihypertensive, lipid lowering, and kidney disease treatment) and the different study centres as random intercept to account for regional clustering (table 8.2). In a further approach, we pre-specified possible interactions of variables presented in the DAG, namely sex-age, sex-BMI and sex-mean arterial pressure, and interactions between PA and sex or age and calculated the respective mixed linear regression models according to model 3 adding these interactions (model 4 in table 8.2).

We performed pathway analyses to describe the single hypothesised pathway direction presented in the DAG (figure 8.2). All pathway analyses were adjusted for study centre (treated as fixed effect factor) and association estimates between vigorous PA intensity, distensibility and the covariates were presented as standardised betas.

We repeated the main analyses considering other carotid stiffness parameters, namely beta stiffness index, Peterson's elastic modulus and Young's elastic modulus to investigate whether the conclusions were consistent among carotid stiffness parameters. Therefore, we performed multivariate regression analyses with a four-dimensional outcome using the same predictor variables as in the model for distensibility and treating study centre as a fixed effect. For a description of the additional mentioned carotid stiffness parameters and the methodology of these analyses we refer to previous extensive analyses [29]. The conclusions drawn from these combined indices did not differ from distensibility and therefore only the analyses on distensibility - an often used carotid stiffness measure - are presented in this

article. All analyses were performed using the statistical software STATA (StataCorp, Release 12. Statistical Software, College Station, TX: StataCorp LPTexas, USA). The analytical data set and the statistical code are available from the corresponding author upon request, since ethics approval and participants consent does not allow public sharing of data.

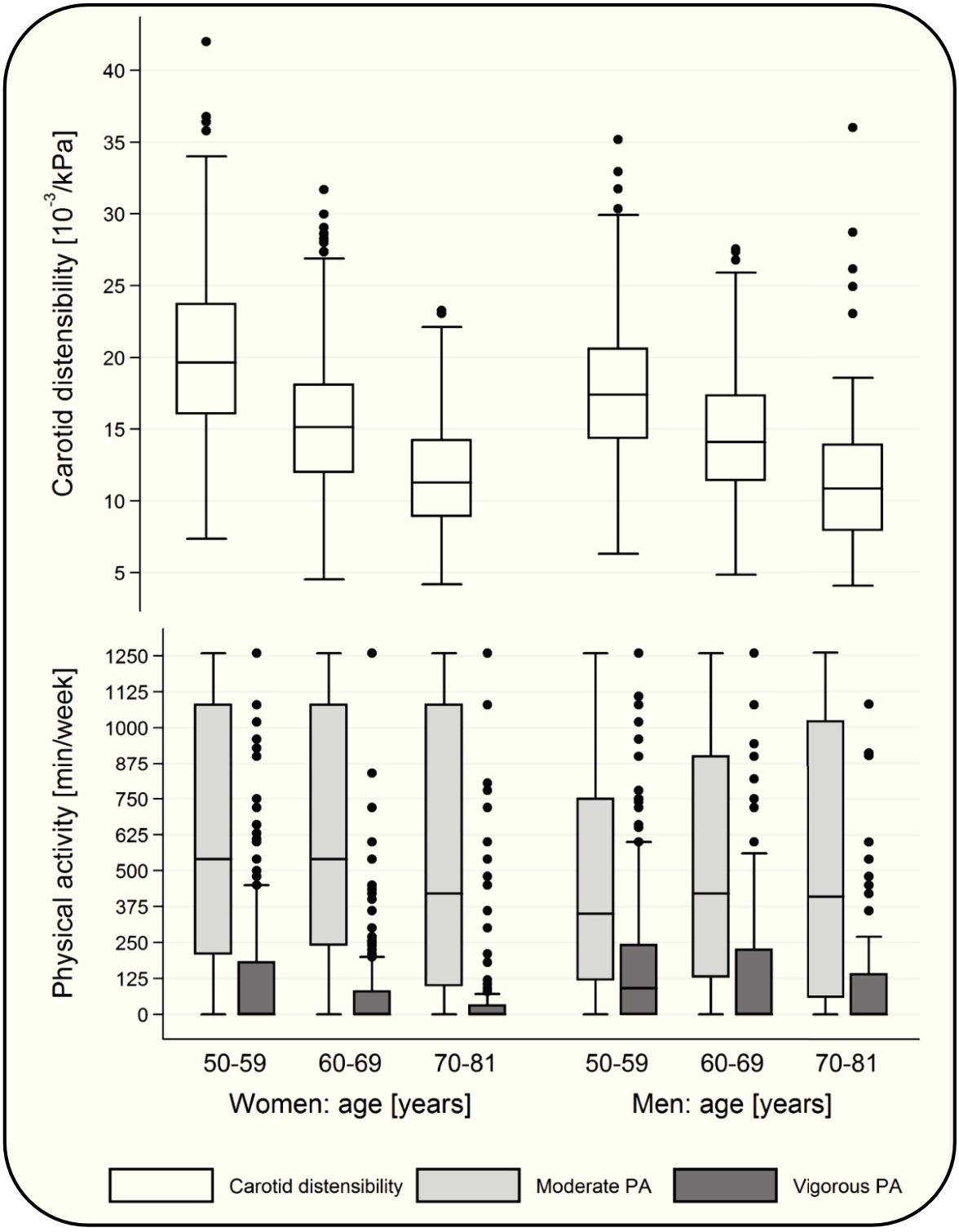

Figure 8.2: Box plots of carotid distensibility values and amount of moderate and vigorous physical activity (PA) for both sexes across three 10-year age categories

Results

Descriptive analyses

Characteristics of the study participants for males and females are shown in table 8.1. Box plots of distensibility values and moderate and vigorous PA for both sexes and across three 10-year age categories from 50 to 81 years are presented in figure 8.2. Compared to the youngest age group, distensibility and vigorous PA were lower in older age groups for both sexes ($p<0.05$). Moderate PA did not show an age-associated decline. Within each age group, vigorous PA was significantly lower in women than in men ($p<0.05$). No differences for distensibility and moderate PA values were found in the oldest age group for both sexes ($p>0.1$). In the youngest and middle age groups, women had on average a higher distensibility and moderate PA compared to men ($p<0.05$).

Multiple linear models

Detailed estimates of the associations between PA intensities and distensibility from different mixed linear models (models 1-4) are depicted in table 8.2. We found significant univariate associations (model 1) between vigorous PA and distensibility, but not for total PA and moderate PA (percent change in the geometric mean per 10min/week increase in vigorous PA = 0.11, $p<0.01$; in total PA = 0.03, $p=0.08$; in moderate PA = 0.02, $p=0.45$). In all mixed linear regression analyses, associations of different PA determinants with distensibility disappeared when age was included in the model (data not shown, see also model 2 adjusted for age and sex, and other PA determinants $p>0.7$). Significant associations between confounders or mediators of distensibility and distensibility were found for age, mean arterial BP, BMI, heart rate, smoking and medication intake across all different PA determinants (model 3). Including further interaction terms (model 4), we observed a significant interaction between sex and BMI ($p<0.01$). Based on the 95% confidence interval, a trend was found for the interactions of sex with age and sex with mean arterial BP. We found no interactions between physical activity intensities and sex or age ($p>0.8$).

Pathway analyses

The strongest predictor of distensibility was age (standardised beta = -0.48) followed by mean arterial BP (standardised beta = -0.35) and heart rate (standardised beta = -0.18). All direct or intermediate pathways of vigorous PA were marginal and statistically non-significant. Detailed results can be found in the DAG presented in figure 8.3.

Table 8.2: Estimates of associations between different PA determinants and carotid distensibility adjusted for different covariates

	Parameter	Units	Model 1		Model 2		Model 3		Model 4	
			CGM (95% CI) [%]	p-value	CGM (95% CI) [%]	p-value	CGM (95% CI) [%]	p-value	CGM (95% CI) [%]	p-value
Moderate PA	**Moderate PA**	10 min/week	0.02 (-0.02; 0.06)	0.45	0.00 (-0.03; 0.04)	0.83	0.01 (-0.02; 0.04)	0.54	0.01 (-0.02; 0.04)	0.49
	Sex	female vs male			8.27 (5.01; 11.63)	<0.01	2.56 (-0.15; 5.34)	0.06	18.76 (-13.05; 62.19)	0.28
	Age	years			-2.68 (-2.87; -2.49)	<0.01	-2.32 (-2.49; -2.15)	<0.01	-2.16 (-2.41; -1.91)	<0.01
	Medication	yes vs no*					-6.57 (-9.36; -3.69)	<0.01	-6.19 (-8.99; -3.29)	<0.01
	Mean arterial BP	mmHg					-1.15 (-1.26; -1.03)	<0.01	-1.02 (-1.20; -0.85)	<0.01
	BMI	kg/m^2					-0.66 (-0.98; -0.34)	<0.01	-1.28 (-1.85; -0.71)	<0.01
	Heart rate	bpm					-0.72 (-0.85; -0.59)	<0.01	-0.71 (-0.84; -0.58)	<0.01
	Smoking	packyears					0.14 (0.07; 0.20)	<0.01	0.13 (0.07; 0.20)	<0.01
	Interaction sex-age	female age							-0.28 (-0.61; 0.05)	0.10
	Interaction sex-bpmap	female bpmap							-0.21 (-0.45; 0.03)	0.08
	Interaction sex-bmi	female bmi							0.91 (0.22; 1.59)	<0.01
Vigorous PA	**Vigorous PA**	10 min/week	0.11 (0.03; 0.19)	<0.01	-0.01 (-0.08; 0.06)	0.76	-0.02 (-0.08; 0.03)	0.43	-0.02 (-0.07; 0.04)	0.51
	Sex	female vs male			8.24 (4.98; 11.61)	<0.01	2.51 (-0.21; 5.29)	0.07	19.14 (-12.75; 62.69)	0.27
	Age	years			-2.69 (-2.88; -2.50)	<0.01	-2.33 (-2.50; -2.16)	<0.01	-2.17 (-2.42; -1.91)	<0.01
	Medication	yes vs no*					-6.65 (-9.44; -3.78)	<0.01	-6.28 (-9.08; -3.39)	<0.01
	Mean arterial BP	mmHg					-1.14 (-1.26; -1.03)	<0.01	-1.02 (-1.20; -0.85)	<0.01
	BMI	kg/m^2					-0.66 (-0.98; -0.34)	<0.01	-1.27 (-1.84; -0.70)	<0.01
	Heart rate	bpm					-0.72 (-0.85; -0.59)	<0.01	-0.71 (-0.84; -0.58)	<0.01
	Smoking	packyears					0.13 (0.07; 0.20)	<0.01	0.13 (0.06; 0.20)	<0.01
	Interaction sex-age	female age							-0.29 (-0.62; 0.05)	0.09
	Interaction sex-bpmap	female bpmap							-0.21 (-0.44; 0.03)	0.09
	Interaction sex-bmi	female bmi							0.89 (0.20; 1.57)	0.01

Table 8.2 continued

	Parameter	Units	Model 1 CGM (95% CI) [%]	p-value	Model 2 CGM (95% CI) [%]	p-value	Model 3 CGM (95% CI) [%]	p-value	Model 4 CGM (95% CI) [%]	p-value
Moderate and vigorous PA	**Moderate PA**	10 min/week	0.18 (-3.89; 4.42)	0.93	0.55 (-2.87; 4.09)	0.76	1.27 (-1.65; 4.28)	0.40	1.34 (-1.58; 4.34)	0.37
	Vigorous PA	10 min/week	0.01 (0.00; 0.02)	<0.01	-0.00 (-0.01; 0.01)	0.71	-0.00 (-0.01; 0.00)	0.33	-0.00 (-0.01; 0.00)	0.39
	Sex	female vs male			8.17 (4.87; 11.56)	<0.01	2.36 (-0.37; 5.16)	0.09	18.46 (-13.26; 61.79)	0.29
	Age	years			-2.69 (-2.88; -2.50)	<0.01	-2.33 (-2.50; -2.16)	<0.01	-2.17 (-2.43; -1.92)	<0.01
	Medication	yes vs no*					-6.58 (-9.37; -3.70)	<0.01	-6.20 (-9.01; -3.31)	<0.01
	Mean arterial BP	mmHg					-1.15 (-1.26; -1.03)	<0.01	-1.03 (-1.20; -0.85)	<0.01
	BMI	kg/m^2					-0.66 (-0.98; -0.34)	<0.01	-1.28 (-1.84; -0.71)	<0.01
	Heart rate	bpm					-0.72 (-0.85; -0.59)	<0.01	-0.71 (-0.84; -0.58)	<0.01
	Smoking	packyears					0.14 (0.07; 0.20)	<0.01	0.13 (0.07; 0.20)	<0.01
	Interaction sex-age	female age							-0.28 (-0.61; 0.05)	0.10
	Interaction sex-bpmap	female bpmap							-0.21 (-0.44; 0.03)	0.08
	Interaction sex-bmi	female bmi							0.90 (0.21; 1.58)	<0.01
Total PA	**Total PA**	10 min/week	0.03 (0.00; 0.06)	0.08	0.00 (-0.03; 0.03)	0.96	0.00 (-0.02; 0.03)	0.86	0.00 (-0.02; 0.03)	0.77
	Sex	female vs male			8.30 (5.06; 11.65)	<0.01	2.63 (-0.07; 5.40)	0.06	19.09 (-12.80; 62.64)	0.27
	Age	years			-2.68 (-2.87; -2.49)	<0.01	-2.32 (-2.49; -2.15)	<0.01	-2.16 (-2.41; -1.91)	<0.01
	Medication	yes vs no*					-6.61 (-9.40; -3.73)	<0.01	-6.22 (-9.03; -3.33)	<0.01
	Mean arterial BP	mmHg					-1.15 (-1.26; -1.03)	<0.01	-1.02 (-1.20; -0.85)	<0.01
	BMI	kg/m^2					-0.66 (-0.98; -0.34)	<0.01	-1.28 (-1.85; -0.71)	<0.01
	Heart rate	bpm					-0.72 (-0.85; -0.59)	<0.01	-0.71 (-0.84; -0.58)	<0.01
	Smoking	packyears					0.14 (0.07; 0.20)	<0.01	0.13 (0.07; 0.20)	<0.01
	Interaction sex-age	female age							-0.28 (-0.62; 0.05)	0.10
	Interaction sex-bpmap	female bpmap							-0.21 (-0.45; 0.03)	0.08
	Interaction sex-bmi	female bmi							0.90 (0.22; 1.59)	<0.01

Results are expressed as percentage change in the geometric mean (CGM) per unit increase in the respective parameter with 95% confidence interval (CI), interactions between physical activity parameters and sex or age were not significantly associated p>0.8 (data not shown), *second mentioned = reference group, BMI = body mass index, bpmap = mean arterial blood pressure

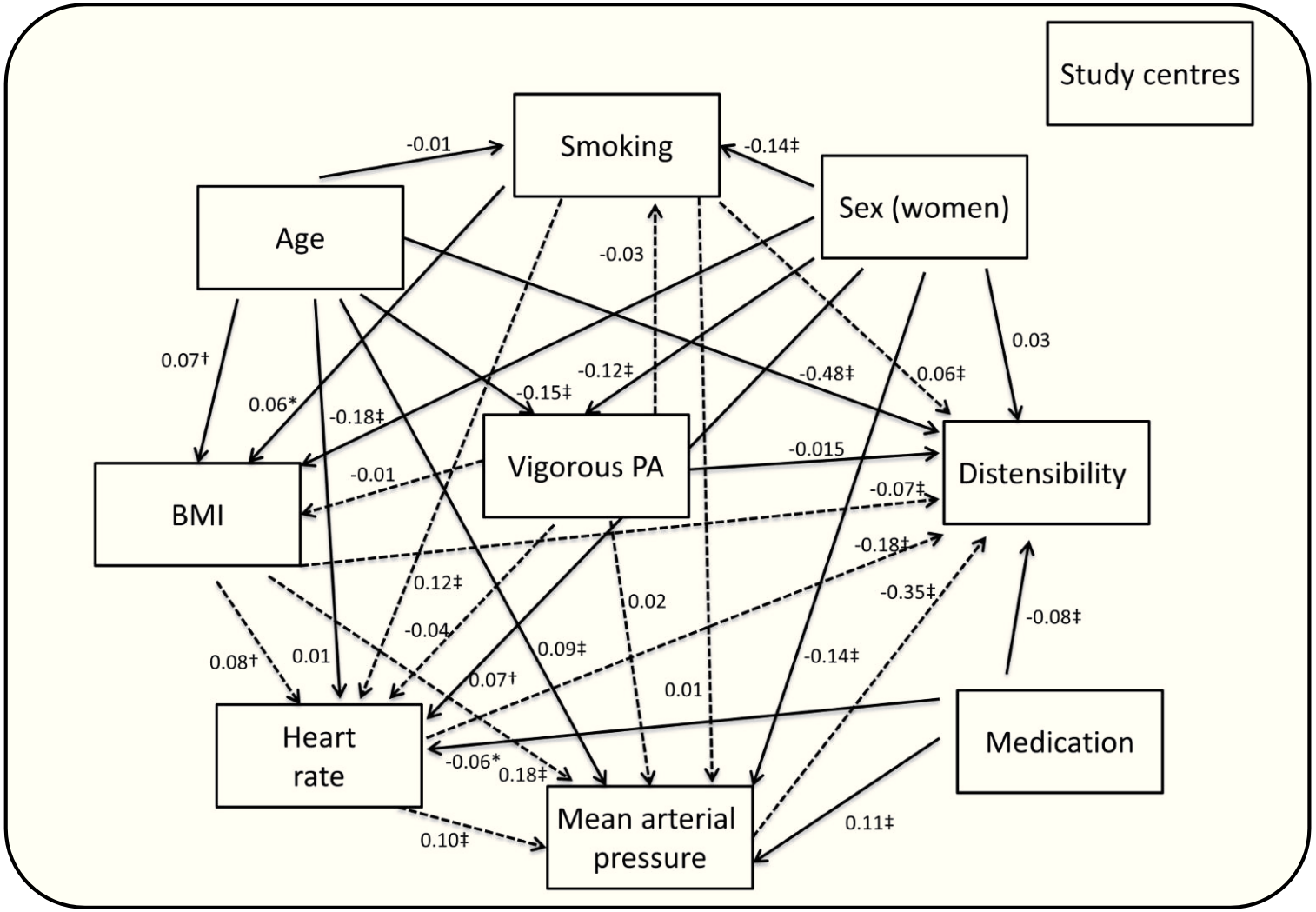

Figure 8.3: Pathway analyses based on the Directed Acyclic Graph (DAG) of vigorous physical activity (PA) and distensibility using standardised betas
BMI = body mass index, PA = physical activity, arrows = hypothesised effect directions; dashed lines = mediator pathways of PA, * p<0.05, † p<0.01, ‡ p<0.001

Discussion

We observed no association of self-reported PA with distensibility in individuals aged 50 to 81 years of the SAPALDIA cohort free of CVD diagnosis. Only vigorous PA was significantly associated with reduced carotid stiffness in univariate analyses. However, these associations disappeared after adjustment for age.

We used an a priori generated DAG to disentangle the complex interrelation of PA and distensibility with several classical risk factors, sex and age. Based on plausible physiological and clinical assumptions the DAG helped to unravel directions and strengths of associations between potentially influencing factors. The pathway analyses presented in figure 8.3 clearly identifies age as the dominant determinant of carotid stiffness (-0.48) followed by hemodynamic variables (-0.18 for heart rate and -0.35 for mean arterial pressure) and all remaining classical risk factors (< 0.08) including vigorous PA.

Age is a well-known strong determinant of carotid stiffness [10,22,30,31]. Nevertheless, it is challenging to distinguish between age as a classical cardiovascular risk factor, e.g. representing the accumulation of life-style risks, and age as a normal physiological process known as 'vascular ageing' [10]. The concept of 'vascular ageing' reflects changes of the arterial wall properties, on one hand due to normal vascular remodelling with advancing age and on the other hand also due to changes due to an early exposure to risk factors [10]. The influence of age treated as a risk factor (e.g. time to unavoidable strain such as normal heart rate) might change over lifetime and arterial segment [32]. Thus, it is not clear whether PA has a comparable impact on arterial stiffness at different ages. More precisely, does PA influence carotid stiffness differently in elderly subjects compared to children and young adults since vascular ageing is already advanced in elderly people. However, since we found no interaction between PA and age, our results suggest that there are no specific differences in the effect of PA on arterial stiffness in individuals above an age of 50 years.

Our results are principally in line with a report of 'The Atherosclerosis Risk in Community' (ARIC) cohort study also cross-sectionally investigated [19]. They found no association of PA with carotid stiffness in the overall population. Only in a sub analysis vigorous PA was weakly associated with reduced carotid stiffness independent of risk factors and age [19]. In contrast, cross-sectional studies with far smaller study samples seemed to show much stronger inverse associations of increased PA with reduced carotid stiffness parameters [13–19]. However, these findings were mostly obtained by comparing extremes of PA. Thus, middle-aged to older trained athlete swimmers, runners and rowers showed reduced stiffness assessed as compliance and beta-stiffness index compared with aged-matched sedentary peers [14,15]. Beta-stiffness index even lost significance after adjustment for heart rate [15]. Middle-aged to older trained male athletes exercising vigorously had a lower carotid compliance and beta-stiffness index while light to moderately exercise was associated only with favourable carotid compliance compared to their sedentary peers [16]. Furthermore, carotid stiffness in postmenopausal women at a similar age as our cohort was correlated with moderate to vigorous PA but not with light PA [18]. These studies indicate a

certain dose-dependent reduction of carotid stiffness with more vigorous PA even at older age [16,18]. However, the fact that some of the very strong effects were found in small studies raises concern about publication bias. Furthermore, some differences in baseline fitness level between sedentary and trained people were very large, e.g. maximal oxygen consumption were ~30 ml/kg/min for untrained and 34-50 ml/kg/min for trained individuals, equivalent to a percentage difference of 13 to 66% [14,16]. The expected gain of maximal oxygen consumption by training is as much as 25% on average [33], so that it is questionable whether these differences could be achieved by exercise training alone. Instead, exercise capacity and arterial stiffness are likely determined by genetic differences. Overall, except for ARIC, these small studies with highly selected samples probably do not reflect the general population, limiting the transfer of their results from athletes to normal individuals.

Based on extensive evidence from the literature on favourable mechanisms of PA on vascular function via the nitric oxide pathway [34–37] and conserving effect on elastin and collagen content against adverse wall remodelling [37,38], we were convinced to find a clear independent favourable contribution of PA on carotid stiffness described in the DAG. Positive effects of PA on cardiovascular risk factors and CVD were already widely investigated [3–8] and different plausible associations of cardiovascular risk factors and carotid stiffness were shown by our group [29]. Even more, we were astonished that we were not able to show any effect of PA on arterial stiffness in contrast to previous findings.

Strengths and limitations

This study performed ultrasound examinations to assess carotid stiffness in a large sample size with highly standardised procedures and investigated its association with PA assessed by IPAQ. The IPAQ was already validated with objective measurements describing a moderate reasonable agreement between accelerometry and questionnaire [26,27]. A separate IPAQ validation within the SAPALDIA 3 setting including also middle age to elderly individuals confirmed the previous findings (paper submitted). However, self-reported PA over the last seven days might lead to bias since PA intensity is based on subjective assessment. People might consider themselves active based on their life-long activity level, which may has changed with age. Overall, the degree of misclassification might be strong enough to prevent small effects of PA from being detected. However, measurement of PA in a large sample is challenging and costly and a validated questionnaire often seems to be the only feasible solution to assess PA in a cohort. In line with other larger studies investigating PA and carotid stiffness, our study was limited by its cross-sectional design. Although we generated a DAG emphasising to reduce bias by identifying a priori possible confounders, the presented DAG and the cross-sectional design do not solve the challenge of possible reverse causality. Even though we excluded participants with known pre-existing CVD, there is still the chance that an unknown unfavourable health status affects the association between PA and carotid stiffness. Thus, to addressing reverse causality, we suggest to assess PA activity longitudinally before measuring carotid stiffness

Conclusion and perspectives

This is only the second epidemiological study investigating the associations of PA, risk factors and age on carotid stiffness assessed in an ageing population without diagnosed CVD. Although we hypothesized an independent beneficial effect of PA on arterial stiffness in the CCA, only an increasing amount of vigorous PA (and not total or moderate PA) was associated with reduced carotid stiffness and the association was no longer present after adjusting for age. Among the classical risk factors of vascular health, age had the dominant effect on carotid stiffness followed by mean arterial pressure and heart rate. Since only very small intervention studies not representative for the general population showed a beneficial effect of PA on carotid stiffness, we recommend to investigate PA determinants in correlation with carotid stiffness longitudinally including a representative study sample.

Acknowledgments

The authors have no conflicts of interest to disclose. This study could not have been done without the help of the study participants, technical and administrative support, the medical teams and field workers at the local study sites, the SAPALDIA team and the members of the Department of Sport, Exercise and Health. The authors thank them for their excellent work.

Conflicts of Interest and Source of Funding

The authors have no conflicts of interest to disclose. The Swiss Study on Air Pollution and Lung and Heart Diseases In Adults (SAPALDIA) was supported by the Swiss National Science Foundations (grants no 33CSCO-134276/1, 33CSCO-108796, 3247BO-104283, 3247BO-104288, 3247BO-104284, 3247-065896, 3100-059302, 3200-052720, 3200-042532, 4026-028099, PMPDP3_129021/1, PMPDP3_141671/1), the Federal Office for Forest, Environment and Landscape, the Federal Office of Public Health, the Federal Office of Roads and Transport, the canton's government of Aargau, Basel-Stadt, Basel-Land, Geneva, Luzern, Ticino, Valais, and Zürich, the Swiss Lung League, the canton's Lung League of Basel Stadt/ Basel Landschaft, Geneva, Ticino, Valais, Graubünden and Zurich, Stiftung ehemals Bündner Heilstätten, SUVA, Freiwillige Akademische Gesellschaft, UBS Wealth Foundation, Talecris Biotherapeutics GmbH, Abbott Diagnostics, European Commission 018996 (GABRIEL), Wellcome Trust WT 084703MA.

References

1 Lee, I.-M., Shiroma, E. J., Lobelo, F., Puska, P., Blair, S. N. and Katzmarzyk, P. T. (2012) Effect of physical inactivity on major non-communicable diseases worldwide: an analysis of burden of disease and life expectancy. Lancet. **380**: 219-229

2 World Health Organization. (2009) Global health risks: mortality and burden of disease attributable to selected major risks, World Health Organization, Geneva. Retrieved March 27, 20124, from http://www.who.int/healthinfo/global_burden_disease/global_health_risks/en/

3 World Health Organization. (2010) Global recommendations on physical activity for health, World Health Organization, Geneva, Switzerland. Retrieved January 7, 2014, from http://www.who.int/dietphysicalactivity/publications/9789241599979/en/

4 Physical Activity Guidelines Advisory Committee. (2008) Physical Activity Guidelines Advisory Committee Report, U.S. Department of Health and Human Services, Washington, DC. Retrieved November 14, 2013, from http://onlinelibrary.wiley.com/doi/10.1111/j.1753-4887.2008.00136.x/pdf

5 Li, J. and Siegrist, J. (2012) Physical activity and risk of cardiovascular disease - a meta-analysis of prospective cohort studies. Int. J. Environ. Res. Public. Health. **9**, 391–407

6 Federal Office of Sport FOSPO, Federal Office of Public Health FOPH, Health Promotion Switzerland, bfu – Swiss Council for Accident Prevention, Swiss Accident Insurance Fund (Suva), Health and Physical Activity and Network Switzerland. (2013) Health - Enhancing Physical Activity Core Document for Switzerland, Maggingen: FOSPO. Retrieved January 6, 2014, from http://www.hepa.ch/internet/hepa/de/home/dokumentation/grundlagendokumente.html

7 Wen, C. P., Wai, J. P. M., Tsai, M. K., Yang, Y. C., Cheng, T. Y. D., Lee, M.-C., Chan, H. T., Tsao, C. K., Tsai, S. P. and Wu, X. (2011) Minimum amount of physical activity for reduced mortality and extended life expectancy: a prospective cohort study. Lancet. **378**, 1244–1253

8 Sattelmair, J., Pertman, J., Ding, E. L., Kohl, H. W., Haskell, W. and Lee, I.-M. (2011) Dose response between physical activity and risk of coronary heart disease: a meta-analysis. Circulation. **124**, 789–795

9 Ross, R. (1999) Atherosclerosis - an inflammatory disease. N. Engl. J. Med. **340**, 115–126

10 Nilsson, P. M., Boutouyrie, P. and Laurent, S. (2009) Vascular aging: a tale of EVA and ADAM in cardiovascular risk assessment and prevention. Hypertension. **54**, 3–10

11 Adji, A., O'Rourke, M. F. and Namasivayam, M. (2011) Arterial stiffness, its assessment, prognostic value, and implications for treatment. Am. J. Hypertens. **24**, 5–17

12 McDonnell, B. J., Maki-Petaja, K. M., Munnery, M., Yasmin, Wilkinson, I. B., Cockcroft, J. R. and McEniery, C. M. (2013) Habitual exercise and blood pressure: age dependency and underlying mechanisms. Am. J. Hypertens. **26**, 334–341

13 Schmidt-Trucksäss, A., Schmid, A., Brunner, C., Scherer, N., Zäch, G., Keul, J. and Huonker, M. (2000) Arterial properties of the carotid and femoral artery in endurance-trained and paraplegic subjects. J. Appl. Physiol. **89**, 1956–1963

14 Nualnim, N., Barnes, J. N., Tarumi, T., Renzi, C. P. and Tanaka, H. (2011) Comparison of central artery elasticity in swimmers, runners, and the sedentary. Am. J. Cardiol. **107**, 783–787

15 Cook, J. N., DeVan, A. E., Schleifer, J. L., Anton, M. M., Cortez-Cooper, M. Y. and Tanaka, H. (2006) Arterial compliance of rowers: implications for combined aerobic and strength training on arterial elasticity. Am. J. Physiol. Heart Circ. Physiol. **290**, H1596–1600

16 Tanaka, H., Dinenno, F. A., Monahan, K. D., Clevenger, C. M., DeSouza, C. A. and Seals, D. R. (2000) Aging, habitual exercise, and dynamic arterial compliance. Circulation. **102**, 1270–1275

17 Laar, R. J. van de, Ferreira, I., Mechelen, W. van, Prins, M. H., Twisk, J. W. and Stehouwer, C. D. (2010) Lifetime vigorous but not light-to-moderate habitual physical activity impacts favorably on carotid stiffness in young adults: The Amsterdam Growth and Health Longitudinal Study. Hypertension. **55**, 33–39

18 Sugawara, J., Otsuki, T., Tanabe, T., Hayashi, K., Maeda, S. and Matsuda, M. (2006) Physical activity duration, intensity, and arterial stiffening in postmenopausal women. Am. J. Hypertens. **19**, 1032–1036

19 Schmitz, K. H., Arnett, D. K., Bank, A., Liao, D., Evans, G. W., Evenson, K. R., Stevens, J., Sorlie, P. and Folsom, A. R. (2001) Arterial distensibility and physical activity in the ARIC study. Med. Sci. Sports Exerc. **33**, 2065–2071

20 Ackermann-Liebrich, U., Kuna-Dibbert, B., Probst-Hensch, N. M., Schindler, C., Felber Dietrich, D., Zemp Stutz, E., Bayer-Oglesby, L., Baum, F., Brändli, O., Brutsche, M., Downs, S. H., Keidel, D., Gerbase, M. W., Imboden, M., Keller, R., Knöpfli, B., Künzli, N., Nicod, L., Pons, M., Staedele, P., Tschopp, J.-M., Zellweger, J.-P. and Leuenberger, P. (2005) Follow-up of the Swiss Cohort Study on Air Pollution and Lung Diseases in Adults (SAPALDIA 2) 1991-2003: methods and characterization of participants. Soz.-Präventivmedizin. **50**, 245–263

21 Martin, B. W., Ackermann-Liebrich, U., Leuenberger, P., Künzli, N., Zemp Stutz, E., Keller, R., Zellweger, J. P., Wüthrich, B., Monn, C., Blaser, K., Bolognini, G., Bongard, J. P., Brändli, O., Braun, P., Defila, C., Domenighetti, G., Grize, L., Karrer, W., Keller-Wossidlo, H., Medici, T. C., Peeters, A., Perruchoud, A. P., Schindler, C., Schoeni, M. H., Schwartz, J., Solari, G., Tschopp, J.-M. and Villiger, B. (1997) SAPALDIA: methods and participation in the cross-sectional part of the Swiss Study on Air Pollution and Lung Diseases in Adults. Soz.- Präventivmedizin. **42**, 67–84

22 Nichols, W. W., O'Rourke, M. F. and Vlachopoulos, C. (2011) McDonald's blood flow in arteries: theoretical, experimental and clinical principles 6th ed., Hodder Arnold, London. 1-768

23 Teynor, A., Caviezel, S., Dratva, J., Künzli, N. and Schmidt-Trucksäss, A. (2012) An automated, interactive analysis system for ultrasound sequences of the common carotid artery. Ultrasound Med. Biol. **38**, 1440–1450

24 Caviezel, S., Dratva, J., Schaffner, E., Teynor, A., Baumstark, M. W., Schindler, C., de Groot, E., Burdet, L., Rothe, T., Pons, M., Gaspoz, J.-M., Rochat, T., Künzli, N., Probst-Hensch, N. and Schmidt-Trucksäss, A. (2013) Variability and reproducibility of carotid structural and functional parameters assessed with transcutaneous ultrasound – results from the SAPALDIA cohort study. Atherosclerosis. **231**, 448–455

25 Van Bortel, L. M., Duprez, D., Starmans-Kool, M. J., Safar, M. E., Giannattasio, C., Cockcroft, J., Kaiser, D. R. and Thuillez, C. (2002) Clinical applications of arterial stiffness, Task Force III: recommendations for user procedures. Am. J. Hypertens. **15**, 445–452

26 Craig, C. L., Marshall, A. L., Sjöström, M., Bauman, A. E., Booth, M. L., Ainsworth, B. E., Pratt, M., Ekelund, U., Yngve, A., Sallis, J. F. and Oja, P. (2003) International physical activity questionnaire: 12-country reliability and validity. Med. Sci. Sports Exerc. **35**, 1381–1395

27 Kim, Y., Park, I. and Kang, M. (2013) Convergent validity of the international physical activity questionnaire (IPAQ): meta-analysis. Public Health Nutr. **16**, 440–452

28 International Physical Activity Questionnaire (IPAQ) web site - Guidelines for Data Processing and Analysis (2005). Retrieved January 6, 2014, from www.ipaq.ki.se

29 Caviezel, S., Dratva, J., Schaffner, E., Schindler, C., Zemp Stutz, E., de Groot, E., Burdet, L., Rothe, T., Pons, M., Gaspoz, J.-M., Rochat, T., Künzli, N., Probst-Hensch, N. and Schmidt-Trucksäss, A. (2014) Sex-specific associations of cardiovascular risk factors with carotid stiffness – results from the SAPALDIA cohort study. Atherosclerosis. **235**, 576–584

30 Vaidya, D., Heckbert, S. R., Wasserman, B. A. and Ouyang, P. (2012) Sex-specific association of age with carotid artery distensibility: Multi-Ethnic Study of Atherosclerosis. J. Womens Health. **21**, 516–520

31 Schmidt-Trucksäss, A., Grathwohl, D., Schmid, A., Boragk, R., Upmeier, C., Keul, J. and Huonker, M. (1999) Structural, functional, and hemodynamic changes of the common carotid artery with age in male subjects. Arterioscler. Thromb. Vasc. Biol. **19**, 1091–1097

32 Van der Heijden-Spek, J. J., Staessen, J. A., Fagard, R. H., Hoeks, A. P., Boudier, H. A. S. and Van Bortel, L. M. (2000) Effect of age on brachial artery wall properties differs from the aorta and is gender dependent: a population study. Hypertension. **35**, 637–642

33 Bouchard, C. and Rankinen, T. (2001) Individual differences in response to regular physical activity. Med. Sci. Sports Exerc. **33**, S446–51; discussion S452–3

34 Hambrecht, R., Fiehn, E., Weigl, C., Gielen, S., Hamann, C., Kaiser, R., Yu, J., Adams, V., Niebauer, J. and Schuler, G. (1998) Regular physical exercise corrects endothelial dysfunction and improves exercise capacity in patients with chronic heart failure. Circulation. **98**, 2709–2715

35 Monahan, K. D., Tanaka, H., Dinenno, F. A. and Seals, D. R. (2001) Central arterial compliance is associated with age- and habitual exercise–related differences in cardiovagal baroreflex sensitivity. Circulation. **104**, 1627–1632

36 Taddei, S., Galetta, F., Virdis, A., Ghiadoni, L., Salvetti, G., Franzoni, F., Giusti, C. and Salvetti, A. (2000) Physical activity prevents age-related impairment in nitric oxide availability in elderly athletes. Circulation. **101**, 2896–2901

37 Chatzizisis, Y. S., Coskun, A. U., Jonas, M., Edelman, E. R., Feldman, C. L. and Stone, P. H. (2007) Role of endothelial shear stress in the natural history of coronary atherosclerosis and vascular remodeling: molecular, cellular, and vascular behavior. J. Am. Coll. Cardiol. **49**, 2379–2393

38 Dabagh, M., Jalali, P., Konttinen, Y. and Sarkomaa, P. (2008) Distribution of shear stress over smooth muscle cells in deformable arterial wall. Med. Biol. Eng. Comput. **46**, 649–657

CHAPTER 9

Synthesis, discussion and perspectives

Vascular damage plays a key role in the development of atherosclerosis and cardiovascular disease (CVD). Therefore, we measured carotid structure and function (carotid stiffness) as markers of vascular damage. A summary of the background and a detailed description of the respective goals can be found in chapter 2. This thesis aimed to summarise the state of the art of ultrasound measurements of the common carotid artery (CCA) and the respective relevance for the atherosclerotic risk management; to validate a new ultrasound analysis program; to investigate the reproducibility of structural and functional parameters of the CCA; to clarify the single associations between different cardiovascular risk factors and carotid stiffness indices; and to elucidate the multifarious relationship between physical activity (PA), distensibility and cardiovascular risk factors (chapter 4-8).

SUMMARY OF THE MAIN RESULTS

Carotid intima media thickness (CIMT) is an established biomarker of subclinical atherosclerosis. However, standardised preparation, performance and evaluation of CIMT measurements are essential (methods) to generate valid and reproducible results and thus also for the evaluation and interpretation of the predictive values and subsequent potential treatment options (chapter 4). Our first review outlined the state of the art of CIMT measurements using ultrasound. Aside from homogeneous external conditions such as lying- or head-position, image acquisition needs to be done by trained sonographers in pre-specified regions, ideally using sequential-based records across several heart cycles or at least three images during the end-diastolic phase in which the the structure of the carotid artery is depicted with sufficient visibility. Image analysis using automatic or semiautomatic software enhance reproducibility of measurements, especially if landmarks such as the opening of the bulb of the CCA were used to tag the start of ultrasound measurements. Overall, valid measurements are essential for distinguishing between normal and pathological structures. CIMT has been shown to be a suitable approach to improve risk stratification in subjects with intermediate risk. Despite the positive findings, using CIMT to assure or reclassify CVD risk stratification in daily clinical practice is still under discussion.

Based on the gained knowledge, we performed standardised measurements of CIMT and lumen diameter (LD) in sequential images across the complete heart cycle using an automatic analysing system called DYARA (DYnamic ARtery Analysis), which also allows manual interaction (chapter 5). To validate the system, all measurements were analysed twice. The intra- and inter-reader results were highly consistent with slightly higher bias for analyses with manual interactions compared to the automatic detection. Among the carotid structure parameters (average CIMT and LD across heart cycle, single images in diastole and systole), average CIMT and LD showed lowest variability between the two analyses, with smaller relative differences in the latter one (LD). DYARA tackles the challenge of being able to analyse ultrasound images of variable quality and therefore can be recommended for epidemiological research, diagnostics and clinical practice on grounds of its high precision.

The new validated DYARA program was then used to investigate carotid structure and function of participants from the SAPALDIA cohort. To assess the quality of the study data, we evaluated the reproducibility and the factors attributing to variability of structural and functional parameters in a representative sample of 165 SAPALDIA 3 participants (for details see chapter 6). Apart from the fact that we can report a good to excellent reproducibility for structural and functional indices, our results revealed that the subject itself is the source of greatest ultrasound measure variability. In addition, the use of different statistical approaches and the relatively large sample size was unique compared to other population-based studies within the same research field. On the grounds of these results, we can provide a solid data basis for further research on vascular health.

Focusing on carotid function, the first step was to examine associations between several cardiovascular risk factors assessed within the first SALPALDIA follow-up period (SALPALDIA 2, 2001-2003) and parameters for carotid stiffness, assessed in the SALPALDIA 3 study period (2010-2011), separately for men and women (chapter 7). In both sexes, most cardiovascular risk factors in SAPALDIA 2 were associated with increased carotid stiffness ten years later, except PA and high-density lipoprotein cholesterol (HDL-C). HDL-C was the only protective vascular determinant and no relation was observed for PA. Sex differences were found for heart rate, low-density lipoprotein cholesterol (LDL-C) and triglyceride with stiffer arteries in men compared to women for the former two risk factors and in women for the latter one. This might suggest a sex-specific burden of atherosclerotic risk factors that might be worthwhile to investigate it in more detail.

In a second step, we examined whether the effects of a given risk factor on carotid stiffness were heterogenic among the six carotid stiffness parameters. The analyses suggested comparable effects for distensibility, β-stiffness index and Peterson's elastic modulus across the different cardiovascular risk factors. Young's elastic modulus differed in associations with lipids, strain with hemodynamic parameters and compliances with various cardiovascular risk factors. While the mathematical formulas of Young's elastic modulus and strain might explain the resulting deviances in some association patterns, we question whether compliance is a comparable parameter for cardiovascular risk assessment compared to the other carotid stiffness indices.

Given the results that most carotid stiffness parameters could be used in an interchangeable manner, we used only distensibility for further analyses. Although we had already found out that PA in SAPALDIA 2 was not significantly associated with carotid stiffness, we assumed that health changes within the ten years of the first and the second follow-up might have masked the positive effect of PA on carotid stiffness. However, PA assessed in SAPALDIA 3 showed no beneficial effect on distensibility (see chapter 8). This was the second population-based study reporting null-results for PA and since protective reports were mostly found in smaller studies, this raises the concern of publication bias. Overall, detailed pathway analyses between PA, distensibility and cardiovascular risk factors indicated that age was the strongest predictor for distensibility, followed by mean arterial pressure and heart rate.

SYNTHESIS AND GENERAL DISCUSSION

The outlined results were based on a well-conceived framework within the SAPALDIA cohort. An intensive planning phase lead to high procedural standardisations in ultrasound measures, the collection of other biological biomarkers and questionnaire based assessments, which emphasised to increase the SAPALDIA data quality. However, it is at least as important to investigate and communicate the study data quality in a transparent manner [1]. The TREND, the CONSORT, the PRISMA or the STROBE statements and the Cochrane Collaboration provide very important information for improved reporting quality for clinical trials, observational study designs, systematic reviews and meta-analyses [2–7]. However, study data quality does not only reflect methodological issues and procedures (as intensively discussed in chapter 4); it also includes the reporting of validity and reliability tests for instruments (chapter 5 and 6) [8]. This leads to the question, which is the appropriate way to assess the study data quality. Although some of the statements might be transferable to validation or reliability studies, a general guideline for assessing the study data quality is missing (e.g. number of needed observations, possible statistical methods). Although we did not develop a guideline, (data) quality management is a fundamental SAPALDIA principle, since data quality has a considerable impact on a proper interpretation of the results and thus on the strength of recommendations [1].

Establish a solid data base for carotid stiffness parameters – tasks and duties

Since DYARA was a newly developed ultrasound analysis program, validating the new research tool was inevitable. However, we were not able compare DYARA to another system, since there is no gold-standard in analysing ultrasound images and manual tracing has been a common way to validate new analysis programs [9,10]. Although automatic devices would have been preferable (as outlined in chapter 4), we either could not afford costly automatic systems or devices were not compatible with our native format of ultrasound images. Since there was no data set from a former validation study available, we compared our system to completely manually traced ultrasound images. Results showed a far smaller bias for automatic and semiautomatic tracings compared to manual traced clips. Thus, we accurately measured what we intended to measure (validity) and increased the measurement precision (reliability). We provide our entire validation data set free of charge upon request to enable a comparison with all existing and future systems based on the very same data from the same patients, ultrasound equipment, and sonographers as described in chapter 5.

In addition to the validation study, we performed the reproducibility study in a relatively large subsample of the SAPALDIA cohort (chapter 6) to establish the reliability of the whole ultrasound examination process (not only the reading part). Therefore, we visualised different concepts of variability and reliability (e.g. dispersion or precision) by depicting Bland-Altman plots. Even though relevant information provided by Bland-Altman plots

depends on subjective decisions and judgment [11], they deliver much valuable information about the investigated data set (e.g systematic bias or heteroscedasticity). However, none of the population-based studies mentioned in chapter seven, presented Bland-Altman plots [12–19].

Overall, comparison of reliability measures remains challenging not only due to different statistical approaches, but also due to initially specified study methods and study designs (e.g. differences in vascular measurement regions or missing study sample description and different reader or observer comparisons, see overview in table 6.4). In order to address the adjustable issues and to enhance comparability with other reliability studies, we used different statistical approaches to assess the reproducibility (mixed model analysis to determine the sources of variability, coefficient of variation (CV) and intraclass correlation coefficient (ICC)). Since the precision of obtained estimates also influences the interpretation of the results, we presented ICC and CV as average estimate with 95% confidence interval [20]. Figure 9.1 illustrates the 95% confidence area for the reproducibility of carotid structural and functional parameters schematically. Although the degree of precision is fundamental, we were the only population-based study within our research area reporting confidence intervals for reproducibility results [12–19].

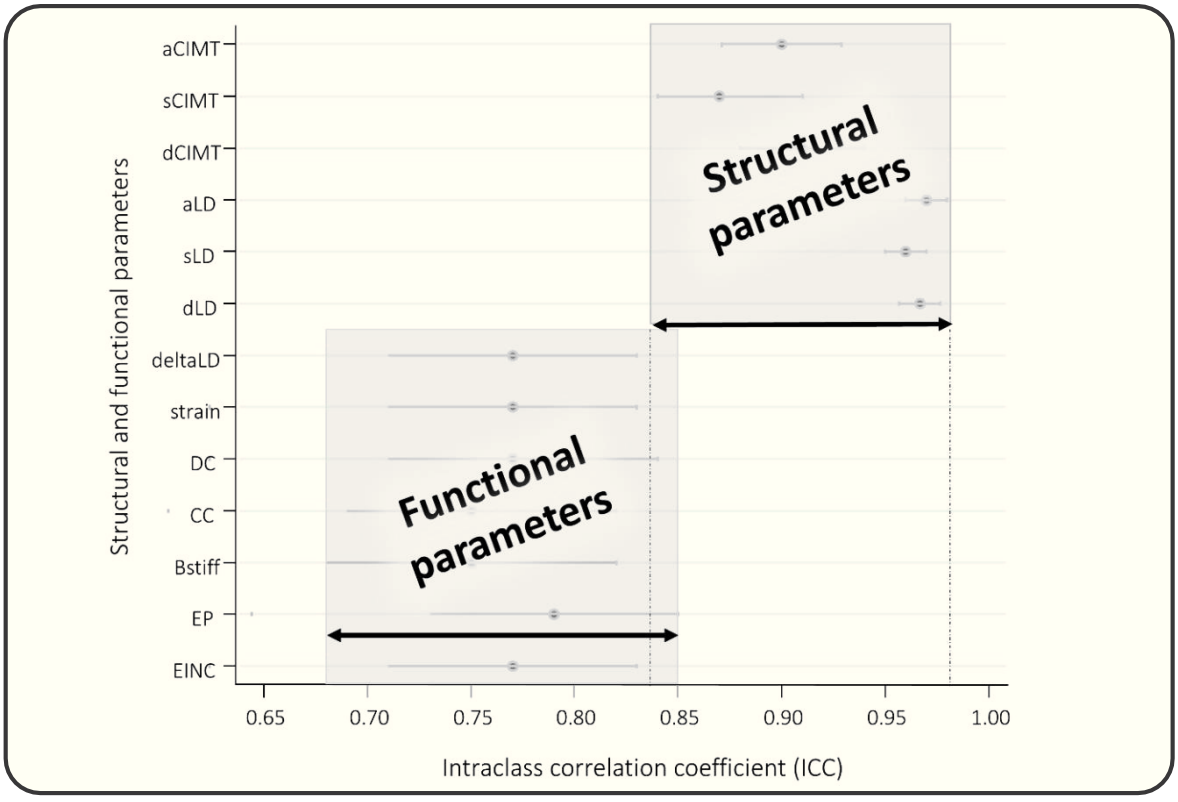

Figure 9.1: Illustrational diagram of the 95% confidence area for the intraclass correlation coefficients (ICC) of structural and functional parameters

To increase the quality of reported reliability studies, official validation and reliability guidelines addressing the outlined issues are needed. In addition, journals should promote publishing validation and reliability studies and assure that at least the characteristics of the validation sample, point estimates and confidence interval of the analyses are described.

Different carotid stiffness parameters – do they provide similar information?

While the reliability study results indicated that reproducibility is comparable across the different carotid stiffness parameters (figure 9.1) and while most cardiovascular risk factors were highly associated with carotid stiffness (see chapter 7), it is unknown whether all carotid stiffness parameters are similarly strong associated within each cardiovascular risk factor. This merits particular attention, since we have noticed that some studies, which used two or more carotid stiffness indices, rendered more or less divergent results of associations between carotid stiffness parameters and cardiovascular risk factors [21–23] or CVD events [24,25]. Even more questionable was the fact that the presented results of several carotid stiffness parameters within the same study were based on different statistical approaches [22,23].

Investigating this question for the first time in general, correlation analyses yielded a moderate to high correlation across the different carotid stiffness indices (chapter 7). More detailed analyses showed similar association patterns across cardiovascular risk factors for Peterson's elastic modulus, β-stiffness index, distensibility and with exception to certain risk factors also for strain and Young's elastic modulus, but not for compliance (figure 7.3). The reported deviances of association patterns might be attributed to parameter omission or amendment in the different carotid stiffness parameter formulas and/or to mathematical formula modifications (table 6.1). Summarising the formulas, all carotid stiffness parameters contain information about the diameter change in diastole and systole and the respective blood pressure (BP) values (except strain) to describe the (in)ability of the arterial structure to adapt to pressure changes [26]. Carotid wall thickness is only included by Young's elastic modulus [21]. Since strain does not account for BP values [26], hemodynamic parameters have a direct mechanical impact on LD change and this clarifies the strong associations of BP values on strain (figure 7.3).

The discrepancy in the association of Young's elastic modulus and lipids might be explained by the inclusion of CIMT and therefore describes the vascular remodelling of the arterial structure in a slightly different manner. As outlined in the introduction, the underlying mechanisms of atherosclerotic wall alterations involve multiple processes, which are complex and interrelated [27]. The interaction between lipid accumulation and their inflammatory response is beside other triggers such as endothelial dysfunction or direct mechanical stress an established atherosclerotic characterisation [28–30].

Among all carotid stiffness parameters, results of association patterns of compliance suggest partially strong heterogeneity in many cardiovascular risk factors with greatest deviances in anthropometric parameters. Based on the formulas, compliance provides as solely parameter a two dimensional information of the CCA (cross-sectional area of LD instead of the diameter as one-dimensional parameter) [31]. Since the diameter is affected by many cardiovascular risk factors and especially by the body morphology [32], this might explain the apparent heterogeneity of compliances compared to the other carotid stiffness indices. However, the heterogeneity in compliance association estimates is current in many

cardiovascular risk factors compared to the other carotid stiffness indices. Thus, it is questionable whether statements based on compliance investigations can be generalised as carotid stiffness conclusions.

In conclusion, regarding the association patterns between carotid stiffness and different cardiovascular risk factors, we were the first to document existing heterogeneity among specific association estimates. However, beside these defined association estimates, most carotid stiffness parameters yielded very similar directions in association estimates within each cardiovascular risk factor for both sexes, this indicates that most carotid stiffness parameters could be used in an exchangeable manner (with certain exceptions e.g. strain and hemodynamic parameters).

Associations of cardiovascular risk factors and carotid stiffness in men and women – differences or similarities?

Comparing not only methodological issues, carotid stiffness as a marker of vascular damage might offer additional information about cardiovascular risk beyond classical risk factors [33]. It is acknowledged that cardiovascular morbidity and mortality are highly dependant on an adverse risk factor profile [34]. We showed that most unfavourable cardiovascular risk factors were associated with increased carotid stiffness in both sexes, even in the long-term. These findings are widely established by various research studies, at least in the short term, as already outlined in the introduction (figure 1.1) [16,35–37]. However, manifest CVD and lifetime risk are higher among middle-aged men than among women with a comparable risk factor profile [38,39].

We aimed to determine sex differences in carotid stiffness as a possible biomarker of CVD risk, since they might indicate a sex-specific susceptibility to certain cardiovascular risk factors. Therefore, we investigated possible sex-interactions of cardiovascular risk factors across all six carotid stiffness parameters and significant sex-specific discrepancies in effect sizes were found for heart rate, LDL-C and triglycerides (table 7.2 and figure 7.2). Increased heart rate and LDL-C was more strongly associated with increased carotid stiffness among men than women while the opposite was the case for triglycerides. However, comparisons of sex-specific results in the literature were hindered, since sex-specific interactions of cardiovascular risk factors and carotid stiffness have rarely been investigated. Although different studies examined the direct pathways of major cardiovascular risk factors and carotid stiffness (figure 1.1), the majority of these studies either investigated only in male or female study populations [40–42], adjusted for sex as a possible confounder or performed stratified analyses, but they did not investigate sex-specific interactions [36,37,43–47]. This leads to the paradoxial situation that a similar database might be present, but specific results needed for literature comparisons are not available due to different research aims.

Lipids, the metabolic syndrome and cardiovascular diseases in men and women

Among all cardiovascular determinants in SAPALDIA 2, HDL-C was the only predictor, which was significantly associated with reduced carotid stiffness in both sexes. Looking at the determinants of the basic lipid profile, our results showed that increased LDL-C and triglyceride were sex-specifically related to stiffer carotid arteries. Women seem to absorb and synthesize less cholesterol than men resulting in a lower non-HDL-C concentration [48].

With regard to the metabolic syndrome, HDL-C and triglyceride are two of the five components to establish the diagnosis [49]. Groupings of at least three parameters of the metabolic syndrome have been shown to be associated with increased carotid stiffness in participants without clinical CVD [49]. These analyses were adjusted for sex (amongst other carotid stiffness parameters), but did not investigate sex-risk factor interactions [49]. A similar study observed a significant increase in carotid stiffness with increasing number of metabolic syndrome risk factors only in women [35]: sex only modified the association of glucose and carotid stiffness [35]. Glucose might play an important role as an intermediate phenotypic trait of diabetes mellitus and according to other studies, the diabetes-related influence on cardiovascular risk is elevated in women compared to men due to a stronger sex-specific impact of high BP, low HDL-C and high triglycerides [50]. However, it has already been shown that diabetes, stroke and myocardial infarction differently influence the cardiovascular risk in men and women [37,38,51]. Even though the sex-specific predisposition of triglycerides is partly reproduced through our findings, since carotid stiffness is a predictor of CVD [52], we decided at the beginning of our study to exclude individuals with clinically manifest CVD to avoid additional modification effects.

Heart rate, blood pressure and underlying pathways

Beside LDL-C and triglyceride, sex differences were also found for heart rate, which remains a major risk factor for cardiovascular events and mortality [53]. In the general population, increased heart rate is associated with increased carotid stiffness [36]. These analyses were amongst other parameters adjusted for sex, but they did not report results for the adjustments [36]. Nevertheless, enhanced heart rate due to increased pacing has been shown to be a direct modulator of carotid stiffness independent of BP changes [54]. The underlying pathways are multifactorial and reflect the complex interplay between heart rate, BP and carotid stiffness. Although they are not yet fully understood, this section aims to focus on possible functional interactions by heart rate.

Heart rate pacing studies could offer the opportunity to elucidate the underlying short-term mechanisms by heart rate alone. Heart rate pacing at rest showed a reduced stroke volume and a relatively constant cardiac output with increasing heart rate [55]. At the same time, central venous pressure was slightly reduced, pulmonary mean pressure was unchanged and mean arterial pressure was increased [55]. This indicates that heart rate modulates the stroke volume by reducing the cardiac preload resulting in a reduced end-diastolic volume

[55,56]. Although an increased heart rate might to some extent compensate for the reduced stroke volume to maintain the cardiac output [54], the raised mean arterial pressure indicates an increased cardiac afterload [55]. The underlying mechanisms might be explained by the left-ventricular ejection fraction and the resulted pulse wave [37]. Each ejection results in a pulse wave, which travels along the arterial tree and is reflected when arteries bifurcate [37]. Even though a higher heart rate reduces the stroke volume and might therefore reduce the magnitude of the forward pulse wave, the time spent in end-diastolic phase is also shorter [37]. This relates together with stiffer arteries to the circumstances that the reflected pulse wave overlap in an earlier phase of the forward travelling pulse wave resulting in increased amplitude in the systolic phase while at the same time the diastolic pressure is not augmented (increased pulse pressure) [37]. In the short-term, the increased pulse pressure affects the direct myogenic response of the artery also called Bayliss effect to counteract the mechanical impact [26]. Therefore, an increased mean arterial pressure suggests an interplay between a shorter diastolic phase and/or an increased vascular resistance due to vasoconstriction based on enhanced cyclic stress [54].

However, the interrelationships between heart rate, cardiac output, BP and carotid stiffness might occur differently based on divergent physiological needs and responses [55]. Interestingly, these results during exercise in general agree with the previous findings [55,56]. As expected, cardiac output increased with enhancing intensity of PA, but cardiac output did not differ with or without atrial pacing [55,56]. In contrast to the findings at rest, mean arterial pressure was maintained [55,56] probably due to higher availability of endothelial vasodilators, since PA affects the endothelial function [57]. In both, pacing and non-pacing exercise interventions, stroke volume increased to about 40% of the maximal work load with a subsequent steady state and a following decrease [56]. Thus, it seems to be reasonable that heart rate modifies the interaction between stroke volume and carotid stiffness to control for BP values and to maintain the homeostasis [55,56]. To compensate for the smaller stroke volume [58,59] and in line with our results, heart rate is higher among women than among men. This possibly explains the stronger relative effect of heart rate among men compared to women.

There is evidence that BP values differ between men and women during all stages of life, but differences are reduced with advancing age (especially in systolic BP) [60]. In line with the literature, men showed higher BP values than women (table 7.1). Therefore, one could assume that associations to carotid stiffness might also differ by sex. We found no significant sex differences for continuous variables in multivariate analyses with additional Bonferroni-Holm correction. However, in sub-analyses investigating the five BP categories according to the hypertension management guidelines [61], women in the optimal category (systolic/diastolic BP lower than 120/80 mmHg, respectively) had a significantly reduced carotid stiffness compared to men in the same category [62]. While carotid stiffness was increasing with increasing BP category, no further sex differences were found for all other categories [62]. This suggests that the impact of increased BP on vascular function is similar among men and women.

Other explanations and possible mechanisms of sex-differences?

To our knowledge, we were the first to investigate the long-term associations and sexual dimorphisms of prior single cardiovascular risk factors and carotid stiffness measured about ten years later in a population-based study in elderly. This population based observation revealed sex differences in CVD risk occurrence assessed by carotid stiffness but this might be not enough to explain the complex underlying mechanisms themselves. Reasons for sexual dimorphisms of atherosclerotic diseases may lie in the hormonal status, but investigations of sex differences particularly with respect to hormonal and menopausal status were beyond the scope of this thesis. Nevertheless, the possible impact of hormonal status on the atherosclerotic process will be outlined briefly. Beside the differential exposure to risk factors leading to a lower cumulative risk factor burden in women, which might explain sexual dimorphisms in cardiovascular risk, it might be plausible that sex hormones have a direct effect on pathophysiological changes in vascular function [63]. The question rises whether hormonal status might be responsible for the differences in lipid absorption. Wang et al. concluded that the lipid mechanism is only partially dependent on hormonal status and cannot sufficiently explain sex differences [64]. Other possible anti-atherosclerotic benefits of oestrogen have been shown by regulating NO activity especially in the premenopausal stage, but the protective effect attenuated or disappeared with advancing age in postmenopausal women [63]. This results in an increased vascular remodelling independent of cardiovascular risk factors [65,66] and leads to an assimilation of the carotid atherosclerosis in men and women [67]. In the second SAPALDIA follow-up survey, 75% of all female study participants were over 56 years old and 50% older than 63 years. This means that women were most likely in the post-menopausal phase and thus, the pre-menopausal effect might be minimized.

Another sex-specific pathway might be due to medication intake [68]. It has been shown that women are more likely to be under-estimated in risk assessment or under-treated in pharmacotherapy in comparison with men [68]. Therefore, we generally adjusted for medication intake (antihypertensive, lipid lowering, and glucose lowering treatment) in all analyses to avoid confounding by medication. Medication intake in SAPALDIA 2 includes two different sources. First of all, participants reported the specific drug names and secondly, information is derived from the health care matrix, which included general answers to medication intake for detailed doctor diagnosed diseases or symptoms (e.g. high BP). Both sources together enhance the completeness of medication intake information and enable the correct adjustment for confounding. According to the descriptive analyses, women took less medication than men (see table 7.1). When we investigated the sex differences between risk factors and carotid stiffness, we additionally included a sex-medication interaction term to adjust for possible sex differences in association of carotid stiffness and medication intake (data not shown). Based on our data, we cannot support the hypothesis that the described sex differences were based on prescribed medications.

Which is the most important trigger of carotid stiffness?

Extending the aim of long-term associations and looking at the complex interrelationship of different cardiovascular risk factors as presented in figure 1.2, the atherosclerotic burden seemed to be more than a cumulative load of single cardiovascular exposures. Therefore, we investigated the direct associations and thus, the particular strengths of single risk factors on vascular changes.

Our results revealed that age was the strongest predictor of increased carotid stiffness (standardised betas are displayed in directed acyclic graph (DAG) in figure 8.3). This suggested that vascular changes were predominantly based on a normal physiological process. Looking at the direct pathways of risk factors and distensibility, mean arterial BP and heart rate were beside age the strongest predictors. To set the normal physiological process and the direct associations of other risk exposures into relation, which might help to interpret the relevance of the findings, the relative proportions of each mean standardised risk factor estimate and age are presented here. The strength of direct association of mean arterial pressures was 27% smaller compared to the direct association of age on distensibility, heart rate was 62% smaller, and all other parameters were more than 83% smaller, respectively. However, the strength of direct association of age might be overestimated since age is the time being alive and possibly exposed to other influences. Therefore, age as a covariate might also account for the burden of other unmeasured exposures (mediators). Nevertheless, these results impressively demonstrate the high direct impact of age compared to the other risk factors.

Since age induces remodelling of the carotid artery based on a normal vascular process, we presumed that the association of different risk factors might differ with advancing age. However, we found no indication for an interaction between age and different risk factors on distensibility in further analyses and therefore we suggested that there are no specific interaction effects on distensibility in individuals aged 50 years or more. Whether accumulation of risk exposure differently affects the arterial system in children or younger adults compared to the elderly population remains to be investigated in trans-generational, longitudinal study designs.

Interestingly, the adjusted R^2 of the pathway analyses model investigating the direct effect estimates on distensibility was 0.52 (data not shown). This is rather high for an epidemiological study, but given the fact that age alone already explains 30% of the variation in univariate analysis, this might explain the high R^2 in the final model (data not shown). The univariate analysis for age seemed to be appropriate to investigate the overall effect estimate of age on distensibility since age can be assumed as an un-confounded exposure. Thus, this underlines that vascular changes assessed by carotid stiffness is predominantly affected by age, which might reflect the atherosclerotic and/or arteriosclerotic progression. Nevertheless, age is an immutable exposure and other assessed cardiovascular risk factors contributed to the prediction of carotid stiffness, which might affect the application of further treatment (further information see ‘outlook and perspectives’).

Focus physical activity - disentangle the complex cardiovascular risk factor relationship

We will shortly return to the long-term analyses analysis (chapter 7). In contrast to most other cardiovascular risk factors, increased PA in SAPALDIA 2 based on the short questions was not significantly associated with reduced carotid stiffness in SAPALDIA 3. The self-reported short questions were related to duration, intensity ("a bit out of breath" and "out of breath or sweat"), and frequency of PA and might be not capable to identify small differences in carotid stiffness. The failure to detect a significant association might additionally be due to the fact that PA behaviour may have changed between the two surveys. However, sub-analyses investigating the longitudinal effect of PA behaviour on carotid stiffness yielded in the same conclusion (data not shown). Being physically active or inactive in either surveys or getting active or inactive from SAPALDIA 2 to SAPALDIA 3 was not significantly associated with carotid stiffness.

Returning to the pathway analyses based on SAPALDIA 3 data (chapter 8): We calculated not only the direct pathways of different risk factors and carotid stiffness, but also focused on the complex interrelationship of PA and distensibility. PA was assessed with the long form of the International PA Questionnaire (IPAQ) in SAPALDIA 3, which promised more detailed information on PA compared to the short questions. However, age confounded the association between vigorous PA and distensibility substantially.

As discussed in chapter 8, we have expected to find associations based on previous findings, although most of the studies were based on a small sample size, or researchers investigated trained versus age-matched sedentary peers and not a population-based sample [47,69]. Even though the general results of 'The Atherosclerosis Risk in Community' Study' (ARIC) were comparable to our findings [70], in contrast to ARIC we found no indication of any associations in further sub-group analyses (data not shown). Comparing the lowest to the highest tertiles of PA, total PA and also intensity stratified analyses lead to the same inference.

Since PA is an acknowledged modifier of cardiovascular risk factors [71], the relative overall impact of PA on carotid stiffness might be attenuated. Nevertheless, the other possible pathways were also non-significant. Time effects, long-term lifestyle modifications and influence of prior or present risk factors are potential modifiers of carotid stiffness (other limitations regarding the PA assessment are discussed in the methodological section). Although our pathway analyses showed very nicely the different relationships between and within the cardiovascular risk factors and distensibility (figure 8.3), directions were based on a DAG, which in turn relied on a priori-defined hypothesis. The cross-sectional design does not allow drawing causal conclusions and it does not solve the issue of reverse causality [72]. The latter one is especially true for BP.

Reverse causality – blood pressure and carotid stiffness

Even though BP and vascular changes were longitudinally investigated in normotensive subjects [73], there is no evidence, whether the development of hypertension may have occurred due to an increased arterial stiffness or the other way around ('Chicken-or-the-Egg' question) [74]. Figure 9.2 shows the modified carotid stiffness circle originally generated by McEniery and Wilkinson [75] referring to the challenge of reverse causation of vascular function and BP leading to atherosclerosis and/or arteriosclerosis. Even though BP always has a short-term direct mechanical impact on carotid stiffness (action and reaction), it re-affects BP itself [26], either by auto regulation (e.g. in terms of the Bayliss effect) and/or by acute effects as it can be induced by exercise interventions [76,77]. Short-term changes are assumed to be reversible and aim to maintain blood flow and to preserve homeostasis within the artery. In the long-term, cyclic stress induced by heart rate might adapt the cardiac and arterial structure and function (e.g. left ventricular mass or the cardiac contractility and CIMT, LD or the carotid function) and other main factors (e.g. cardiac pre- and afterload or BP) [75,78].

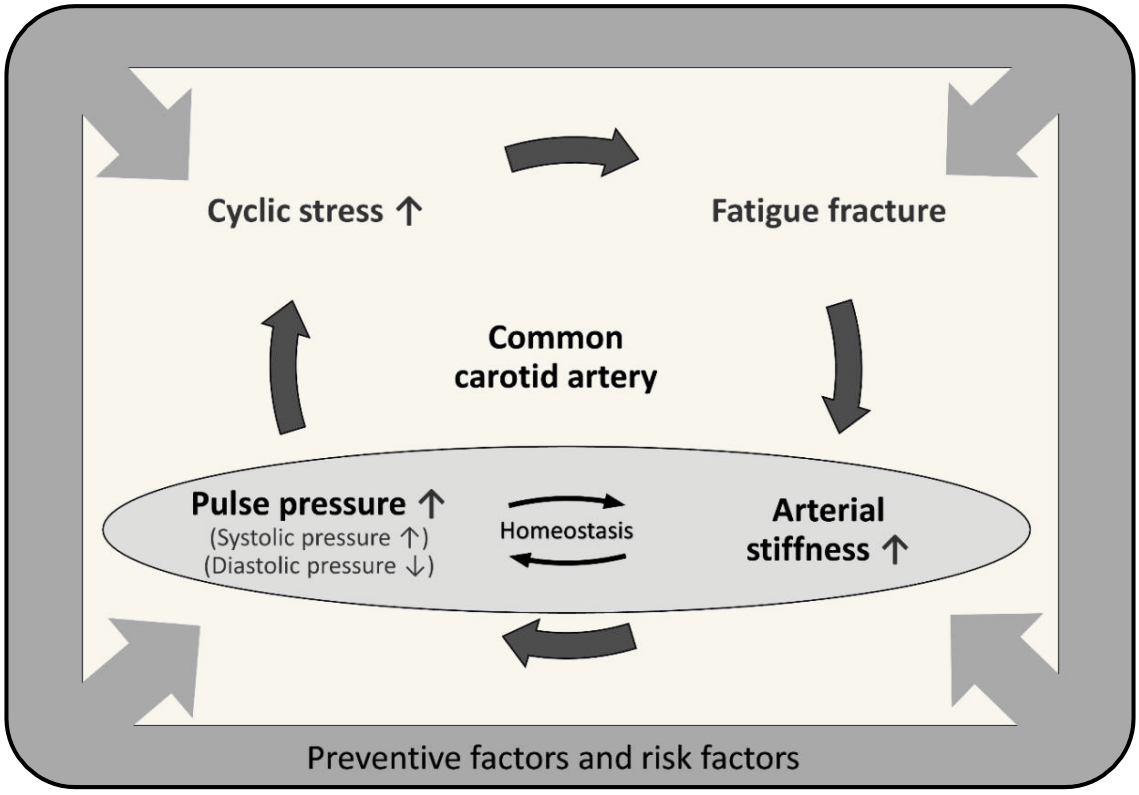

Figure 9.2: Modified circular adaption of the common carotid artery (McEniery & Wilkinson, 2013) [68]

The circular adaption of BP and vascular function covers the determination of the cause and the consequence. The interpretation is additionally aggravated due to possible varying circumstances (interactions of preventive factors and risk factors). For instance, the impact of heart rate differs from short-term to long-term. While heart rate is a short term modifier of BP, which in turn induces a mechanical stress, in the long-term it is a direct risk factor of carotid stiffness since it reflects the extent of exposure to mechanical stress (cyclic stress) [79].

METHODOLOGICAL ISSUES – DECISIONS, POSSIBILITIES AND CHALLENGES

Research is always a balancing act between optimal and feasible scientific research. Statistical analyses rely on models, which aim to reflect the real situation, but also rely on certain assumptions. Although our research is based on solid research using highly standardised and validated methods and our findings are reasonable in the light of current literature, some research methodology restrict the generalisation of the results and need to be discussed.

Central versus brachial blood pressure

Even though the difference between brachial and central BP decreases with advancing age [26], and it has been shown that brachial BP can be used to calculate carotid stiffness parameters in elderly people [52], pulse pressure might differ between central and peripheral locations [80]. The difference between central and brachial BP not only depends on the process of arterial stiffening based on arteriosclerosis and atherosclerosis, but also on the vessel properties (e.g. radius), blood flow (e.g. density and velocity), and determinants of the cardiac function [81–83]. Since vessel function changes along the arterial tree characterised by higher elastic wall properties and lager diameter in central arteries compared to the periphery, blood flow is reduced in the latter one and resistance is increased, respectively (Hagen-Poiseuille equation and Laplace's law) [26]. To overcome the higher flow resistance, a higher pressure difference is needed in the periphery compared to the central pulse pressure [26].

The underlying parameters, which cause differences between the central and brachial BP values additionally vary between sexes and may modify to some extent the sex-specific associations of cardiovascular risk factors and carotid stiffness [32,59,84,85]. In agreement with literature, we found that body and diameter size, BP values and all carotid stiffness parameters were reduced among women compared to men while heart rate was contrarily increased (see table 7.1, diameter size not shown) [59,60,85–87]. As outlined before, heart rate has an additional impact on BP. Increased heart rate reduces the time of the cardiac cycle due to a shorter end-diastolic phase [37]. In combination with stiffer arteries, the superposition of the forward and backward pulse wave is more likely to be in the systolic phase, which leads to higher pulse pressure, especially in the periphery [37]. Thus, the pulse pressure amplification between the brachial and central pulse pressure is also dependent on heart rate [82].

By using brachial BP for carotid stiffness, investigations may overestimate carotid stiffness. Also, the question whether sex-specific variability of BP values has an impact on our estimates, is not clarified [88,89]. Since we have not measured central BP, this might affect the outlined investigations and it should be implemented in future carotid stiffness examinations.

Carotid parameters

Whenever a plaque occurred in the measurement region of interest, we excluded this specific observation. We did not implement plaque as an additional criterion for atherosclerosis because the sonographers were not trained to detect plaque occurrence in the carotid artery and we would therefore have implemented a detection bias. This is also reflected by our descriptive statistics. Inclusion of plaque might have added important additional information on atherosclerosis, since plaque always reflects a pathological atherosclerotic process, while an increased intima media thickness does not necessarily [90]. The resulting blood flow reduction of plaques is dependent on the stage of atherosclerotic stenosis [91]. However, focal narrowing induces blood flow reduction, but it is not necessarily associated with ischemic neurological symptoms [91]. But plaques still induce blood flow disturbance and reduction [91].

SAPALDIA ultrasound examinations were performed in 3489 participants. Data of 65 participants (1.9%) could not be analysed due to poor ultrasound data quality. A plaque was only found in 174 participants (5%), whereby the expected prevalence in middle to older populations would be assumed to lie above 50% (increasing prevalence with advancing age) [92]. Thus, all conclusions in this thesis are based on plaque-free detection results according to the Mannheim Consensus [93].

Not only does inclusion or exclusion of plaques influence carotid parameter values. Carotid diameter variations during cardiac cycle are well examined [94] and all carotid stiffness parameters include specifically diameter measures at diastolic and systolic phase (see table 6.1). Therefore, it seems to be logical that similar definitions are available for the carotid wall thickness, since significant differences between systolic and diastolic CIMT were reported [13]. In line with our results, CIMT is smallest in systole and largest in diastole while the average of all analysed CIMT measures across heart cycle lay in-between (table 6.2). However, in terms of Young's elastic modulus, which was the only carotid stiffness parameter considering CIMT (table 6.1), it is not specified which time point or measure of CIMT should be used for its calculation. Considering the earlier perspective that previous devices were not capable or were limited in measuring CIMT in predefined time points, we decided to use the average of all CIMT measurements as a stable outcome to calculate Young's elastic modulus (chapter 6, table 6.1). Further research is needed to evaluate the clinical impact of different carotid structures (e.g. CIMT or roughness) on carotid functions, especially since carotid structure and function represents the atherosclerotic process in a slightly different way.

Physical activity assessment

The use of questionnaire to assess PA is still very common - especially in epidemiological studies with large sample sizes [95]. A questionnaire is easy to apply, the organisation and distribution is rather simple, it is relatively inexpensive and the self-administered tool is

commonly well accepted by the participants [95]. This being said, several methodological issues regarding the PA assessment may still have influenced the accuracy of the results, which in turn might reduce the sensitivity to detect changes.

In the first follow-up, only the short questions were posed. In the second follow-up, the short questions were repeated and the long version of the IPAQ was introduced. Both questionnaires were self-reported assessments of PA behaviour. Especially in subjective measures, recall or desirability bias might be induced when asking about past PA [95,96]. In a sub-sample, there was a tendency to over report the total minutes spent in light to moderate PA and to under report the vigorous intensity compared to accelerometers (with a greater bias for men compared to women) [97]. In addition, older individuals (> 65 years) tended to over report their PA compared to younger people [97]. The degree of misclassification might be strong enough to prevent small differences due to PA from being detected.

Although reliability and validity of both assessments were examined [97,98], reliability of subjective measures (e.g. questionnaires) are likely reduced compared to objective measurements (accelerometer), whereby correlations were around 0.45 [97]. The IPAQ was separately validated within the SAPALDIA 3 setting, also including middle aged to elderly participants, and the results verified the previous findings (paper submitted by Wanner et al.). We also found similar correlations when comparing the short questions and IPAQ information within the study sample used in chapter 8 (data not shown). Despite the fact that these correlations were relatively low to moderate, such thresholds are at the moment well accepted in the respective research community [96].

Both questionnaires offered the opportunity to investigate the frequency, duration and total amount of PA during the last seven days. After examination and discussion about the advantages and disadvantages of absolute and relative intensities, we decided to use intensities in minutes per week and not metabolic equivelent's (METs) mainly to avoid misunderstanding of the definition of MET. This will be shortly outlined. Moderate and vigorous intensity is dependent on a person's cardiorespiratory fitness level (VO_2max) and it is known that this intensity may vary between individuals performing the same exercise [95]. In contrast, one MET corresponds to the energy expenditure at rest ($\sim 3.5\ ml\ O_2 \times kg^{-1} \times min^{-1}$) and is therefore an absolute value due to a specific performance ('objective MET') [99]. Interpersonal variation of MET's may still exists due to dependent covariates such as sex and age [96], but it is presumably negligible compared to the fitness level variation. Depending on different PA guidelines, moderate intensity relates to approximately 3-6 MET's and vigorous intensity to 6-8 MET's [99,100]. However, when MET's are calculated based on duration and subjective intensities (e.g. out of breath or sweat), one can assume that the 'objective MET' based on energy expenditure might differ from the 'subjective MET' based on intensities [95]. To explain this statement in more detail, we consider a fictive example including person A (trained) and person B (untrained). In the week before they fill in the questionnaire, they did exactly the same exercise (e.g. running

5mph ~ 8 MET [101], 2 times per week for 20 minutes). Since the IPAQ or the short questions (as most other PA questionnaires) did not assess the type of exercise (running at 5 mph) but rather the total amount of moderate or vigorous intensity performed in the last seven days, person A considered the personal exercise intensity as moderate while person B exercised at a vigorous intensity. By using the guidelines (e.g. the Guidelines for Data Processing and Analysis of the IPAQ [102]) the calculated 'subjective MET' differs between person A and B and do not correspond to the 'objective MET' value (8 MET = 5 mph). Misunderstanding might occur by interpreting the 'subjective MET' as 'objective MET'. Since the IPAQ-MET's were only transformed values of the subjective intensities, our conclusions regarding PA and carotid stiffness did not change. However, in terms of comparability to other different studies, MET's might be an option for future investigations.

Despite the mentioned limitations of the PA assessments, the choice of which method to use was a compromise between feasibility and accuracy. A lower accuracy of PA assessment methods may lower the variation of the exposure. This implies that the expected carotid stiffness effects of PA could lie beyond the detection limit. For future studies, objective measures of PA such as accelerometers would likely enhance the accuracy of the assessment. And the complementary inclusion of cardiorespiratory fitness, which is strongly correlated to cardiovascular health [103], might be a crucial determinant for future research to detect small differences in carotid function. However, for longitudinal data, it is assumed that a certain individual threshold is needed to induce a physiological response (e.g. a change in carotid stiffness) [96]. Therefore, subjective PA might still play a key role in future research.

Study designs and biases – implications for the interpretation of study results

SAPALDIA is to-date a 'closed' cohort traced since 1991 with the initial purpose to investigate air pollution and the occurrence of lung and heart disease during follow-up. 'Closed cohort' means that no further members were added [104]. The advantages of this are that all members were observed over time and longitudinal data are available. This enables the investigation of prevalence but also of incidence and progression of diseases and other outcomes [72,104]. The potential downside of a 'closed cohort' is that the cohort size gets smaller with passing time [72,104]. A bias might be induced especially when dropouts differ from persistent members [72,104]. Loss to follow-up should therefore be prevented, particularly since cohorts are likely to suffer from a healthy survivor effect, which in turn affects the general interpretation of the study results [72,104]. A key part of identifying healthy survivor bias is intensive and prospective examination of participant's health and environment information - a real strength of the SAPALDIA cohort study. This constitutes a basis for identifying determinants of healthy survivor bias. Since the magnitude of healthy survivor effect also depends on the research question (the number of healthy survivor determinants influence the degree of bias), this enables a more precise interpretation of the results.

All data used in this thesis were collected at the first and second SAPALDIA follow-up. Although SAPALDIA participants were highly committed to the study, loss to follow-up occurred. Internal statistical analysis showed that SAPALDIA 3 participants were more likely to be young, not obese, non-smokers (never or former smokers), of Swiss nationality, higher educated and without clinical symptoms compared to SAPALDIA 2 participants, indicating a certain healthy survivorship (Meier et al., paper in preparation). But what does this mean for the presented research? For instance, we excluded a priori participants with known manifest CVD, which means that this specific healthy survivor determinant might therefore be less capable to influence our results. Although the study sample represented the Swiss population in 1991 (sample recruitment based on randomization), the generalisation of SAPALDIA 3 results might be more transferable to younger people with generally better health characteristics (non-obese, non-smokers etc.) due to the loss of follow-up. However, healthy survivor effect might also help to explain why we have not found the expected association. We anticipated to find socioeconomic influences, but since SAPALDIA 3 participants were more homogenously represented in terms of the socioeconomic status, this might explain why socioeconomic status was not a determinant of carotid stiffness.

When interpreting the study results, one should be aware of the most common study errors [72]. Errors can generally be distinguished as systematic or random errors [104]. While random error decreases with increasing study size sample, (a further advantage of cohorts), systematic errors, also called bias, are unaffected by study size enlargement, since they refer to a specific source of error [104]. Besides the healthy survivor bias – which was already introduced – selection bias, information bias and confounding are the most prominent sources of error in observational studies [104, 105]. Selection bias may be reduced in the SAPALDIA cohort since the initial study sample was selected by randomization, although due to loss of follow-up healthy survivor bias was introduced. Forms of information bias are measurement errors, interviewer bias or recall bias [104,105]. Conducted validation and reproducibility studies help to identify systematic measurement errors. Intensive staff training and standard operating procedures may reduce bias introduced by fieldworkers. In addition, separated assessments of diseases, behaviour and symptoms may reduce recall bias (although a smoker might be more aware of smoking related symptoms than a non-smoker). And DAG's and intensive analyses may help to identify and adjust for possible confounders.

Except for validation and reproducibility studies, which were separately conducted within the SAPALDIA 3 survey, the presented studies in this thesis were based on cross-sectional design. The study about prior cardiovascular risk factors and current carotid stiffness (chapter 7) was a special case of cross-sectional investigation since the exposure and outcome had a long-term relationship. Let us bear in mind that an assessment conducted at one specific time point is a snapshot and does not reflect change in time. Thus, the discussion about probable causes and effects (e.g. pathway analyses) were based on a priori defined hypotheses, since a cross-sectional design does not allow conclusions on this specific issue [104]. Therefore, only associations and no causal relationships can be confirmed.

Relevance of the study results

To comprehend the relevance of the study results, it is important to define the framework condition in which the study results should be interpreted. Considering the common research process [106], which is not always a straight forward approach, the following stages can be determined: 1) Development of new tools or new ideas, which allows the investigation of basic pathways and relations within a new research field based on the current state of the art. 2) Conducting additional studies and collecting further information on the same research object, 3) achieving evidence, 4) creating knowledge, 5) adapting and applying the new knowledge (e.g. into policy) and finally 1') developing new research questions within the new research field - leading again to stage one but on a higher level. The different stages are of course the result of the competitive work among various research centres and may partially overlap.

Setting the conducted studies within this thesis into the context of the research spiral, we are currently switching between phase one and two: Carotid stiffness is assumed to be an early biomarker of cardiovascular risk [52]. Therefore, we examined the state of the art of ultrasound assessments (chapter 4, stage 1') with the aim to enhance future measurement quality, which would then allow a more exact investigation of basic relations to carotid stiffness exposures. Ensuingly, the new ultrasound analysis program was developed and validated (chapter 5 and 6, stage 1) and first basic pathways were investigated within an epidemiological study (chapter 7 and 8, stage 1/2). Thus, relevance of the study results should be discussed in relation to the possibilities in stage 1 or 2. Potential implications with regard to e.g. public health or treatment are discussed in the section 'outlook and perspectives'.

The relevance of results in any study not only depend on the measured magnitude (effect size), but also on the statistical precision of the measured estimates (e.g. confidence interval), the reproducibility of analyses (e.g. ICC) and the critical reflection of methodological issues (strengths and limitations) [1]. To summarize the strengths and limitations: carotid structure and function measurements were validated and revealed good to excellent reproducible results. Associations of prior and current cardiovascular risk factors showed consistent relations, even though effect sizes seemed to be small in comparison to age as non-modifiable exposure. However, long latency periods of small exposures can also cumulatively expand the burden of disease. Therefore, it is of significance that most cardiovascular risk factors were still associated in the long term with carotid stiffness for both sexes (independent of age, education level, mean arterial pressure, medication and study area) and keeping in mind that exposures could have changed over time and inclusion of brachial BP may have affected the strength of association.

To summarise the relevance of the study results, we can conclude that our analyses provides a powerful basis to continue the research and to collect further information on determinants of carotid stiffness and its underlying pathways.

OUTLOOK AND PERSPECTIVES

Classical Epidemiological studies investigate health determinants and/or disease occurrence in the population [104]. Therefore, epidemiological research is a crucial element of public health activities, which aim to preserve, promote and improve the population's health and quality of life [107,108]. Since health determinants in relation to carotid stiffness were investigated in the population-based SAPALDIA cohort, the long- and short-term perspectives rely on clarifications of important questions, such as: what are the short-term perspectives and are there any other aspects, which could further elucidate the complex relationship of risk factors and carotid stiffness? Which treatments and interventions might be implemented in the future to reduce atherosclerotic burden? What is the long-term role of atherosclerosis research for public health and why is it relevant?

Short-term prospects

We assessed prior cardiovascular risk factors in SAPALDIA 2 and also current cardiovascular risk factors in SAPALDIA 3, therefore the question needs to be answered: why did we not investigate the longitudinal changes in these risk factors? The reason is mainly due to the fact that blood parameters of SAPALDIA 3 remain to be determined due to unexpected delay. We assume that the associations of the 10-year risk exposure (longitudinal design) would be even stronger.This would strengthen our findings and therefore will be an aim for future investigation.

Addressing the aspect of further research possibilities within the SAPALDIA cohort to clarify the complex relationship of different risk factors, other diseases or exposures were assessed within the surveys. With regard to SAPALDIA's origin, air pollution has already been shown to be related to carotid intima media thickness [109,110], while associations with carotid stiffness remain to be evaluated. Other exposure effects such as noise, nutrition or other diseases and its intermediate phenotypes could be excellently investigated in relation to carotid stiffness. Family history might give additional insights to identifying possible transgenerational determinants. Complementary to the physical risk factors of carotid stiffness, psychological and psychosocial characteristics (e.g. mental disorders, stress or social support) may influence the development of atherosclerosis since it has already been shown to affect cardiovascular events and mortality [111]. An additional research field might be the gender related investigation beyond biological sex-specific differences as examined in chapter 7.

In addition, there might also be the possibility to stratify by culture or environment. Switzerland not only has different cultural aspects (German, French and Italian); but it also encompasses both rural and urban characteristics. Therefore, the SAPALDIA database offers an excellent opportunity to examine such aspects in detail. In the presented studies, we commonly adjusted for areas as random effects but investigations by language or environment were beyond the scope of this thesis.

Returning to the unused capabilities of the raw database of ultrasound measurements, expert readers analysed all ultrasound images and the respective raw data contour detection results were saved in Hierarchical Data Format files (HDF5). At the moment, only the most widely used carotid structural parameters were extracted (CIMT and LD in diastole, systole and the respective average across heart cycle). These data were needed to calculate carotid stiffness parameters. However, HDF5 files contain a lot more information than those mentioned. Each clip, which was used in this thesis, includes at least around 90 single frames per heart cycle (10.7ms per frame, up to tree heart cycles were analysed). Overall, about 17'000 clips and 17'000 images leading to a total number of about 2'500'000 single images (including SAPALDIA sample examinations, validation study and certifications) were analysed by DYARA and expert readers. From these, other parameters of interest could be calculated. For instance, CIMT variability [112] or intima media roughness [113,114] could be extracted from the HDF5 files. Since both LD and CIMT were assessed across the whole heart cycle, this could be done not only over a certain segment, but also over the whole heart cycle, which might provide additional information about atherosclerotic wall alterations. Therefore, HDF5 files provide an excellent opportunity for future research, not excluding evaluation and further improvement of methodological and technological aspects, which could minimise measurement variations.

Besides the examination of carotid structure and function, another measure of arterial stiffness was assessed within the SAPALDIA 3 survey – pulse wave velocity. While carotid stiffness is a direct measure of local arterial stiffness, pulse wave velocity quantifies the systemic arterial stiffness [88]. Since these measurements of arterial stiffness involve different segments of the artery and since elasticity (and therefore also pulse wave reflection) changes along the arterial tree, this suggests that different mechanisms interact with both forms of arterial stiffness [52]. Therefore, contribution of different risk factor may also vary between these two measurements [37,115]. Comparing these carotid stiffness and pulse wave velocity and investigating the respective effects of cardiovascular risk factor may help to understand the underlying pathways in more detail.

While normal values already exist for pulse wave velocity and CIMT [116,117], no reference values have been published (up-to-date) for carotid stiffness. Normal values are the basis to identify individuals with potential deviating characteristics towards an increased cardiovascular risk. While established risk scores depend on a snapshot of current exposure to cardiovascular risk factors, carotid stiffness may overcome this weakness since it reflects the accumulated vascular damage based on the whole burden of exposure. First studies on reclassifications of Framingham Scores due to vascular ageing were performed indicating that a combined approach may prevent underestimation of individual's cardiovascular risk [33]. However, whether a combination of pulse wave velocity, CIMT and carotid stiffness would even better predict cardiovascular events or re-classify people at risk remains to be evaluated in further studies.

Possible treatment and intervention perspectives

All presented studies (chapter 4-8) aim to increase the amount of information to understand the complex relationship of different cardiovascular risk factors and carotid stiffness. Knowing the impact of certain cardiovascular risk factors on the atherosclerotic burden may affect the respective interventions or treatment, which may leads then to an optimal vascular ageing. The concept of 'early vascular ageing' (EVA) was presented in chapter 1. The counteracting approach of EVA is called 'ADAM' and it is defined as the 'aggressive decrease of atherosclerosis modifiers' [118]. A targeted use of ADAM by intensifying the respective treatment of a certain risk factor aims to reduce or minimize vascular changes. Thus, based on our results and on the direct pathways, main treatment targets seem to be BP and heart rate, which has also been confirmed by previous studies [36,75,79]. Considering now the interrelationships of the different cardiovascular risk factors (figure 1.1, figure 1.2 and figure 8.3), it is of crucial importance for cardiovascular health and a preventive perspective to avoid unfavourable lifestyle behaviours and other modifiable risk factors in general [79,119–121]. In the case of present cardiovascular risk factors (or even latent CVD), it is also of significance to apply ADAM and to treat patients with the best practice approach [122]. While the former perspective describes more the primary CVD prevention, the latter aims to optimise the secondary preventive effort [122].

The question remains: which individuals should be targeted to receive preventive medicine and how could carotid stiffness assessment influence this decision? The decision is often based on cost-effectiveness by targeting those individuals at high risk [123]. In case of hypertension, already a more sophisticated approach is available, which considers different interventions and treatment depending on the individual's risk of hypertension and cardiovascular exposure based on risk factors or organ damage [61]. Let us remember that carotid stiffness data includes no plaque occurrence since plaques reflect a different shape of atherosclerotic process [124]. Considering the crude overall range of the atherosclerotic process starting with non-pathological vascular remodelling, followed by pathological manifestation of plaques and ending with occurrence of cardiovascular events, our measurement of carotid stiffness refers to the first stage. Therefore, normal values of carotid stiffness might help to identify individuals at high risk for non-pathological vascular changes. This means that with regard to the whole atherosclerotic process, carotid stiffness might even enable an early diagnosis of cardiovascular risk. However, identification of early vascular damage even in asymptomatic individuals may not lead directly to a reduced or delayed development of CVD, but it potentially creates an important basis for further research attempting to reach this goal. From a preventive point of view, we should take this time advantage and implement early interventions [125]. Whether high-risk or population-based strategies should be implemented remains to be evaluated [123].

Long-term investment - public health relevance

The 'Global Burden of Diseases 2013 Mortality and Causes of Death Collaborators' recently published their new analysis for the global burden of disease with a sex- and age-specific focus [126]. Global life expectancy has increased since 1991 compared to 2013 (life expectancy at birth in Switzerland: males from 74 to 80 years and females from 81 to 85 years), but mortality rate has decreased more in women compared to men, and this was the case in all age groups except the oldest one [126]. However, age-standardised death rates of CVD showed a decrease of 22% from 1990 to 2013 [126]. This seems to be confusing at a first glance, since one would assume that a population with increased life expectancy, death due to CVD would also increase. However, age-standardisation considers the differences in age distribution at the time point 1990 and 2013. This means, that the 'standard population' has improved since 1990, possibly due to health related promotion and lifestyle changes such as PA [126]. PA has been shown to be a suitable non-pharmacological treatment for primary or secondary CVD prevention – especially on the population level [127].

However, looking at the total number of deaths from CVD, death has globally increased by 40% (from 1990 to 2013) [126]. By comparing the total and the age-standardised value, we nicely see the impact of the ageing population. The three main causes of years of life lost in Switzerland in 2013 were ischaemic heart diseases, lung cancer and stroke [126]. Knowing the actual burden of the diseases is one aspect, but assuming a further increase in life expectancy, the burden of age-associated diseases will in particular increase [128]. According to the Swiss Federal Statistical Office, the population demography in people aged 65 or more will change from 17.6% (2013) to 27.7% in 2050 [129]. From a cost-effectiveness perspective, the larger the incidence and prevalence of the disease (as CVD is assumed to occur more often with advancing age), the smaller the number needed to treat in order to avoid one event [128]. However, the relevance of primary prevention is not solely the prolongation of life expectancy, but more importantly, to increase the health related quality of life [130]. Thus, reduction or at least to postpone the burden of CVD is of greatest public health relevance and therefore, current research on atherosclerosis as ageing related disease is an investment for the future.

CONCLUSION

In order to investigate the multidimensional pathways of cardiovascular risk factors on the atherosclerotic burden, which will be an upcoming challenge in the near future, a new ultrasound analyses system was developed and validated and we investigated the long- and short-term associations of cardiovascular risk factors and carotid stiffness in elderly participants in the multicentre SAPALDIA cohort. Carotid stiffness indices can provide information on the elastic carotid properties and therefore reflect vascular damage accumulated over time [31].

Given the good reproducible results, these studies showed for the first time that most carotid stiffness parameters could be used interchangeably. In addition, the past preload of single cardiovascular exposures has added important information to the overall vascular damage assessed by carotid stiffness separately for both sexes. Since also sex-specific attributions to the atherosclerotic burden were found for heart rate, LDL-C and triglyceride, this indicates a different susceptibility among men and women, which should be further examined. Shifting our focus from the long-term to the short-term observations, we gained valuable insights in the multifactorial pathways of cardiovascular risk factors and carotid stiffness. Even though the strongest predictor of carotid stiffness was age, indicating that most alterations depend on normal vascular ageing, strengths of association regarding other risk factors such as heart rate or mean arterial pressure, were still substantial. However, age has also been shown to be a strong confounder and therefore, the assumed positive associations of PA could not be supported by our findings. Nevertheless, previous beneficial effects of PA on carotid stiffness were based on small intervention studies not representative of the general population. Since this was only the second epidemiological study examining the complex relationship of PA, cardiovascular risk factors and carotid stiffness in a cross-sectional design, we recommend further investigating PA determinants in correlation with carotid stiffness longitudinally, including a representative study sample.

In conclusion, the presented studies within this thesis provide an important basis towards future investigations targeting the early and late consequences of atherosclerosis and its progression. In addition, evaluation of preventive or prognostic determinants of carotid structural and functional parameters may help to improve CVD management and may facilitate implementation of carotid ultrasound in clinical practice.

References

1 Guyatt, G. H., Oxman, A. D., Vist, G. E., Kunz, R., Falck-Ytter, Y., Alonso-Coello, P. and Schünemann, H. J. (2008) GRADE: an emerging consensus on rating quality of evidence and strength of recommendations. BMJ. **336**, 924–926

2 Schulz, K. F., Altman, D. G., Moher, D. and for the CONSORT Group. (2010) CONSORT 2010 Statement: updated guidelines for reporting parallel group randomised trials. BMJ. 340, c332–c332

3 Des Jarlais, D. C., Lyles, C. and Crepaz, N. (2004) Improving the reporting quality of nonrandomized evaluations of behavioral and public health interventions: the TREND statement. Am. J. Public Health. **94**, 361–366

4 Armstrong, R., Waters, E., Moore, L., Riggs, E., Cuervo, L. G., Lumbiganon, P. and Hawe, P. (2008) Improving the reporting of public health intervention research: advancing TREND and CONSORT. J. Public Health. **30**, 103–109

5 Moher, D., Liberati, A., Tetzlaff, J., Altman, D. G. and The PRISMA Group. (2009) Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLOS Med. 6, e1000097

6 Higgins JPT, Green S (editors) (2011). Cochrane Handbook for Systematic Reviews of Interventions Version 5.1.0 [updated March 2011]: The Cochrane Collaboration. Retrieved August 23, 2014, from www.cochrane-handbook.org

7 PLOS Medicine Editors. (2014) Observational studies: getting clear about transparency. PLOS Med. **11**, e1001711

8 Higgins, P. A. and Straub, A. J. (2006) Understanding the error of our ways: mapping the concepts of validity and reliability. Nurs. Outlook. **54**, 23–29

9 Molinari, F., Zeng, G. and Suri, J. S. (2010) An integrated approach to computer-based automated tracing and its validation for 200 common carotid arterial wall ultrasound images a new technique. J. Ultrasound Med. **29**, 399–418

10 Faita, F., Gemignani, V., Bianchini, E., Giannarelli, C., Ghiadoni, L. and Demi, M. (2008) Real-time measurement system for evaluation of the carotid intima-media thickness with a robust edge operator. J Ultrasound Med. **27**, 1353–1361

11 Bland, J. M. and Altman, D. G. (1986) Statistical methods for assessing agreement between two methods of clinical measurement. The Lancet. **327**, 307–310

12 Arnett, D. K., Chambless, L. E., Kim, H., Evans, G. W. and Riley, W. (1999) Variability in ultrasonic measurements of arterial stiffness in the atherosclerosis risk in communities study. Ultrasound Med. Biol. **25**, 175–180

13 Polak, J. F., Meisner, A., Pencina, M. J., Wolf, P. A. and D'Agostino, R. B. (2012) Variations in common carotid artery intima-media thickness during the cardiac cycle: implications for cardiovascular risk assessment. J. Am. Soc. Echocardiogr. **25**, 1023–1028

14 Henry, R. M. A., Kostense, P. J., Spijkerman, A. M. W., Dekker, J. M., Nijpels, G., Heine, R. J., Kamp, O., Westerhof, N., Bouter, L. M. and Stehouwer, C. D. A. (2003) Arterial stiffness increases with deteriorating glucose tolerance status: the Hoorn Study. Circulation. **107**, 2089–2095

15 Fernandes, V. R. S., Polak, J. F., Cheng, S., Rosen, B. D., Carvalho, B., Nasir, K., McClelland, R., Hundley, G., Pearson, G., O'Leary, D. H., Bluemke, D. A. and Lima, J. A. C. (2008) Arterial stiffness is associated with regional ventricular systolic and diastolic dysfunction: the Multi-Ethnic Study of Atherosclerosis. Arterioscler. Thromb. Vasc. Biol. **28**, 194–201

16 Della-Morte, D., Gardener, H., Denaro, F., Boden-Albala, B., Elkind, M. S. V., Paik, M. C., Sacco, R. L. and Rundek, T. (2010) Metabolic syndrome increases carotid artery stiffness: the Northern Manhattan Study. Int. J. Stroke. **5**, 138–144

17 Rundek, T., Elkind, M. S., Pittman, J., Boden-Albala, B., Martin, S., Humphries, S. E., Juo, S. H. and Sacco, R. L. (2002) Carotid intima-media thickness is associated with allelic variants of stromelysin-1, interleukin-6, and hepatic lipase genes the Northern Manhattan Prospective Cohort Study. Stroke. **33**, 1420–1423

18 Van Popele, N. M., Grobbee, D. E., Bots, M. L., Asmar, R., Topouchian, J., Reneman, R. S., Hoeks, A. P. G., van der Kuip, D. A. M., Hofman, A. and Witteman, J. C. M. (2001) Association between arterial stiffness and atherosclerosis: the Rotterdam Study. Stroke. **32**, 454–460

19 Mattace-Raso, F. U. S., van der Cammen, T. J. M., Hofman, A., van Popele, N. M., Bos, M. L., Schalekamp, M. A. D. H., Asmar, R., Reneman, R. S., Hoeks, A. P. G., Breteler, M. M. B. and Witteman, J. C. M. (2006) Arterial stiffness and risk of coronary heart disease and stroke: the Rotterdam Study. Circulation. **113**, 657–663

20 Clayton, D. and Hills, M. (2013) Statistical models in epidemiology Auflage: Reprint., Oxford University Press, Usa, Oxford. 1-376

21 Leone, N., Ducimetière, P., Gariépy, J., Courbon, D., Tzourio, C., Dartigues, J.-F., Ritchie, K., Alpérovitch, A., Amouyel, P., Safar, M. E. and Zureik, M. (2008) Distension of the carotid artery and risk of coronary events: the Three-City Study. Arterioscler. Thromb. Vasc. Biol. **28**, 1392–1397

22 Tseke, P., Grapsa, E., Stamatelopoulos, K., Samouilidou, E., Protogerou, A., Papamichael, C. and Laggouranis, A. (2006) Atherosclerotic risk factors and carotid stiffness in elderly asymptomatic HD patients. Int. Urol. Nephrol. **38**, 801–809

23 Roman, M. J., Devereux, R. B., Schwartz, J. E., Lockshin, M. D., Paget, S. A., Davis, A., Crow, M. K., Sammaritano, L., Levine, D. M., Shankar, B.-A., Moeller, E. and Salmon, J. E. (2005) Arterial

stiffness in chronic inflammatory diseases. Hypertension. **46**, 194–199

24 Störk, S., van den Beld, A. W., von Schacky, C., Angermann, C. E., Lamberts, S. W. J., Grobbee, D. E. and Bots, M. L. (2004) Carotid artery plaque burden, stiffness, and mortality risk in elderly men: a prospective, population-based cohort study. Circulation. **110**, 344–348

25 Yang, E. Y., Chambless, L., Sharrett, A. R., Virani, S. S., Liu, X., Tang, Z., Boerwinkle, E., Ballantyne, C. M. and Nambi, V. (2012) Carotid arterial wall characteristics are associated with incident ischemic stroke but not coronary heart disease in the Atherosclerosis Risk in Communities (ARIC) Study. Stroke. **43**, 103–108

26 Nichols, W. W., O'Rourke, M. F. and Vlachopoulos, C. (2011) Mcdonald's blood flow in arteries: theoretical, experimental and clinical principles 6th ed., Hodder Arnold, London

27 Weber, C. and Noels, H. (2011) Atherosclerosis: current pathogenesis and therapeutic options. Nat. Med. **17**, 1410–1422

28 Paoletti, R., Gotto, A. M. and Hajjar, D. P. (2004) Inflammation in atherosclerosis and implications for therapy. Circulation. **109**, III–20–III–26

29 Wick, G. and Grundtman, C. (2012) Inflammation and Atherosclerosis, Springer-Verlag, Wien

30 Ross, R. (1999) Atherosclerosis - an inflammatory disease. N. Engl. J. Med. **340**, 115–126

31 Van Bortel, L. M., Duprez, D., Starmans-Kool, M. J., Safar, M. E., Giannattasio, C., Cockcroft, J., Kaiser, D. R. and Thuillez, C. (2002) Clinical applications of arterial stiffness, Task Force III: recommendations for user procedures. Am. J. Hypertens. **15**, 445–452

32 Polak, J. F., Wong, Q., Johnson, W. C., Bluemke, D. A., Harrington, A., O'Leary, D. H. and Yanez, N. D. (2011) Associations of cardiovascular risk factors, carotid intima-media thickness and left ventricular mass with inter-adventitial diameters of the common carotid artery: the Multi-Ethnic Study of Atherosclerosis (MESA). Atherosclerosis. **218**, 344–349

33 Łoboz-Rudnicka, M., Jaroch, J., Bociąga, Z., Kruszyńska, E., Ciecierzyńska, B., Dziuba, M., Dudek, K., Uchmanowicz, I. and Łoboz-Grudzień, K. (2013) Relationship between vascular age and classic cardiovascular risk factors and arterial stiffness. Cardiol. J. **20**, 394–401

34 World Health Organization. (2009) Global health risks: mortality and burden of disease attributable to selected major risks, World Health Organization, Geneva. 1-63

35 Koskinen, J., Magnussen, C. G., Viikari, J. S. A., Kähönen, M., Laitinen, T., Hutri-Kähönen, N., Lehtimäki, T., Jokinen, E., Raitakari, O. T. and Juonala, M. (2012) Effect of age, gender and cardiovascular risk factors on carotid distensibility during 6-year follow-up. The cardiovascular risk in Young Finns Study. Atherosclerosis. **224**, 474–479

36 Whelton, S. P., Blankstein, R., Al-Mallah, M. H., Lima, J. A. C., Bluemke, D. A., Hundley, W. G., Polak, J. F., Blumenthal, R. S., Nasir, K. and Blaha, M. J. (2013) Association of resting heart rate with carotid and aortic arterial stiffness: Multi-Ethnic Study of Atherosclerosis. Hypertension. **62**, 477–484

37 Stehouwer, C. D. A., Henry, R. M. A. and Ferreira, I. (2008) Arterial stiffness in diabetes and the metabolic syndrome: a pathway to cardiovascular disease. Diabetologia. **51**, 527–539

38 Berry, J. D., Dyer, A., Cai, X., Garside, D. B., Ning, H., Thomas, A., Greenland, P., Van Horn, L., Tracy, R. P. and Lloyd-Jones, D. M. (2012) Lifetime risks of cardiovascular disease. N. Engl. J. Med. **366**, 321–329

39 Lerner, D. J. and Kannel, W. B. (1986) Patterns of coronary heart disease morbidity and mortality in the sexes: a 26-year follow-up of the Framingham population. Am. Heart J. **111**, 383–390

40 Aggoun, Y., Bonnet, D., Sidi, D., Girardet, J. P., Brucker, E., Polak, M., Safar, M. E. and Levy, B. I. (2000) Arterial mechanical changes in children with familial hypercholesterolemia. Arterioscler. Thromb. Vasc. Biol. **20**, 2070–2075

41 Tanaka, H., Dinenno, F. A., Monahan, K. D., Clevenger, C. M., DeSouza, C. A. and Seals, D. R. (2000) Aging, habitual exercise, and dynamic arterial compliance. Circulation. **102**, 1270–1275

42 Schmidt-Trucksäss, A., Grathwohl, D., Frey, I., Schmid, A., Boragk, R., Upmeier, C., Keul, J. and Huonker, M. (1999) Relation of leisure-time physical activity to structural and functional arterial properties of the common carotid artery in male subjects. Atherosclerosis. **145**, 107–114

43 Miao, D.-M., Ye, P., Xiao, W.-K., Gao, P., Zhang, J.-Y. and Wu, H.-M. (2011) Influence of low high-density lipoprotein cholesterol on arterial stiffening and left ventricular diastolic dysfunction in essential hypertension. J. Clin. Hypertens. **13**, 710–715

44 Cunha, R. S., Pannier, B., Benetos, A., Siche, J.-P., London, G. M., Mallion, J. M. and Safar, M. E. (1997) Association between high heart rate and high arterial rigidity in normotensive and hypertensive subjects. J Hypertens. **15**, 1423–1430

45 Redheuil, A., Yu, W.-C., Wu, C. O., Mousseaux, E., de Cesare, A., Yan, R., Kachenoura, N., Bluemke, D. and Lima, J. A. C. (2010) Reduced ascending aortic strain and distensibility: earliest manifestations of vascular aging in humans. Hypertension. **55**, 319–326

46 Brinkley, T. E., Hsu, F.-C., Carr, J. J., Hundley, W. G., Bluemke, D. A., Polak, J. F. and Ding, J. (2011) Pericardial fat is associated with carotid stiffness in the Multi-Ethnic Study of Atherosclerosis. Nutr. Metab. Cardiovasc. Dis. **21**, 332–338

47 Cook, J. N., DeVan, A. E., Schleifer, J. L., Anton, M. M., Cortez-Cooper, M. Y. and Tanaka, H. (2006) Arterial compliance of rowers: implications for combined aerobic and strength training on arterial elasticity. Am. J. Physiol. Heart Circ. Physiol. **290**, H1596–1600

48 Matthan, N. R., Zhu, L., Pencina, M., D'Agostino, R. B., Schaefer, E. J. and Lichtenstein, A. H. (2013) Sex-specific differences in the predictive value of cholesterol homeostasis markers and 10-year cardiovascular disease event rate in Framingham Offspring Study participants. J. Am. Heart Assoc. **2**, e005066

49 Scuteri, A., Najjar, S. S., Muller, D. C., Andres, R., Hougaku, H., Metter, E. J. and Lakatta, E. G. (2004) Metabolic syndrome amplifies the age-associated increases in vascular thickness and stiffness. J. Am. Coll. Cardiol. **43**, 1388–1395

50 Juutilainen, A., Kortelainen, S., Lehto, S., Rönnemaa, T., Pyörälä, K. and Laakso, M. (2004) Gender difference in the impact of type 2 diabetes on coronary heart disease risk. Diabetes Care. **27**, 2898–2904

51 Lee, C., Joseph, L., Colosimo, A. and Dasgupta, K. (2012) Mortality in diabetes compared with previous cardiovascular disease: a gender-specific meta-analysis. Diabetes Metab. **38**, 420–427

52 Van Sloten, T. T., Schram, M. T., van den Hurk, K., Dekker, J. M., Nijpels, G., Henry, R. M. A. and Stehouwer, C. D. A. (2014) Local stiffness of the carotid and femoral artery is associated with incident cardiovascular events and all-cause mortality: the Hoorn Study. J. Am. Coll. Cardiol. **63**, 1739–1747

53 Lang, C. C., Gupta, S., Kalra, P., Keavney, B., Menown, I., Morley, C. and Padmanabhan, S. (2010) Elevated heart rate and cardiovascular outcomes in patients with coronary artery disease: clinical evidence and pathophysiological mechanisms. Atherosclerosis. **212**, 1–8

54 Giannattasio, C., Vincenti, A., Failla, M., Capra, A., Cirò, A., De Ceglia, S., Gentile, G., Brambilla, R. and Mancia, G. (2003) Effects of heart rate changes on arterial distensibility in humans. Hypertension. **42**, 253–256

55 Bada, A. A., Svendsen, J. H., Secher, N. H., Saltin, B. and Mortensen, S. P. (2012) Peripheral vasodilatation determines cardiac output in exercising humans: insight from atrial pacing. J. Physiol. **590**, 2051–2060

56 Munch, G. D. W., Svendsen, J. H., Damsgaard, R., Secher, N. H., González-Alonso, J. and Mortensen, S. P. (2014) Maximal heart rate does not limit cardiovascular capacity in healthy humans: insight from right atrial pacing during maximal exercise. J. Physiol. **592**, 377–390

57 Seals, D. R., Desouza, C. A., Donato, A. J. and Tanaka, H. (2008) Habitual exercise and arterial aging. J. Appl. Physiol. Bethesda Md 1985. **105**, 1323–1332

58 Santos, M. A. A., Sousa, A. C. S., Reis, F. P., Santos, T. R., Lima, S. O. and Barreto-Filho, J. A. (2013) Does the aging process significantly modify the mean heart rate? Arq. Bras. Cardiol. **101**, 388–398

59 Hayward, C. S., Kalnins, W. V. and Kelly, R. P. (2001) Gender-related differences in left ventricular chamber function. Cardiovasc. Res. **49**, 340–350

60 Sandberg, K. and Ji, H. (2012) Sex differences in primary hypertension. Biol. Sex Differ. **3**, 7

61 Mancia, G., Fagard, R., Narkiewicz, K., Redon, J., Zanchetti, A., Böhm, M., Christiaens, T., Cifkova, R., Backer, G. D., Dominiczak, A., Galderisi, M., Grobbee, D. E., Jaarsma, T., Kirchhof, P., Kjeldsen, S. E., Laurent, S., Manolis, A. J., Nilsson, P. M., Ruilope, L. M., Schmieder, R. E., Sirnes, P. A., Sleight, P., Viigimaa, M., Waeber, B., Zannad, F., Redon, J., Dominiczak, A., Narkiewicz, K., Nilsson, P. M., Burnier, M., Viigimaa, M., Ambrosioni, E., Caufield, M., Coca, A., Olsen, M. H., Schmieder, R. E., Tsioufis, C., Borne, P. van de, Zamorano, J. L., Achenbach, S., Baumgartner, H., Bax, J. J., Bueno, H., Dean, V., Deaton, C., Erol, C., Fagard, R., Ferrari, R., Hasdai, D., Hoes, A. W., et al. (2013) 2013 ESH/ESC guidelines for the management of arterial hypertension: the task force for the management of arterial hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). Eur. Heart J. **34**, 2159–2219

62 Caviezel, S., Dratva, J., Schaffner, E., Schindler, C., Zemp Stutz, E., Künzli, N., Gaspoz, J.-M., Rochat, T., Probst-Hensch, N. and Schmidt-Trucksäss, A. (2013) Sex-specific associations between carotid distensibility and prior blood pressure categories – results from the SAPALDIA cohort. Artery Res. **7**, 133

63 Meadows, J. L. and Vaughan, D. E. (2011) Endothelial biology in the post-menopausal obese woman. Maturitas. **69**, 120–125

64 Wang, X., Magkos, F. and Mittendorfer, B. (2011) Sex differences in lipid and lipoprotein metabolism: it's not just about sex hormones. J. Clin. Endocrinol. Metab. **96**, 885–893

65 El Khoudary, S. R., Wildman, R. P., Matthews, K., Thurston, R. C., Bromberger, J. T. and Sutton-Tyrrell, K. (2013) Progression rates of carotid intima-media thickness and adventitial diameter during the menopausal transition. Menopause N. Y. N. **20**, 8–14

66 Muscelli, E., Kozàkovà, M., Flyvbjerg, A., Kyriakopoulou, K., Astiarraga, B. D., Glintborg, D., Konrad, T., Favuzzi, A. and Petrie, J. (2009) The effect of menopause on carotid artery remodeling, insulin sensitivity, and plasma adiponectin in healthy women. Am. J. Hypertens. **22**, 364–370

67 Stamatelopoulos, K. S., Armeni, E., Georgiopoulos, G., Kazani, M., Kyrkou, K., Stellos, K., Koliviras, A., Alexandrou, A., Creatsa, M., Papamichael, C. and Lambrinoudaki, I. (2012) Recently postmenopausal women have the same prevalence of subclinical carotid atherosclerosis as age and traditional risk factor matched men. Atherosclerosis. **221**, 508–513

68 Birkemeyer, R., Schneider, H., Rillig, A., Ebeling, J., Akin, I., Kische, S., Paranskaya, L., Jung, W., Ince, H. and Nienaber, C. A. (2014) Do gender differences in primary PCI mortality represent a different adherence to guideline recommended therapy? a multicenter observation. BMC Cardiovasc. Disord. **14**, 71

69 Nualnim, N., Barnes, J. N., Tarumi, T., Renzi, C. P. and Tanaka, H. (2011) Comparison of central artery elasticity in swimmers, runners, and the sedentary. Am. J. Cardiol. **107**, 783–787

70 Schmitz, K. H., Arnett, D. K., Bank, A., Liao, D., Evans, G. W., Evenson, K. R., Stevens, J., Sorlie, P. and Folsom, A. R. (2001) Arterial distensibility and physical activity in the ARIC study. Med. Sci. Sports Exerc. **33**, 2065–2071

71 Janiszewski, P. M. and Ross, R. (2009) The utility of physical activity in the management of global cardiometabolic risk. Obesity. **17**, S3–S14

72 Carlson, M. D. A. and Morrison, R. S. (2009) Study design, precision, and validity in observational studies. J. Palliat. Med. **12**, 77–82

73 Dernellis, J. and Panaretou, M. (2005) Aortic stiffness is an independent predictor of progression to hypertension in nonhypertensive subjects. Hypertension. **45**, 426–431

74 Glasser, S. P. and Arnett, D. K. (2008) Vascular stiffness and the "Chicken-or-the-Egg" question. Hypertension. **51**, 177–178

75 McEniery, C. M. and Wilkinson, I. B. (2013) The pressures of aging. Hypertension. 1–2

76 Bianchini, E., Bruno, R. M., Corciu, A. I., Faita, F., Gemignani, V., Ghiadoni, L., Picano, E. and Sicari, R. (2012) Assessment of carotid elasticity during exercise: a reproducibility study. Ultrasound Med. Biol. **38**, 223–230

77 Myers, C. W., Farquhar, W. B., Forman, D. E., Williams, T. D., Dierks, D. L. and Taylor, J. A. (2002) Carotid distensibility characterized via the isometric exercise pressor response. Am. J. Physiol. Heart Circ. Physiol. **283**, H2592–2598

78 Myung, Y., Seo, H.-S., Jung, I. H., Lee, N.-H., Suh, J., Choi, J. H. and Cho, Y. H. (2012) The correlation of carotid artery stiffness with heart function in hypertensive patients. J. Cardiovasc. Ultrasound. **20**, 134–139

79 Thorin, E. and Thorin-Trescases, N. (2009) Vascular endothelial ageing, heartbeat after heartbeat. Cardiovasc. Res. **84**, 24–32

80 McEniery, C. M., Cockcroft, J. R., Roman, M. J., Franklin, S. S. and Wilkinson, I. B. (2014) Central blood pressure: current evidence and clinical importance. Eur. Heart J. **35**, 1719-1725

81 Hahn, C. and Schwartz, M. A. (2009) Mechanotransduction in vascular physiology and atherogenesis. Nat. Rev. Mol. Cell Biol. **10**, 53–62

82 Avolio, A., Butlin, M. and Tan, I. (2013) Importance of pressure pulse amplification in the association of resting heart rate and arterial stiffness. Hypertension. **62**, e46

83 Avolio, A. P., van Bortel, L. M., Boutouyrie, P., Cockcroft, J. R., McEniery, C. M., Protogerou, A. D., Roman, M. J., Safar, M. E., Segers, P. and Smulyan, H. (2009) Role of pulse pressure amplification in arterial hypertension: experts' opinion and review of the data. Hypertension. **54**, 375–383

84 O'Rourke, M. F. and Hayward, C. S. (2003) Arterial stiffness, gender and heart rate. J. Hypertens. **21**, 487–490

85 Reckelhoff, J. F. (2001) Gender differences in the regulation of blood pressure. Hypertension. **37**, 1199–1208

86 Vaidya, D., Heckbert, S. R., Wasserman, B. A. and Ouyang, P. (2012) Sex-specific association of age with carotid artery distensibility: Multi-Ethnic Study of Atherosclerosis. J. Womens Health. **21**, 516–520

87 Marlatt, K. L., Kelly, A. S., Steinberger, J. and Dengel, D. R. (2013) The influence of gender on carotid artery compliance and distensibility in children and adults. J. Clin. Ultrasound. **41**, 340–346

88 Laurent, S., Cockcroft, J., Van Bortel, L., Boutouyrie, P., Giannattasio, C., Hayoz, D., Pannier, B., Vlachopoulos, C., Wilkinson, I. and Struijker-Boudier, H. (2006) Expert consensus document on arterial stiffness: methodological issues and clinical applications. Eur. Heart J. **27**, 2588–2605

89 McEniery, C. M., Yasmin, McDonnell, B., Munnery, M., Wallace, S. M., Rowe, C. V., Cockcroft, J. R. and Wilkinson, I. B. (2008) Central pressure: variability and impact of cardiovascular risk factors the Anglo-Cardiff Collaborative Trial ii. Hypertension. **51**, 1476–1482

90 Negi, S. I. and Nambi, V. (2012) The role of carotid intimal thickness and plaque imaging in risk stratification for coronary heart disease. Curr. Atheroscler. Rep. 115–123

91 Mughal, M. M., Khan, M. K., DeMarco, J. K., Majid, A., Shamoun, F. and Abela, G. S. (2011) Symptomatic and asymptomatic carotid artery plaque. Expert Rev. Cardiovasc. Ther. **9**, 1315–1330

92 Joakimsen, O., Bønaa, K. H., Stensland-Bugge, E. and Jacobsen, B. K. (1999) Age and sex differences in the distribution and ultrasound morphology of carotid atherosclerosis: the Tromsø Study. Arterioscler. Thromb. Vasc. Biol. **19**, 3007–3013

93 Touboul, P.-J., Hennerici, M. G., Meairs, S., Adams, H., Amarenco, P., Bornstein, N., Csiba, L., Desvarieux, M., Ebrahim, S., Fatar, M., Hernandez Hernandez, R., Jaff, M., Kownator, S., Prati, P., Rundek, T., Sitzer, M., Schminke, U., Tardif, J.-C., Taylor, A., Vicaut, E., Woo, K. S., Zannad, F. and Zureik, M. (2007) Mannheim carotid intima-media thickness consensus (2004-2006): an update on behalf of the advisory board of the 3rd and 4th Watching the Risk Symposium 13th and 15th European Stroke Conferences, Mannheim, Germany, 2004, and Brussels, Belgium, 2006. Cerebrovasc. Dis. **23**, 75–80

94 Giannattasio, C., Salvi, P., Valbusa, F., Kearney-Schwartz, A., Capra, A., Amigoni, M., Failla, M., Boffi, L., Madotto, F., Benetos, A. and Mancia, G. (2008) Simultaneous measurement of beat-to-beat carotid diameter and pressure changes to assess arterial mechanical properties. Hypertension. **52**, 896–902

95 Strath, S. J., Kaminsky, L. A., Ainsworth, B. E., Ekelund, U., Freedson, P. S., Gary, R. A., Richardson, C. R., Smith, D. T. and Swartz, A. M.

(2013) Guide to the Assessment of Physical Activity: Clinical and Research Applications A Scientific Statement From the American Heart Association. Circulation. **128**, 2259–2279

96 Shephard, R. and Vuillemin, A. (2003) Limits to the measurement of habitual physical activity by questionnaires. Br. J. Sports Med. **37**, 197–206

97 Wanner, M., Probst-Hensch, N., Kriemler, S., Meier, F., Bauman, A. and Martin, B. W. (2013) What physical activity surveillance needs: validity of a single-item questionnaire. Br. J. Sports Med. bjsports. **21**, 1570-1576

98 Craig, C. L., Marshall, A. L., Sjöström, M., Bauman, A. E., Booth, M. L., Ainsworth, B. E., Pratt, M., Ekelund, U., Yngve, A., Sallis, J. F. and Oja, P. (2003) International physical activity questionnaire: 12-country reliability and validity. Med. Sci. Sports Exerc. **35**, 1381–1395

99 World Health Organization. (2010) Global recommendations on physical activity for health, World Health Organization, Geneva, Switzerland

100 Physical Activity Guidelines Advisory Committee. (2008) Physical Activity Guidelines Advisory Committee Report, U.S. Department of Health and Human Services, Washington, DC. Retrieved December 3, 2012, from http://nutritionreviews.oxfordjournals.org/content/nutritionreviews/67/2/114.full.pdf

101 Ainsworth, B. E., Haskell, W. L., Whitt, M. C., Irwin, M. L., Swartz, A. M., Strath, S. J., O'Brien, W. L., Bassett, D. R., Jr, Schmitz, K. H., Emplaincourt, P. O., Jacobs, D. R., Jr and Leon, A. S. (2000) Compendium of physical activities: an update of activity codes and MET intensities. Med. Sci. Sports Exerc. **32**, S498–504

102 International Physical Activity Questionnaire (IPAQ). (2005) Guidelines for Data Processing and Analysis of the International Physical Activity Questionnaire (IPAQ). Retrieved January 6, 2014, from https://drive.google.com/viewerng/viewer?a=v&pid=sites&srcid=ZGVmYXVsdGRvbWFpbnx0aGVpcGFxfGd4OjE0NDgxMDk3NDU1YWRlZTM

103 Minder, C. M., Shaya, G. E., Michos, E. D., Keenan, T. E., Blumenthal, R. S., Nasir, K., Carvalho, J. A. M., Conceição, R. D., Santos, R. D. and Blaha, M. J. (2014) Relation Between Self-Reported Physical Activity Level, Fitness, and Cardiometabolic Risk. Am. J. Cardiol. **113**, 637–643

104 Rothman, K. J. (2012) Epidemiology: An Introduction, Oxford University Press. 1-286

105 Hammer, G. P., du Prel, J.-B. and Blettner, M. (2009) Avoiding bias in observational studies: part 8 in a series of articles on evaluation of scientific publications. Dtsch. Ärztebl. Int. **106**, 664–668

106 Kumar, R. (2005) Research Methodology: A Step-by-Step Guide for Beginners, SAGE Publications. 1-332

107 World Health Organization. (1998) WHO | The WHO Health Promotion Glossary. WHO. Retrieved December 30, 2014, from http://www.who.int/healthpromotion/about/HPG/en/

108 Smith, B. J., Tang, K. C. and Nutbeam, D. (2006) WHO Health Promotion Glossary: new terms. Health Promot. Int. **21**, 340–345

109 Künzli, N., Jerrett, M., Garcia-Esteban, R., Basaga\ na, X., Beckermann, B., Gilliland, F., Medina, M., Peters, J., Hodis, H. N. and Mack, W. J. (2010) Ambient air pollution and the progression of atherosclerosis in adults. PLOS ONE. **5**, e9096

110 Künzli, N., Jerrett, M., Mack, W. J., Beckerman, B., LaBree, L., Gilliland, F., Thomas, D., Peters, J. and Hodis, H. N. (2005) Ambient air pollution and atherosclerosis in Los Angeles. Environ. Health Perspect. **113**, 201-206

111 Rutters, F., Pilz, S., Koopman, A. D., Rauh, S. P., Te Velde, S. J., Stehouwer, C. D., Elders, P. J., Nijpels, G. and Dekker, J. M. (2014) The association between psychosocial stress and mortality is mediated by lifestyle and chronic diseases: The Hoorn Study. Soc. Sci. Med. **118**, 166–172

112 Saba, L., Mallarini, G., Sanfilippo, R., Zeng, G., Montisci, R. and Suri, J. (2011) Intima Media Thickness Variability (IMTV) and its association with cerebrovascular events: a novel marker of carotid atherosclerosis? Cardiovasc. Diagn. Ther. **2**, 10–18

113 Sandrock, M., Schulze, C., Schmitz, D., Dickhuth, H.-H. and Schmidt-Trucksaess, A. (2008) Physical activity throughout life reduces the atherosclerotic wall process in the carotid artery. Br. J. Sports Med. **42**, 839–844

114 Schmidt-Trucksäss, Sandrock, M., Cheng, D., Muller, H. M., Baumstark, M. W., Rauramaa, R., Berg, A. and Huonker, M. (2003) Quantitative measurement of carotid intima-media roughness-effect of age and manifest coronary artery disease. Atherosclerosis. **166**, 57–66

115 Paini, A., Boutouyrie, P., Calvet, D., Tropeano, A.-I., Laloux, B. and Laurent, S. (2006) Carotid and Aortic Stiffness: Determinants of Discrepancies. Hypertension. **47**, 371–376

116 Arterial Stiffness Collaboration. (2010) Determinants of pulse wave velocity in healthy people and in the presence of cardiovascular risk factors: "establishing normal and reference values." Eur. Heart J. **31**, 2338–2350

117 Touboul, P. J., Labreuche, J., Vicaut, E., Belliard, J. P., Cohen, S., Kownator, S., Pithois-Merli, I. and Amarenco, P. (2009) Country-Based Reference Values and Impact of Cardiovascular Risk Factors on Carotid Intima-Media Thickness in a French Population: The "Paroi Artérielle et Risque Cardio-Vasculaire"(PARC) Study. Cerebrovasc. Dis. **27**, 361–367

118 Nilsson, P. M., Boutouyrie, P. and Laurent, S. (2009) Vascular aging: a tale of EVA and ADAM in cardiovascular risk assessment and prevention. Hypertension. **54**, 3–10

119 Versari, D., Daghini, E., Virdis, A., Ghiadoni, L. and Taddei, S. (2009) Endothelial dysfunction as

a target for prevention of cardiovascular disease. Diabetes Care. **32**, S314–S321

120 Brunner, H., Cockcroft, J. R., Deanfield, J., Donald, A., Ferrannini, E., Halcox, J., Kiowski, W., Luscher, T. F., Mancia, G., Natali, A., Oliver, J. J., Pessina, A. C., Rizzoni, D., Rossi, G. P., Salvetti, A., Spieker, L. E., Taddei, S. and Webb, D. J. (2005) Endothelial function and dysfunction. Part II: association with cardiovascular risk factors and diseases. A statement by the Working Group on Endothelins and Endothelial Factors of the European Society of Hypertension. J. Hypertens. Febr. **23**, 233–246

121 Nilsson, P. M. (2008) Early vascular aging (EVA): consequences and prevention. Vasc. Health Risk Manag. **4**, 547–552

122 Perk, J., De Backer, G., Gohlke, H., Graham, I., Reiner, Z., Verschuren, M., Albus, C., Benlian, P., Boysen, G., Cifkova, R., Deaton, C., Ebrahim, S., Fisher, M., Germano, G., Hobbs, R., Hoes, A., Karadeniz, S., Mezzani, A., Prescott, E., Ryden, L., Scherer, M., Syvänne, M., Scholte op Reimer, W. J. M., Vrints, C., Wood, D., Zamorano, J. L. and Zannad, F. (2012) European guidelines on cardiovascular disease prevention in clinical practice (version 2012). The fifth joint task force of the European Society of Cardiology and other societies on cardiovascular disease prevention in clinical practice (constituted by representatives of nine societies and by invited experts). Developed with the special contribution of the European Association for Cardiovascular Prevention & Rehabilitation (EACPR). Eur. Heart J. **33**, 1635–1701

123 Rose, G. (2008) Rose's Strategy of Preventive Medicine Auflage: Updated., Oxford University Press, USA, Oxford ; New York. 1-190

124 Touboul, P.-J. (2015) Intima-Media Thickness of Carotid Arteries. In Frontiers of Neurology and Neuroscience (Alonso, A., Hennerici, M. G., and Meairs, S., eds.), pp 31–39, S. KARGER AG, Basel

125 Sniderman, A. D. and Furberg, C. D. (2008) Age as a modifiable risk factor for cardiovascular disease. The Lancet. **371**, 1547–1549

126 GBD 2013 Mortality and Causes of Death Collaborators. (2014) Global, regional, and national age-sex specific all-cause and cause-specific mortality for 240 causes of death, 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet. Retrieved December 28, 2014, from http://www.sciencedirect.com/science/article/pii/S0140673614616822

127 Swift, D. L., Lavie, C. J., Johannsen, N. M., Arena, R., Earnest, C. P., O'Keefe, J. H., Milani, R. V., Blair, S. N. and Church, T. S. (2013) Physical activity, cardiorespiratory fitness, and exercise training in primary and secondary coronary prevention. Circ. J. **77**, 281–292

128 Prince, M. J., Wu, F., Guo, Y., Gutierrez Robledo, L. M., O'Donnell, M., Sullivan, R. and Yusuf, S. (2014) The burden of disease in older people and implications for health policy and practice. The Lancet. Retrieved November 24, 2014, from http://www.sciencedirect.com/science/article/pii/S0140673614613477

129 Bundesamt für Statistik. (2013) Bundesamt für Statistik - Bevölkerung nach Alter. Retrieved January 27, 2013, from http://www.bfs.admin.ch/bfs/portal/de/index/themen/01/02/blank/key/alter/gesamt.html

130 Song, M., Lee, H.-W. and Kang, D. (2014) The potential application of personalized preventive research. Jpn. J. Clin. Oncol. **44**, 1017–1024

APPENDIX

Contribution to the PhD project

Table A: Appendix - Contribution to the PhD project

Topic	Time	Contribution by the PhD student*	Collaboration and contribution by the SAPALDIA Team
Sonographer training	2009-2010		Prof. Dr. A. Schmidt-Trucksäss, Dr. J. Dratva, PD Dr. H. Hanssen
Data collection	2010-2011		Ultrasound examinations done by trained and certified SAPALIDA fieldworkers
Coordination and transfer of ultrasound raw data	2010-2012	Administration and coordination to assure a fluent raw data transfer from Swiss TPH to DSBG. Strong collaboration with Dr. J. Dratva, M. Tarantino, Dr. M. Baumstark.	Dr. J. Dratva, M. Tarantino, Dr. M. Baumstark
DYARA	2010-2012	Reader feedback and strong collaboration with the computer scientist Prof. Dr. Alexandra Teynor to improve user-friendly handling of the new ultrasound analysis program (DYARA)	Prof. Dr. A. Teynor
Ultrasound data management and image analysis at DSBG	2010-2012	a. Completion of DSBG reading SOP's b. Systematic training of DSBG reader c. Coordination of reading procedure at DSBG and managing the reading center team (10 Readers) d. Monthly report / feedback of reading procedures with Swiss TPH (Dr. J. Dratva) e. Coordination of backup procedures with Dr. M. Baumstark (University of Freiburg, D) f. Coordination of data transfer (analysed ultrasound data) from DSBG Server to Swiss TPH Server (M. Tarantino, Dr. M. Baumstark)	M. Tarantino, Dr. J. Dratva, Dr. M. Baumstark, The DSBG Reading Center Team

Table A continued

Topic	Time	Contribution by the PhD student*	Collaboration and contribution by the SAPALDIA Team
Data entry, controlling, cleaning	2012	a. Generation of first ultrasound data set (analysed data) in October 2011 b. Reception of new data from Swiss TPH in December 2011 c. Generation of final data set in February 2012 d. Data cleaning of DSBG ultrasound data in February 2012 e. Data cleaning of blood pressure data in March 2012 f. Reception of final joined ultrasound dataset including blood pressure data from Swiss TPH in June 2012 g. Data entry of 100 IPAQ's in April 2013 h. Generation of CIMT and carotid stiffness codebook in strong collaboration with Dr. J. Dratva. i. Controlling: Single structural and functional parameters, were separately generated by E. Schaffner and on my part according to prior defined definitions in the SAPALDIA codebook. We compared our results minutely detailed and redefined some definitions due to unexpected deviations in the generated variables due to the complex CIMT methodology.	E. Schaffner, Dr. J. Dratva and the SAPALDIA data management team
Statistical analyses	2013-2014	a. Research analysis plan and first analyses (paper and conference projects) were generated by the PhD student b. Discussion of research analysis plan and first results at the CIMT Working Groups meetings. c. Detailed discussion about statistical analyses (PD. Dr. C. Schindler, E. Schaffner)	CIMT Working Group (Prof. Dr. A. Schmidt-Trucksäss, Prof. Dr. N. Künzli, Prof. Dr. N. Probst-Hensch, PD Dr. C. Schindler, Dr. Julia Dratva, S. Endes, E. Schaffner)
Paper projects	2012-2014	**In all papers:** all authors contributed to the interpretation of the results and have read, revised and approved the final version of the submitted manuscript. **Additional specific tasks by the PhD student** - Review (chapter 4): formatting and submitting the manuscript - DYARA validation paper (chapter 5), Reproducibility paper (chapter 6), sex-specific associations (chapter 7), IPAQ and carotid stiffness (chapter 8): Study conception and design, acquisition of data, contributed to the data analysis plan, analysis and interpretation of data, drafting of manuscript, formatting and submitting the manuscript (except DYARA validation paper).	(Co-)authors

Table A continued

Topic	Time	Contribution by the PhD student*	Collaboration and contribution by the SAPALDIA Team
Doctoral training	2010-2014	The academic training was primarily oriented to achieve statistical and epidemiological skills in order to manage SAPALDIA2 and SAPALDIA3 database analysis. The absolved courses exceed the formal requirements for PhD thesis. A detailed course list can be found in the curriculum vitae. Overall, 34.25 credit points were acquired in 27 courses. The gained knowledge was summarised in internal presentations (DSBG)	Mainly SSPH+ courses, MPH courses, Swiss Epidemiology Winter School
Presentations excluding conferences	2010-2014	Regular presentations (> 20) about past courses or status updates and research plans of the PhD project were given at the SAPALDIA workshops, in the CIMT Working Group Meetings, in the DSBG journal club or the DSBG research colloquium.	
Past conference abstracts and presentations	2011-2014	Overall, the PhD student attendet in 10 national and international conferences. With exception of the first conference, 9 different projects were submitted and presented by the PhD student. As a result, statistical, methodological, writing and presenting skills were progressed due to new challenges within each project.	CIMT Working Group Team
Teaching activities by the PhD student	2011-2014	Please find the teaching activity list in the curriculum vitae. Some of the teaching activities within the DSBG were specifically related to the PhD project ("Angewandte Gefässphysiologie in Bewegung und Gesundheit"). In a broader perspective, teaching activities for "Writing a master thesis in exercise and health sciences: a practical approach" was an application of the generated knowledge about general project management in SAPALDIA. The PhD student built up a new semester course. It was a win-win situation for both - the master students and the PhD student.	Prof. Dr. A. Schmidt-Trucksäss, PD Dr. H. Hanssen
PWV database	2010-2011	Data management - data entry, controlling and cleaning of pulse wave velocity data was done analogously to ultrasound data. Since the PWV database is now part of another PhD project, I did not analyse the data.	

* supervised by Prof. Dr. med. Arno Schmidt-Trucksäss, CIMT = carotid intima media thickness; DSBG = Department of Sport, Exercise and Health; DYARA = DYnamic ARtery Analysis program; SOP = standard operating procedures; MPH = Master of Public Health; SSPH+ = Swiss School of Public Health; Swiss TPH = Swiss Tropical and Public Health Institute,