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Small steps for large arteries

Bringing arterial structure and function measurements to daily clinical practice

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Abbreviations

AA	Ascending aorta
AIx	Augmentation index
AP	Augmented pressure
ATP	Adult treatment panel
AUC	Area under the curve
BMI	Body mass index
BSA	Body surface area
CA	Carotid artery
CC	Cross-sectional compliance
Cf	Carotid-femoral
CHD	Coronary heart disease
CI	Cardiac index
CKD	Chronic kidney disease
CO	Cardiac output
CSA_{ao}	aortic cross-sectional area
CV	Cardiovascular
CVD	Cardiovascular disease
CV-RFs	Cardiovascular risk factors
DBP	Diastolic blood pressure
DC	Distensibility coefficient
eGFR	Estimated glomerular filtration rate.
E_{inc}	Incremental elastic modulus
ESC	European Society of Cardiology
ESH	European Society of Hypertension
FA	Femoral artery
FF	Form factor
FP	Fractional polynomial

FRS	Framingham risk score
FVI	Flow velocity integral
HDL	High-density lipoprotein
HR	Heart rate
ICD	International Classification of Diseases
IFG	Impaired fasting glycaemia
IMT	Intima-media thickness
IOP	Intra-ocular pressure
LDL	Low-density lipoprotein
LVH	Left ventricular hypertrophy
MAP	Mean arterial pressure
MRI	Magnetic resonance imaging
NRI	Net reclassification improvement
NTG	Normal-tension glaucoma
PP	Pulse pressure
PWF	Pressure waveform
PWV	Pulse wave velocity
RM	Reflection magnitude
SAC	Total arterial compliance
SBP	Systolic blood pressure
SCORE	Systematic COronary Risk Evaluation
SPARTE	Statégie de Prévention Cardiovasculaire Basée sur la Rigidité Arterielle
SV	Stroke volume
TB	Truncus brachiocephalicus
TOD	Target organ damage
TPRI	Total peripheral resistance index
WCSA	Wall Cross-Sectional Area
WHO	World Health Organization

Chapter 1 Introduction

According to WHO (World Health Organization) statistics, cardiovascular disease (CVD) is the main cause of morbidity and mortality worldwide.[1] In 2008, 17.3 million people worldwide died of CVD. It is estimated that in 2030 this number will reach 23 million. In Belgium the same pattern emerges: in 2009, 32 599 out of 103 816 deaths, were attributable to CVD, making it also here the leading cause of death.[2]

Based on the International Classification of Diseases (ICD-10), the most frequent types of CVD and their corresponding ICD-codes are[3]:

- Ischemic heart disease (I20-I25): e.g. angina pectoris, myocardial infarction, coronary atherosclerosis
- Other heart diseases (I30-I52): e.g. heart valve disease, cardiomyopathy, heart failure, left ventricular hypertrophy
- Cerebrovascular diseases (I60-I69): e.g. stroke (cerebral hemorrhage or infarction)
- Diseases of the arteries, arterioles and capillaries (I70-I79): e.g. peripheral artery disease (PAD), aneurysm, arterial embolism and thrombosis

Despite reaching epidemiological proportions, CVD is a non-communicable disease, meaning it is non-infectious and non-transmissible among people. CVD is also largely preventable and treatable, providing many routes for intervention. The latter is a consequence of the slow but progressive nature of the disease, building with time. The pathophysiology of CVD represents a continuum, including an early *preclinical* phase, which is hard to diagnose but more easy to reverse, and a late *clinical* phase, which can be easily diagnosed but is often irreversible.[4]

1.1 Pathophysiology of CVD

By definition, CVD involves diseases of the heart (*cardio*) and the blood vessels (*vascular*). However, since all organs (including the heart) rely on blood vessels to be nourished, at the root of CVD often lies a *vascular* problem. As a result, atherosclerosis is the leading cause of cardiovascular morbidity and mortality.[5]

Atherosclerosis is a disease of the innermost layer of the arterial wall or endothelium. It involves a gradual process, in which some key stages can be distinguished: (1) endothelial dysfunction, (2) lipoprotein deposition and formation of 'foam cells', (3) inflammation and plaque growth (4) fibrous cap formation and (5) plaque rupture (Figure 1.1).[6]

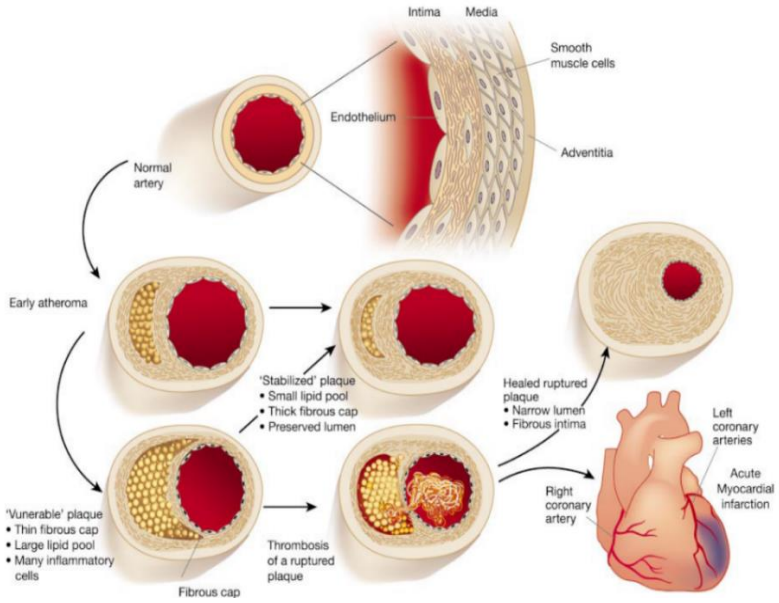


Figure 1.1 The pathogenesis of atherosclerosis. Adopted with permission from *Nature (2002)*[6]

Rupture or erosion of plaque material is the final and crucial stage in this process. This leads to a cascade of events, including activation of clotting factors and platelet aggregation, creating a thrombotic (hypercoagulable) state. The end-result is either a narrowed lumen (healing response), or worse, thrombus formation. The latter is responsible for the majority of fatal cardiovascular (CV) events.[7] When a thrombus completely blocks an artery, blood supply to downstream organs is restricted, causing ischemia and eventually loss of function. The implications depend on the location of the blockage, such as myocardial infarction (when coronary arteries are affected) or a stroke (cerebral arteries). In addition to thrombus formation, the arterial wall may also be significantly weakened to the extent an aneurysm forms, carrying a significant risk of rupture and internal bleeding.[8]

Whatever the outcome, all types of atherosclerotic disease are characterized by a silent development, often only revealing itself on an unexpected, fatal event before becoming symptomatic (e.g. angina pectoris, claudication). Fortunately, various tools are available to track the progression of atherosclerosis in its preclinical phase. Those are called *risk markers* or *biomarkers*. Ideally, a biomarker can be measured non-invasively (i.e. without significantly harming the human body) and yet shows a strong correlation with outcome. Furthermore, when a risk marker is also considered to play a more causative role, contributing to the disease process, it is called a *risk factor*.[9] Cardiovascular risk factors (CV-RFs) constitute the mainstay for CVD prevention, diagnosis, treatment and prognosis. Most of them are modifiable (e.g. smoking), while others are genetically determined (e.g. sex, family history), or a combination of the two (e.g. hypertension).

1.2 Risk factors for CVD

CV-RFs adopted by the European Society of Hypertension (ESH) are listed in Table 1.1.[10]

Table 1.1

Risk factors for CVD according to 2013 ESH guidelines.
Non-modifiable risk factors
Age: men ≥55 years; women ≥65 years
Sex: male
Family history of premature CVD: men aged <55 years; women aged <65 years
Modifiable risk factors
Hypertension: SBP ≥ 140 mmHg and/or DBP ≥ 90 mmHg
Smoking
Dyslipidemia: Total cholesterol > 190 mg/dL, and/or LDL cholesterol >115 mg/dL, and/or HDL cholesterol: men < 40 mg/dL, women < 46 mg/dL, and/or triglycerides > 150 mg/dL
Fasting plasma glucose: >102 mg/dL
Abnormal glucose tolerance test
Obesity: BMI ≥30 kg/m²
Abdominal obesity: waist circumference: men ≥102 cm; women ≥88 cm (in Caucasians)

SBP = systolic blood pressure. DBP = diastolic blood pressure. HDL = High-density lipoprotein. LDL = Low-density lipoprotein. BMI = body mass index

If an individual carries one or more of these CV-RFs, this indicates he is at greater risk for developing CVD. However, it may not necessarily be the *absolute value* of a single risk factor, but rather the *total number* of risk factors that matters.[11] To illustrate, a subject with very high cholesterol levels but otherwise no risk factors may have a much better prognosis than someone having mild hypertension *and* mild abdominal obesity. This synergistic effect of risk factors (i.e. the sum being greater than its parts) has led to the development of risk scores which integrate several risk factors into a single value. The classical example of this is the Framingham risk score (FRS), developed in the United States.[12] The FRS indicates the risk of cardiovascular disease in the next 10 years. In Europe, a similar system called "Systematic

COronary Risk Evaluation" (SCORE), indicates the probability of dying of CVD within the next 10 year.[13] Based on this evaluation, a physician can then decide whether to advise lifestyle modifications, medication, or more severe treatment strategies. SCORE charts have been calibrated for high-risk and low-risk countries, and sometimes even tailored to national mortality statistics and risk factor distributions, as was done in Belgium (Figure 1.2).

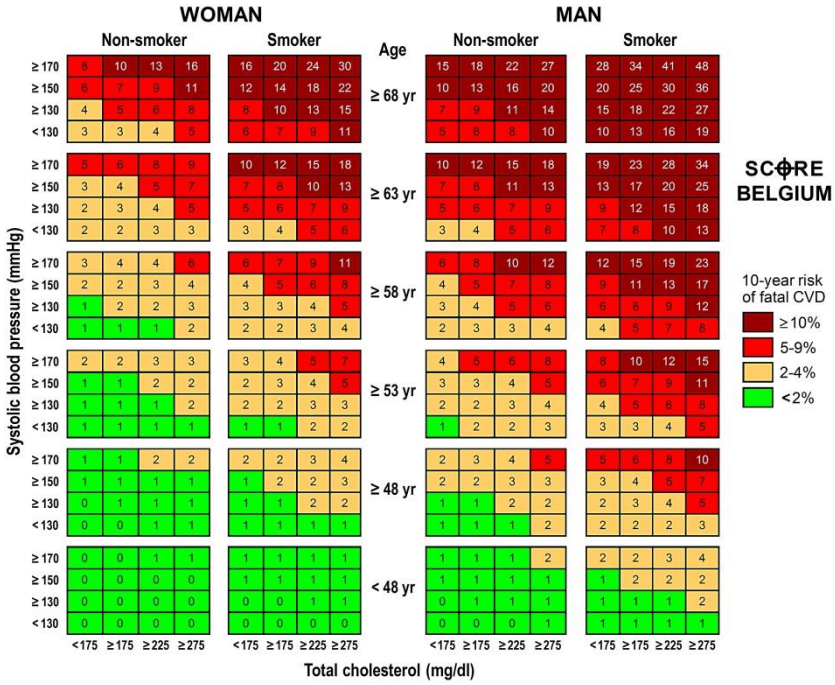


Figure 1.2 Belgian SCORE risk chart indicating the probability of dying from CVD within the next 10 year. Adopted with permission from the *International journal of cardiology.*[13]

Although this system has been validated on a large prospective study sample, it is still imperfect.[13] If a 5% risk score was used as a cut-off point for intervention, this would yield a sensitivity/specificity ratio of 72%/78%. Although superior to many other systems, this means a large number of subjects would still be misclassified. In particular, 28% of subjects at increased risk would not be classified as such, and hence be deprived of nec-

essary treatment. Conversely 22% of subjects at low risk would wrongly end up in the high-risk group, receiving unnecessary treatment. Anecdotal evidence supports this limitation. Everybody knows the apparently healthy runner dying of an unexpected CV event, and the chain smoker living on for decades. One of the reasons for these inconsistencies is that not everybody responds in the same way to his or her risk profile.[14] While some people are extremely sensitive to the effect of certain CV-RFs, others seem to be immune to them and escape their predicted fate. What is needed, therefore, is a better way to quantify the true *damage* from exposure to CV-RFs. In other words, measures have to be established to identify those subjects in which CV-RFs are translated into real risk. This led to the development of target organ damage (TOD) as a tool for risk stratification.

1.3 Target organ damage

Asymptomatic organ damage is now considered an intermediate end-point in the continuum of CVD. It represents a state in which long-term exposure to CV-RFs has led to significant damage to a certain organ (e.g. the heart, brain or kidneys), but without any clear symptoms or complaints. Measures of TOD adopted by the ESH are listed in Table 1.2.

Table 1.2

Measures of TOD according to 2013 ESH guidelines.
Pulse pressure: (in the elderly) ≥60 mmHg
Electrocardiographic LVH: Sokolow–Lyon index >3.5 mV; RaVL >1.1 mV; Cornell voltage duration product >244 mV*ms
Echocardiographic LVH: LVM index: men >115 g/m²; women >95 g/m² (BSA)
Carotid wall thickening: IMT >0.9 mm or plaque
Carotid–femoral PWV: >10 m/s
Ankle-brachial index: <0.9
CKD with eGFR: 30–60 ml/min/1.73 m^2 (BSA)
Microalbuminuria: 30–300 mg/24h, or albumin–creatinine ratio: 30–300 mg/g; 3.4–34 mg/mmol

LVH = left ventricular hypertrophy. LVM = left ventricular mass. BSA = body surface area. IMT = intima-media thickness. PWV = pulse wave velocity. CKD = chronic kidney disease. eGFR = estimated glomerular filtration rate.

Four markers [microalbuminuria, increased carotid-femoral pulse-wave velocity (PWV), left ventricular hypertrophy (LVH) and carotid plaques] have been shown to carry additional predictive value above and beyond SCORE classification.[15] Moreover, similar to the cumulative effect of CV-RFs, risk increases as the number of damaged organs goes up.[16] As such, asymptomatic organ damage is explicitly included in the ESH/ESC guidelines, and is receiving increasingly more attention with every update.[10]

This manuscript will focus on two specific types of vascular TOD, i.e. (1) carotid wall thickening (or more broadly, 'arterial wall thickening'), and (2) carotid-femoral PWV (or more broadly, 'arterial stiffness'). Both will be discussed in more detail in the following sections, including their definition and predictive value.

1.3.1 Arterial wall thickening

1.3.1.1 Definition

When an atherosclerotic plaque is growing, this automatically leads to thickening of the arterial wall. Hence, measuring wall thickness allows directly assessing the atherosclerotic damage at a certain vascular site. Since the presence of atherosclerosis at one location correlates with atherosclerosis elsewhere along the arterial tree (e.g. at the coronary arteries), local wall thickening can be used as a proxy for systemic atherosclerosis.[17] Arterial wall thickening is usually assessed at the carotid artery, and to a lesser degree at the femoral artery. Different phenotypes include intima-media thickness (IMT) and plaque (Figure 1.3).

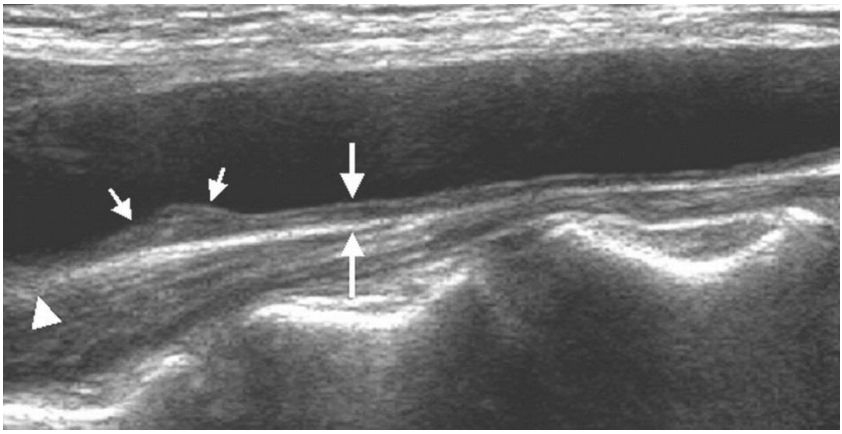


Figure 1.3 Example of a carotid ultrasonogram. Indications represent common carotid artery-IMT (*large arrows*), carotid plaque (*small arrows*) and the carotid bifurcation (*arrowhead*). Adopted with permission from *American Journal of Neuroradiology*.[18]

IMT is a measure of the thickness of the two innermost layers of the arterial wall, i.e. the intima and media. The concept was first described in 1986 by Panoli *et al*,[19] employing ultrasound. By taking advantage of the echogenic properties of the adventitia and intima, it is possible to measure the thick-

ness of the layer in between adventitia and the lumen, which is the intima+media. Nowadays, high-resolution echotracking is used for this purpose, yielding a resolution of 21μm.[20] The rationale behind measurement of IMT is that, at the carotid artery, intima-medial thickening is closely related to intimal thickening which may be an early feature of atherosclerosis. Microscopic examinations on samples derived from autopsy confirm IMT is a valid surrogate for intimal thickness, at least for the carotid artery.[19] However, intimal thickening is not always malignant, as in intimal hyperplasia and intimal fibrocellular hypertrophy.[21] In addition, particularly at the femoral artery, medial thickening may also be responsible for increased IMT (e.g. in resistance-trained athletes, whose arteries are often under extremely high pressures).[22] An overview of the possible confounding factors leading to an increased carotid IMT is provided in Table 1.3. The existence of these confounders represents the major limitation of IMT as a measure of vascular TOD. However, the cut-off value for increased carotid IMT as proposed by consensus guidelines, and adopted by the ESH, (i.e. > 900 μm) is of such large size that at least some degree of atherosclerosis is assumed to be present. For femoral IMT, no such threshold has been established, making it hard to distinguish benign from malignant arterial remodeling.

Table 1.3

Possible confounding factors linked with an increased carotid IMT.
Increased tensile stress (e.g. in athletes): carotid IMT is greater in elite footballers.[23]
Blood flow effects: carotid IMT is associated with low levels of shear stress.[24]
Heritability: IMT is more heritable than plaque score and maximal stenosis, suggesting IMT reflects (genetic) differences in structure, rather than the (environmental) impact of atherosclerosis.[25]
Response to radiotherapy: IMT is increased following radiation of the neck. However, this increase diminishes with time and may therefore reflect acute rather than chronic (i.e. atherosclerotic) changes.[26]

Plaque refers to a thickening of the intima-media layer to the extent a significant lesion protrudes into the lumen. According to consensus guidelines, a lesion must fulfil one of the following three criteria to be considered a plaque[27]

- Encroaching at least 0.5 mm into the arterial lumen
- Exceeding the surrounding IMT value by 50%
- Demonstrating an absolute thickness of 1.5 mm

In contrast to IMT, plaque is a more direct measure of atherosclerosis,[28] less confounded by the secondary factors listed in Table 1.3.

However, overall, ultrasound imaging may not be the best way to investigate the extent of atherosclerotic burden, as deep arteries are not well suited, and so are calcifications.[29]

1.3.1.2 Predictive value

The predictive value of arterial wall thickening is highly dependent on the arterial territory (carotid or femoral).

At the ***carotid artery***, both increased IMT and presence of plaques predict incident CVD, independent of each other.[30] In addition, progression over time may be deterred by targeted interventions.[31] However, measurement

of carotid IMT in clinical practice is questionable, since it does not lead to a significant reclassification above FRS or SCORE.[32] In addition, two meta-analyses have been published showing no association between carotid IMT and cardiovascular risk.[33,34] Plaques, on the other hand, more accurately predict coronary artery disease,[35–37] and provide additional information on top of FRS or SCORE.[16] This may reflect the notion that a plaque constitutes a more advanced stage in the continuum of an atherosclerotic lesion,[38] while the meaning of an increased IMT is still controversial.[39]

For the ***femoral artery***, outcome studies are scarce, and the incremental value of femoral IMT and femoral plaque has been demonstrated only once.[40] As a result, the predictive value is weaker compared to the carotid artery. However, femoral IMT and femoral plaques have been shown to correlate with atherosclerosis of the coronary arteries,[41–43] and with LV mass.[44] In the Asklepios study, femoral artery plaque showed the strongest association with oxidized LDL levels, independent of carotid artery plaque or IMT.[45]

1.3.2 Arterial stiffness

1.3.2.1 Definition

Arteries provide the circuit for the heart to distribute blood. But besides their conduit function, they also act as a buffer to cushion large pulsations generated by the heart and transform these into a steady blood flow.[46] This is particularly relevant for the large elastic arteries, such as the aorta and the carotid arteries.[47] However, through repetitive cycles and aggravated by oxidative stress,[48] arteries may show signs of 'material fatigue', characterized by a loss of elasticity.[49] This phenomenon has several unfavorable implications for the human body as a whole. When arteries are stiffened, this puts an increased burden on the heart, which has to work harder against an

elevated afterload.[50] In addition, the loss of buffering function results in transmission of large pulsations into the microcirculation, which may induce remodeling of arterioles[51] or cause damage to the capillaries of e.g. the brain, kidney or eye.[52]

Thus, arterial stiffness is at the same time a *consequence* of damage done to the vasculature, but also a *cause* of further harm, constituting an intermediate end-point.[53] Therefore, the importance of assessing arterial stiffness for risk classification cannot be overestimated. However, there is no single measure of a person's arterial stiffness. Because the arterial tree is composed of heterogeneous arteries, varying in histologic and/or elastic properties,[54] the 'arterial stiffness' will differ depending on the specific location.[54] When interpreting stiffness measurements, the context (i.e. its location) is therefore of critical importance. For example, stiffness measured at the carotid artery is often referred to as 'elastic artery stiffness', while the same measurement done at the femoral artery is considered 'muscular artery stiffness'. Comparing femoral to carotid stiffness would be like comparing apples to oranges. The same holds true for e.g. carotid-femoral and brachial-ankle stiffness, which span different segments with differing elastic properties, and therefore should not be used interchangeably.

Another (more terminological) distinction that deserves some attention is the one between compliance and distensibility as measures of arterial stiffness. Both terms are used quite randomly (and often incorrect) in literature. However, although compliance is related to arterial stiffness, it is actually a measure of the buffering capacity of the artery, which is also dependent on the vessel caliber. Therefore, arterial distensibility, which is less dependent on arterial dimensions, can be considered a better marker for (the inverse of) arterial stiffness.

In what follows, arterial stiffness measures will be further classified depending on the length of the segment under consideration. As such, we distinguish ***local***, ***regional*** and ***global*** stiffness measures.

Local stiffness is defined as the arterial stiffness of a particular cross-sectional site. It can be determined on almost all superficial large and medium-sized arteries (e.g. on the brachial, carotid and femoral artery) using ultrasound. Because of limited resolution, ultrasound is not well suited to measure local stiffness of deeper lying arteries (e.g. the aorta).[55] However, by reducing the distance between transducer and artery (e.g. using transesophageal echocardiography, TEE)[56] or by using non-ultrasound-based methods (e.g. magnetic resonance imaging, MRI) it is possible to measure local stiffness of the aorta.[57] Nevertheless, these techniques are not widely applied and local stiffness is most frequently examined using ultrasound on the carotid artery (to measure elastic artery stiffness) and femoral artery (to measure muscular artery stiffness). Elastic arteries are probably the most interesting to consider, since these are abundant in elastin molecules, which are prone to degeneration due to ageing or oxidative stress.[58] However, knowing the stiffness of the femoral (muscular) artery may as well provide complementary information. Muscular artery stiffness may reflect the status of smooth muscle cells, regulating vascular tone.[59] In addition, it has been postulated that when elastic arteries lose their elasticity, their buffering function is transferred to muscular (e.g. femoral) arteries, which limit the loss of compliance through an increase in diameter.[60,61]

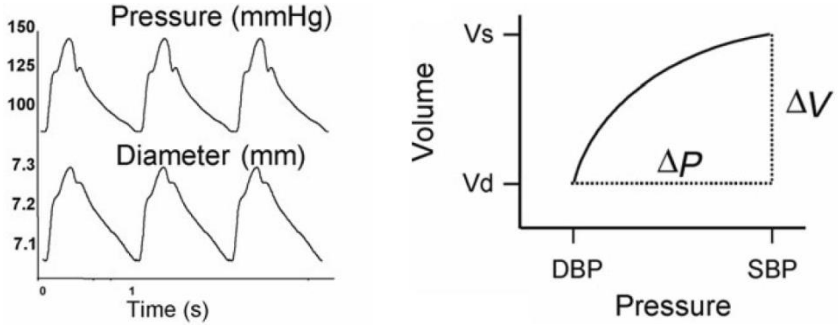


Figure 1.4 Overview of pressure and distension waveforms aligned in time (left), resulting in an approximation of the pressure-volume relationship between diastole and systole (right).

Measuring local stiffness of an artery requires knowing the relative change in volume, for a given change in pressure (Figure 1.4). This yields a complete description of the pressure-volume relationship (or more correctly, the pressure-cross-sectional area relationship, as we will see further)

Because of the phenomenon of pulse pressure amplification[1], brachial pulse pressure (PP) should not be used for calculation of local stiffness. However, at most superficial arteries, it is impossible to measure the local PP using conventional methods. Instead applanation tonometry is employed, which allows to capture the arterial pressure wave shape at a particular arterial (e.g. femoral or carotid) site. This, however, only yields a curve without reliable absolute levels of arterial pressure. To overcome this, a calibration scheme is employed:

[1] The pulse pressure (PP) at a given location is the amplitude of the blood pressure wave. This wave has a forward and backward component, the latter arising from wave reflections. The closer to the reflection sites (i.e., the further in the periphery), the earlier the forward and backward waves interact, boosting the amplitude of the blood pressure wave. Hence, PP will physiologically increase going from central (e.g. carotid artery (ca)) to peripheral (e.g. brachial artery (ba)) arteries. The central-to-brachial pulse pressure amplification is calculated as PPba/PPca.

1/ Blood pressure is measured at the brachial artery, yielding the brachial PP.

2/ Waveforms are measured at the brachial artery, yielding a brachial form factor (FF), which is a measure of how peaked the waveform is (see Figure 2.2 on p32).[62] Brachial FF is calculated using formula 1.1

$$\text{Brachial FF} = (\text{MAP} - \text{DBP}) / \text{Brachial PP} \quad (1.1)$$

In which FF = form factor, MAP = mean arterial pressure, DBP = diastolic blood pressure, PP = pulse pressure.

3/ Waveforms are also measured at the local (e.g. carotid or femoral) level, yielding a local FF.

$$\text{Local FF} = (\text{MAP} - \text{DBP}) / \text{Local PP} \quad (1.2)$$

4/ Assuming (MAP - DBP) will hardly change across the arterial tree,[63] equations (1.1) and (1.2) can be merged, yielding formula (1.3) to calculate local PP

$$\text{Local PP} = (\text{brachial FF} / \text{local FF}) \times \text{Brachial PP} \quad (1.3)$$

Since applanation tonometry is not applicable in all individuals (particularly due to obesity),[64] arterial distension waves can also be used as an alternative to calculate local PP. The approach is similar, with the only exception that the FF's from equations (1.1) and (1.2) are then derived from distension curves instead of pressure curves.[65]

Volume and volume change are approximated by cross-sectional area and cross-sectional area change respectively, assuming that longitudinal movement of the vessel wall is negligible.[66] These can be determined using ultrasound. In particular, algorithms based on echotracking have been devel-

oped, which allow to accurately (resolution = 1.7 µm)[67] follow displacement of arterial wall in time. This yields measures of diastolic diameter, systolic diameter, and distension, which is the difference between these two. When diameter, distension and PP are known, functional wall properties can be calculated. As noted above, it is important to make a clear distinction between cross-sectional compliance (CC), which is an indicator of the buffering capacity, and the distensibility coefficient (DC) as a measure of elasticity (or the inverse of stiffness). From (1.4) and (1.5) we can deduce that CC relates to DC as CC = DC x A. In other words, compliance is the product of elasticity and total cross-sectional area.[61]

$$CC = \frac{\Delta A}{\Delta P} = \frac{\pi \text{ x } (Ds^2 - Dd^2)}{4 \text{ x } \Delta P} \tag{1.4}$$

$$DC = \frac{\frac{\Delta A}{A}}{\Delta P} = \frac{(Ds^2 - Dd^2)}{Dd^2 \text{ x } \Delta P} \tag{1.5}$$

In which, ΔA = change in arterial cross-sectional area at a given location; ΔP = local pulse pressure (PP) at a given location; D_s = arterial diameter at end-systole; D_d = arterial diameter at end-diastole; A = arterial cross-sectional area at end-diastole.

Regional stiffness corresponds with the stiffness of a large or medium-sized segment, often containing multiple arterial beds. It always refers to a measure of (the inverse of) distensibility. The stiffness of a certain arterial region can be quantified using the concept of pulse-wave velocity (PWV), which is based on the assumption that waves are transmitted faster through a segment with stiff vessel walls than through a segment with distensible walls. From the Moens-Korteweg[68] (1.6) and Bramwell-Hill[69] (1.7) equations,

it follows that PWV is inversely proportional to the elasticity of the vessel wall.

$$PWV = \sqrt{\frac{h \times E_{inc}}{\rho \times D}} \quad (1.6)$$

In which, h = wall thickness, E_{inc} = incremental elastic modulus, ρ = blood density and D = lumen diameter

$$PWV = \sqrt{\frac{1}{\rho \times DC}} \quad (1.7)$$

In which, ρ = blood density and DC = cross-sectional distensibility coefficient

PWV can be measured between any two arterial sites, but the vast majority of the studies focus on carotid-femoral PWV (cf-PWV). This is because 1) carotid and femoral arteries are easily accessible and 2) in between carotid and femoral artery lies the aorta, which is of major interest. Indeed, the aorta and its primary branches are what the heart *sees* and is thus most affected by.[70] In addition, the aorta is made up of (mainly) elastic tissue (with exception of the abdominal aortic and iliac part), which is more sensitive to the effects of ageing and CV-RFs compared to muscular wall material.[71] Therefore, the gold standard method for assessing regional stiffness is carotid-femoral PWV. (Figure 1.5)

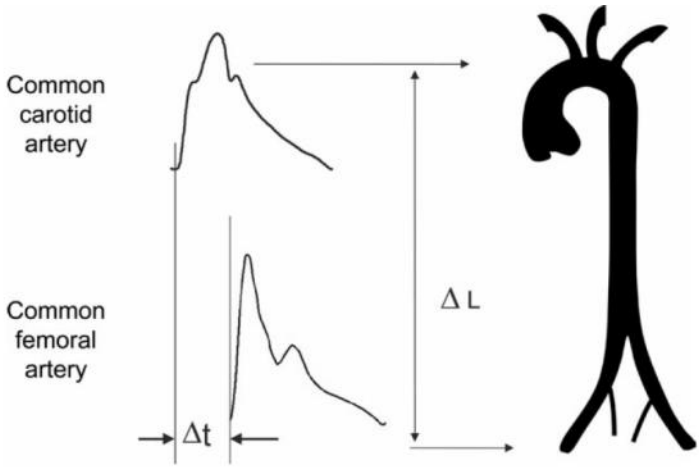


Figure 1.5 Overview of the measurement locations along the arterial tree, accompanied by sample pressure waveforms (left) obtained at either site. ΔL represents the direct distance, Δt the transit time.

To measure cf-PWV, travelled distance (ΔL) is divided by transit time (Δt), or

$$\mathrm{PWV} = \frac{\Delta \mathrm{L}}{\Delta \mathrm{t}} \tag{1.8}$$

Transit time can be measured non-invasively by detection of pressure, flow or distension waves at each respective site.[72] This can be done simultaneously or sequentially, by gating both signals to the R-top of an electrocardiogram. Travelled distance is harder to estimate non-invasively. The arterial travelled paths cannot be seen from the outside and have to be approximated by superficial measurements. In addition, a correction factor needs to be incorporated, since waves are travelling at the same time in opposite directions.[73] This is dealt with by measuring the direct distance between femoral and carotid measurement sites, and then multiplying this number with 0.8, which is known as the "80%-rule".[74]

Global stiffness refers to the stiffness of the entire arterial tree. However, the term 'stiffness' is actually a misnomer here, since this always corresponds with a measure of the total arterial compliance. Therefore, the term 'systemic arterial compliance' (SAC) would be more appropriate. SAC can be approximated by looking at the pressure change (PP) for a given stroke volume (SV), or

$$\mathrm{SAC} = \frac{\mathrm{SV}}{\mathrm{PP}} \tag{1.9}$$

However, this is merely an approximation, since it assumes the entire stroke volume is stored in the large elastic arteries, neglecting peripheral outflow. To more accurately determine TAC, three- or four-element Windkessel models should be employed.[75,76] This strategy is used by a commercially available device, which combines information of blood flow, pressure and pressure decay to obtain the SAC.[77] Another device provides estimates of large artery compliance (C1) and small artery compliance (C2), based on the decay of the radial pressure waveform alone.[78,79] However, its working principle has been called into question.[80] A more detailed description of techniques to measure SAC and their limitations is beyond the scope of this thesis.

1.3.2.2 Predictive value

Regional stiffness, and in particular cf-PWV, has the most firmly established predictive value of all stiffness measures. This was demonstrated in large epidemiological studies[81] and in two meta-analyses.[82,83] Recently, it has been shown that cf-PWV improves incident CVD prediction (both stroke, CHD and CV death) beyond classical risk factors.[82] In addition, Guerin et al. have shown that reduction of cf-PWV (or rather the absence of a reduction) predicts outcome in end-stage renal disease patients, suggesting cf-PWV is a

good surrogate end-point for all-cause and CV mortality.[84] Therefore, cf-PWV is considered the gold standard measure of aortic stiffness.[85]

Local stiffness has also been shown to carry prognostic value, but this depends on the type of artery under consideration (elastic/muscular).

Most studies so far have focused on the carotid artery stiffness. The ARIC[86] and Hoorn studies[87] showed associations between lower carotid DC (i.e. increased stiffness) and incident stroke,[86] incident CV events[87] and all-cause mortality,[87] independent of CV-RFs and cf-PWV. In addition, some earlier studies in small samples[88–90] or in specific (patient) populations[91–93] have shown predictive value of carotid DC for CVD and/or mortality, whereas others did not.[94–97]

Femoral artery stiffness is not commonly incorporated in vascular measurement protocols, and is in general less extensively studied. As such, its predictive value is less well established. However, recent data from the Hoorn study showed that the predictive value of local carotid artery stiffness was extended to the femoral artery.[87] Furthermore, in patients in the early stages of metabolic disease, such as diabetes[98] and mild non-familiar hypercholesterolemia,[99] femoral stiffness has been reported to be increased whereas aortic and carotid stiffness were not altered. In addition, femoral artery stiffness is closely associated with prevalent lower limb arterial disease,[100,101] which is a clinically important cardiovascular outcome.

A meta-analysis on the predictive value of local stiffness has not been published so far. Therefore, all available outcome studies that measured carotid and/or femoral artery stiffness are listed in Table 1.4.

Table 1.4 Overview of outcome studies measuring local arterial stiffness.

First author (year, country)	Follow-up (year)	Type of patient (number)	Mean age (year)	Low DC group	Outcome measure	Adj. HR (95% CI)	Ref
Carotid artery							
Blacher (1998, Fr)	2.1	ESRD (79)	58	Q1 (lower quartile)	All-cause mortality	6.4 (1.8-23.3)	90
Barenbrock (2001, Ge)	7.9	ESRD (68)	43	- 1 x 10^{-3} kPa^{-1}	CV events	1.27 ($p<0.05$)	89
Störk (2004, Nl)	2.0	Elderly (367)	78	- 1 x 10^{-3} kPa^{-1}	All-cause mortality	1.01 (0.95-1.08)	93
					CV mortality	1.05 (0.94-1.16)	
Haluska (2010, Au)	7.3	General population (373)	55	< 24 x 10^{-3} kPa^{-1}	All-cause mortality	1.85 (1.10-3.13)	91
Karras (2012, Fr)	5.0	CKD (439)	60	- 1 SD	mortality	1.62 (1.17-2.23)	88
					CV events	1.69 (1.31-2.17)	
Yang (2012, Fr)	13.8	General population (10 407)	55	- 1 SD	Stroke	1.19 (1.02-1.39)	86
					CHD	1.01 (0.94-1.09)	
van Sloten (2014, Nl)	7.6	General population (579)	67	- 1 SD	All-cause mortality	1.51 (1.11; 2.06)	87
					CV mortality	1.22 (0.95-1.56)	
Femoral artery							
van Sloten (2014, Nl)	7.6	General population (579)	67	- 1 SD	All-cause mortality	1.27 (0.90-1.79)	87
					CV mortality	1.39 (1.06-1.83)	

ESRD = End-stage renal disease. CKD = chronic kidney disease. CHD = Coronary heart disease. Adj. HR = Adjusted hazard ratio. CI = confidence interval. SD = standard deviation.

Systemic stiffness, in the form it is represented here, has the least number of studies showing predictive value. However, SV/PP predicts CV morbidity in hypertensives,[102] and CHD mortality in elderly men.[103] In the Hoorn study, SAC was calculated using the SV/PP and the pressure decay method, but neither was predictive for cardiovascular or all-cause mortality. In the MESA study,[87] decreased C2 (small artery compliance), but not C1 (large artery compliance) correlated with CVD.[104] However, the exact meaning of these parameters is not fully understood and debated.

1.4 Problem statement and aims of the thesis

Both arterial wall thickening and arterial stiffness have predictive value and can be measured with high reproducibility.[105] However, their clinical applicability is limited. Nowadays primary prevention is still based on classical CV-RFs and in general focused on normalizing arterial blood pressure and lipid profiles. Even the most advanced measure, cf-PWV, has barely made its way into the doctor's office. Among other factors, obstacles impeding translation to clinical practice include the (often) time-consuming measurement procedure, lack of methodological consensus and the absence of reference values. Therefore, the general aim of this thesis is to bring non-invasive measurements of vascular TOD to routine clinical practice, by helping them overcome current obstacles. This aim will be approached from five different angles, corresponding with five specific study objectives. (Table 1.5)

Table 1.5

Five specific study objectives of the thesis.
1) to investigate the impact of body *side* and *size* on carotid-femoral-PWV.
2) to investigate the left-right distribution of carotid and femoral atherosclerosis.
3) to establish normal values for carotid artery stiffness.
4) to establish normal values for femoral artery stiffness.
5) to examine the utility of cardiovascular structure and function measurements in patients with normal-tension glaucoma.

Study objective n°1

In recent years, much energy has been invested in making cf-PWV more applicable to the clinic and to research in general. Reference intervals exist[106] and consensus operating procedures have been established.[74] The purpose of this study is therefore limited to merely fine-tuning of these already quite detailed guidelines. To provide context, current guidelines are tabulated on the next page (Table 1.6).[74]

Table 1.6

Consensus guidelines for measurement of cf-PWV.[74]
1) Measurements should be performed in a quiet room with stable room temperature.
2) Perform measurements in supine position after at least 10 min of rest.
3) Measurements should preferentially be done at the right common carotid and common femoral arteries.
4) Because of diurnal variations repeated measurements should be done at the same time of the day.
5) No meal, caffeine or smoking is allowed within 3 h before measurement.
6) Speaking and sleeping are not allowed during measurements.
7) Data should be mean of registrations during at least one respiratory cycle (about 5–6 s).
8) Be aware of possible white coat effects.
9) Measure distance in a straight line. If not possible with a tape measure, the upside-down use of an infantometer may be helpful.
10) Take mean of at least two measurements; if difference between the two measurements is more than 0.5 m/s, perform a third measurement and take the median value.
11) Situations in which measurement of cfPWV should not be performed: arrhythmia, unstable clinical situation, high-grade stenosis of carotid artery, carotid sinus syndrome.

As can be interpreted from guideline 3 (Table 1.6), measurements should be done on the right side of the body. However, this is an arbitrary choice, and there are no indications left-sided arteries are less well suited for measurements. It is conceivable that both sides cannot be used interchangeably, (bearing in mind the asymmetric architecture of the arterial tree), but this should be investigated. Indeed, a substantial difference in anatomy exists between the left and right common carotid artery, which differ in their origin, and between right and left iliac-femoral path, the latter of which is expected to be shorter (since the aortic midline is on average oriented to the left of the body).[107] However, the impact of these asymmetries on the difference in total real travelled path length between left and right side is currently unknown. Apart from these bilateral differences in arterial geometry, another source of error in cf-PWV may be introduced by differences in body contours. Particularly in obese people, large abdomen and/or breast size may have a substantial impact on the distance

measured by tape measure, while the real (intra-arterial) distance is considered unaffected. As pointed out in guideline 9 (Table 1.6), it is therefore recommended to make use of a sliding caliper such as an anthropometer if measurement in a straight line is impossible with a tape. The size of the error introduced when using a tape has been examined in only one study, in which the authors concluded that it is crucial to *always* use a sliding caliper for distance measurements.[108] This finding is (partially) in contrast with the guidelines, which only offer the *option* to use an anthropometer, but do not necessitate this in all subjects. Therefore, this topic deserves further investigation, including a comparison with the 'real' intra-arterial distances. As such, study objective n°1 is to investigate the impact of body *side* and body *size* on cf-PWV.

Study objective n°2

For arterial wall thickening (IMT/plaque), the level of clinical applicability is similar to that of cf-PWV. Reference values have been established,[109] and updated guidelines are available for measuring carotid IMT and plaque.[27] To provide context, these guidelines are tabulated on the next page (Table 1.7).

Table 1.7

Consensus guidelines for measurement of IMT.[27]
1) Edge detection systems provide accurate measurements of IMT, and should be preferred above manual measurements, which are more observer dependent and more time-consuming
2) Inter-adventitial and intraluminal diameters should also be measured as IMT is significantly correlated with arterial diameter.
3) Mean IMT values averaged across the entire distance are less susceptible to outliers, whereas the maximal IMT may reflect more advanced stages with focal thickening or plaque formation. IMT values from the left and right side can be averaged although there is a significant difference between the left and right CCA IMT, with higher values on the left side.
4) Vascular laboratories should always report intra-class correlation coefficients for intra- and inter-observer variability, both for IMT and plaque measurements.

However, there is still room for improvement. Guideline 3 (Table 1.7), providing instructions on the measurement side, may cause confusion among operators. If there is indeed a significant difference between right and left carotid IMT, then a clear distinction should be made between measurements done on the left, right or both sides. Standardization with regard to body side is often poor: although some studies report measurements of only one side of the body,[110,111] others show both values,[112,113] or average out the IMT from left and right side.[114] The suggestion of a higher left common carotid IMT, as proposed by the guidelines (Table 1.7), was based on two studies.[115,116] However, other studies do not show a significant left-right difference in carotid IMT,[112,117] or plaque prevalence.[118] Reference values for carotid IMT also do not make a distinction between left and right side IMT,[109] although they should if there is indeed a substantial difference. Therefore, this topic deserves further investigation. In addition, no guidelines have been established for femoral IMT, for which some studies also noted a difference between left and right side.[42,119] Therefore, study objective n°2 is to investigate the left-right distribution of atherosclerosis at the carotid and femoral artery.

In addition, comparing left to right-sided arteries provides a paired test to assess the influence of local geometry on atherosclerosis. Results of such comparison may improve our mechanistic understanding of how local factors influence the progression of an atherosclerotic lesion.

Study objective n°3

Measuring local arterial stiffness is more cumbersome than regional stiffness (cf-PWV), and its predictive value is less well established. As a result, local stiffness measurements are much further away from implementation in routine clinical practice. Accordingly, more work needs to be done towards clinical implementation. In particular, at present, no normal or reference values have been established for carotid stiffness. This makes it hard to interpret individual measurements obtained in clinical practice or research. Therefore, study objective n°3 is to establish normal values for carotid artery stiffness, based on a pooled dataset of various European cohorts.

Study objective n°4

With the same underlying motivation as described for study objective n°3, study objective n°4 is to establish normal values for femoral artery stiffness. In addition, such analysis may significantly improve our understanding of arterial physiology. By setting up normal values for carotid and femoral artery stiffness, in a synchronized and uniform fashion, a standardized comparison can be made between two histologically quite distinct arteries.[54] Identifying similarities and discrepancies between muscular and elastic artery stiffness, including their relationship with age, CV risk factors, and underlying mechanisms, is therefore a secondary goal of these studies.

Study objective n°5

The 5th and final specific study objective of this thesis can be regarded as an application of all of the above. In particular, this study involves looking at the utility of cardiovascular structure and function measurements in patients with vascular dysregulation. To this aim, a specific population of Normal-tension glaucoma (NTG) patients will be recruited for which there are indications they might have an altered vascular phenotype.[120] Glaucoma is the second leading cause of blindness worldwide[121] and is characterized by typical damage to the optic nerve head, termed 'glaucomatous optic neuropathy'. NTG, a subtype of the disease, represents a challenge to researchers.[122] Unlike the majority of glaucoma sufferers, NTG patients exhibit intra-ocular pressures within the normal range, suggesting other (systemic) factors are involved.[123] However, there is a lot of controversy around the true identity of these factors. It is now accepted that NTG patients exhibit vascular dysregulation, i.e. an inappropriate response to certain stimuli.[124] But the actual mechanism has not been revealed yet. Therefore, study objective n°5 is to examine the utility of cardiovascular structure and function measurements in patients with normal-tension glaucoma.

Chapter 2 Methods

To answer the research questions and study goals posed in chapter 1, several methods and population samples were used, which are described in more detail in sections 2.1 and 2.3 respectively. Table 2.1 provides an overview of when each method/population is applied in the following chapters.

Table 2.1 Methods and populations used in this thesis.

	Chapter 3	Chapter 4	Chapter 5	Chapter 6	Chapter 7
Methods					
Standardized measurement conditions					X
Blood pressure					X
Local arterial stiffness			X	X	X
Local pulse pressure			X	X	X
Regional stiffness					X
Wave reflections					X
Augmentation index					X
Reflection magnitude					X
Total peripheral resistance					X
Cardiac output					X
Intima-media thickness		X			X
Plaque		X			
Real travelled path lengths	X				
Magnetic resonance imaging	X				
MRI post-processing	X				
Populations					
Asklepios population		X	X	X	
NTG patients and matched controls					X
Healthy volunteers eligible for MRI	X				
Various other population samples			X	X	

MRI = magnetic resonance imaging. NTG = Normal-tension glaucoma.

2.1 Description of the methods

2.1.1 Standardized measurement conditions

Hemodynamic measurements were done in supine position and under standardized conditions (derived from the Task Force III, clinical applications for arterial stiffness)[125] in a temperature controlled room (22±1°C). Subjects were asked not to eat, smoke, and drink caffeine containing beverages for at least 3h before and during the measurements. They also had to refrain from drinking alcohol for at least 10h before measurements.

2.1.2 Blood pressure

Supine brachial SBP and DBP and heart rate (HR) were recorded at the dominant arm with a validated semi-automated oscillometric device (OMRON M6, OMRON Healthcare, Hoofddorp, The Netherlands). Mean arterial pressure (MAP) was calculated by taking the area under the curve (AUC) of scaled brachial artery pressure waveforms (PWFs) obtained by applanation tonometry.

2.1.3 Local arterial stiffness and buffering capacity

Arterial cross-sectional compliance (CC, a measure of the buffering capacity) and distensibility coefficient (DC, the inverse of the stiffness) were calculated using the formulas (1.4) and (1.5), shown in section 1.3.2.1

Diastolic external diameter (D) and change in diameter during the heart cycle were measured on the right common carotid artery and on the right common femoral artery, 2 cm proximal to the bifurcation. For this purpose, a 10 MHz pulsed ultrasound echotracking system (Wall Track system®, AU5, Esaote Pie Medical, Maastricht, The Netherlands) was used. This system allows analyzing radiofrequency signals originating from an M line perpendicular to the longitudinal axis of the artery, selected on the two-dimensional B-mode image.[126]

2.1.3.1 Local pulse pressure

Carotid and femoral PP were obtained by recording local pressure waveforms (PWFs) non-invasively and calibrating them using brachial artery DBP and MAP.[127] PWFs were obtained using applanation tonometry (Sphygmocor®, AtCor Medical, Sydney, Australia). Pulse-pressure amplification was calculated by dividing peripheral (brachial) over central (carotid) PP.

2.1.4 Regional stiffness

Regional stiffness was quantified by the carotid-to-femoral pulse wave velocity (cf-PWV). Cf-PWV was calculated using the 80%-rule, i.e. 0.8 x direct carotid-femoral distance/transit time. To calculate the transit time, pressure waveforms were obtained non-invasively at the common carotid artery and the common femoral artery using applanation tonometry (Sphygmocor®, AtCor Medical, Sydney, Australia). The transit time was then the time delay between the feet of the 2 waveforms, which were identified using the intersecting tangents algorithm.[128] The travelled distance was estimated by taking the surface distance between the recording sites in the supine position using a tape measure, or anthropometer (Figure 2.1) if a straight line could not be obtained.

Figure 2.1 Image of a sliding caliper or anthropometer.

2.1.5 Wave reflections

Pressure waves have a forward and backward component, the latter arising from wave reflections.

2.1.5.1 Augmentation index

Augmentation index (AIx) has been proposed as a surrogate measure for wave reflection. Central augmentation index (AIx) was calculated from the carotid PWFs as the ratio of the amplitude of the pressure wave above its systolic shoulder, or P2/P1. Carotid AIx is a surrogate for aortic AIx.[129] Although AIx is being widely used, it is a rather poor measure of the magnitude of wave reflections (due to its dependency on wave speed, heart rate and height).[130]

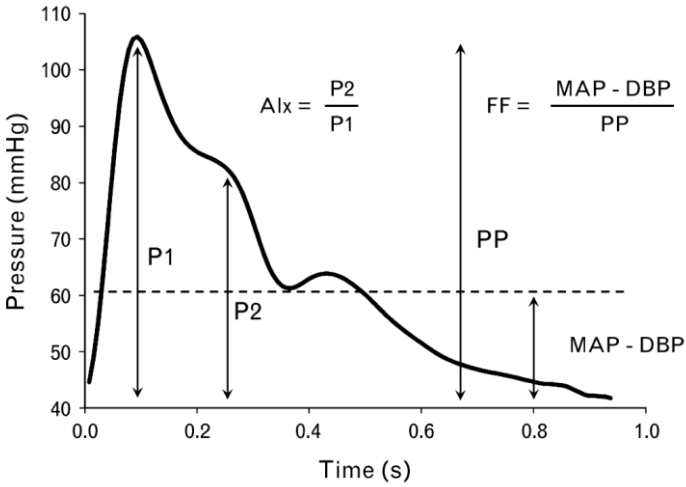


Figure 2.2 Arterial pressure wave, indicating the first (P1) and second (P2) inflection points used to calculate the augmentation index (AIx) [131]

2.1.5.2 Reflection magnitude

The reflection magnitude (RM) has been proposed as a more accurate measure, with a better prognostic value than AIx.[132] The RM is calculated by taking the

ratio of the backward and forward pressure wave (2.1), obtained through wave separation analysis.

$$RM = P_b/P_f \tag{2.1}$$

In which P_b is the amplitude of the backward pressure wave, and P_f the amplitude of the forward pressure wave.

Wave separation requires knowing both pressure and flow, preferably measured simultaneously on the same location. When no flow data is available, RM can also be estimated based on information of the pressure curve alone, as in the 'triangulation method'.[133] However, as Kips *et al.* have shown, better results are obtained when using an averaged physiological waveform for all subjects,[134] which is the method used in this thesis.

2.1.6 Total peripheral resistance

Total peripheral resistance (TPR) determines the relationship between mean arterial pressure (MAP) and cardiac output (CO), as shown in formula (2.2). As such, it represents the state of the microcirculation (TPR is mainly regulated by arterioles).

$$TPR = MAP / CO \tag{2.2}$$

By normalizing for body surface area (BSA), the total peripheral resistance index (TPRI) is obtained. BSA was obtained through the Gehan and George formula (2.3).[135]

$$BSA\ (m^2) = 0.0235 \times Height(cm)^{0.42246} \times Weight(kg)^{0.51456} \tag{2.3}$$

2.1.6.1 Cardiac output

Cardiac output (CO) was measured using echocardiography (AU5, Esaote, Genoa, Italy). Aortic diameter (D) was measured at least three times using pulsed ultrasound at 2.5 MHz from a standard two-dimensional long-axis parasternal view at the site of the aortic annulus. Aortic blood velocity profiles (at least five beats) were measured across the aortic valve with continuous ultrasound using an apical window. Stroke volume (SV) was calculated from aortic cross-sectional area ($CSA_{ao} = \pi \times (D/2)^2$) multiplied by the flow velocity integral (FVI). CO was calculated by multiplying SV with HR and divided by BSA to obtain the cardiac index (CI).

2.1.7 Preclinical atherosclerosis

2.1.7.1 Intima-media thickness

In the Glaucoma study, wall thickness (IMT) was measured on the right common carotid artery and on the right common femoral artery, during diastole, 2 cm proximal to the bifurcation. For this purpose, a 10 MHz pulsed ultrasound echotracking system (Wall Track system®, AU5, Esaote Pie Medical, Maastricht, The Netherlands) was used. This system allows analyzing radiofrequency signals originating from a single (cross-sectional) M-line perpendicular to the longitudinal axis of the artery, selected on the two-dimensional B-mode image.

Because IMT is influenced by the variations in internal diameter, due to the (near) incompressibility of the wall material, Wall Cross-Sectional Area (WCSA) is a better parameter for evaluating arterial remodeling (assuming that remodeling has a negligible effect on the length of the artery).[136] WCSA (mm^2) is calculated by subtracting luminal area [$\pi \times (D/2- IMT)^2$] from total arterial circumference [$\pi \times (D/2)^2$], or

$$WCSA = [\pi \times (D/2)^2] - [\pi \times (D/2 - IMT)^2] \quad (2.4)$$

In the ASKLEPIOS study, wall thickness (IMT) was measured at the left and right carotid and femoral arteries (VIVID 7, GE Vingmed Ultrasound).[27,137–139] Analysis of the ECG-gated cineloops was performed offline by a single, measurement-dedicated reader (Ernst Rietzschel), who was blinded for patient characteristics.[27] IMT was defined as the distance from the leading edge of the lumen-intima interface to the leading edge of the media-adventitia interface, measured in end diastole, at the far wall, in the common carotid artery, over a segment from 0 mm to 15 mm proximal to the bifurcation.[27,137–139] The reported number corresponds with the maximum value over the observed segment. Intra-observer variability of IMT was 5%.[139] Presence of plaque at an IMT measuring site precludes IMT measurement at that site. This, together with difficult imaging (often related to obesity) resulted in missing IMT values in 8, 168, and 198 subjects for the right carotid, left femoral and right femoral arteries, respectively. Carotid IMT-values were categorized using the cut-off value of 0.9 mm.[10] In the absence of an established cut-off for femoral IMT, the same threshold (0.9 mm) was used to categorize femoral IMT measurements.

2.1.7.2 Plaque

In the ASKLEPIOS study, carotid and femoral arteries were scanned bilaterally by a single skilled operator (Ernst Rietzschel) for the presence of plaque (a focal protrusion >50% compared to adjacent sites with an absolute thickness >1.5 mm or with a protrusion into the lumen of >0.5 mm). The variable "vascular target organ damage" (TOD) was defined as either IMT > 0.9 mm or presence of a plaque.

2.1.8 Real travelled path lengths of cf-PWV

MRI examinations were performed on a 1.5 T Magnetom Avanto scanner (Siemens Medical Solutions, Erlangen). All images were scanned with a slice thick-

ness of 6 mm and an interslice gap of 0.6 mm. In order to image the complete vasculature from carotid to femoral artery, two image series of the neck, thorax and abdomen with different table position were acquired per subject. Both series were merged to one dataset for post-processing.

Vitamin A pearls were placed at the carotid and femoral artery sites where the pressure pulse was recorded. These sites were used for distance measurements using tape measure. The Vitamin A pearls were identified on the MRI images and made it possible to visualize the exact body surface measurement points. The exact position was later used for the reconstruction of the real travelled aortic path length.

MRI post-processing was performed using a custom-developed Matlab®program (The MathWorks™, Natick, Massachusetts USA). To calculate the reference distance for the real travelled aortic path length, centerpoints were put manually in each slice. A centerline was reconstructed from those manually determined centerpoints. Using this centerline, different distances were calculated: the distance between ascending aorta (AA; from aortic valve) and the branching-off of the truncus brachiocephalicus (TB), the branching-off of TB and carotid artery (CA), the branching-off of TB and the right femoral artery (FA). At the time the pulse wave arrives at the carotid artery, this same pulse wave is already further down in the aorta. Presuming the same pulse wave velocity in the thoracic aorta and the carotid artery, the real travelled distance has to be calculated as the AA to FA distance (AA-FA) minus AA to CA distance (AA-CA). This distance will further be called “reference distance”. (Figure 2.3)

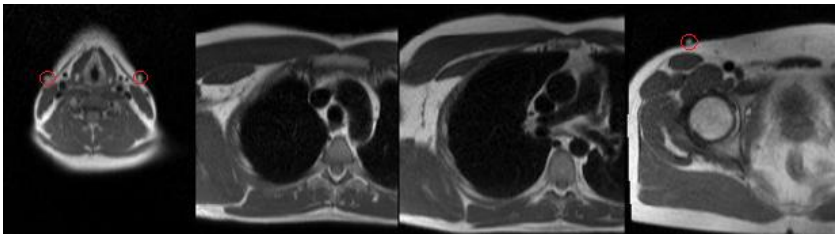


Figure 2.3 Four examples of cross-sectional MRI images used to trace the reference distance: from left to right: carotid artery, truncus brachiocephalicus, aortic bifurcation, femoral artery. The position of the vitamin A pearls (body surface measurement points) is indicated by red circles.

In addition the software program was used to reconstruct the straight distance between different anatomical points from the MRI images: carotid artery (Vitamin A pearl), suprasternal notch, umbilicus and femoral artery (Vitamin A pearl). These distances mimic distances obtained with an anthropometer and will further be called “straight MRI measured distances”.

2.2 Reproducibility of measurements

Prior to all studies, intra-observer reproducibility tests have been performed for cardiac output, AIx, IMT, cf-PWV, femoral and carotid diameter and femoral and carotid distension. These tests consisted of two sessions of triplicate measurements, separated in time (>1h), on 10 subjects, yielding intra-and intersession coefficients of variation. The results of these tests are tabulated in Table 2.2.

The measurements in the Asklepios study were carried out by a different operator (Ernst Rietzschel). The intra-observer reproducibility of IMT measurements in Asklepios was tested in 150 subjects (coefficient of variation 5.24%).[138]

Table 2.2 Results of reproducibility tests.

	Intra-session CV	Inter-session CV
Cardiac output	4.36 %	5.34 %
Cf-PWV	4.26 %	2.36 %
Carotid artery		
Augmentation index (tonometry)	3.62 %	3.69 %
diameter	1.91 %	1.59 %
distension	8.54 %	4.77 %
IMT	1.37 %	1.63 %
Femoral artery		
Diameter	2.01 %	3.18 %
Distension	8.97 %	9.52 %
IMT	2.36 %	2.01 %
Brachial artery		
Augmentation index (tonometry)	5.37 %	6.36 %

CV = coefficient of variation. Cf-PWV = carotid-femoral pulse wave velocity.

IMT = Intima-media thickness.

2.3 Description of the populations

2.3.1 Asklepios study participants

A cohort of 2524 apparently healthy, male and female volunteers aged 35 to 55 years was randomly sampled from the twinned Belgian communities of Erpe-Mere and Nieuwerkerken. They were all free from overt cardiovascular disease. Exclusion criteria were: 1, clinical evidence of atherosclerotic or atherothrombotic disease; 2, major concomitant illness; 3, diabetes mellitus type 1 and 2 with proven clinical macro-vasculopathy or significant renal impairment; 4, pregnancy; 5, inability to provide informed consent.[137]

2.3.2 Normal-tension glaucoma patients and healthy controls

32 patients diagnosed with NTG were recruited from the University hospital ophthalmology department. NTG was defined as neuroretinal rim loss assessed

by stereo disc assessment and photography, with a typical visual field defect, despite normal intraocular pressure (IOP, < 21 mm Hg). 35 Healthy control subjects, matched for age and sex, were recruited from the local community. Exclusion criteria were: (1) history of cardiovascular disease, (2) modest or severe arterial hypertension [i.e. systolic blood pressure (SBP) > 160 and/or diastolic blood pressure (DBP) > 100 mmHg], (3) diabetes mellitus, (4) severe hypercholesterolemia (defined as total cholesterol > 290 mg/dl), (5) pregnancy or lactation.

2.3.3 Healthy volunteers eligible for the MRI study

98 Healthy (male and female) volunteers aged 18 to 80 years were recruited from the Flemish community. Subjects not eligible for magnetic resonance imaging were excluded, yielding the following exclusion criteria: (1) wearing a pacemaker, aneurysm clip, cochlear implant, epicardial pacemaker wires or neural stimulators, (2) significant claustrophobia, (3) significant obesity, (4) large tattoos, (5) pregnancy or breastfeeding.

2.3.4 Various other population samples

Chapter 5 and 6 used pooled data from various other population samples (including the Asklepios sample) scattered across Europe. These populations are listed in tables 5.1 and 6.1 respectively.

Chapter 3 Cf-PWV: the influence of body side and body contours.

Adapted from:

Bossuyt J, Van de Velde S, Azermai M, Vermeersch SJ, De Backer TLM, Devos DG, Heyse C, Filipovsky J, Segers P, Van Bortel LM

Noninvasive assessment of carotid-femoral pulse wave velocity: the influence of body side and body contours. J Hypertens 2013; 31:946–951.

3.1 Abstract

Background: Recently an expert group advised to measure carotid-femoral (cf) pulse wave velocity (PWV) on the right side of the body, and to use a sliding caliper when tape measure distance cannot be obtained in a straight line. The present study investigates the evidence for these advices by comparing the real travelled cf path lengths at both body sides and comparing the straight distance (as can be obtained with a sliding caliper) with the tape measure distance.

Methods: Real travelled cf path lengths were measured with Magnetic Resonance Imaging (MRI) in 98 subjects (49 men, age 21-76 years). Path lengths from the aortic arch to the carotid (AA-CA) and femoral (AA-FA) sites were determined. Real travelled cf path lengths was calculated as (AA-FA)-(AA-CA) and compared between both sides. Real travelled cf path lengths were compared with 80% of the direct cf distance using a tape measure and the straight cf distance obtained from MRI images.

Results: Real travelled cf path length was slightly longer [11 mm (12), $p<0.001$] at the right side. The 80%-rule overestimated the real travelled cf path length with 0.5% at the right and 2.7% at the left side. Straight MRI distance tended ($p=0.09$) to perform slightly better than tape measure distance.

Conclusions: The travelled cf path is slightly longer at the right than at the left body side and the straight MRI distance tends to perform better than tape measure distance. The present study supports the advice of the expert consensus group to measure cf-PWV at the right body side using a sliding caliper when tape measure distance cannot be obtained in a straight line.

3.2 Introduction

A recent expert consensus document advocates to measure cf-PWV at the right side and to use a sliding caliper when no straight tape measure distance can be obtained.[74] Although these two advices are likely justified, they were based on expert opinion. The present study investigates the evidence for these two advices by 1) comparing the travelled distance at right and left body side and 2) by investigating whether the distance measured using a sliding caliper is more accurate than using a tape measure.

3.3 Methods

Ninety-eight apparently healthy subjects underwent body surface measurements and magnetic resonance imaging (MRI), as described in section 2.1.8 (p35). The carotid and femoral artery sites where the pressure pulse was maximally palpable were used as measurement sites for tape measurement of the direct distance. At these sites vitamin A pearls were placed, which could be identified on the MRI images and enabled us to calculate the straight distance between carotid and femoral measurement points, mimicking the distance that would be obtained using a sliding caliper. For calculation of the real travelled distance, post processing of the MRI images allowed to draw a centerline in the arteries, as previously described.[140]

The anatomical path travelled by the pulse wave was measured as the distance from the aortic arch to the femoral artery site (AA-FA) minus the distance from aortic arch to carotid measurement site (AA-CA). This was calculated for left- and right-sided carotid and femoral arteries.

All analyses were done using PASW19® (SPSS inc, Chicago, Illinois USA). Distances on left and right body side were compared using a paired samples t-test. Pearson's correlation coefficients were determined to assess the

association of participants' characteristics with differences between distance estimates. Levels of agreement between right and left real travelled distances were assessed by constructing scatter plots and Bland–Altman plots. Values of $p<0.05$ are considered significant. Data are reported as mean (SD) or frequencies (percentages).

3.4 Results

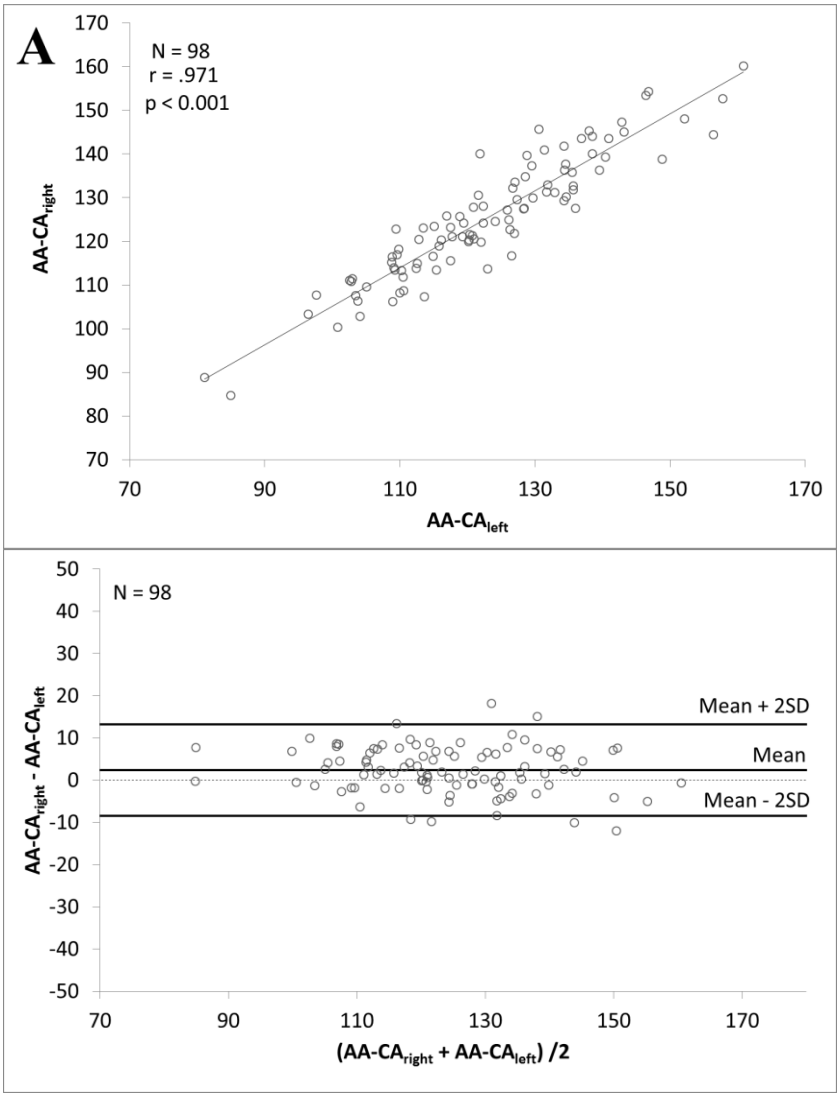
All participants were equally divided across age decade and gender. Subject characteristics are summarized in Table 3.1. As the majority of the participants (95 %) had a Body Mass Index (BMI) < 30 kg/m², we considered this a generally non-obese population sample.

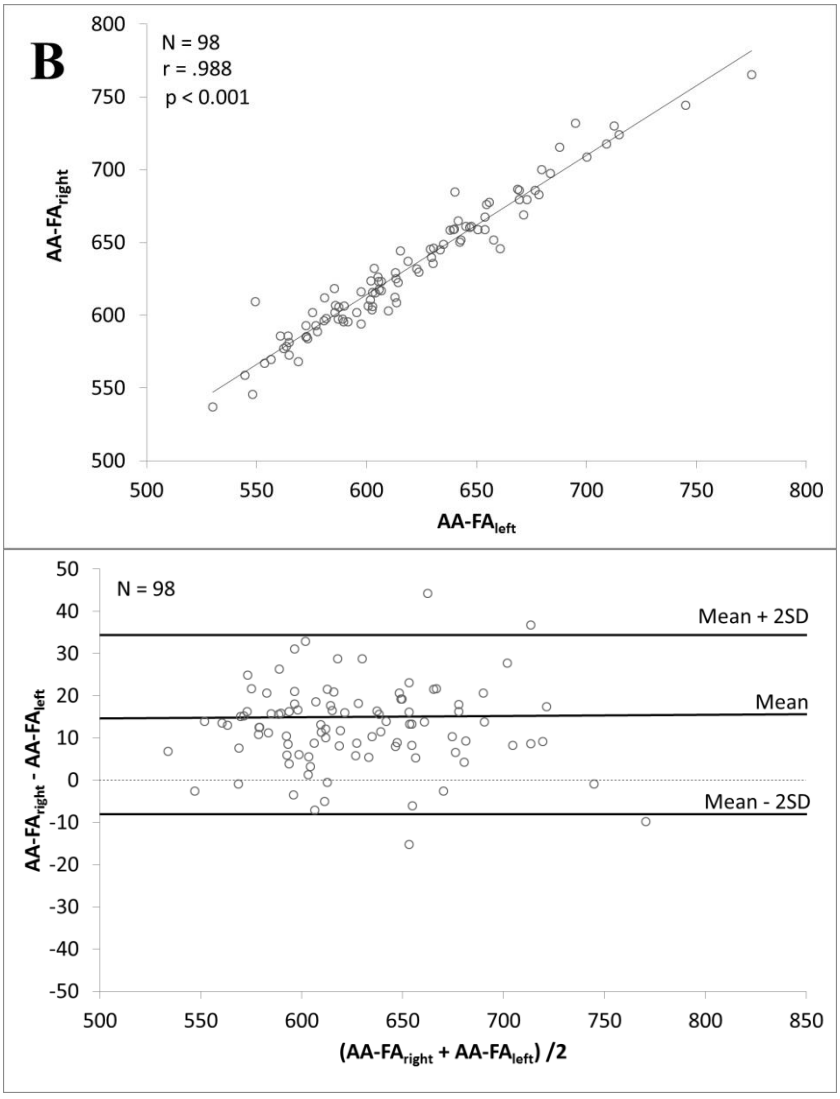
Table 3.1 Subject characteristics (n=98)		
Men (%)		50
Age (years, range)		47.8 (21-76)
Age decade	20-29 (%)	18.4
	30-39 (%)	19.4
	40-49 (%)	15.3
	50-59 (%)	15.3
	60-69 (%)	15.3
	70-79 (%)	16.3
Mean Height (cm, range)		171.6 (150-199)
Mean Weight (kg, range)		72.0 (51-115)
Mean BMI (kg/m², range)		24.5 (19-35)
Normal	18.5-24.9 (%)	56.3
Increased	25.0-29.9 (%)	38.5
Obesity	≥ 30.0 (%)	5.2
Mean SBP (mmHg, SD)*		134 (15)
Mean DBP(mmHg, SD)*		76 (10)
Mean Heart rate (bpm, SD)*		71 (11)

*Mean of 3 measurements after 10 minutes of supine rest using a validated oscillometric device (OMRON 705 IT, OMRON Healthcare, Kyoto, Japan); BMI: Body Mass Index; bpm: Beats per minute; DBP: Diastolic Blood Pressure; SBP: Systolic Blood Pressure

3.4.1 Comparison between right and left real travelled distances

The results of the MRI based real travelled distances are depicted in Table 3.2. Scatter plots and Bland-Altman plots of right versus left path lengths are presented Figure 3.1. For each arterial segment there was a small but statistically significant length difference between the left and the right path. This resulted in a travelled carotid-femoral path being slightly longer [Δ 11 (12) mm, $p<0.001$] at the right side compared to the left.


A
N = 98
r = .971
p < 0.001
AA-CA$_{right}$
AA-CA$_{left}$
170
160
150
140
130
120
110
100
90
80
70
70
90
110
130
150
170
N = 98
Mean + 2SD
Mean
Mean - 2SD
AA-CA$_{right}$ - AA-CA$_{left}$
(AA-CA$_{right}$ + AA-CA$_{left}$) /2
50
40
30
20
10
0
-10
-20
-30
-40
-50
70
90
110
130
150
170


B
N = 98
r = .988
p < 0.001
AA-FA$_{right}$
AA-FA$_{left}$
N = 98
Mean + 2SD
Mean
Mean - 2SD
AA-FA$_{right}$ - AA-FA$_{left}$
(AA-FA$_{right}$ + AA-FA$_{left}$) /2

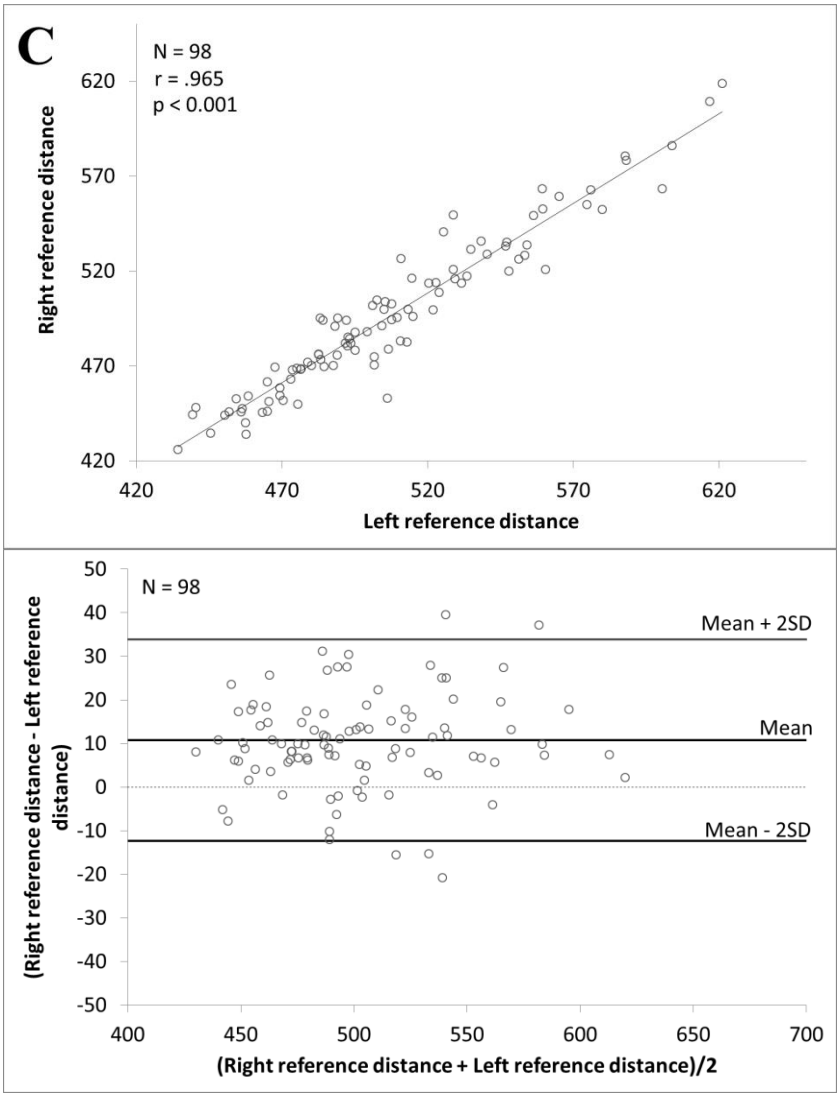


Figure 3.1 Scatterplots and Bland-Altman plots. **A:** Comparison between the distance of aortic arch to right carotid artery (AA-CA_{right}) and aortic arch to left carotid artery (AA-CA_{left}), **B:** comparison between the distance of aortic arch to right femoral artery (AA-FA_{right}) and aortic arch to left femoral artery (AA-FA_{left}). **C:** comparison between right reference distance and left reference distance.

Table 3.2 Travelled path lengths obtained from MRI imaging.

	Right (mm)	Left (mm)	Right – Left (mm)
(AA-CA)	125 (14)	123 (15)	2 (6)*
(AA-FA)	632 (46)	618 (47)	13 (11)*
(AA-FA) - (AA-CA)	**506 (42)**	**496 (41)**	**11 (12)***

Data are shown as mean (SD). AA: aortic arch at the branching off of the brachiocephalicus; AA-CA: distance between aortic arch and the measurement point at the common carotid artery; AA-FA: distance between aortic arch and the measurement point at the common femoral artery. * $p<0.001$ statistical difference between left and right body side

The tape measure distance according to the 80 % rule overestimated the real travelled distance at the left side by 2.7%, while this was only 0.5% at the right side (Table 3.3).

Table 3.3 Path lengths estimated by tape measure and straight MRI distance using the 80% rule.

	Right	Left
Real travelled distance (mm)	**506 (42)**	**496 (41)**
Tape measure distance x 0.8 (mm)	509 (35)	509 (35)
(Tape measure distance x 0.8 – real travelled distance)/ real travelled distance (%)	**0.5**	**2.7***
Straight MRI distance x 0.8 (mm)	505 (35)	505 (35)
(Straight MRI distance x 0.8 – real travelled distance)/ real travelled distance (%)	**-0.2**	**1.9**

Data are shown as mean (SD) or %; Tape measure distances were measured on the right side and were considered to be the same on the left side. * $p<0.05$ statistical difference from real travelled distance

3.4.2 Accuracy of tape measure and straight distances

Straight MRI distances - taken as surrogate for distances measured with a sliding caliper - did not differ substantially between left and right body side [Δ 0.28 (1.1) mm, $p<0.05$]. At both body sides the straight MRI distance was shorter than the one obtained by tape measure, although no statistical significance was reached [right: Δ 4.6 (2.6) mm, $p=0.09$; left Δ 4.8 (2.6) mm, $p=0.07$]. Straight MRI distances multiplied by 0.8 (80% rule) showed a statistically non-significant ($p=0.09$) more accurate approximation of the real travelled distance, compared with tape measure multiplied by 0.8. (Table 3.3 and Figure 3.2)

Linear regression (Table 3.4) revealed that in the present population sample the difference in length between straight MRI and tape measure distance was only influenced by gender (right: $r=0.269$, $p=0.008$; left: $r=0.280$, $p=0.005$). As gender was the single significant univariate correlate, no multivariate analysis was carried out. In men, straight MRI distance did not differ from tape measure distance [right: Δ -2.6 (3.0) mm, $p=0.547$; left: Δ -2.4 (3.0) mm, $p=0.793$)], while in women, significantly longer distances were obtained using a tape measure [right: Δ 11.7 (1.9) mm, $p< 0.001$; left: Δ 12.1 (1.9) mm, $p<0.001$].

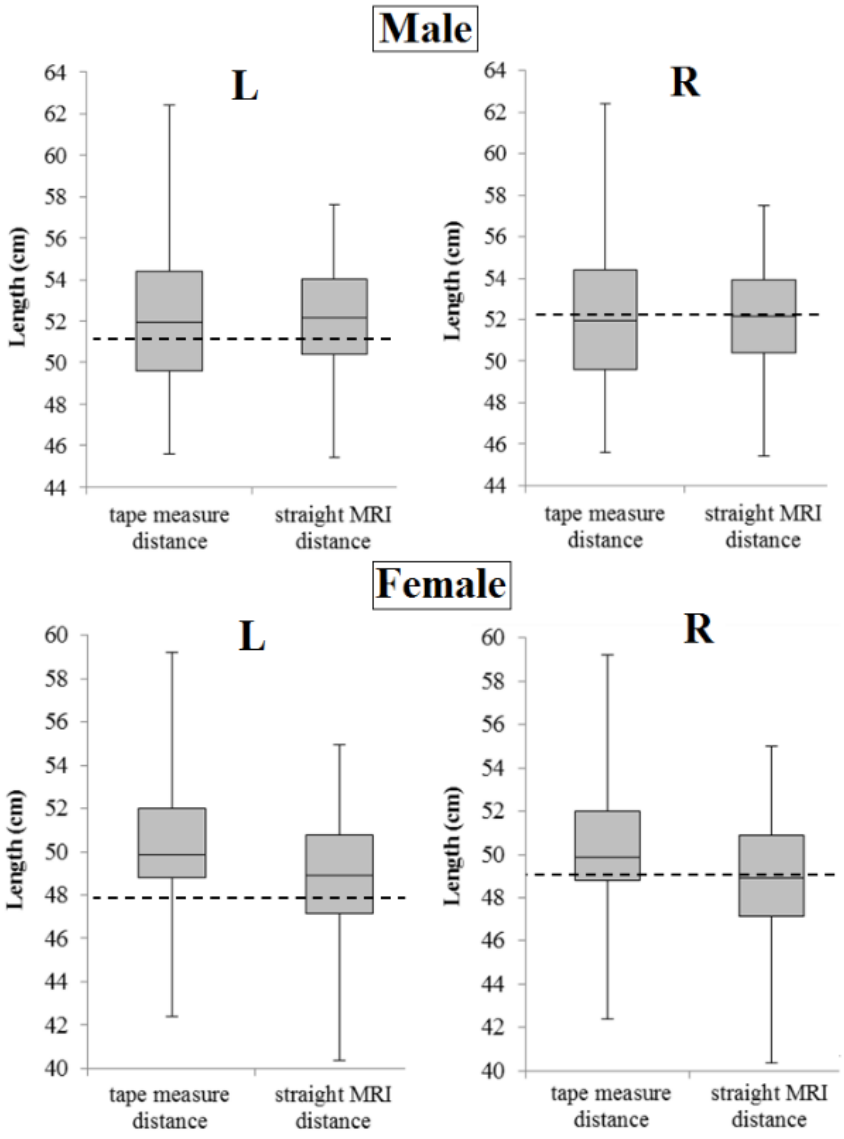


Figure 3.2 Boxplots: Comparison between tape measure and straight MRI distance, relative to the real travelled distance. R = right body side, L = left body side. Top = male, bottom = female. Tape measure and straight MRI distances are obtained after application of the 80%-rule (measured distance x 0.8). Dashed horizontal lines represent mean right and left real travelled path length

Table 3.4 Associations of subject characteristics with the difference between tape measure distance and straight MRI distance.

	Right		Left	
	r	**95% CI**	**r**	**95% CI**
Age	0.126	-0.07 to 0.33	0.134	-0.07 to 0.33
Height	-0.141	-0.34 to 0.06	-0.151	-0.35 to 0.05
Weight	0.050	-0.26 to 0.15	0.051	-0.26 to 0.15
BMI	0.048	-0.14 to 0.27	0.052	-0.15 to 0.26
Male	-0.269*	-0.47 to -0.07	-0.280*	-0.48 to -0.09

Univariate regression model with the difference between tape measure and straight MRI distance as dependent variable, for right and left body side. r = regression coefficient. CI = confidence interval. * $p<0.05$ statistically significant correlation.

3.5 Discussion

The present study showed a slightly longer travelled path length (11 mm) between carotid and femoral arteries at the right side compared to the left side. This left-right difference in carotid-femoral travelled path length was mainly due to a longer right than left iliac and femoral path (from bifurcation to the measurement point at the common femoral artery; 13 mm), while the also slightly longer right arch-to-carotid path (from aortic arch at the branching off of the brachiocephalicus to the measurement point at the common carotid artery; 2 mm) limited the left-right difference in real travelled carotid-femoral path length.

The consensus document showed that the 80% rule by tape measurement slightly overestimated the travelled right carotid-femoral path length by 0.5% in the total population sample (all age groups). The present study shows that the 80% rule overestimates the travelled left carotid-femoral path with 2.7%. It is not clear to what extent this will influence the calculation of cf-PWV at the left side, as it is not clear to what extent this shorter travelled path length also translates into a shorter transit time. Dzeko *et al.*

showed that different PWV values are obtained when switching between left and right carotid artery, leaving the femoral measurement site constant.[141] However, this small difference was comparable with the inter-observer difference.[142] So, should we also apply the 80% rule to measurements at the left side? One should realize however that the discrepancy definitely still falls within the limits of error of the cf-PWV assessment and may therefore be of less importance for single measurements in the clinic and that it can be used if measurement at the right side is not possible.

The present study favors the distance measurement using a sliding caliper (although this was demonstrated only indirectly, using MRI measurements as a surrogate for caliper measurements), which after application of the 80% rule yields the closest approximation of the real travelled distance. In the present non-obese population sample, the use of the sliding caliper would be particularly important in women, who showed the largest deviation of tape measurement distance from the real travelled distance, probably due to breast contours. A larger study including obese subjects could show to what extent obesity may interfere with the (straight) distance measurement.

The present study has some limitations. Huybrechts *et al.* already acknowledged the physical constraints of the MRI scanner, posing a limit on the degree of obesity of the subjects (i.e. < 140-150 kg).[140] This may explain why BMI was not a determinant of the difference between tape measure and straight MRI distances in the present study. Furthermore, because this study was initially not designed to investigate the influence of body side, tape measure distances from carotid to femoral artery were only available for the right body side, and were considered the same on the left body side. Nevertheless, with respect to the MRI-measured distances, no assumptions had to be made, because the entire left path could be constructed using exactly the

same MRI images as for the right path. In addition, the same MRI planes at carotid and femoral arteries were used to calculate straight MRI distances at both sides. Therefore, this is the first study comparing the complete carotid-to-femoral path length between the two body sides, using an accurate and reproducible MRI-based method. Finally, the study was not designed to analyze the influence of different cardiovascular risk factors on the path lengths. The MRI study showed a mild but acceptable influence of age on the accuracy of the measurement at the right body side.[140] It is not likely that this influence would substantially be different at the left body side, but this has not been studied yet.

In conclusion, the present study shows that the travelled carotid-femoral path is longer at the right than at the left side, causing the 80% direct distance rule to be less accurate at the left side, which may influence accuracy of cf-PWV. The difference in distance falls within the error of the cf-PWV assessment and might be less important for a single measurement, but can add to other inaccuracies. The size of error can further be reduced by a straight distance measurement as can be obtained with a sliding caliper instead of a tape measure. Therefore, the present study supports the advice of the consensus document to preferentially measure cf-PWV at the right side, measuring the distance in a straight line from carotid to femoral measurement points and to make use of a sliding caliper if a straight line cannot be obtained (due to belly or breast size). However, these advices were derived from a relatively small and healthy population. Further validation in larger and other populations should be advised.

Chapter 4 Left-right prevalence of femoral and carotid atherosclerosis

Adapted from:

Bossuyt J, Van Bortel LM, De Backer TLM, Van de Velde S, Azermai M, Segers P, De Buyzere M, Van daele C, Rietzschel E, on behalf of the Asklepios Investigators.

Asymmetry in prevalence of femoral but not carotid atherosclerosis. Journal of Hypertension. 2014;32(7):1429–1434.

4.1 Abstract

Objective(s): Atherosclerotic disease is caused by a combination of systemic and local factors (e.g. geometry) affecting local flow conditions. In contrast to the carotid artery, at the iliac-femoral artery region, a large degree of bilateral asymmetry exists. Therefore, we aimed to determine the influence of body side on the prevalence of atherosclerosis (i.e. plaque and intima-media thickening; IMT) at the carotid and femoral arteries.

Methods: Data were used from the ASKLEPIOS study, including 2524 apparently healthy subjects with a mean age of 46 year (range 35-55). Echographic images were obtained bilaterally of the carotid and femoral arteries. A single observer approach was used for the acquisition and quantification of plaques and IMT.

Results: The carotid artery displays no significant left-right difference in IMT values nor plaque prevalence (right: 12.0 % vs. left 13.3 %; p=0.18). In contrast, for the femoral artery, the IMT distribution at the right common femoral artery is more skewed (P90 right: 1.11 mm, left 1.01 mm; p<0.001), which is mirrored by a significantly higher plaque prevalence (right: 21.9 % vs. left: 15.7 %; p<0.001).

Conclusions: In the present study, atherosclerotic lesions are more prevalent at the right than at the left femoral artery. This finding highlights the possible role of local arterial geometry in the development of atherosclerosis, and underscores the importance of the choice of body side when assessing vascular health.

4.2 Introduction

The link between arterial geometry and atherosclerosis, mediated through changes in local hemodynamics and shear stress, has long been recognized.[143] We hypothesized that if a left-right difference in arterial geometry (as demonstrated in Chapter 3) translates into an asymmetric distribution of atherosclerosis, then this should reveal itself at the population level. Therefore, our aim was to compare the prevalence of atherosclerosis (looking at both plaques and intima-media thickness, IMT) between the left and right carotid and femoral arteries, in a large population sample.

4.3 Methods

The ASKLEPIOS study protocol, methodology, and baseline population characteristics have been described elsewhere in detail.[137] The vascular imaging protocol and the study population are described in more detail in sections 2.1.7. and 2.3.1. respectively.

4.3.1 Statistical analyses

For continuous variables, median, 80^{th}, 85^{th}, 90^{th} and 95^{th} percentiles are reported. Distributions were compared between left and right side by performing quantile regression on median and individual percentile values. Categorical variables were summarized as absolute values and percentages. The influence of body side and (possible) confounding effect of sex was tested using logistic regression, adding side, sex and an interaction term (body side*sex) as fixed factors into the model. For each test $p<0.05$ was considered statistically significant. Odds ratios (OR) and corresponding 95% confidence intervals (CI) were calculated. The OR represents the ratio of the odds of finding a plaque at the right side, over the odds of finding a plaque at the left side. As a sensitivity analysis we checked whether the results

would be maintained when looking at mean IMT (i.e. the average IMT over the observed segment) instead of maximal IMT, and when subjects with atherosclerosis at two or more locations were excluded (i.e. looking at "single-site atherosclerosis", including only those subjects with a lesion at one single site).

4.4 Results

Demographics, anthropometric data, lifestyle, and ultrasonographic data are provided in Table 4.1.

4.4.1 Intima-media thickness (IMT)

Median IMT was not significantly different between left and right carotid arteries. Quantile regression also revealed no significant differences for the 80^{th}, 85^{th}, 90^{th} and 95^{th} percentiles between right and left side ($p>0.05$ for all percentiles). Median femoral artery IMT was identical for the left and right side. Comparison of upper percentiles however, showed increasingly higher values at the right femoral artery ($p<0.05$), indicating a more skewed distribution (Figure 4.1). This effect was also demonstrated by the significantly higher number of subjects exceeding the 0.9 mm cut-off value for femoral artery IMT on the right side. (Table 4.2)

Table 4.1. Baseline characteristics of the ASKLEPIOS Study population.

Variable	Women (n=1301; 51.5%)	Men (n=1223; 48.5%)
Age, y	45.7 (40.8–51.1)	45.9 (41.2–50.9)
BMI, kg/m²	25.1±4.7	26.5±3.7
Waist circumference, cm	80.5±11.3	93.8±10.4
Obesity, BMI ≥30 kg/m² (%)	13.5	17.3
Abdominal obesity, ATP III (%)	20.7	19.2
Hemodynamic parameters		
Systolic BP, mm Hg	123±14	131±13
Diastolic BP, mm Hg	78±10	82±10
Pulse pressure, mm Hg	45±9	48±7
Heart rate, bpm	72±10	68±12
Biochemical parameters		
Total cholesterol, mg/dl	214±36	219±38
HDL-cholesterol, mg/dl	71±17	56±14
LDL-cholesterol, mg/dl	125±33	137±34
Diabetes mellitus/IFG, %	1.1/6.9	2.1/14.5
Lifestyle variables		
Smoking: Active/Ex, %	17.7/21.8	24.1/34.3
Pack-years, of ever smokers	8.1 (2.4–17.7)	11.3 (4.8–22.2)
Physical activity: None, %	66.0	53.6
Ultrasonographic measures		
Right carotid IMT, mm*	0.67 (0.60–0.76)	0.71 (0.63–0.82)
Left carotid IMT, mm*	0.67 (0.60-0.76)	0.73 (0.63–0.83)
Right femoral IMT, mm*	0.59 (0.51–0.71)	0.72 (0.60–0.92)
Left femoral IMT, mm*	0.59 (0.51–0.70)	0.71 (0.60–0.87)

Cut-offs for abdominal obesity (Adult Treatment Panel, ATP III) are: waist >88 cm (females) / >102 cm (males); Impaired fasting glycaemia (IFG): glucose ≥100 mg/dl and <126 mg/dl (diabetes). Data are mean±SD or median (interquartile range) where appropriate. *maximum value over the observed segment.

4.4.2 Plaque

A total of 303 and 335 plaques were found at the left and right carotid artery respectively, revealing no significant influence of the body side

(p=0.92). In contrast, at the level of the femoral artery, substantially more plaques were found on the right side (right: n=552, left: n=396), resulting in an odds ratio significantly different from 1 (OR 1.53, 95% CI: 1.28-1.83; p<0.001). (Table 4.2) When cases of IMT>1.5 mm but without significant protrusion into the lumen (>50% compared to adjacent sites or >0.5 mm) were also classified as plaques, these results were maintained (femoral artery: right: n=573, left: n=423; OR 1.51, 95% CI: 1.27-1.81; p<0.001; carotid artery: right: n=304: left: n=336; OR 1.01, 95% CI: 0.82-1.25; p=0.92).

4.4.3 Target organ damage (TOD)

The combined phenotype, “TOD”, exhibits the same pattern as either plaque prevalence or intima-medial thickening alone, confirming the symmetrical and asymmetrical distributions at the carotid and femoral arteries respectively. (Table 4.2)

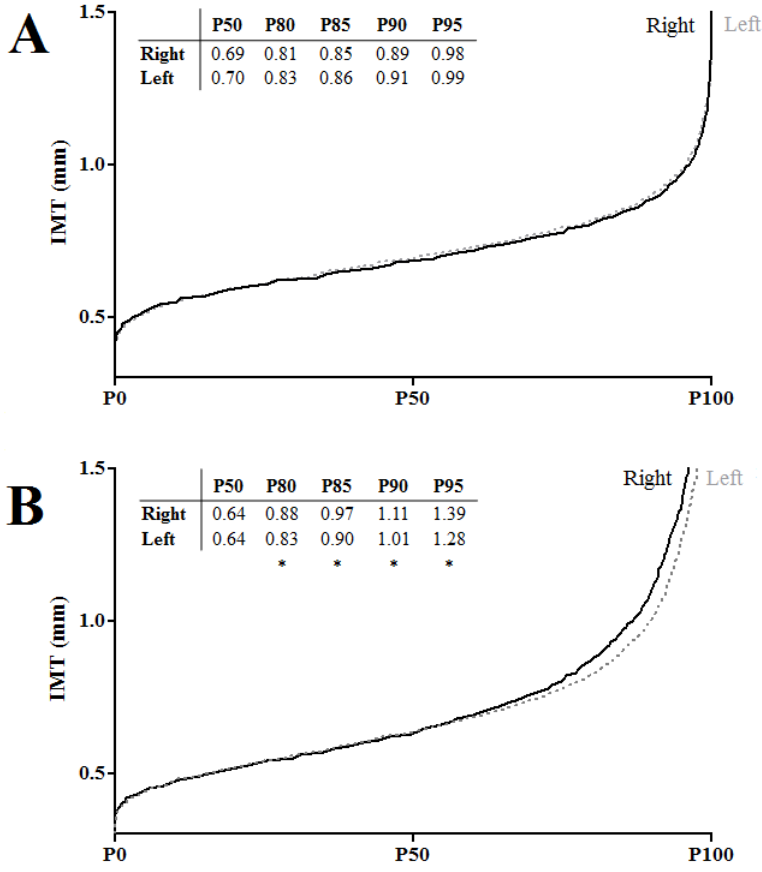


Figure 4.1 Cumulative distribution of maximal IMT at the carotid (A) and femoral (B) arteries. Left and right Carotid IMT distributions largely overlap, while increasingly higher values are found at the right femoral artery compared to the left. Statistically significant ($p < 0.05$) differences between individual percentiles are indicated with an asterisk (*).

Table 4.2. Prevalence of preclinical atherosclerosis in men, women and all subjects.

Carotid artery									
	Men (n=1223)		Women (n=1301)		All (n=2524)		p-value		
	Left	Right	Left	Right	Left	Right	Body side	Sex	Side*sex
IMT > 0.9	185 (15)	161 (13)	72 (6)	61 (5)	257 (10)	222 (9)	0.164	<0.001	0.952
Plaque	210 (17)	212 (17)	93 (7)	123 (9)	303 (12)	335 (13)	0.915	<0.001	0.101
TOD	335 (27)	310 (25)	140 (11)	169 (13)	475 (19)	479 (19)	0.251	<0.001	0.036
Femoral artery									
	Men (n=1223)		Women (n=1301)		All (n=2524)		p-value		
	Left	Right	Left	Right	Left	Right	Body side	Sex	Side*sex
IMT > 0.9	241 (20)	292 (24)	113 (9)	137 (14)	354 (14)	429 (17)	0.013	<0.001	0.846
Plaque	280 (23)	382 (31)	116 (9)	170 (13)	396 (16)	552 (22)	<0.001	<0.001	0.981
TOD	402 (33)	508 (42)	195 (15)	250 (19)	597 (24)	758 (30)	<0.001	<0.001	0.588

Vascular target organ damage (TOD) is defined as IMT ≥0.9 mm and/or presence of a plaque. Data are shown as absolute values and percentages, n (%). P-values were obtained from logistic regression in all subjects, including body side, sex, and an interaction term (body side*sex) as fixed factors.

4.4.4 The influence of sex

The prevalence of lesions was always higher in men than women (p<0.001). However, the modifying effect of sex was limited, since the interaction term (body side*sex) was significant only for carotid TOD (p=0.036), because of a reversed trend in men (right: n=310, left: n=335) compared to women (right: n=169, left: n=140). (Table 4.2)

4.4.5 Sensitivity analyses

4.4.5.1 Single-site atherosclerosis

When only taking into account subjects with atherosclerosis at a single site, the same pattern emerges: an almost identical number of lesions at right and left carotid arteries (right: n=112, left: n=110; OR 1.02; CI: 0.78-1.33; p=0.89), in sharp contrast to the more than twofold difference seen at the femoral arteries (right: n=207; left: n=94, OR 2.31, CI: 1.80-2.97; p<0.001). (Figure 4.2) This also indicates that by measuring both carotid arteries and only right, only left or none of the femoral arteries, 94 (4%), 207 (8%) and 474 (19%) subjects would be wrongly classified without TOD respectively.

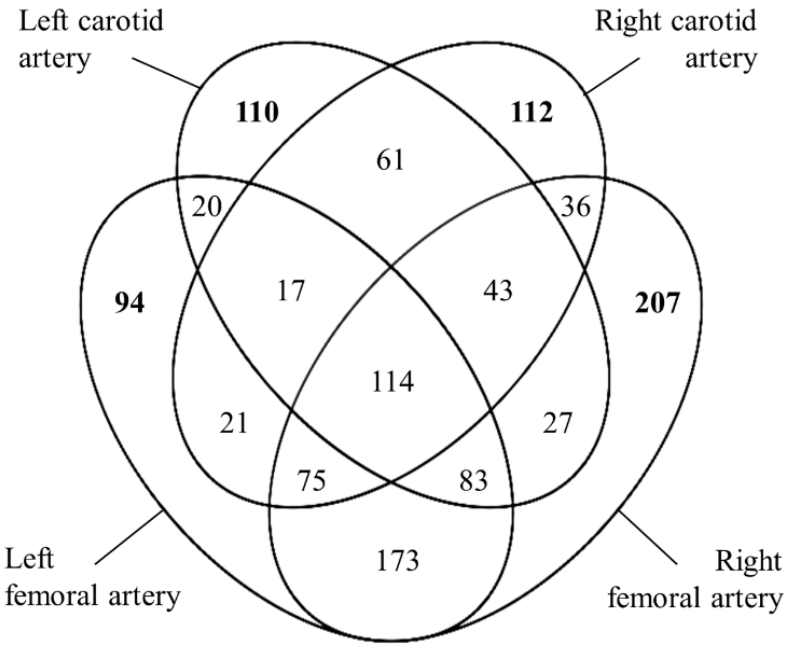


Figure 4.2 Distribution of preclinical atherosclerosis in all subjects. Venn-diagram displaying all possible combinations of target organ damage distribution (TOD, IMT >0.9 mm or presence of a plaque), emphasizing cases of single-site atherosclerosis (bold).

4.4.5.2 Using mean IMT instead of maximal IMT

When maximal IMT was replaced with mean IMT, this shifted IMT distributions towards lower values, reducing the total number of individuals exceeding the 0.9 mm cut-off value. However, this had no impact on our conclusions: P90 percentiles were again divergent for the femoral artery (right: 0.90 mm, left 0.85 mm; $p<0.05$), while equal for the carotid artery (right: 0.75 mm, left 0.75 mm). Left-right distributions of TOD prevalence also remained unchanged when TOD was defined using mean instead of maximum IMT (femoral artery: left: n=488, right: n=633; $p<0.001$; carotid artery: left: n=333, right: n=368; $p=0.15$) adding to the robustness of our findings.

4.5 Discussion

The present study investigated the influence of body side on the prevalence of atherosclerosis at the carotid and femoral arteries. Our main finding was that atherosclerosis, examined through maximal IMT and the presence of plaque, was distributed symmetrically at the carotid artery while asymmetrically at the femoral artery. For the carotid artery, these observations are in line with results from some previous epidemiological studies, showing small[115] or insignificant[113] differences between both sides. With regard to the femoral artery, data are rather scarce but also tend to confirm our observations. Plaque thickness[42] and IMT[119] tend to be higher when measured on the right side.

The results of this study also mirror anatomical features described in literature.[144] The equal distribution of carotid artery atherosclerosis reflects the limited amount of morphological asymmetry between left and right side at the level of the measurement location, proximal to the bifurcation, while the skewed distribution of femoral artery atherosclerosis can be explained by more pronounced differences in arterial anatomy. Moreover, the finding that the most frequently affected femoral artery is the one lying on the right side resonates with the current hemodynamic principles. It is known that regions of curvature, bifurcation or branching are associated with low longitudinal or high oscillatory shear stress, creating flow conditions which promote the development of atherosclerotic lesions.[145–148] Therefore, the higher prevalence of atherosclerosis seen at the right femoral artery may be explained by its generally more curved, bended nature. Although the anatomy of left and right carotid artery is also not completely comparable, particularly differing in terms of their origin, we speculate that the impact of this anatomical variability on the hemodynamics at the more distal measurement location is buffered by the relatively long straight segment in be-

tween. Indeed, at the common carotid artery, no bilateral difference is found in blood flow characteristics,[149] including timing and velocity of the flow waveform,[150] and shear stress distributions.[151] In addition, it can be speculated that the difference in vascular wall properties (the more elastic carotid and the more muscular femoral arteries), the more bended iliac-femoral trajectory and the higher pressures at the femoral arteries in standing position might contribute to a possible difference in sensitivity to local atherogenic factors between the common carotid and common femoral arteries. However, to provide more conclusive evidence for this hypothesis, information on the arterial anatomy on an individual basis is warranted. Instead, our assumptions are based on averaged anatomical data found in literature, including our own MRI-based research in a different healthy population.[144]

4.5.1 Study limitations

As a general remark, we emphasize this study was carried out in an apparently healthy population, exhibiting only *preclinical* signs of atherosclerosis. It remains to be established whether these lesions will actually become *clinically* apparent later in life. Prospective studies are needed to show the clinical relevance of the possible difference of atherosclerosis in right and left femoral arteries.

Other limitations of the study include the absence of bilateral diameter and flow measurements, and some limitations inherent to the non-invasive assessment of atherosclerosis. Ultrasound imaging provides only a two-dimensional view of the vessel wall and also fails to distinguish intimal from medial thickening.[39] However, to deal with this latter point, we reported maximal (and not mean) IMT values, which may be considered to represent more closely the first signs of an atherosclerotic lesion.[152,153] In our study, the difference in maximal IMT values between left and right side was indeed

closely mirrored by the difference in plaque prevalence, confirming this assumption.

In addition, this study provides a systematic and robust measurement of atherosclerosis at the left and right carotid and femoral arteries, excluding any bias due to variations in operator or measurement device. While similar experiments have been repeatedly carried out on the carotid artery, this is the first study making a rigorous comparison of the prevalence of atherosclerosis between left and right femoral artery.

4.5.2 Implications

For the carotid artery, our data provide additional support for the current consensus advice of not taking into account the body side (on a population level). For the femoral artery, guidelines are at present non-existing, leaving operators free in their choice on which body side to measure upon. However, researchers should be aware of the possibility that femoral artery atherosclerosis may more likely affect the right side of the body, as observed in this study population. A second implication may relate to the mechanisms responsible for the observed differences in atherosclerosis prevalence. While unable to provide conclusive evidence for the causal role of anatomical asymmetries, our data do provide an interesting starting point for further research. Characterizing also left-right differences in shear stress and blood flow patterns at the same time may yield more insight in the underlying processes behind this phenomenon, unraveling the critical anatomical features responsible for driving the asymmetrical distribution observed in our sample. Follow-up studies (including outcome of patients) could be of help to identify the clinical relevance.

4.5.3 Conclusion

In this healthy, middle-aged population sample, subclinical atherosclerosis is equally distributed between right and left carotid arteries. In contrast, the right femoral artery is significantly more affected than the left femoral artery. These results may reflect differences in anatomy between left and right side and therefore suggest a causal role for variations in local hemodynamics.

Chapter 5 Reference values for carotid artery stiffness.

Engelen L, Bossuyt J, Ferreira I, Van Bortel LM, Boutouyrie P, Laurent S, Segers P, Reesink K, Stehouwer CDA.

Reference values for local arterial stiffness. Part A: Carotid artery. (in preparation)

5.1 Abstract

Aims: Non-invasive measures of common carotid artery properties, such as diameter and distension, and pulse pressure, have been widely used to determine carotid artery distensibility coefficient (DC), a measure of carotid stiffness (stiffness~1/DC). Carotid stiffness has been associated with incident cardiovascular disease (CVD) and may therefore be a useful intermediate marker for CVD. We aimed to establish age- and sex-specific reference intervals of carotid stiffness.

Methods and results: We combined data on 22,708 individuals (age range 15-99; 54% men) from 24 research centres worldwide. Individuals without CVD and established cardiovascular risk factors (CV-RFs) constituted a healthy subpopulation (n=3,601, 48% men) and were used to establish sex-specific equations for percentiles of carotid DC across age. In the subpopulation without CVD and treatment (n=12,906, 52% men), carotid DC Z-scores based on these percentile equations were independently and negatively associated, in men and women respectively, with diabetes [-0.28 (95% CI: -0.41; -0.15) and -0.27 (-0.43; -0.12)], mean arterial pressure [-0.26 (-0.29; -0.24) and -0.32 (-0.35; -0.29)], total-to-HDL cholesterol ratio [-0.05 (-0.09; -0.02) and -0.05 (-0.011; 0.01)] and body mass index [-0.06 (-0.09; -0.04) and -0.05 (-0.08; -0.02)], whereas these were positively associated with smoking [0.30 (0.24; 0.36) and 0.24 (0.18; 0.31)].

Conclusion: We estimated age- and sex-specific percentiles of carotid stiffness in a healthy population and assessed the association between CV-RFs and carotid DC Z-scores, which enables comparison of carotid stiffness values between (patient) groups with different cardiovascular risk profiles, helping interpretation of such measures.

5.2 Introduction

As described in *Chapter 1*, carotid artery stiffness (or its inverse, the carotid artery distensibility coefficient, DC_{car}) carries added predictive value for all-cause and cardiovascular mortality.[87] However, the interpretation of DC_{car} values measured across different age, sex and risk groups has been hampered by the absence of reference values. In view of these considerations, we aimed 1) to establish age- and sex-specific normal values using percentiles of local DC_{car} obtained in individuals without prior CVD, treatment and established cardiovascular risk factors (CV-RFs) and 2) to investigate associations between known CV-RFs and these DC_{car} percentiles in individuals with or without CV-RFs, treatment and prior CVD.

5.3 Methods

5.3.1 Study population

With a systematic literature review all cohort studies using echotracking for DC_{car} measurement were identified. Next, the principal investigators of the cohorts (n=57) were personally contacted to inform them about the project and inviting them to participate. Finally, subject-level data was compiled from 24 research centres/research groups – corresponding to 30 distinct cohorts – distributed across 13 countries worldwide (Table 5.1).[109] A total of 23,007 individuals with data on carotid artery diameter and distension obtained using echotracking systems, blood pressure (BP), age (range 5-99 years), sex (12,390 men/10,617 women), CVD status, and important CV-RFs were available for analysis. For the present study we excluded 299 (54% girls) individuals who were aged <15 years because their data lacked sufficient variability with age (primarily concentrated at the age of five[154]) leaving 22,708 (46% women) individuals for analyses.

To generate age- and sex-specific normative tables for DC_{car}, we selected a healthy sub-population composed of individuals who did not meet any of the following criteria: 1) history of CVD; 2) use of BP-, lipid- and/or glucose-lowering medication; 3) hypertension [i.e. SBP ≥140 mm Hg and/or diastolic blood pressure (DBP) ≥90 mm Hg];[10] 4) current smoking; 5) diabetes (defined as self-reported diabetes and/or fasting plasma glucose ≥7.0 mmol/L (if available) and/or postload plasma glucose ≥11.0 mmol/L (if available)];[155] 6) total cholesterol >6.2 mmol/L;[156] 7) HDL cholesterol <1.17 mmol/L (for men) and <1.30 (for women); [156] and 8) BMI ≥30 kg/m^2.[157] This healthy sub-population consisted of 3,601 (52% women) individuals, who originated from 19 out of the 24 research centres (Table 5.1). The cut-off values used to define the healthy sub-population were chosen, whenever possible, to be similar to those used to indicate increased risk in current guidelines (or risk algorithms) to enable optimal comparison with other studies.

To investigate the relation of CV-RFs with individuals' levels of DC_{car} percentiles we stratified the total population according to history of CVD and, in individuals without prior CVD only, by use of BP-, lipid- and/or glucose-lowering medication. This resulted in three reference sub-populations consisting of: 1) 12,906 (48% women) individuals without prior CVD and without use of BP-, lipid- and/or glucose-lowering medication; 2) 5,137 (52% women) individuals without prior CVD and who used BP-, lipid- and/or glucose-lowering medication; and 3) 4.665 individuals (34% women) with prior CVD irrespective of medication use. A flowchart describing the selection of the healthy and reference sub-populations and exact numbers per sex is presented in Figure 5.1.

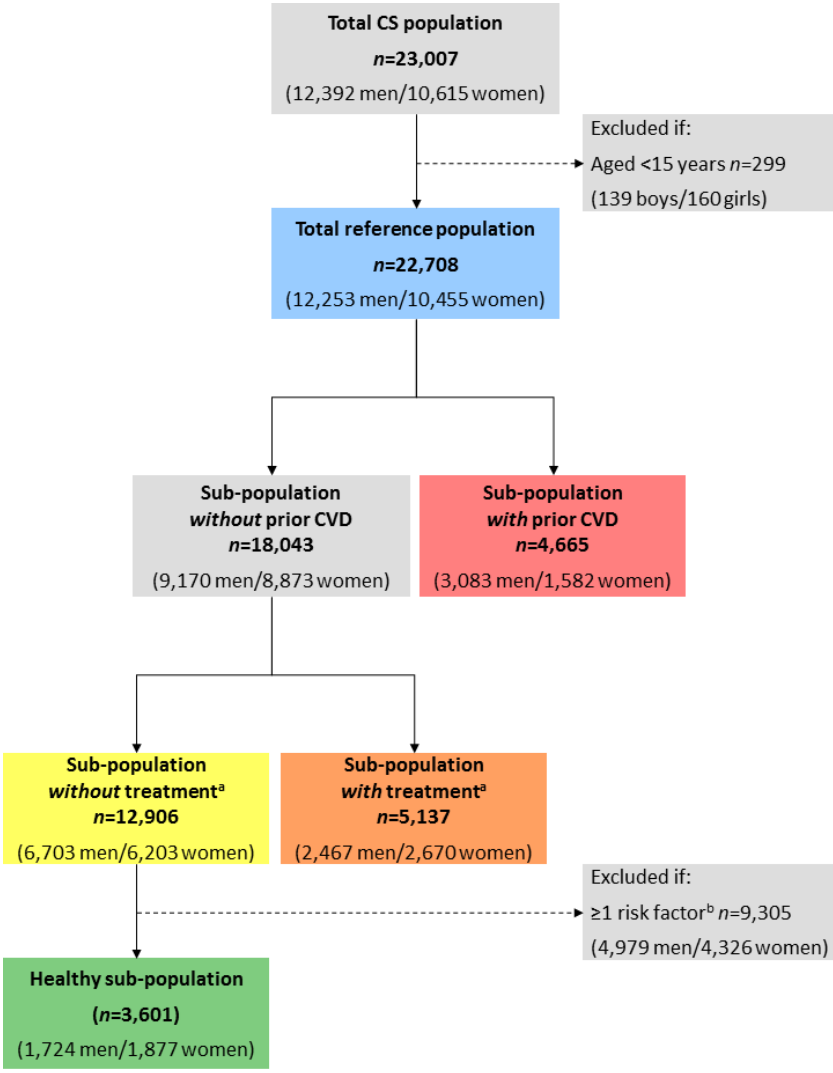


Figure 5.1 Study flowchart describing the selection and categorization of individuals from the total carotid stiffness (CS) to the reference and healthy sub-populations.

[a]BP-, lipid-, and/or glucose-lowering medication. [b]Risk factors considered were hypertension (systolic blood pressure/diastolic blood pressure ≥140/90 mmHg), current smoking, diabetes [self-reported diabetes and/or fasting plasma glucose ≥7.0 mmol/L and/or post-load plasma glucose ≥11.0 mmol/L (if available)], total cholesterol >6.2 mmol/L, HDL cholesterol <1.17 mmol/L (for men) and <1.30 mmol/L (for women), and body mass index ≥30 kg/m^2.

Table 5.1 Contributing centres (in order of decreasing number of participating individuals) and respective carotid artery measurement techniques used.

Total n	Healthy sub-population n		Centre	Study name/ acronym	Echotracking system	Anatomical location*	(Local) PP measure-ment	MAP calculation for local PP
4,892	-		Rotterdam (NL)	Rotterdam Study	WTS	1 cm	Brachial PP	-
4,772	1,059	810	Paris-HEGP (F)	PPS3 (*n*=3,762)	ART.LAB[a]	1 cm	Distension waveforms	Distension waveforms
		201		HEGP studies (*n*=622)	WTS[b]	2 cm	Carotid tonometry/	Radial tonometry
		48		CASHMERE (*n*=388)	WTS	2 cm	brachial PP (277/304)	Radial tonometry
							Carotid tonometry	
3,423	14	14	Utrecht (NL)	SMART (*n*=3,296)	WTS	2 cm	Brachial PP	-
		-		Whistler Cardio (*n*=127)	ART.LAB	1 cm	Brachial PP	-
2,027	742		Ghent (BE)	ASKLEPIOS	Echopac	1-2 cm	Carotid tonometry	Brachial tonometry
1,597	279	45	Maastricht/	Hoorn study (*n*=717)	WTS	1 cm	Distension waveforms	Distension waveforms
		192	Amsterdam (NL)	AGAHLS (*n*=406)	WTS	1 cm	Distension waveforms	Distension waveforms
		42		CODAM 1 (*n*=474)	WTS	1-2 cm	Brachial PP	-
1,367	338		Leuven (BE)	FLEMENGHO	WTS	2 cm	Carotid tonometry	Maximal oscillometry
854	398		Shanghai (CN)	Ningbo Working place	ART.LAB	0-1 cm	Radial tonometry[f]	Constant
664	158	37	Pisa (I)	CATOD (*n*=369)	Carotid	1 cm	Carotid tonometry	Brachial tonometry
		121		Other (*n*=295)	Studio[e]		/Radial tonometry	Brachial tonometry/
							(241/54)	Constant (241/54)
570	74		Mannheim (D)	MIPH Industrial Cohort Study	ART.LAB	1 cm	Brachial PP	-
472	83		Vilnius (LT)	LitHir	ART.LAB	1 cm	Carotid tonometry/	Radial tonometry
							brachial PP (249/223)	
359	11		Antwerp (BE)		WTS	2 cm	Brachial PP	-

307	65	São Paulo (BR)	CHEST-BR, GeneHy	WTS	1 cm	Brachial PP	-
300	32	Nancy (F)	ARTEOS study	WTS	2 cm	Brachial PP	-
248	71	Bern (CH)		ART.LAB	1 cm	Carotid tonometry	Brachial tonometry
223	29	Milano/Monza (I)		ART.LAB	2 cm	Radial tonometry	Constant
222	43	Maastricht-VitaK (NL)		ART.LAB	2 cm	Brachial PP	-
176	127	Budapest (H)		ART.LAB	1 cm	Carotid tonometry	Radial tonometry
136	36	Rouen (F)		ART.LAB	1 cm	Carotid tonometry	Radial tonometry
121	2	Paris-Foch (F)		ART.LAB	1 cm	Carotid tonometry	Maximal oscillometry
85	-	Gdansk (PL)	CareNorth	ART.LAB	1 cm	Carotid tonometry	Constant (0.33)
43	-	Pilsen (CZ)	SAS study	ART.LAB	1 cm	Brachial PP	-
32	-	Québec (CDN)		ART.LAB	1 cm	Carotid tonometry	Radial tonometry
21	-	Montreal (CDN)		ART.LAB	1 cm	Carotid tonometry	Brachial tonometry

*Anatomical location of the measurement is expressed as distance (in cm) proximal to the carotid bifurcation; [a]ART.LAB echotracking system (ESAOTE, Maastricht, the Netherlands); [b]Wall Track System [WTS (former version of ART.LAB), ESAOTE, Maastricht, the Netherlands]; [c]Vivid-7 US system (GE Vingmed Ultrasound, Horten, Norway) with Echopac post-processing; [d]Aloka SSD-650 US system (Aloka, Tokyo, Japan) with post-processing in dedicated software (M'ATHS, Metris, France); [e]Carotid Studio (Institute of Clinical Physiology, National Research Council, Pisa, Italy); [f]Radial tonometry plus transfer function (Sphygmocor, Atcor Medical, Australia).

5.3.2 Estimation of carotid artery stiffness: preliminary methodological considerations

Level of carotid artery stiffness was expressed by the distensibility coefficient (DC_{car}), calculated as described earlier (section 1.3.2.1).

Estimates of local carotid PP were only available in a subsample of 50% (n=11,458) of the total study population. Among them, the correlation between brachial and estimated local PP was strong both in men (r=0.73, p<0.001) and women (r=0.80, p<0.001), though strongest for those in the oldest tertile (youngest: r=0.57, middle: r=0.79, oldest: r=0.82). This indicates that the rank between individuals within the study population will not change much when DC_{car} will be estimated using one or the other PP. Therefore, we chose to use DC_{car} as estimated with brachial PP to preserve the largest population sample, though we acknowledge that such values would expectedly be slightly higher than when using local PP in the calculation due to the amplification in PP between central and peripheral arterial sites. Nevertheless, and for completeness, we have also estimated the reference intervals for DC_{car} calculated with local carotid PP, though these data were confined to 3,123 individuals out of the 3,601 individuals meeting the criteria for a healthy sub-population, i.e., free from CV-RFs, prior CVD and not on medication.

5.3.2.1 Measurement of diameter and distension

Only external (diastolic) carotid diameter and distension data obtained by means of echotracking was included (either pure echotracking or related techniques).

Different types of ultrasound systems were used across centres:

(1) the ART.LAB system (*n*=6,841; advanced version of WTS; ESAOTE, Maastricht, The Netherlands);
(2) the Wall Track System (*n*=13,176; WTS, ESAOTE, Maastricht, The Netherlands[158]);
(3) the Vivid-7 US system, with Echopac post-processing, which has been validated against the WTS.[159] (*n*=2,027; GE Vingmed Ultrasound, Horten, Norway)
(4) Carotid Studio (*n*=664; Institute of Clinical Physiology, National Research Council, Pisa, Italy).[20]

The exact anatomical location of the measurement of carotid artery diameter and distension differed across centres:

(1) 0-1 cm proximal to the carotid artery bifurcation (*n*=854);
(2) 1 cm proximal to the carotid artery bifurcation (*n*=12,528);
(3) at 1-2 cm proximal to the carotid artery bifurcation (*n*=2,601)
(4) at 2 cm proximal to the carotid artery bifurcation (*n*=6,725)

Therefore, prior to the calculation of DC, all carotid diameter and distension values obtained with different echotracking systems and anatomical location were standardized. To this aim, original carotid diameter and distension values were rescaled to the same metric of the most recent system and the mostly used anatomical location, i.e. measurements with the ART.LAB system and centred at 1 cm proximal to the carotid bifurcation (Table 5.2).

5.3.2.2 Measurement of local pulse pressure

Different methods to determine local carotid PP were used. First, carotid distension waveforms were obtained and rescaled using brachial distension waveforms (*n*=4,807). Second, carotid tonometry was performed and the obtained pressures were rescaled with brachial MAP calculated using brachial tonometry (*n*=2,940), radial tonometry (*n*=1,247), maximal oscillometry

(n=1,384) or the equation MAP=DBP+1/3*PP (n=71). Third, radial tonometry was performed to obtain carotid pressures using a transfer function (Sphygmocor, Atcor Medical, Australia; n=1,009) (Table 5.3). Similar to the calibration analyses for diameter and distension, multiple linear regression analyses with included dummy variables for each method (with carotid distension waveforms + brachial distension waveforms as reference) was used to obtain 'calibration factors' to rescale individual carotid PP values to the reference technique (Table 5.3). We used these rescaled carotid PP values in all further analyses.

5.3.3 Statistical analyses

Statistical analyses were performed using the Statistical Package for Social Sciences, version 20 (SPSS, Inc, Chicago, IL, USA) unless specified otherwise.

5.3.3.1 *Multiple imputation of missing values*

A total of 3,165 individuals (14% of the total reference population) had missing values for one (n=3,044) or more (n=121) of the co-variables of interest. The percentage missing values per variable varied from 0.3% (BMI) to 13% (total cholesterol). We used multiple imputation chained equations to impute those values rather than perform complete case analyses in order to decrease bias and increase the power of the analyses.[160,161]

Table 5.2 Calibration factors for carotid diameter and distension values as obtained with different measurement devices and locations

	Carotid diameter			Carotid distension		
	β	95% CI	p	β	95% CI	p
Echotracking system [reference=ART.LAB* (n=6,841)]						
Wall Track system (n=13,176)	0.220	0.191; 0.250	<0.001	0.019	0.014; 0.024	<0.001
Vivid-7 (n=2,027)	0.191	0.109; 0.273	<0.001	0.185	0.172; 0.198	<0.001
Carotid studio (n=664)	-0.082	-0.149; -0.015	0.016	0.113	0.102; 0.123	<0.001
Anatomical location [reference=centered at 1 cm** (n=12,528)]						
0-1 cm (n=854)	0.910	0.849; 0.970	<0.001	-0.015	-0.024; -0.005	0.002
1-2 cm (n=2,601)	-0.068	-0.139; 0.003	0.062	-0.115	-0.126; -0.103	<0.001
2 cm (n=6,725)	-0.125	-0.155; -0.095	<0.001	0.004	-0.001; 0.009	0.105

Regression coefficients β represent the mean difference in carotid artery diameter (in mm) or distension (in mm) when using each of the echotracking systems, and/or anatomical locations vs. the reference one (as indicated above) at mean levels of age, sex, MAP, total-HDL cholesterol ratio, smoking, diabetes, BMI, history of CVD, and use of BP- and/or lipid-lowering medication in the total reference population (n=22,812).

*In contrast to the Wall Track system, Vivid-7 and Carotid Studio, which select a single M-line, ART.LAB takes measures over an arterial width of >10 mm, comprising multiple M-lines, which may yield considerably more precise measurements.

**Anatomical location is expressed as distance (in cm) proximal to the carotid bifurcation.

On the basis of this equation, to rescale diameter values obtained by, for instance, the Wall Track System (WTS) to values of ART.LAB, 0.220 mm needs to be *subtracted* from the original WTS values. In addition, the appendix (p203) contains reference tables calibrated to each specific device.

Table 5.3 Calibration factors for local carotid artery pulse pressure values as obtained with different methods

	Carotid artery pulse pressure		
	β	95% CI	p
Reference method	-	-	-
Carotid tonometry + brachial tonometry	0.8	0.0; 1.5	0.049
Carotid tonometry + radial tonometry	1.9	1.2; 2.7	<0.001
Carotid tonometry + maximal oscillometry	-0.9	-3.7; 1.8	0.505
Carotid tonometry + constant	-5.7	-6.5; -4.9	<0.001
Radial tonometry + transfer function	5.4	4.7; 6.0	<0.001

Regression coefficients β represent the mean difference in local carotid pulse pressure (in mm Hg) when using each of the local PP measurement techniques vs. the reference one (carotid distension waveforms + brachial distension waveforms) at mean levels of age, sex, MAP, heart rate, total-HDL cholesterol ratio, BMI, history of CVD, and use of BP- and/or lipid-lowering medication only in individuals in whom a measure of local carotid PP was performed (n=11,558).

On the basis of this equation, to rescale local carotid PP values obtained by for instance radial tonometry + transfer function to values of carotid distension waveforms + brachial distension waveforms (i.e. to the values presented in Table 5.10), to the original radial tonometry + transfer function values 5.6 mm Hg needs to be *added*.

5.3.3.2 *Standardization of carotid artery diameter and distension measurements*

We performed multiple linear regression analyses that included dummy variables for each echotracking system (with ART.LAB as reference) and anatomical location of the measurement (with measurements centred at 1 cm proximal to the carotid bifurcation as reference) as independent determinants of carotid diameter and distension. These analyses were conducted in the total population (n=22,708) and included adjustments for all CV-RFs, history of CVD and use of BP- and/or lipid-lowering medication. The regression coefficients (β) for the dummy variables hereby obtained were used as 'calibration factors' to rescale individual carotid diameter and distension values to the refer-

ence technique (for details, see Table 5.2). We used these rescaled carotid diameter and distension values in all further analyses.

5.3.3.3 *Definition of age- and sex-specific reference intervals of DC_{car}*

Calculation of age-specific reference intervals for DC_{car} [and additionally for the individual components of the DC_{car} (i.e., carotid diameter, carotid distension and brachial PP)] was conducted in the healthy sub-population (*n*=3,601), and in men and women separately. To this aim we used a parametric regression method based on fractional polynomials (FPs) as described by Royston and Wright and implemented in STATA software (version 11.0 Stata Corp., College Station, TX, USA).[162–164] Briefly, carotid DC data were assumed to be normally distributed, conditional on age. With the STATA command xrigls, the best fitting FPs for the age-specific mean and standard deviation (SD) regression curves were defined using an iterative procedure (generalised least squares - GLS). Results of these analyses enable estimation of the age-specific mean and SD of DC_{car} as $mean_{DC} = a + b*age^{p} + c*age^{q} + \ldots$, where *a*, *b*, *c*, … are the coefficients, and *p*, *q*, … are the powers with numbers selected from the set [-2, -1, -0.5, 0, 0.5, 1, 2, 3] estimated from the regression for the $mean_{DC}$ curve and, likewise, from the regression for the SD_{DC} curve. For example, FPs with powers [1 2], that is, with *p*=1 and *q*=2, illustrate an equation with the form $a + b*age + c*age^{2}$. Estimated *mean*, *SD* and *Z-scores* [i.e., $(observed_{DC} - mean_{DC})/SD_{DC}$] were all stored in the dataset. The *Z-scores* were used to assess the model fit, which was deemed appropriate if these were normally distributed with a mean of 0 and an SD of 1, and randomly scattered above and below 0 when plotted against age. Finally, age-specific 2.5th, 10th, 25th, 50th, 75th, 90th, and 97.5th percentile curves were calculated as *meanDC + Zp* SD*, where *Zp* assumed the values of -1.96, -1.28, -0.67, 0, 0.67, 1.28, and 1.96, respectively.

5.3.3.4 *Association with risk factors*

Based on the equations estimated as described above, we computed expected 'normal' mean DC_{car} (and additionally carotid diameter, carotid distension an brachial PP) values for each individual in the reference sub-populations (i.e. those with and without CVD and/or medication) and calculated age- and sex-specific DC_{car} Z-scores as ($observed_{DC}$ – $expected_{DC}$)/$SDexpected_{DC}$; this allows for a standardized comparison between observed DC values vs. those from healthy individuals of the same age and sex, expressed by the number of SDs that an individual measurement lies above or below the healthy population median (i.e., 50^{th} percentile).

The associations between known CV-RFs and the DC_{car} Z-scores were then investigated in the different reference sub-populations to enable interpretation of DC_{car} values across different risk groups. We performed (multiple) linear regression analyses, unadjusted (model 1), adjusted for mean arterial pressure (MAP; model 2) and additionally adjusted for the other CV-RFs (model 3) to assess to which extent the associations between the individual CV-RFs and DC_{car} Z-scores were independent of MAP and the other CV-RFs.

The fully adjusted analyses were additionally performed for Z-scores of the individual components of DC_{car} (i.e., of carotid diameter, carotid distension and brachial PP). In these analyses, we estimated standardized regression coefficients to enable comparison of the magnitude of the associations and interpretation of the association between CV-RFs and DC_{car} Z-scores in view of the associations between CV-RFs and the individual components, i.e., to enable understanding of the driving forces behind the associations between CV-RFs and DC_{car} Z-scores.

In addition, we added interaction terms between sex and each of the CV-RFs to the models to assess potential effect modification.

Table 5.4 Risk factors and clinical characteristics of the total, healthy and reference sub-populations in *men*.

	Total Reference population	Healthy sub-population	Sub-population without CVD		Sub-population with CVD
			without treatment[a]	with treatment[a]	
N	12,253	1,724	6,703	2,467	3,083
Carotid diameter (mm)	7.6 ± 1.0	7.0 ± 0.7	7.3 ± 0.9	7.7 ± 1.0	8.0 ± 1.2
Carotid distension (mm)	0.42 ± 0.17	0.49 ± 0.19	0.45 ± 0.17	0.40 ± 0.15	0.38 ± 0.14
PP (mm Hg)	56 ± 14	51 ± 9	54 ± 12	59 ± 15	60 ± 15
DC_{car} (10^{-3} kPa^{-1})	16.6 ± 8.5	22.1 ± 9.8	18.9 ± 9.2	14.4 ± 6.7	13.3 ± 6.4
Age [years (range)]	56 (15-99)	45 (15-81)	51 (15-99)	58 (16-98)	63 (23-97)
Body mass index (kg/m^2)	26.2 ± 3.7	23.9 ± 2.7	25.6 ± 3.5	27.5 ± 3.9	26.4 ± 3.4
SBP (mm Hg)	135 ± 19	122 ± 10	131 ± 17	142 ± 19	140 ± 20
DBP (mm Hg)	79 ± 11	73 ± 8	78 ± 11	82 ± 12	80 ± 10
MAP (mm Hg)	102 ± 13	92 ± 8	99 ± 12	106 ± 13	104 ± 13
Hypertension [n (%)]	6,363 (52)	-	1,920 (29)	2,032 (83)	2,410 (78)
Total cholesterol (mmol/L)	5.4 ± 1.1	5.0 ± 0.8	5.5 ± 1.1	5.4 ± 1.1	5.3 ± 1.1
LDL cholesterol (mmol/L)	3.4 ± 1.0	3.0 ± 0.7	3.5 ± 1.0	3.4 ± 1.0	3.4 ± 1.0
HDL cholesterol (mmol/L)	1.3 ± 0.4	1.5 ± 0.3	1.3 ± 0.4	1.2 ± 0.4	1.2 ± 0.3
Total-to-HDL cholesterol	4.6 ± 2.1	3.3 ± 0.7	4.4 ± 1.6	4.7 ± 2.7	4.9 ± 2.3
Triglycerides (mmol/L)	1.4 (1.0-2.0)	0.9 (0.7-1.3)	1.2 (0.9-1.8)	1.5 (1.1-2.1)	1.5 (1.1-2.1)
Fasting glucose (mmol/L)	5.9 ± 1.7	5.1 ± 0.7	5.4 ± 1.1	6.4 ± 2.3	6.3 ± 1.9
Diabetes [n (%)]	1,442 (12)	-	253 (4)	580 (24)	609 (20)
Current smoking [n (%)]	2,952 (24)	-	1,518 (23)	509 (21)	924 (30)
BP-lowering drugs [n (%)]	3,646 (30)	-	-	1,810 (74)	1,836 (60)
Lipid-lowering drugs [n (%)]	2,221 (18)	-	-	988 (40)	1,233 (40)
Glucose-lowering drugs [n (%)]	689 (6)	-	-	404 (16)	285 (9)
History of CVD [n (%)]	3,083 (25)	-	-	-	3,083 (100)

Data are presented as means ± SD, medians [interquartile ranges] or numbers (percentages), as appropriate. [a]BP-, lipid- and/or glucose-lowering treatment.

Table 5.5 Risk factors and clinical characteristics of the total, healthy and reference sub-populations in *women*.

	Total Reference population	Healthy sub-population	Sub-population without CVD		Sub-population with CVD
			without treatment[a]	with treatment[a]	
N	10,455	1,877	6,203	2,670	1,582
Carotid diameter (mm)	6.9 ± 0.9	6.4 ± 0.7	6.7 ± 0.8	7.2 ± 0.9	7.2 ± 0.9
Carotid distension (mm)	0.37 ± 0.14	0.44 ± 0.16	0.39 ± 0.15	0.34 ± 0.13	0.35 ± 0.13
PP (mm Hg)	56 ± 15	48 ± 9	52 ± 13	61 ± 16	64 ± 17
DC_{car} (10^{-3} kPa^{-1})	16.4 ± 9.4	23.8 ± 11.3	18.7 ± 10.1	13.0 ± 6.7	13.0 ± 6.9
Age [years (range)]	57 (15-95)	44 (15-85)	53 (15-95)	62 (17-93)	63 (20-95)
Body mass index (kg/m^2)	25.7 ± 4.6	22.7 ± 2.7	24.6 ± 4.1	27.6 ± 4.9	26.4 ± 4.5
SBP (mm Hg)	132 ± 21	116 ± 11	127 ± 19	141 ± 21	140 ± 21
DBP (mm Hg)	76 ± 11	71 ± 8	74 ± 10	79 ± 12	77 ± 11
MAP (mm Hg)	99 ± 13	89 ± 9	95 ± 12	104 ± 14	102 ± 13
Hypertension [n (%)]	4,862 (47)	-	1,467 (24)	2,306 (87)	1,089 (69)
Total cholesterol (mmol/L)	5.8 ± 1.1	5.1 ± 0.7	5.7 ± 1.1	5.9 ± 1.2	5.8 ± 1.1
LDL cholesterol (mmol/L)	3.6 ± 1.0	2.8 ± 0.7	3.5 ± 1.0	3.7 ± 1.1	3.6 ± 1.0
HDL cholesterol (mmol/L)	1.6 ± 0.4	1.9 ± 0.4	1.7 ± 0.4	1.5 ± 0.4	1.5 ± 0.4
Total-to-HDL cholesterol	3.9 ± 1.5	2.8 ± 0.6	3.6 ± 1.2	4.2 ± 1.9	4.2 ± 1.5
Triglycerides (mmol/L)	1.2 (0.9-1.7)	0.8 (0.7-1.1)	1.1 (0.8-1.5)	1.4 (1.0-2.0)	1.4 (1.0-1.9)
Fasting glucose (mmol/L)	5.6 ± 1.6	4.9 ± 0.6	5.2 ± 0.9	6.1 ± 2.0	6.1 ± 1.9
Diabetes [n (%)]	976 (9)	-	182 (3)	498 (19)	296 (19)
Current smoking [n (%)]	1,861 (18)	-	1,090 (18)	402 (15)	369 (23)
BP-lowering drugs [n (%)]	2,902 (28)	-	-	2,118 (79)	785 (50)
Lipid-lowering drugs [n (%)]	1,327 (13)	-	-	875 (33)	452 (28)
Glucose-lowering drugs [n (%)]	412 (4)	-	-	285 (11)	127 (8)
History of CVD [n (%)]	1,582 (15)	-	-	-	1,582 (100)

Data are presented as means ± SD, medians [interquartile ranges] or numbers (percentages), as appropriate. [a]BP-, lipid- and/or glucose-lowering treatment.

5.4 Results

Table 5.4 and Table 5.5 show the participants' characteristics of the total, healthy and reference sub-populations, in men and women, respectively. In the total reference population, women had, on average, a slightly more favorable cardiovascular risk profile than men. Values for CV-RFs were more unfavorable for men and women from the sub-populations with treatment and/or with prior CVD compared to those from the population without treatment and CVD (p-values for trend were 0.001 for all comparisons).

5.4.1 Age- and sex-specific reference intervals for DC_{car} in the healthy sub-population

The best fitting FPs' powers (*p, q…*) for the $mean_{DC}$ curves were *p*=-0.5 for men and *p*=-2 *q*=-2 for women and for the SD_{DC} curves were *p*=-1 for men and *p*=-0.5 for women, indicating non-linear negative relations between age and DC_{car} in both men and women. Accordingly, the equations derived on the basis of the estimated coefficients were, for men:

- $Mean_{DC}$ (in 10^{-3} kPa^{-1}) = -12.85 + 70.85 x $(age/10)^{-0.5}$
- SD_{DC} (in 10^{-3} kPa^{-1}) = 2.510 + 15.43 x $(age/10)^{-1}$

and, for women:

- $Mean_{DC}$ (in 10^{-3} kPa^{-1}) = 4.958 + 1.399 x $(age/10)^{-2}$ + 218.2 x $(age/10)^{-2}$ x ln(age/10)
- SD_{DC} (in 10^{-3} kPa^{-1}) = -3.664 + 21.55 x $(age/10)^{-0.5}$

The estimated Z-scores had a mean value of 0 and an SD of 1 and, when plotted against age, were randomly distributed above and below 0 (Figure 5.2), indicating good model fit and no residual dependency on age.

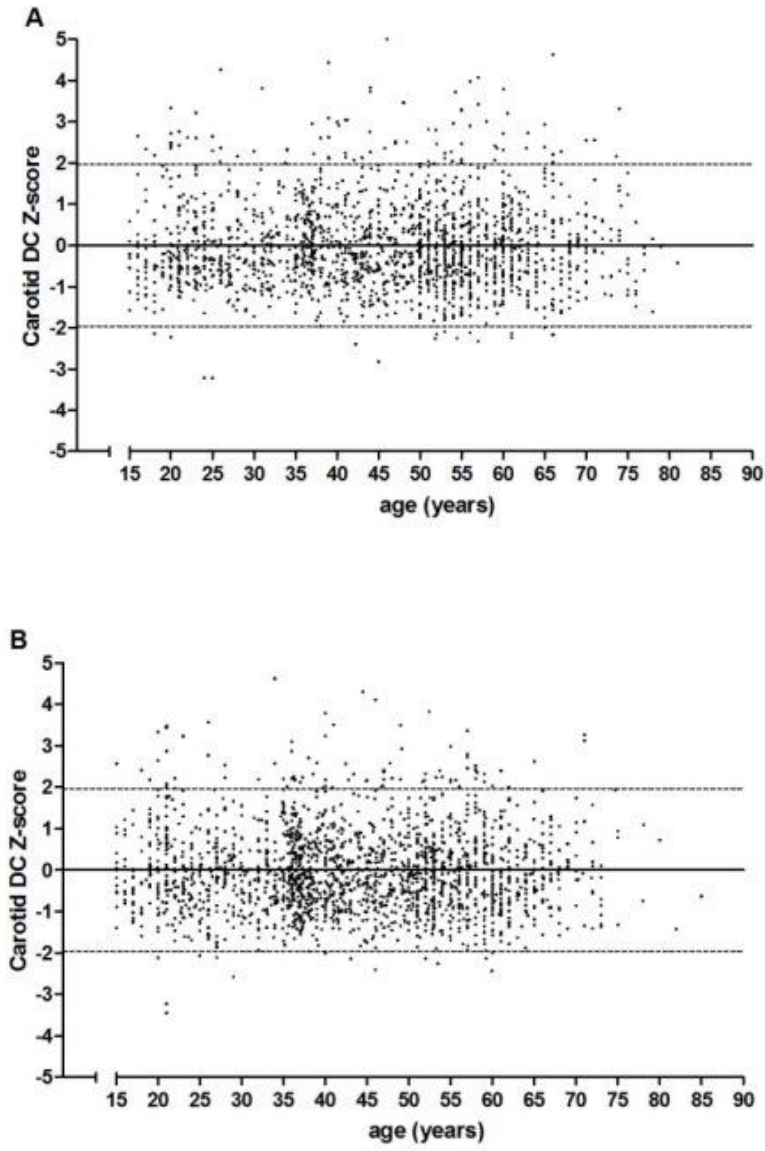


Figure 5.2 Scatter plot of DC_{car} Z-scores by age, showing the mean (horizontal line) and +/- 1.96 SD (dotted lines), from the fitted model for DC_{car} data for men (**A**) and women (**B**)

Sex-specific percentile lines superimposed on the raw data are shown in Figure 5.3 and the respective levels by age category are presented in Table 5.6.

In addition, the appendix (p203) contains reference tables (Table 0.1-0.3) calibrated to devices other than the reference method (Art.lab)

Table 5.6 Age- and sex-specific percentiles of DC_{car} (in 10^{-3} kPa^{-1}) in the healthy sub-population.

		percentiles						
	Age (years)	2.5th	10th	25th	50th	75th	90th	97.5th
Men (n=1,724)	20	17.2	24.2	30.3	37.2	44.2	50.3	57.3
	30	13.1	18.3	22.9	28.1	33.2	37.9	43.1
	40	10.1	14.4	18.3	22.6	26.9	30.7	35.1
	50	7.9	11.7	15.1	18.8	22.6	26.0	29.8
	60	6.1	9.6	12.6	16.1	19.5	22.6	26.0
	70	4.7	7.9	10.7	13.9	17.1	20.0	23.2
Women (n=1,877)	20	20.4	28.3	35.3	43.1	50.9	57.9	65.8
	30	14.5	20.5	25.8	31.7	37.7	43.0	49.0
	40	10.0	14.8	19.2	24.0	28.8	33.1	37.9
	50	7.4	11.4	15.0	19.1	23.1	26.7	30.8
	60	5.8	9.3	12.4	15.9	19.3	22.4	25.9
	70	4.9	7.9	10.6	13.7	16.7	19.4	22.4

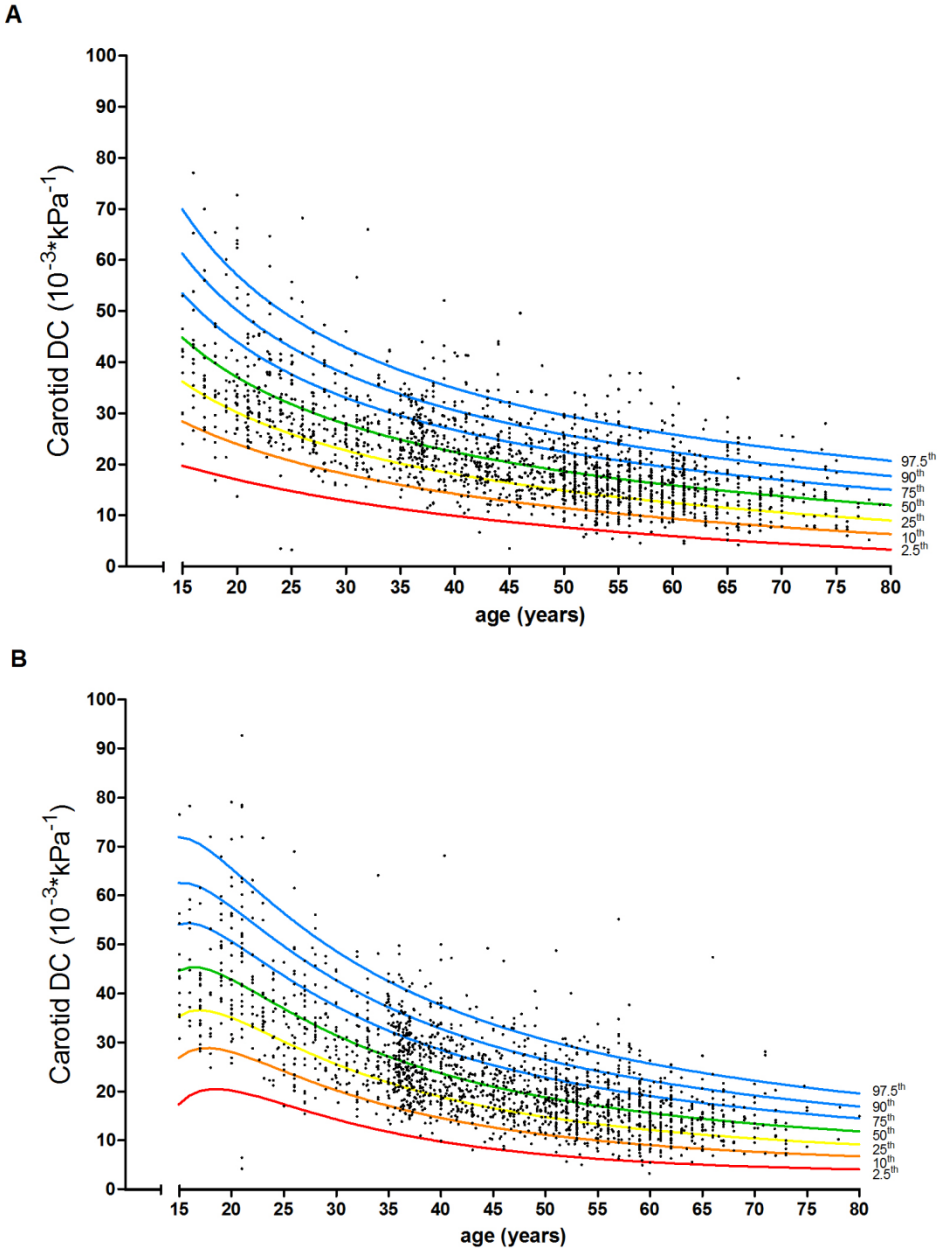


Figure 5.3 Age-specific percentiles of DC_{car} in the healthy sub-population. ***A***, men; ***B***, women.

5.4.2 Age- and sex-specific reference intervals for carotid PWV in the healthy sub-population

To enable comparison with carotid-femoral PWV metrics, DC_{car} was converted to local carotid PWV (in m/s) through the Bramwell-Hill equation.[69]

The best fitting FPs' powers (p, q, …) for the $mean_{PWV}$ curves were p=1 for both men and women and for the SD_{PWV} curves were p=1 for men and p=2 women. Accordingly, the equations derived on the basis of the estimated coefficients were, for men:

- $Mean_{PWV}$ (in m/s) = 4.011 + 0.071 x age
- SD_{PWV} (in m/s) = 0.325 + 0.017 x age

and, for women:

- $Mean_{PWV}$ (in m/s) = 3.391 + 0.082 x age
- SD_{PWV} (in m/s) = 0.640 + 0.022 x $(age/10)^2$

Sex-specific percentile lines superimposed on the raw data are shown in Figure 5.4 and the respective levels of DC_{car} by age category are presented in Table 5.7.

Table 5.7 Age- and sex-specific percentiles of carotid PWV (in m/s) in the healthy sub-population.

		percentiles						
	Age (years)	2.5th	10th	25th	50th	75th	90th	97.5th
Men (n=1,724)	20	4.1	4.6	5.0	5.4	5.9	6.3	6.7
	30	4.5	5.1	5.6	6.1	6.7	7.2	7.8
	40	4.8	5.5	6.2	6.8	7.5	8.1	8.8
	50	5.2	6.0	6.7	7.5	8.3	9.1	9.9
	60	5.6	6.5	7.3	8.3	9.2	10.0	10.9
	70	5.9	7.0	7.9	9.0	10.0	10.9	12.0
Women (n=1,877)	20	3.6	4.1	4.5	5.0	5.5	6.0	6.5
	30	4.2	4.8	5.3	5.8	6.4	6.9	7.5
	40	4.7	5.4	6.0	6.7	7.3	7.9	8.6
	50	5.2	6.0	6.7	7.5	8.3	9.0	9.8
	60	5.5	6.5	7.3	8.3	9.3	10.1	11.1
	70	5.8	6.9	8.0	9.1	10.3	11.3	12.5

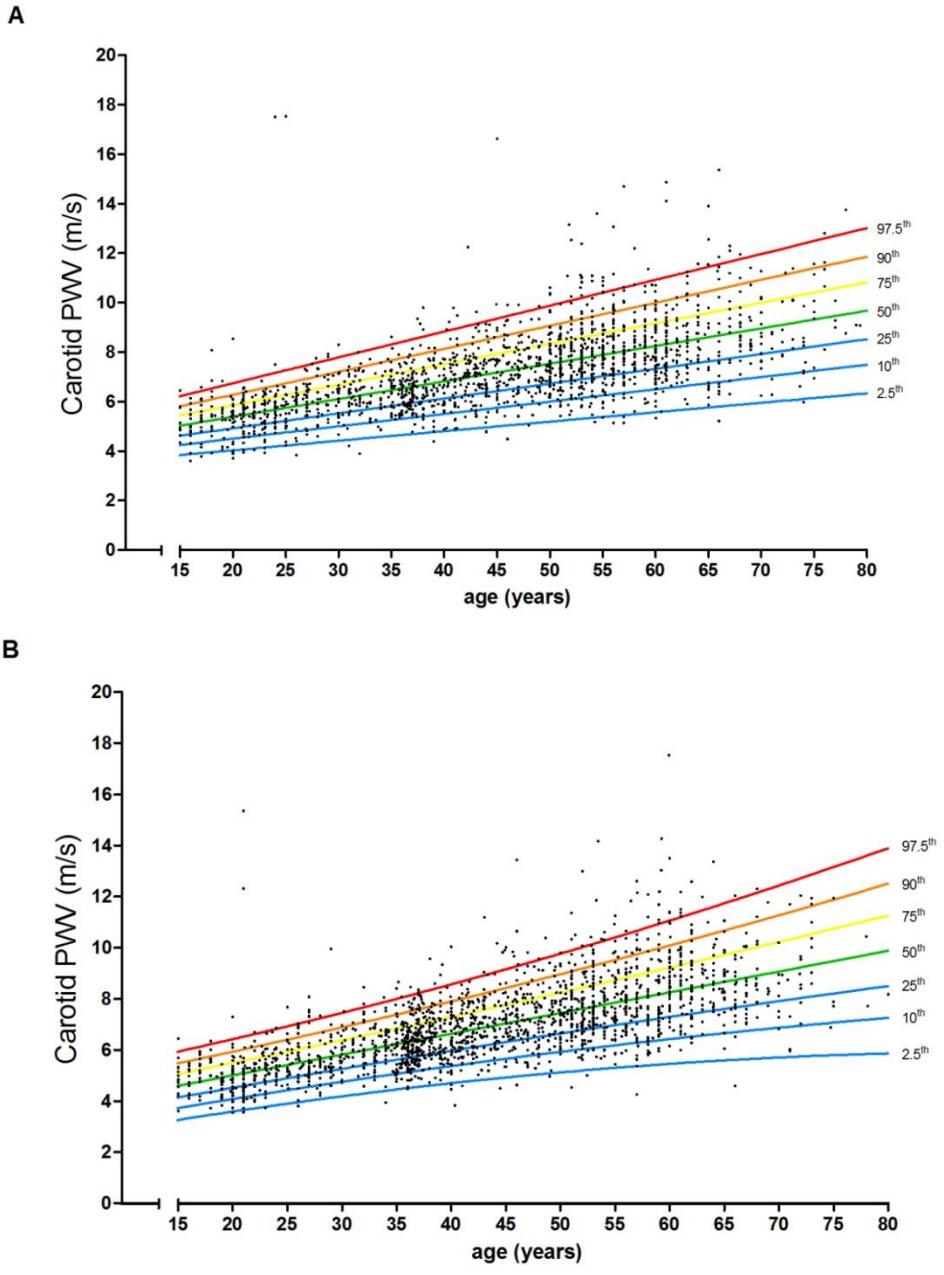


Figure 5.4 Age-specific percentiles of carotid PWV in the healthy sub-population. ***A***, men; ***B***, women.

5.4.3 Associations of cardiovascular risk factors with DC_{car} percentiles derived from the healthy sub-population

In the sub-population without prior CVD and treatment, and both in men and women, diabetes and higher MAP, total-to-HDL-cholesterol ratio and BMI were significantly associated with lower DC_{car} Z-scores (i.e. negative deviation from the healthy population median), whereas smoking was associated with higher DC_{car} Z-scores (Table 5.8 and Table 5.9, Figure 5.5). Although the positive association between smoking (yes vs. no) and DC_{car} Z-scores was stronger in younger individuals, it was positive and significant in the youngest [in the fully adjusted model: 0.36 SD (95%CI: 0.28; 0.44)], middle [0.25 (0.17; 0.32)], and oldest tertiles of age [0.15 (0.08; 0.23)] (data not shown in tables).

Similar results were found in the treated sub-population without CVD although not significantly so for smoking (in men) and total-to-HDL-cholesterol ratio (both in men and women) (Table 5.8 and Table 5.9, Figure 5.6). In the sub-population with prior CVD, smoking was again no longer associated with DC_{car} Z-scores in men and total-to-HDL-cholesterol ratio and additionally use of BP- and/or glucose-lowering medication was not associated with DC_{car} Z-scores in both men and women. However, after full adjustment, the use of lipid-lowering medication was positively associated with DC_{car} Z-scores (Table 5.8 and Table 5.9, Figure 5.7).

Comparisons by sex showed that the associations between CV-RFs and DC_{car} Z-scores were similar in direction and magnitude in men and women (P-values for interaction were all >0.01).

Table 5.8 Relation between known cardiovascular risk factors and DC_{car} Z-scores in the reference sub-populations in *men*

		Sub-population without CVD						Sub-population with CVD (n =3,083)		
		without treatment[a] (n =6,703)			with treatment[a] (n =2,467)					
Risk factor	Model	ß	95%CI	P-value	ß	95%CI	P-value	ß	95%CI	P-value
Mean arterial pressure (10 mmHg)	1	-0.250	-0.271; -0.229	<0.001	-0.285	-0.314; -0.256	<0.001	-0.362	-0.388; -0.336	<0.001
	2	-	-	-	-	-	-	-	-	-
	3	-0.222	-0.244; -0.200	<0.001	-0.271	-0.300; -0.241	<0.001	-0.346	-0.372; -0.320	<0.001
Current smoking (yes)	1	0.284	0.222; 0.346	<0.001	0.135	0.033; 0.236	0.009	0.084	0.003; 0.165	0.043
	2	0.285	0.225; 0.345	<0.001	0.078	-0.018; 0.173	0.111	0.047	-0.026; 0.120	0.204
	3	0.298	0.238; 0.358	<0.001	0.089	-0.007; 0.184	0.068	0.050	-0.025; 0.124	0.190
Diabetes (yes)	1	-0.484	-0.620; -0.347	<0.001	-0.171	-0.267; -0.074	0.001	-0.386	-0.479; -0.294	<0.001
	2	-0.328	-0.460; -0.196	<0.001	-0.188	-0.278; -0.098	<0.001	-0.263	-0.346; -0.179	<0.001
	3	-0.279	-0.411; -0.147	<0.001	-0.162	-0.252; -0.071	<0.001	-0.220	-0.331; -0.108	<0.001
Total-to-HDL cholesterol ratio (unit)	1	-0.070	-0.094; -0.046	<0.001	-0.022	-0.066; 0.022	0.270	-0.026	-0.061; 0.009	0.129
	2	-0.042	-0.063; -0.021	<0.001	-0.013	-0.046; 0.019	0.358	-0.013	-0.040; 0.014	0.309
	3	-0.037	-0.059; -0.014	0.002	-0.011	-0.042; 0.020	0.418	-0.007	-0.034; 0.020	0.587
Body mass index (kg/m^2)	1	-0.047	-0.054; -0.040	<0.001	-0.043	-0.053; -0.033	<0.001	-0.032	-0.043; -0.021	<0.001
	2	-0.024	-0.031; -0.016	<0.001	-0.030	-0.040; -0.020	<0.001	-0.019	-0.029; -0.009	0.001
	3	-0.018	-0.026; -0.010	<0.001	-0.027	-0.037; -0.017	<0.001	-0.014	-0.024; -0.004	0.006
Use of BP-lowering medication (yes)	1	-	-	-	-	-	-	-0.092	-0.168; -0.017	0.017
	2	-	-	-	-	-	-	-0.045	-0.113; 0.023	0.195
	3	-	-	-	-	-	-	-0.031	-0.102; 0.040	0.393
Use of lipid-lowering medication (yes)	1	-	-	-	-	-	-	0.129	0.054; 0.205	0.001
	2	-	-	-	-	-	-	0.068	0.000; 0.136	0.050
	3	-	-	-	-	-	-	0.097	0.025; 0.170	0.009
Use of glucose-lowering medication	1	-	-	-	-	-	-	-0.341	-0.470; -0.213	<0.001
(yes)	2	-	-	-	-	-	-	-0.253	-0.369; -0.138	<0.001
	3	-	-	-	-	-	-	-0.043	-0.193; 0.108	0.579

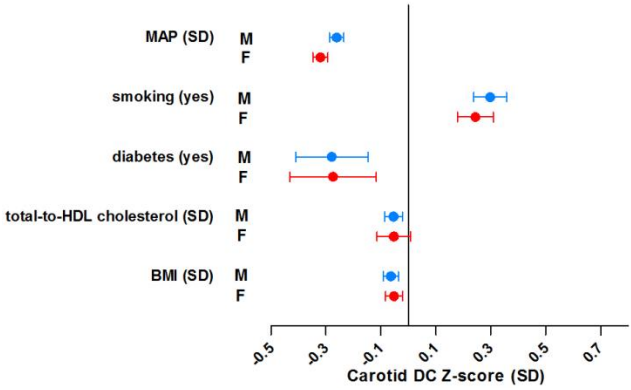
The regression coefficient ß represents the change in DC_{car} (in SD from the healthy population mean among individuals of the same age and sex) per unit increase in each risk factor. Model 1: unadjusted. Model 2: adjusted for MAP. Model 3: adjusted for MAP and all other risk factors. [a]BP-, lipid- and glucose-lowering treatment

Table 5.9 Relation between known cardiovascular risk factors and DC_{car} Z-scores in the reference sub-populations in *women*

		Sub-population without CVD						Sub-population with CVD (n =1,582)		
		without treatment[a] (n =6,203)			with treatment[a] (n =2,670)					
Risk factor	Model	ß	95%CI	P-value	ß	95%CI	P-value	ß	95%CI	P-value
Mean arterial pressure (10 mmHg)	1	-0.288	-0.308; -0.268	<0.001	-0.260	-0.287; -0.232	<0.001	-0.343	-0.380; -0.307	<0.001
	2	-	-	-	-	-	-	-	-	-
	3	-0.258	-0.279; -0.236	<0.001	-0.247	-0.275; -0.220	<0.001	-0.312	-0.349; -0.275	<0.001
Current smoking (yes)	1	0.282	0.214; 0.351	<0.001	0.203	0.089; 0.317	<0.001	0.218	0.094; 0.342	0.001
	2	0.238	0.174; 0.303	<0.001	0.147	0.040; 0.254	0.007	0.208	0.095; 0.320	<0.001
	3	0.244	0.178; 0.309	<0.001	0.133	0.026; 0.240	0.014	0.188	0.075; 0.302	0.001
Diabetes (yes)	1	-0.632	-0.800; -0.464	<0.001	-0.222	-0.323; -0.122	<0.001	-0.629	-0.760; -0.497	<0.001
	2	-0.345	-0.499; -0.191	<0.001	-0.210	-0.305; -0.116	<0.001	-0.436	-0.558; -0.313	<0.001
	3	-0.274	-0.432; -0.116	0.001	-0.166	-0.262; -0.070	0.001	-0.352	-0.511; -0.193	<0.001
Total-to-HDL cholesterol ratio (unit)	1	-0.102	-0.166; -0.038	0.009	-0.046	-0.070; -0.023	<0.001	-0.065	-0.106; -0.025	0.002
	2	-0.052	-0.105; 0.002	0.054	-0.031	-0.053; -0.008	0.008	-0.048	-0.085; -0.010	0.012
	3	-0.042	-0.095; 0.011	0.103	-0.021	-0.042; 0.001	0.061	-0.028	-0.067; 0.010	0.144
Body mass index (kg/m^2)	1	-0.045	-0.051; -0.038	<0.001	-0.029	-0.037; -0.022	<0.001	-0.041	-0.053; -0.030	<0.001
	2	-0.019	-0.025; -0.012	<0.001	-0.020	-0.028; -0.013	<0.001	-0.024	-0.034; -0.013	<0.001
	3	-0.013	-0.020; -0.005	0.001	-0.016	-0.023; -0.008	<0.001	-0.010	-0.021; 0.001	0.084
Use of BP-lowering medication (yes)	1	-	-	-	-	-	-	-0.290	-0.394; -0.185	<0.001
	2	-	-	-	-	-	-	-0.159	-0.256; -0.063	0.001
	3	-	-	-	-	-	-	-0.105	-0.207; -0.002	0.045
Use of lipid-lowering medication (yes)	1	-	-	-	-	-	-	0.015	-0.103; 0.132	0.809
	2	-	-	-	-	-	-	0.073	-0.033; 0.179	0.176
	3	-	-	-	-	-	-	0.115	0.005; 0.225	0.041
Use of glucose-lowering medication	1	-	-	-	-	-	-	-0.576	-0.769; -0.382	<0.001
(yes)	2	-	-	-	-	-	-	-0.393	-0.570; -0.216	<0.001
	3	-	-	-	-	-	-	-0.024	-0.247; 0.199	0.834

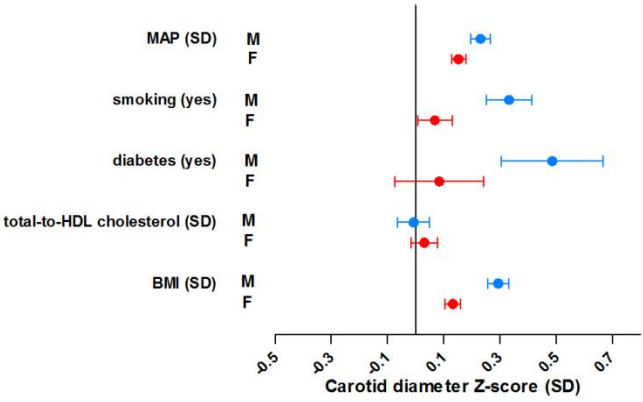
The regression coefficient ß represents the change in DC_{car} (in SD from the healthy population mean among individuals of the same age and sex) per unit increase in each risk factor. Model 1: unadjusted. Model 2: adjusted for MAP. Model 3: adjusted for MAP and all other risk factors. [a]BP-, lipid- and glucose-lowering treatment

A



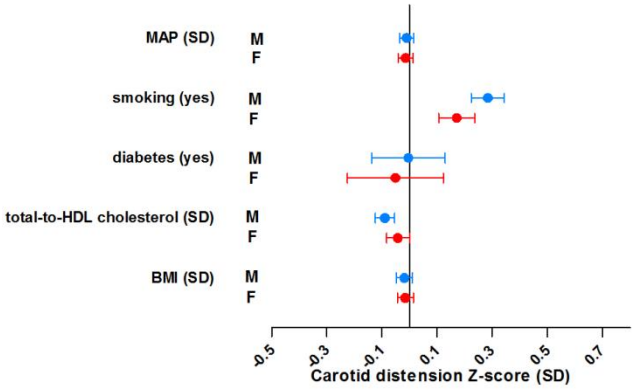
	standardized β (95% CI)	*p*-value for sex interaction
MAP (SD) M	-0.26 (-0.29; -0.24)	0.025
MAP (SD) F	-0.32 (-0.35; -0.29)	
smoking (yes) M	0.30 (0.24; 0.36)	0.227
smoking (yes) F	0.24 (0.18; 0.31)	
diabetes (yes) M	-0.28 (-0.41; -0.15)	0.960
diabetes (yes) F	-0.27 (-0.43; -0.12)	
total-to-HDL cholesterol (SD) M	-0.05 (-0.09; -0.02)	0.818
total-to-HDL cholesterol (SD) F	-0.05 (-0.11; 0.01)	
BMI (SD) M	-0.06 (-0.09; -0.04)	0.302
BMI (SD) F	-0.05 (-0.08; -0.02)	

B



	standardized β (95% CI)	*p*-value for sex interaction
MAP (SD) M	0.23 (0.20; 0.27)	<0.001
MAP (SD) F	0.15 (0.13; 0.18)	
smoking (yes) M	0.33 (0.25; 0.41)	<0.001
smoking (yes) F	0.07 (0.01; 0.13)	
diabetes (yes) M	0.49 (0.31; 0.67)	0.001
diabetes (yes) F	0.08 (-0.07; 0.24)	
total-to-HDL cholesterol (SD) M	-0.01 (-0.06; 0.05)	0.154
total-to-HDL cholesterol (SD) F	0.03 (-0.02; 0.08)	
BMI (SD) M	0.29 (0.26; 0.33)	<0.001
BMI (SD) F	0.13 (0.11; 0.16)	

C



	standardized β (95% CI)	*p*-value for sex interaction
MAP (SD) M	-0.01 (-0.04; 0.02)	0.858
MAP (SD) F	-0.01 (-0.04; 0.01)	
smoking (yes) M	0.28 (0.22; 0.34)	0.014
smoking (yes) F	0.17 (0.11; 0.24)	
diabetes (yes) M	0.00 (-0.14; 0.13)	0.671
diabetes (yes) F	-0.05 (-0.23; 0.12)	
total-to-HDL cholesterol (SD) M	-0.09 (-0.12; -0.06)	0.249
total-to-HDL cholesterol (SD) F	-0.04 (-0.08; 0.00)	
BMI (SD) M	-0.02 (-0.05; 0.01)	0.772
BMI (SD) F	-0.02 (-0.04; 0.01)	

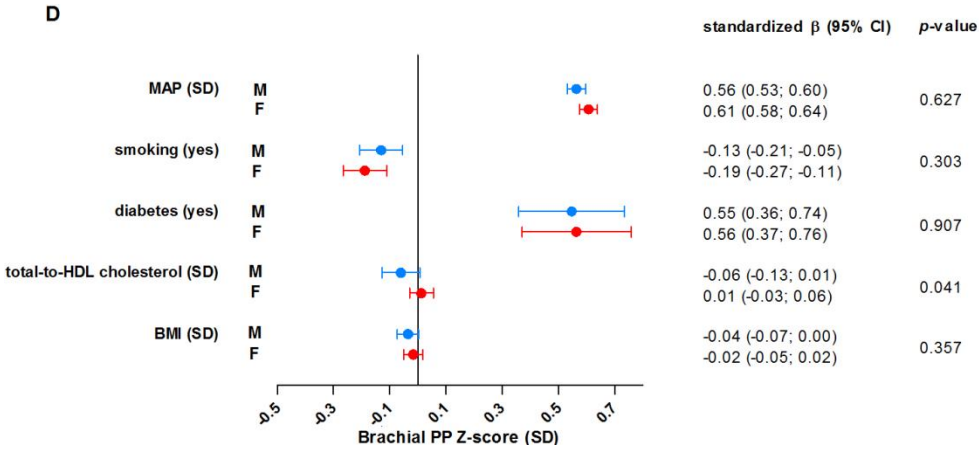
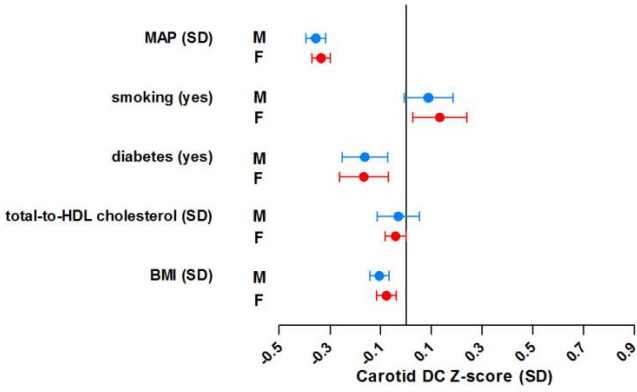
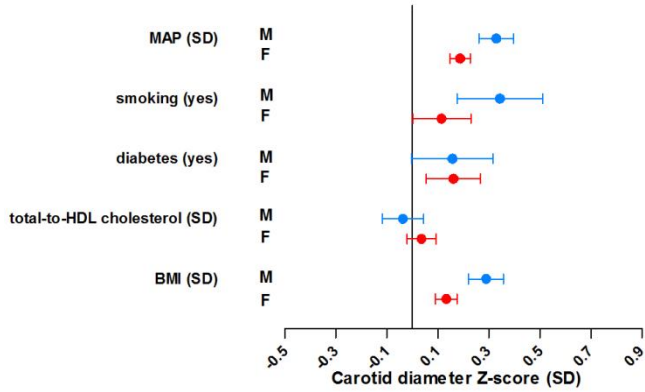


Figure 5.5 Associations between CV-RFs and DC_{car} (***A***), carotid diameter (***B***), carotid distension (***C***) and brachial PP (***D***) Z-scores: ***reference sub-population without CVD or treatment***. Point estimates and 95% confidence intervals represent the increase in the Z-scores (in SD from the healthy population mean) per SD increase (or for presence vs. absence) in risk factor resulting from a multivariable regression model including all risk factors, stratified by sex (male (M) and female (F), respectively). *BMI*, body mass index; *MAP*, mean arterial pressure. SDs in men and women, respectively, were 12 and 12 mm Hg for *MAP*, 1.6 and 1.5 for *total-to-HDL cholesterol ratio*, and 3.5 and 4.1 kg/m^2 for *BMI*. The SD equations in men and women, respectively, were $2.510+15.43*(age/10)^{-1}$ and $-3.664+21.55*(age/10)^{-0.5}$ for *DCcar* (in $10^{-3}*kPa^{-1}$), $0.514+0.001*(age/10)^3$ and $0.555+0.001*(age/10)^3$ for *carotid diameter* (in mm), $0.118+0.221*(age/10)^{-2}$ and $0.089+0.114*(age/10)^{-1}$ for *carotid distension* (in mm), and 9.940-0.035*age and 6.266+0.052*age for *brachial PP* (in mm Hg).

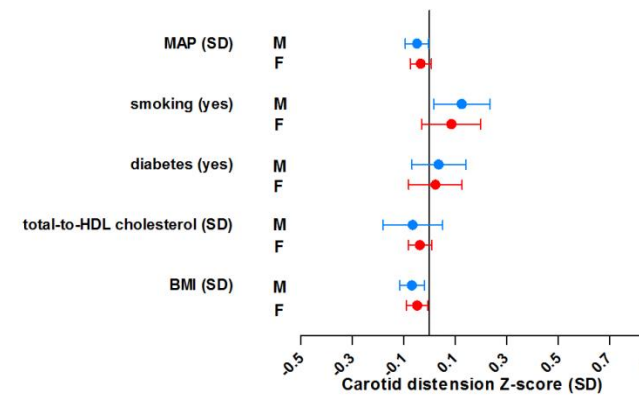
A


standardized β (95% CI)
p-value for sex interaction
MAP (SD)
M
F
-0.36 (-0.40; -0.32)
-0.34 (-0.37; -0.30)
0.244
smoking (yes)
M
F
0.09 (-0.01; 0.18)
0.13 (0.03; 0.24)
0.538
diabetes (yes)
M
F
-0.16 (-0.25; -0.07)
-0.17 (-0.26; -0.07)
0.948
total-to-HDL cholesterol (SD)
M
F
-0.03 (-0.11; 0.05)
-0.04 (-0.08; 0.00)
0.639
BMI (SD)
M
F
-0.11 (-0.14; -0.07)
-0.08 (-0.12; -0.04)
0.084
-0.5
-0.3
-0.1
0.1
0.3
0.5
0.7
0.9
Carotid DC Z-score (SD)

B


standardized β (95% CI)
p-value for sex interaction
MAP (SD)
M
F
0.33 (0.26; 0.40)
0.19 (0.15; 0.23)
<0.001
smoking (yes)
M
F
0.34 (0.18; 0.51)
0.12 (0.00; 0.23)
0.030
diabetes (yes)
M
F
0.16 (0.00; 0.32)
0.16 (0.06; 0.27)
0.963
total-to-HDL cholesterol (SD)
M
F
-0.04 (-0.12; 0.05)
0.04 (-0.02; 0.09)
0.147
BMI (SD)
M
F
0.29 (0.22; 0.36)
0.13 (0.09; 0.18)
<0.001
-0.5
-0.3
-0.1
0.1
0.3
0.5
0.7
0.9
Carotid diameter Z-score (SD)

C


standardized β (95% CI)
p-value for sex interaction
MAP (SD)
M
F
-0.05 (-0.09; 0.00)
-0.03 (-0.07; 0.01)
0.603
smoking (yes)
M
F
0.13 (0.02; 0.24)
0.09 (-0.03; 0.20)
0.616
diabetes (yes)
M
F
0.04 (-0.07; 0.14)
0.02 (-0.08; 0.13)
0.858
total-to-HDL cholesterol (SD)
M
F
-0.06 (-0.18; 0.05)
-0.04 (-0.08; 0.01)
0.803
BMI (SD)
M
F
-0.07 (-0.11; -0.02)
-0.05 (-0.09; -0.01)
0.313
-0.5
-0.3
-0.1
0.1
0.3
0.5
0.7
0.9
Carotid distension Z-score (SD)

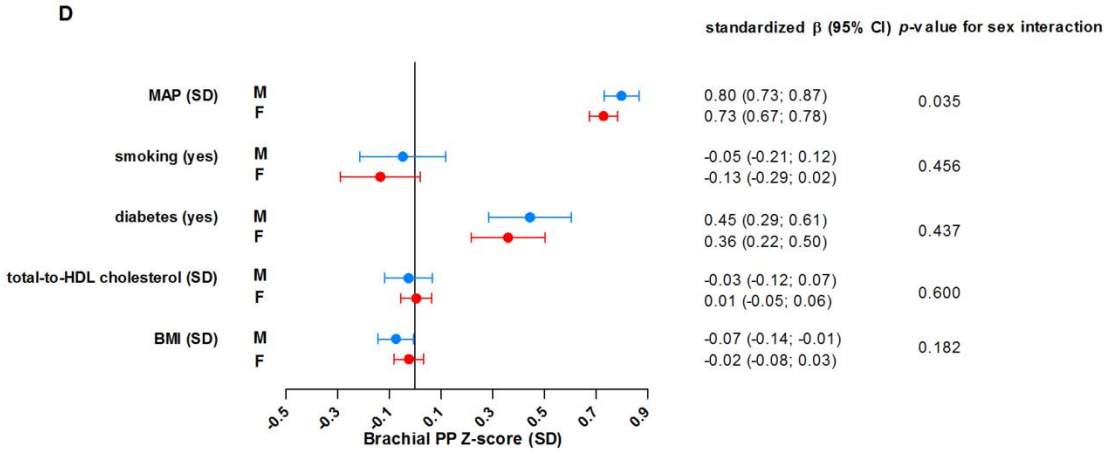
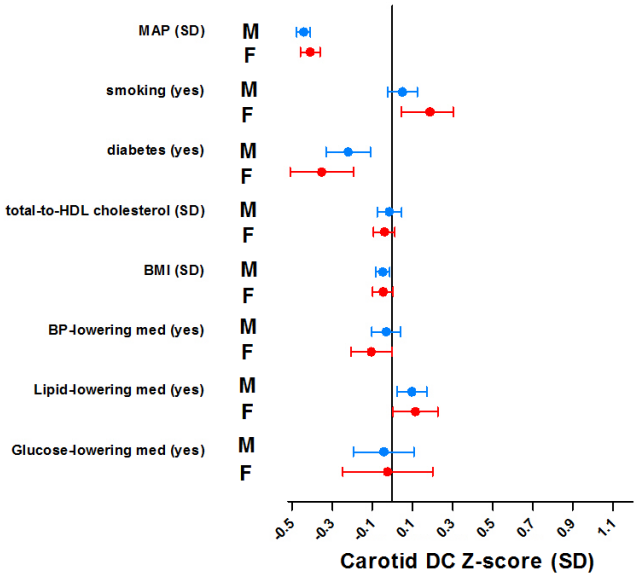
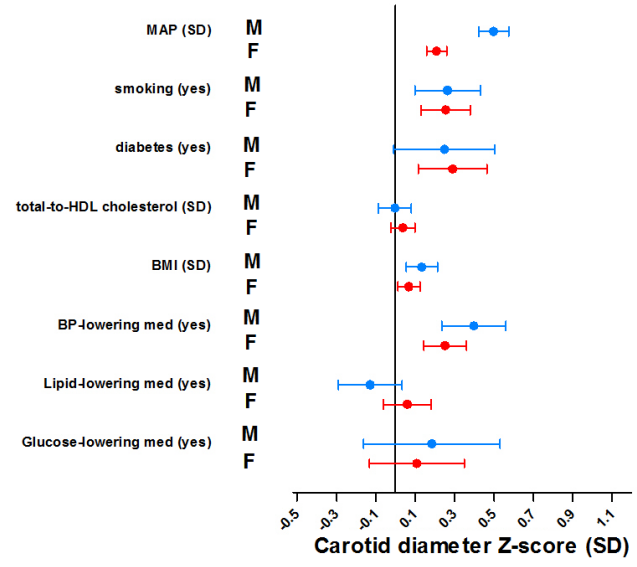


Figure 5.6 Associations between CV-RFs and DC_{car} (*A*), carotid diameter (*B*), carotid distension (*C*) and brachial PP (*D*) Z-scores: ***reference sub-population without CVD with BP-, lipid- and/or glucose-lowering treatment*.** Point estimates and 95% confidence intervals represent the increase in the Z-scores (in SD from the healthy population mean) per SD increase (or for presence vs. absence) in risk factor resulting from a multivariable regression model including all risk factors, stratified by sex (male (M) and female (F), respectively). *BMI*, body mass index; *MAP*, mean arterial pressure. SDs in men and women, respectively, were 13 and 14 mm Hg for *MAP*, 2.6 and 1.9 for *total-to-HDL cholesterol ratio*, and 3.9 and 4.9 kg/m^2 for *BMI*. The SD equations in men and women, respectively, were $2.510+15.43*(age/10)^{-1}$ and $-3.664+21.55*(age/10)^{-0.5}$ for DC_{car} (in $10^{-3}*kPa^{-1}$), $0.514+0.001*(age/10)^3$ and $0.555+0.001*(age/10)^3$ for *carotid diameter* (in mm), $0.118+0.221*(age/10)^{-2}$ and $0.089+0.114*(age/10)^{-1}$ for *carotid distension* (in mm), and 9.940-0.035*age and 6.266+0.052*age for *brachial PP* (in mm Hg).

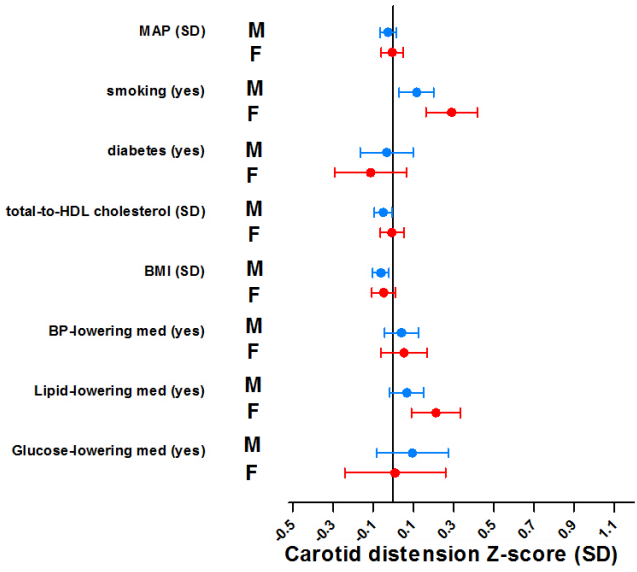
A


standardized β (95% CI)
p-value for sex interaction
MAP (SD)
M
F
-0.44 (-0.48; -0.41)
-0.41 (-0.46; -0.36)
0.136
smoking (yes)
M
F
0.05 (-0.03; 0.12)
0.19 (0.08; 0.30)
0.046
diabetes (yes)
M
F
-0.22 (-0.33; -0.11)
-0.35 (-0.51; -0.19)
0.079
total-to-HDL cholesterol (SD)
M
F
-0.02 (-0.08; 0.05)
-0.04 (-0.09; 0.01)
0.382
BMI (SD)
M
F
-0.05 (-0.08; -0.01)
-0.05 (-0.10; 0.01)
0.599
BP-lowering med (yes)
M
F
-0.03 (-0.10; 0.04)
-0.11 (-0.21; 0.00)
0.240
Lipid-lowering med (yes)
M
F
0.10 (0.03; 0.17)
0.12 (0.01; 0.23)
0.825
Glucose-lowering med (yes)
M
F
-0.04 (-0.19; 0.11)
-0.02 (-0.25; 0.20)
0.409
-0.5
-0.3
-0.1
0.1
0.3
0.5
0.7
0.9
1.1
Carotid DC Z-score (SD)

B


standardized β (95% CI)
p-value for sex interaction
MAP (SD)
M
F
0.50 (0.42; 0.58)
0.21 (0.16; 0.26)
<0.001
smoking (yes)
M
F
0.27 (0.10; 0.43)
0.25 (0.13; 0.38)
0.938
diabetes (yes)
M
F
0.25 (-0.01; 0.51)
0.29 (0.12; 0.47)
0.851
total-to-HDL cholesterol (SD)
M
F
0.00 (-0.09; 0.08)
0.04 (-0.02; 0.10)
0.511
BMI (SD)
M
F
0.13 (0.06; 0.21)
0.07 (0.01; 0.12)
0.109
BP-lowering med (yes)
M
F
0.40 (0.24; 0.56)
0.25 (0.14; 0.36)
0.235
Lipid-lowering med (yes)
M
F
-0.13 (-0.29; 0.04)
0.07 (-0.06; 0.18)
0.155
Glucose-lowering med (yes)
M
F
0.18 (-0.16; 0.53)
0.11 (-0.13; 0.35)
0.812
-0.5
-0.3
-0.1
0.1
0.3
0.5
0.7
0.9
1.1
Carotid diameter Z-score (SD)

C


standardized β (95% CI)
p-value for sex interaction
MAP (SD)
M
F
-0.03 (-0.07; 0.01)
-0.01 (-0.06; 0.05)
0.517
smoking (yes)
M
F
0.12 (0.03; 0.20)
0.29 (0.16; 0.42)
0.028
diabetes (yes)
M
F
-0.03 (-0.17; 0.10)
-0.11 (-0.29; 0.07)
0.325
total-to-HDL cholesterol (SD)
M
F
-0.05 (-0.09; -0.01)
-0.01 (-0.06; 0.05)
0.427
BMI (SD)
M
F
-0.06 (-0.10; -0.02)
-0.05 (-0.11; 0.01)
0.416
BP-lowering med (yes)
M
F
0.04 (-0.04; 0.13)
0.05 (-0.06; 0.17)
0.878
Lipid-lowering med (yes)
M
F
0.07 (-0.02; 0.15)
0.21 (0.09; 0.34)
0.063
Glucose-lowering med (yes)
M
F
0.10 (-0.08; 0.27)
0.01 (-0.24; 0.26)
0.940
-0.5
-0.3
-0.1
0.1
0.3
0.5
0.7
0.9
1.1
Carotid distension Z-score (SD)

D

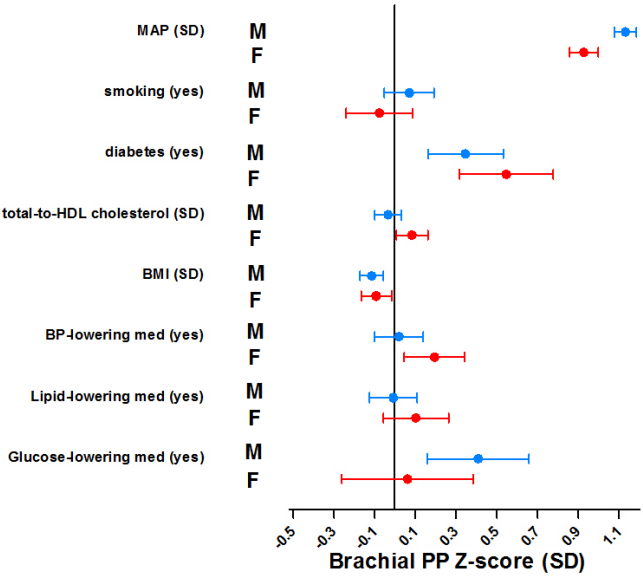

standardized β (95% CI)
p-value for sex interaction
MAP (SD)
M
F
1.13 (1.08; 1.19)
0.93 (0.86; 1.00)
<0.001
smoking (yes)
M
F
0.07 (-0.05; 0.19)
-0.08 (-0.24; 0.09)
0.185
diabetes (yes)
M
F
0.35 (0.16; 0.53)
0.55 (0.32; 0.78)
0.159
total-to-HDL cholesterol (SD)
M
F
-0.03 (-0.10; 0.03)
0.08 (0.01; 0.16)
0.029
BMI (SD)
M
F
-0.11 (-0.17; -0.06)
-0.09 (-0.17; -0.02)
0.296
BP-lowering med (yes)
M
F
0.02 (-0.10; 0.14)
0.19 (0.05; 0.34)
0.085
Lipid-lowering med (yes)
M
F
-0.01 (-0.13; 0.11)
0.10 (-0.06; 0.26)
0.287
Glucose-lowering med (yes)
M
F
0.41 (0.16; 0.66)
0.06 (-0.26; 0.39)
0.069
-0.5
-0.3
-0.1
0.1
0.3
0.5
0.7
0.9
1.1
Brachial PP Z-score (SD)

Figure 5.7 Associations between CV-RFs and DC_{car} (***A***), carotid diameter (***B***), carotid distension (***C***) and brachial PP (***D***) Z-scores: ***reference sub-population with CVD***. Point estimates and 95% confidence intervals represent the increase in the Z-scores (in SD from the healthy population mean) per SD increase (or for presence vs. absence) in risk factor resulting from a multivariable regression model including all risk factors, stratified by sex (male (M) and female (F), respectively). *BMI*, body mass index; *BP*, blood pressure; *med*, medication; *MAP*, mean arterial pressure. SDs in men and women, respectively, were 13 and 13 mm Hg for *MAP*, 2.3 and 1.5 for *total-to-HDL cholesterol ratio*, and 3.4 and 4.5 kg/m^2 for *BMI*. The SD equations in men and women, respectively, were $2.249+16.79*(age/10)^{-1}$ and $-2.894+19.73*(age/10)^{-0.5}$ for DC_{car} in $10^{-3}*kPa^{-1}$), $0.603+0.001*(age/10)^{3}$ and $0.361+1.253*(age/10)^{-1}$ for *carotid diameter* (in mm), $0.117+0.291*(age/10)^{-2}$ and $0.089+0.102*(age/10)^{-1}$ for *carotid distension* (in mm), and 9.820-0.033*age and 5.961+0.056*age for *brachial PP* (in mm Hg).

5.4.4 Additional analyses

5.4.4.1 Reference intervals for carotid artery diameter, distension, and brachial PP

The equations derived from FP analyses on the individual components of DC_{car}, i.e. carotid diameter, carotid distension and brachial PP and the sex-specific percentile lines according to age superimposed on the raw data are provided in Figure 5.8, Figure 5.9 and Figure 5.10 for diameter, distension and PP, respectively. Carotid diameter values increased and carotid distension values decreased non-linearly with age. In men, brachial PP showed a slight decrease with age until the age of 50 after which PP started to increase. In women, brachial PP showed a decrease with age until the age of 25, increasing thereafter in a fairly linear fashion. Of these three components of DC_{car}, carotid distension seemed to be the major driver behind the reduction in DC_{car} with age.

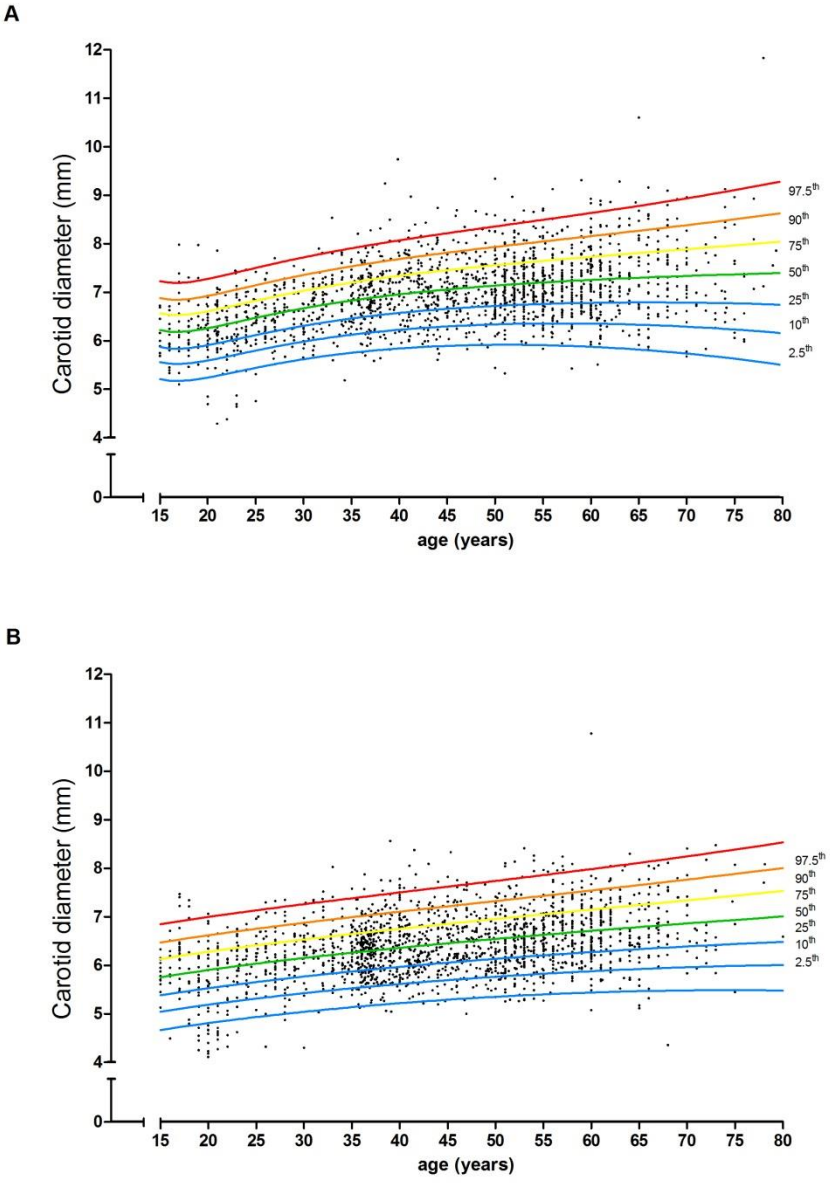


Figure 5.8 Age-specific percentiles of *carotid diameter* in the healthy sub-population. ***A***, men; ***B***, women.

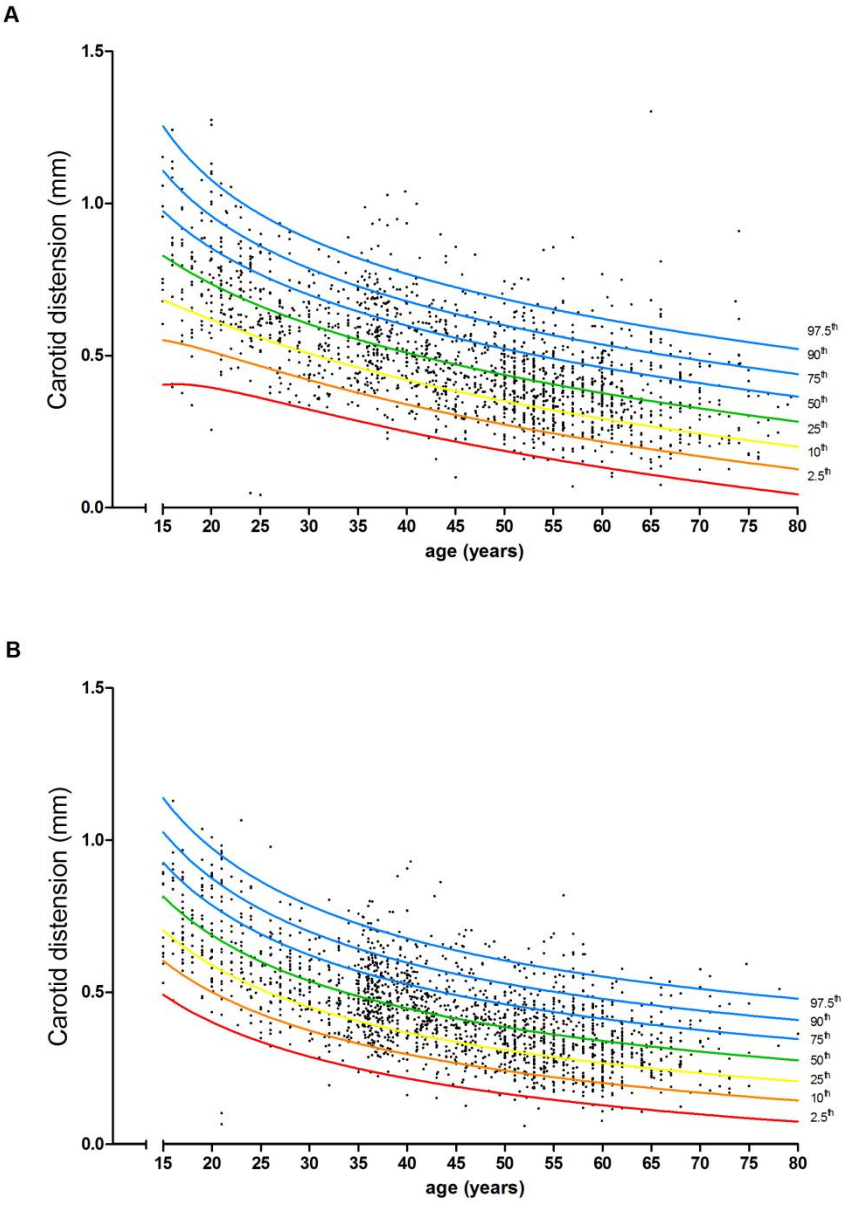


Figure 5.9 Age-specific percentiles of *carotid distension* in the healthy sub-population. ***A***, men; ***B***, women.

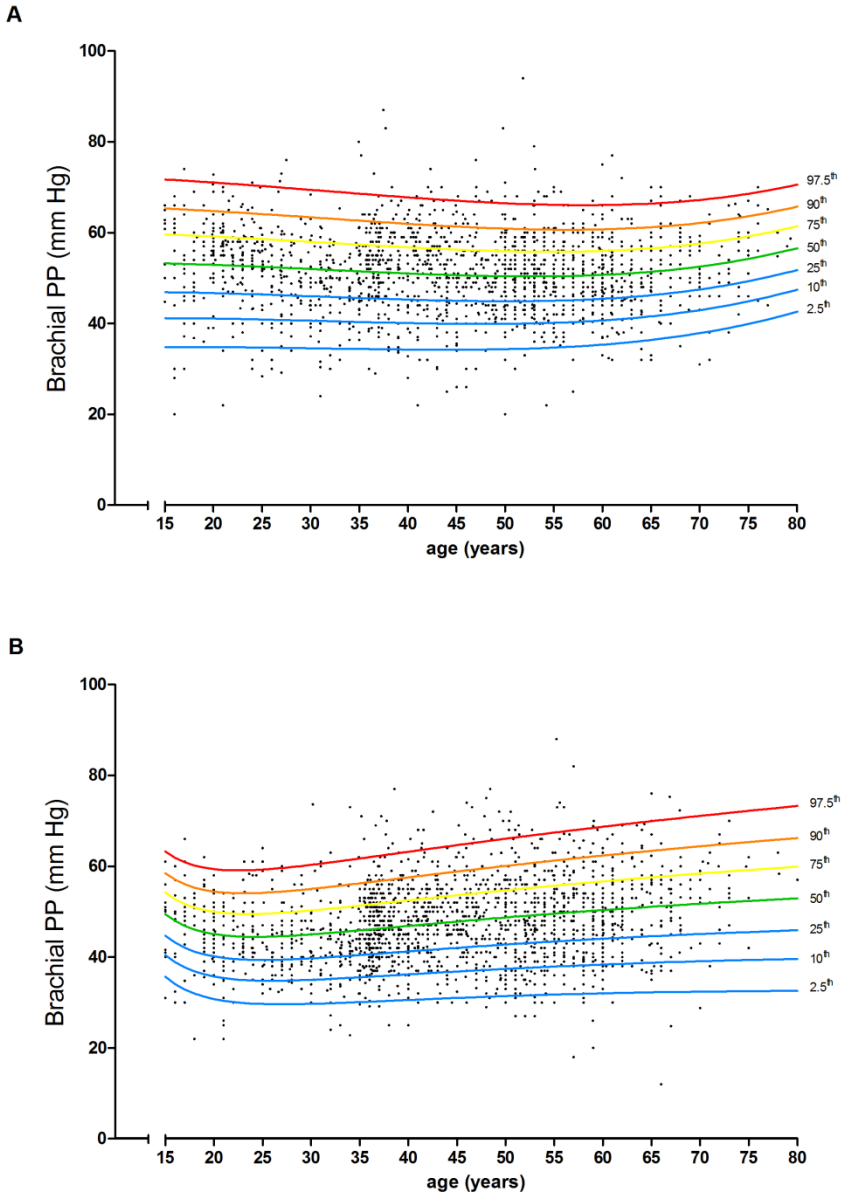


Figure 5.10 Age-specific percentiles of *brachial pulse pressure* in the healthy sub-population. ***A***, men; ***B***, women.

Age- and sex-specific reference intervals for carotid diameter in the healthy sub-population

The best fitting FPs' powers (*p, q, …*) for the $mean_{diameter}$ curves were *p*=-2 *q*=-2 for men and *p*=0.5 for women and for the $SD_{diameter}$ curves were *p*=3 for men and *p*=3 women. Accordingly, the equations derived on the basis of the estimated coefficients were, for men:

- $Mean_{diameter}$ (in mm) = $7.661 + 0.087 \times (age/10)^{-2} - 8.250 \times (age/10)^{-2} \times \ln(age/10)$
- $SD_{diameter}$ (in mm) = $0.514 + 0.001 \times (age/10)^{3}$

and, for women:

- $Mean_{diameter}$ (in mm) = $4.783 + 0.780 \times (age/10)^{0.5}$
- $SD_{diameter}$ (in mm) = $0.555 + 0.001 \times (age/10)^{3}$

Age- and sex-specific reference intervals for carotid distension in the healthy sub-population

The best fitting FPs' powers (*p*, *q*…) for the $mean_{distension}$ curves were p=0 for men and p=-0.5 for women and for the $SD_{distension}$ curves were p=-2 for men and p=-1 women. Accordingly, the equations derived on the basis of the estimated coefficients were, for men:

- $Mean_{distension}$ (mm) = $0.962 - 0.326 \times \ln(age/10)$
- $SD_{distension}$ (in mm) = $0.118 + 0.221 \times (age/10)^{-2}$

and, for women:

- $Mean_{distension}$ (in mm) = $-0.137 + 1.163 \times (age/10)^{-0.5}$
- $SD_{distension}$ (in mm) = $0.089 + 0.114 \times (age/10)^{-1}$

Age- and sex-specific reference intervals for brachial PP in the healthy sub-population

The best fitting FPs' powers (*p*, *q*…) for the $mean_{PP}$ curves were p=3 q=3 for men and p=-2 q=-0.5 for women and for the SD_{PP} curves were p=1 for men and p=1 women. Accordingly, the equations derived on the basis of the estimated coefficients were, for men:

- $Mean_{PP}$ (in mm Hg) = $53.64 - 0.133*(age/10)^3 + 0.067*(age/10)^3 \times \ln(age/10)$
- SD_{PP} (in mm Hg) = 9.940 - 0.035 x age

and, for women:

- $Mean_{PP}$ (in mm Hg) = $72.83 + 55.88 \times (age/10)^{-2} - 59.22 \times (age/10)^{-0.5}$
- SD_{PP} (in mm Hg) = 6.266 + 0.052 x age

5.4.4.2 Associations of cardiovascular risk factors with percentiles of components of DC_{car} (i.e. diameter, distension, PP)

The associations between CV-RFs and the individual components of the DC_{car} are shown in Figure 5.5, Figure 5.6 and Figure 5.7 for the sub-population without prior CVD and treatment, the treated sub-population without prior CVD and the sub-population with CVD, respectively. The *negative* association between MAP and diabetes and the DC_{car} Z-scores seemed to be mainly driven by the strong *positive* association between both MAP and diabetes on the one hand and brachial PP and carotid diameter on the other. The *negative* association between BMI and the DC_{car} Z-scores seemed to be mainly driven by the *positive* association between BMI and carotid diameter. The *negative* association between the total-to-HDL cholesterol ratio and the DC_{car} Z-scores seemed to be mainly driven by the *negative* association between the total-to-HDL cholesterol ratio and carotid distension. Smoking

was *positively* associated with carotid diameter and distension and *negatively* associated with brachial PP (although the latter not significantly so in the treated sub-population without CVD and the sub-population with CVD), resulting in the *positive* association between smoking and the DC_{car} Z-scores.

5.4.4.3 Reference intervals for DC_{car} calculated with local carotid artery PP and the association with CV-RFs

Prior to calculating reference intervals for DC_{car} using local carotid artery PP, we calibrated local carotid PP values obtained with different techniques towards values obtained using the reference method (i.e. calibration using carotid and brachial distension waveforms, for details see Table 5.3).

Absolute values for the 50^{th} percentile of DC_{car} were higher when DC_{car} was calculated using local carotid PP than when using brachial PP. These differences were smaller with increasing age, though. In men, the associations between CV-RFs and DC_{car} Z-scores when calculated using local carotid PP (Table 5.11) were generally somewhat weaker than when brachial PP was used (Table 5.8). Furthermore, in the sub-population without treatment and prior CVD, the association between diabetes and the DC_{car} Z-score was not significant (and positive) when local carotid PP was used, whereas this association was strongly negative when brachial PP was used. In women, the associations between CV-RFs and DC_{car} Z-scores were similar when calculated using local carotid PP (Table 5.12) or using brachial PP (Table 5.9).

Reference intervals were additionally established for DC_{car} calculated with local carotid PP. The best fitting FPs' powers (p, q …) for the $mean_{DC}$ curves were p=-0.5 for men and p=-2 q=-2 for women and for the SD_{DC} curves were p=-2 for both men and women. Accordingly, the equations derived on the basis of the estimated coefficients were,

for men:

- $Mean_{DC}$ (in 10^{-3} kPa^{-1}) =-17.93 + 90.78 x $(age/10)^{-0.5}$
- SD_{DC} (in 10^{-3} kPa^{-1}) = 5.304 + 39.85 x $(age/10)^{-2}$

and, for *women*:

- $Mean_{DC}$ (in 10^{-3} kPa^{-1}) = 8.707 + 117.7 x $(age/10)^{-2}$ + 138.7 x $(age/10)^{-2}$ x ln(age/10)
- SD_{DC} (in 10^{-3} kPa^{-1}) = 3.536 + 87.51 x $(age/10)^{-2}$

Sex-specific percentile lines according to age superimposed on the raw data are shown in Figure 5.11, the respective levels of DC_{car} by age category in Table 5.10 and the associations with CV-RFs in Table 5.11 and Table 5.12.

Table 5.10 Age- and sex-specific percentiles of DC_{car} (in 10^{-3} kPa^{-1}) calculated using local PP in the healthy sub-population.

		percentiles						
	Age (years)	2.5th	10th	25th	50th	75th	90th	97.5th
Men (n=1,532)	20	16.3	26.7	36.0	46.3	56.6	65.8	76.2
	30	15.4	22.0	27.9	34.5	41.1	46.9	53.6
	40	12.2	17.5	22.2	27.5	32.7	37.4	42.7
	50	9.1	13.8	18.0	22.7	27.3	31.5	36.2
	60	6.6	10.9	14.8	19.1	23.5	27.3	31.7
	70	4.4	8.6	12.3	16.4	20.5	24.2	28.4
Women (n=1,591)	20	12.4	29.6	45.0	62.2	79.3	94.7	112.0
	30	12.7	21.7	29.8	38.7	47.7	55.7	64.7
	40	10.4	16.6	22.0	28.1	34.2	39.6	45.7
	50	8.6	13.3	17.6	22.3	27.1	31.4	36.1
	60	7.2	11.2	14.9	18.9	22.9	26.5	30.6
	70	6.2	9.8	13.0	16.6	20.2	23.4	27.0

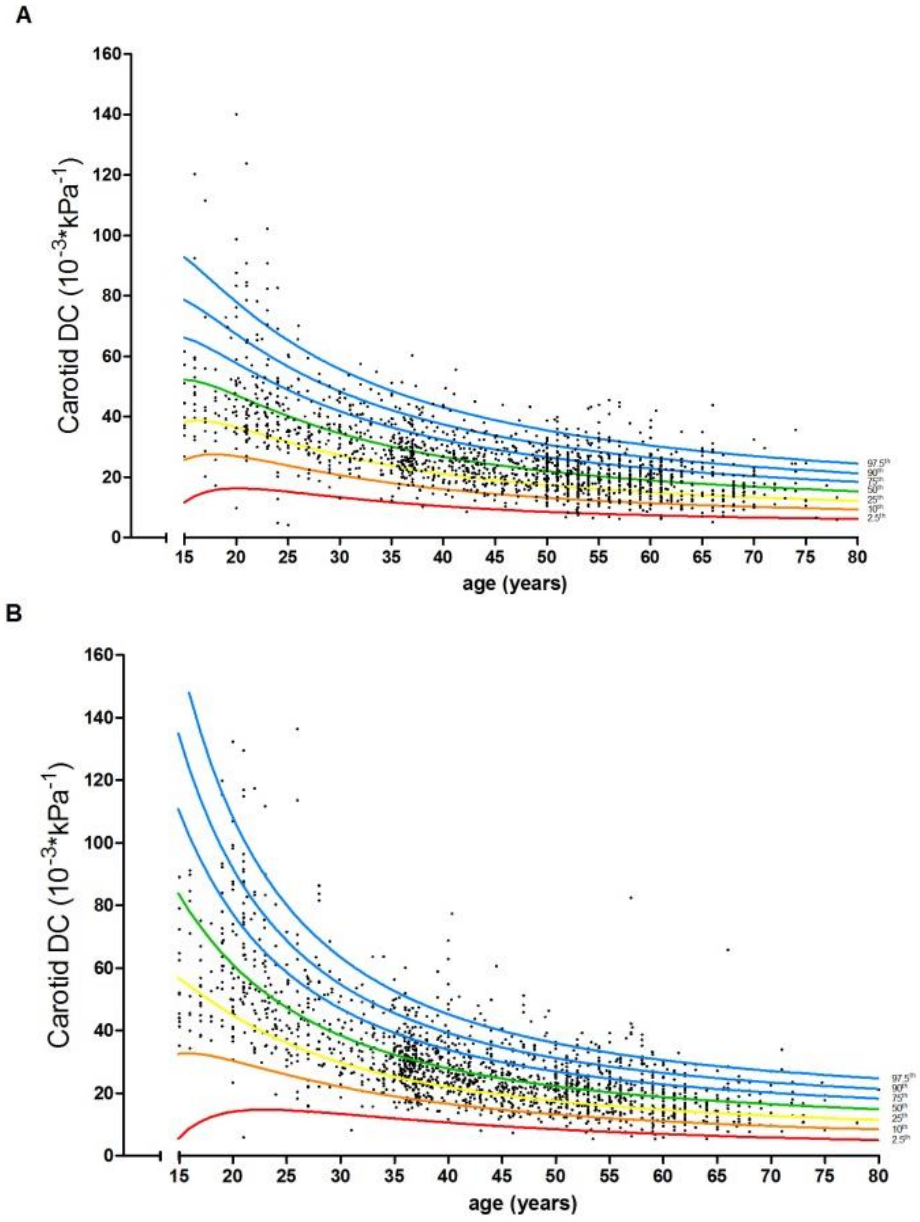


Figure 5.11 Sex-specific percentiles of DC_{car} calculated using local PP according to age in the healthy sub-population. ***A***, men; ***B***, women.

Table 5.11 Relation between known cardiovascular risk factors and DC_{car} Z-scores calculated using local pulse pressure in the reference sub-populations in *men*

		Sub-population without CVD						Sub-population with CVD (n =596)		
		without treatment[a] (n =4,458)			with treatment[a] (n =1,117)					
Risk factor	Model	ß	95%CI	P-value	ß	95%CI	P-value	ß	95%CI	P-value
Mean arterial pressure (10 mmHg)	1	-0.270	-0.298; -0.242	<0.001	-0.349	-0.401; -0.297	<0.001	-0.295	-0.356; -0.235	<0.001
	2	-	-	-	-	-	-	-	-	-
	3	-0.228	-0.257; -0.199	<0.001	-0.330	-0.383; -0.277	<0.001	-0.257	-0.320; -0.194	<0.001
Current smoking (yes)	1	0.002	-0.079; 0.083	0.960	-0.027	-0.201; 0.147	0.763	-0.065	-0.286; 0.156	0.566
	2	0.001	-0.077; 0.079	0.979	-0.043	-0.205; 0.119	0.600	-0.056	-0.263; 0.150	0.592
	3	0.010	-0.069; 0.089	0.808	-0.026	-0.188; 0.137	0.755	-0.019	-0.225; 0.186	0.853
Diabetes (yes)	1	-0.422	-0.665; -0.178	0.001	-0.197	-0.363; -0.032	0.019	-0.618	-0.834; -0.403	<0.001
	2	-0.249	-0.484; -0.013	0.038	-0.127	-0.281; 0.028	0.108	-0.448	-0.655; -0.242	<0.001
	3	-0.188	-0.422; 0.047	0.117	-0.102	-0.259; 0.054	0.200	-0.469	-0.735; -0.204	0.001
Total-to-HDL cholesterol ratio (unit)	1	-0.092	-0.130; -0.053	<0.001	-0.050	-0.135; 0.035	0.197	-0.142	-0.209; -0.075	<0.001
	2	-0.059	-0.099; -0.019	0.007	-0.036	-0.098; 0.026	0.212	-0.114	-0.175; -0.053	<0.001
	3	-0.034	-0.079; 0.010	0.113	-0.032	-0.087; 0.023	0.221	-0.102	-0.166; -0.039	0.002
Body mass index (kg/m^2)	1	-0.063	-0.073; -0.054	<0.001	-0.042	-0.058; -0.025	<0.001	-0.037	-0.060; -0.015	0.001
	2	-0.038	-0.047; -0.028	<0.001	-0.023	-0.038; -0.007	0.004	-0.018	-0.040; 0.004	0.102
	3	-0.033	-0.044; -0.022	<0.001	-0.019	-0.035; -0.002	0.026	-0.003	-0.026; 0.020	0.799
Use of BP-lowering medication (yes)	1	-	-	-	-	-	-	-0.185	-0.349; -0.021	0.027
	2	-	-	-	-	-	-	-0.055	-0.211; 0.101	0.489
	3	-	-	-	-	-	-	-0.005	-0.169; 0.159	0.953
Use of lipid-lowering medication (yes)	1	-	-	-	-	-	-	0.062	-0.118; 0.243	0.498
	2	-	-	-	-	-	-	0.003	-0.164; 0.171	0.969
	3	-	-	-	-	-	-	0.039	-0.139; 0.216	0.670
Use of glucose-lowering medication (yes)	1	-	-	-	-	-	-	-0.394	-0.719; -0.070	0.017
	2	-	-	-	-	-	-	-0.310	-0.614; -0.007	0.045
	3	-	-	-	-	-	-	0.102	-0.279; 0.482	0.600

The regression coefficient ß represents the change in DC_{car} (in SD from the healthy population mean among individuals of the same age and sex) per unit increase in each risk factor. Model 1: unadjusted. Model 2: adjusted for MAP. Model 3: adjusted for MAP and all other risk factors. [a]BP-, lipid- and glucose-lowering treatment

Table 5.12 Relation between known cardiovascular risk factors and DC_{car} Z-scores calculated using local pulse pressure in the reference sub-populations in *women*

		Sub-population without CVD						Sub-population with CVD (n =630)		
		without treatment[a] (n =3,716)			with treatment[a] (n =941)					
Risk factor	Model	ß	95%CI	P-value	ß	95%CI	P-value	ß	95%CI	P-value
Mean arterial pressure (10 mmHg)	1	-0.290	-0.319; -0.261	<0.001	-0.358	-0.413; -0.302	<0.001	-0.438	-0.509; -0.366	<0.001
	2	-	-	-	-	-	-	-	-	-
	3	-0.271	-0.302; -0.240	<0.001	-0.337	-0.394; -0.281	<0.001	-0.356	-0.431; -0.281	<0.001
Current smoking (yes)	1	0.106	0.010; 0.203	0.031	0.219	-0.007; 0.444	0.058	0.523	0.217; 0.828	0.001
	2	0.066	-0.026; 0.158	0.159	0.080	-0.130; 0.291	0.453	0.429	0.152; 0.707	0.002
	3	0.069	-0.024; 0.161	0.145	0.070	-0.140; 0.280	0.513	0.376	0.103; 0.650	0.007
Diabetes (yes)	1	-0.420	-0.932; 0.092	0.102	-0.266	-0.468; -0.063	0.010	-1.037	-1.319; -0.756	<0.001
	2	-0.137	-0.571; 0.298	0.525	-0.195	-0.383; -0.007	0.042	-0.678	-0.946; -0.410	<0.001
	3	-0.061	-0.498; 0.377	0.778	-0.146	-0.337; 0.045	0.135	-0.682	-1.006; -0.358	<0.001
Total-to-HDL cholesterol ratio (unit)	1	-0.092	-0.134; -0.050	<0.001	-0.083	-0.146; -0.020	0.010	-0.035	-0.123; 0.052	0.431
	2	-0.043	-0.085; -0.001	0.045	-0.036	-0.095; 0.023	0.227	-0.010	-0.090; 0.069	0.797
	3	-0.032	-0.077; 0.013	0.157	-0.008	-0.072; 0.055	0.793	0.051	-0.032; 0.135	0.226
Body mass index (kg/m^2)	1	-0.042	-0.051; -0.033	<0.001	-0.039	-0.053; -0.024	<0.001	-0.076	-0.099; -0.052	<0.001
	2	-0.016	-0.025; -0.006	0.001	-0.023	-0.037; -0.009	0.002	-0.040	-0.063; -0.018	<0.001
	3	-0.013	-0.023; -0.002	0.015	-0.020	-0.035; -0.004	0.012	-0.031	-0.055; -0.007	0.012
Use of BP-lowering medication (yes)	1	-	-	-	-	-	-	-0.565	-0.778; -0.351	<0.001
	2	-	-	-	-	-	-	-0.301	-0.502; -0.100	0.003
	3	-	-	-	-	-	-	-0.132	-0.346; 0.082	0.226
Use of lipid-lowering medication (yes)	1	-	-	-	-	-	-	-0.180	-0.464; 0.105	0.215
	2	-	-	-	-	-	-	-0.033	-0.292; 0.226	0.802
	3	-	-	-	-	-	-	0.031	-0.232; 0.294	0.816
Use of glucose-lowering medication (yes)	1	-	-	-	-	-	-	-0.617	-1.129; -0.105	0.018
	2	-	-	-	-	-	-	-0.293	-0.761; 0.174	0.219
	3	-	-	-	-	-	-	0.427	-0.105; 0.960	0.116

The regression coefficient ß represents the change in DC_{car} (in SD from the healthy population mean among individuals of the same age and sex) per unit increase in each risk factor. Model 1: unadjusted. Model 2: adjusted for MAP. Model 3: adjusted for MAP and all other risk factors. [a]BP-, lipid- and glucose-lowering treatment

5.5 Discussion

In the present study we estimated age- and sex-specific percentiles of DC_{car} in healthy individuals aged 15-85 years, based on a large population obtained by combining data at the individual level from 24 research centres worldwide. We additionally assessed the associations between CV-RFs and these DC_{car} percentiles to enable comparison of DC_{car} values across (patient) groups with different cardiovascular risk profiles with those from a healthy population.

Recently, associations between greater levels of carotid stiffness and increased risk of incident stroke, but not coronary heart disease, have been shown in a large sample (>10,000) of middle-aged individuals free from prior CVD (the ARIC study)[86] and associations between greater carotid stiffness and incident CV events and all-cause mortality have been shown in a population-based study among the elderly (the Hoorn study).[87] Importantly, these associations were independent of CV-RFs[86] and carotid-femoral PWV.[87] Earlier studies have also shown carotid stiffness to be associated with incident CVD and/or mortality among patients with chronic kidney disease[88,90,92] and who had received a renal transplant,[89] elderly individuals with and without prior CVD,[93] and healthy individuals.[91] These findings were not corroborated by other studies, however,[94–97] which may be explained, at least in part, by differences in sample size and duration of follow-up, devices and techniques used to process ultrasound signals, the cardiovascular outcomes considered, and the characteristics of the study populations (e.g., old vs. middle aged, diseased vs. apparently healthy). Further studies (e.g., meta-analysis) will determine the predictive association between carotid stiffness and incident CV events and CV mortality. Nevertheless, a major advantage of local stiffness estimation by means of echotracking is that it can be directly determined from changes in pressure driving the change in arte-

rial volume without using any model from the circulation.[85] Moreover, it enables characterization of the arterial structural and functional changes underlying the loss of the elastic properties of the arteries and thus a better understanding of the etiological mechanisms of arterial stiffening in response to risk factor exposure and lifestyle and/or pharmacological interventions.[85] In this line, many studies have now incorporated measures of local stiffness into their vascular characterization protocols. The reference intervals for DC_{car} as currently presented may be helpful in the interpretation of the results on carotid stiffness levels obtained in those studies.

The reference intervals in the healthy sub-population showed a non-linear and negative relation between age and DC_{car}, which was somewhat steeper in women than in men. In contrast, reference values for carotid-femoral PWV did not differ between men and women.[106]

In the sub-population without prior CVD and treatment, we found that diabetes (yes vs. no) and higher MAP, total-to-HDL cholesterol ratio and BMI were significant determinants of lower DC_{car} in men and women, an observation that is largely in line with previous studies.[165,166] Remarkably, smoking (yes vs. no) was a significant determinant of higher DC_{car} in men and women, which has previously been shown in The cardiovascular risk in Young Finns study[167] and in the two smaller case-control studies (although not significantly so for all comparisons).[168,169] A priori, a negative association between smoking and DC_{car} was hypothesized, as smoking may lead to vascular dysfunction (e.g., arterial stiffness) through inflammation, endothelial dysfunction and oxidative stress.[170,171] Such a negative association has also been described previously, although only in two smaller studies on smoking and stiffness index and DC_{car}.[172,173] However, it is possible that the chronic effects of *current* smoking were outweighed by the acute effects of *withdrawal* from smoking, which has an immediate impact on the sympathetic nervous

system activity.[174] Indeed, all measurement protocols of the studies included here institute abstinence from smoking at the time of measurement, at least three hours in advance, which may have confounded the results. In addition, exposure to smoking was cross-sectionally assessed with a yes/no question including the quitters (who may be unhealthier) in the non-smokers group and residual confounding (e.g., by physical activity, alcohol intake) could not be taken into account. Therefore, ideally, the chronic effect of smoking (or quitting smoking) on DC_{car} should still be evaluated in intervention studies that control for these factors.

In addition, in the sub-population with prior CVD regardless of medication, we found that the use of lipid-lowering medication was positively associated with DC_{car} in men and women, which seemed to be driven mainly by its positive association with carotid distension. We assume these lipid-lowering drugs to be mainly statins, but we cannot be sure as specific information regarding the type of drugs is lacking for most included studies. The current findings may reflect the beneficial effects of statin treatment on carotid stiffness that have previously been shown in intervention studies on fluvastatin[175] and atorvastatin.[176–178]

We calculated DC_{car} values both using brachial and local carotid PP. We found that absolute percentile values were lower when DC_{car} was calculated using brachial PP (Table 5.6) than when using local carotid PP (Table 5.10). This may be explained by the principle of PP amplification due to wave reflection at bifurcations resulting in higher PP in peripheral than central arteries.[179] PP amplification decreases with increasing age, showing a plateau from the age of 40 (women) or 50 (men) years onwards in healthy individuals.[180] This is also in line with the current findings of decreasing differences between DC_{car} calculated using brachial vs. local carotid PP with increasing age, similarly showing plateaus (in these differences) from the age of 50

onwards. In men, associations between CV-RFs and DC_{car} were weaker when local carotid PP was used than when brachial PP was used, which may be due to more difficult and/or less precise measurements in the former compared to the latter method. Also, with the use of local carotid PP for the calculation of DC_{car} an additional calibration step was needed to align different techniques to determine local carotid PP, possibly introducing additional error. However, in women, associations between CV-RFs and DC_{car} were similar when either local carotid or brachial PP was used. The difference (found in men) may thus be explained differently.

5.5.1 Limitations

This study has some limitations. First, given the cross-sectional design, the 'increases' of carotid stiffness with age need to be interpreted with caution, because these may misestimate the longitudinal rates of change within individuals. Second, we standardized differences in techniques between studies/centres by first adjusting carotid diameter and distension (and local carotid PP) for all potential physiological/pathological factors supposed to influence these variables, assuming that the residual differences were of methodological origin. However, this calibration may still have been sub-optimal because hidden confounders might have been missed.

5.5.2 Conclusion

In conclusion, we estimated age- and sex-specific percentiles of DC_{car} in a healthy population and assessed the association between CV-RFs and DC_{car} Z-scores, which enables comparison of DC_{car} values for (patient) groups with different cardiovascular risk profiles, helping interpretation of such measures obtained both in research and clinical settings.

Chapter 6 Reference values for femoral artery stiffness.

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Reference values for local arterial stiffness. Part B: Femoral artery. (in preparation)

6.1 Abstract

Aims: Carotid-femoral pulse wave velocity (PWV) is considered the gold standard measure of arterial stiffness, representing mainly aortic stiffness. As compared to the elastic carotid and aorta, the more muscular femoral artery may be differently associated with cardiovascular risk factors (CV-RFs), or, as shown in a recent study, provide additional predictive information beyond carotid-femoral PWV. Still, clinical application is hampered by the absence of reference values. Therefore, our aim was (1) to establish age- and sex-specific reference values for femoral stiffness in healthy subjects and (2) to investigate the associations with CV-RFs.

Methods and results: femoral distensibility coefficient (DC), the inverse of stiffness, was calculated as the ratio of relative diastolic-systolic distension (obtained from ultrasound echo-tracking) and pulse pressure among 5,069 individuals (49.5% men, age range: 15-87 year). Individuals without cardiovascular disease (CVD), CV-RFs and medication use (n=1,489; 43% men) constituted a *healthy sub-population* used to establish sex-specific equations for percentiles of femoral DC across age. In the total population, femoral DC Z-scores were independently associated with body mass index (BMI), mean arterial pressure (MAP), and total-to-HDL cholesterol ratio. Standardized βs, in men and women respectively, were -0.18 (95% CI: -0.23;-0.13) and -0.19 (-0.23; -0.14) for BMI; -0.13 (-0.18; -0.08) and -0.05 (-0.10; -0.01) for MAP; and -0.07 (-0.11; -0.02) and -0.16 (-0.20; -0.11) for total-to-HDL cholesterol ratio.

Conclusion: In young and middle-aged men and women, *normal* femoral stiffness does not change substantially with age up to the 6th decade. CV-RFs related to metabolic disease are associated with femoral stiffness.

6.2 Introduction

As described in *Chapter 1*, femoral artery stiffness (or its inverse, the femoral artery distensibility coefficient, DC_{fem}) carries added predictive value for all-cause and cardiovascular mortality,[87] and can be specifically altered in certain sub-populations.[98,99] In addition, some drugs have been shown to operate exclusively on muscular arteries, providing a window for monitoring pharmacological interventions.[181]

However, the interpretation of DC_{fem} values measured across different age, sex and risk groups is hampered by the absence of reference values. In view of these considerations, we aimed 1) to establish age- and sex-specific normal values using percentiles of local DC_{fem} obtained in individuals without prior CVD, treatment and established cardiovascular risk factors (CV-RFs) and 2) to investigate associations between known CV-RFs and these DC_{fem} percentiles in individuals with or without CV-RFs, treatment and prior CVD.

6.3 Methods

Methods used for data collection, stratification of the population, standardization of methodology and statistical analyses were similar to those used in *Chapter 5*, analysing carotid artery stiffness. For a more detailed description, we therefore refer to *Chapter 5*. Differences are outlined below.

6.3.1 Study population

Of the 31 cohorts included in *Chapter 5*, only 7 contained data on the femoral artery as well. One cohort [Psicofirb, Monza (ITA)] was unique to *Chapter 6* (Table 6.1). A total of 5,069 individuals constituted the femoral artery reference values database, including data on femoral diameter and distension obtained using echotracking systems, blood pressure (BP), age (range 15-87 years), sex (2,510 men/2,559 women), CVD status, and important

cardiovascular risk factors (CV-RFs). The healthy sub-population, meeting the same criteria as outlined in *Chapter 5*, consisted of 1,489 (43% men) individuals. A flowchart describing the selection of the healthy and reference sub-populations and exact numbers per sex is presented in Figure 6.1

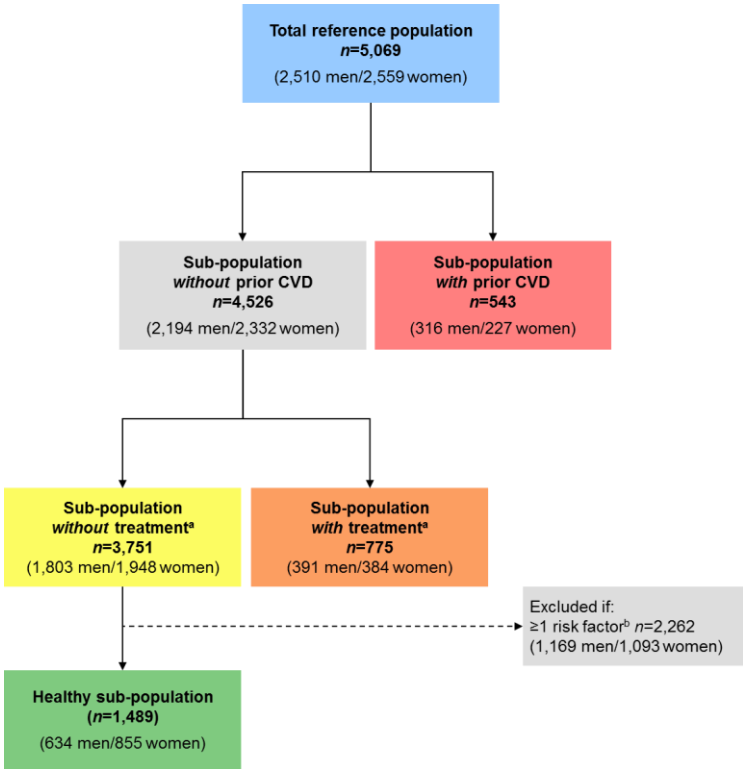


Figure 6.1 Study flowchart describing the selection and categorization of individuals from the total femoral stiffness (FS) to the reference and healthy sub-populations.

[a]BP-, lipid-, and/or glucose-lowering medication. [b]Risk factors considered were hypertension (systolic blood pressure/diastolic blood pressure ≥140/90 mmHg), current smoking, diabetes [self-reported diabetes and/or fasting plasma glucose ≥7.0 mmol/L and/or post-load plasma glucose ≥11.0 mmol/L (if available)], total cholesterol >6.2 mmol/L, HDL cholesterol <1.17 mmol/L (for men) and <1.30 mmol/L (for women), and body mass index ≥30 kg/m^2.

Table 6.1 Contributing centres (in order of decreasing number of participating individuals) and respective femoral artery measurement techniques used.

Total n	Healthy sub-Population n	Centre	Study name/ acronym	Included in *Chapter 5*	Echotracking system	Anatomical location*	(Local) PP measurement	MAP calculation for local PP
2,026	684	Ghent (BE)	Asklepios	Yes	Echopac[c]	1-2 cm	Tonometry	Tonometry
1,486	274	Amsterdam (NL)	Hoorn study (n=664)	Yes	WTS[a]	2 cm	distension curves	distension curves
		/Maastricht (NL)	CODAM 2* (n=414)	Yes	Art.Lab[b]	2 cm	distension curves	distension curves
			AGAHLS** (n=408)	Yes, all	WTS[a]	2 cm	distension curves	distension curves
1,405	458	Leuven (BE)	FLEMENGHO (n=1,305)	Yes	WTS[a]	1 cm	Tonometry	Maximal oscillometry
			Migraine study (n=100)	No	WTS[a]	1-2 cm	Tonometry	Tonometry
100	56	Maastricht (NL)	Migraine	No	WTS[a]	1-2 cm	Tonometry	Tonometry
52	11	Monza (ITA)	Psicofirb	No	Art.Lab[b]	2 cm	distension curves	MAP=SBP+1/3.PP

*Anatomical location of the measurement is expressed as distance (in cm) proximal to the femoral artery bifurcation. [a]Wall Track System (WTS (former version of ART.LAB), ESAOTE, Maastricht, the Netherlands); [b]ART.LAB echotracking system (ESAOTE, Maastricht, the Netherlands); [c]Vivid-7 US system (GE Vingmed Ultrasound, Horten, Norway) with Echopac post-processing;

**in contrast to *Chapter 5* which included CODAM 1 data (baseline examination), data included here are that from CODAM 2 (i.e. 1st follow-up examination), when characteristics of the femoral artery were measured for the first time in this cohort; ***includes independent data from individuals ever measured in this cohort, specifically, n=377 measured for the first time in the 2000 round (at the mean age of 36) + n=31 measured for the first time in the measured round of 2006 (at the mean age of 42);

6.3.2 Estimation of femoral artery stiffness: preliminary methodological considerations

Level of femoral stiffness was expressed by the distensibility coefficient (DC_{fem}), calculated as described earlier (section 1.3.2.1).

Estimates of local PP were available in 85% of all subjects (*n*=4,347). Correlation between brachial and femoral artery PP was strong both in men (r=0.82, p<0.001) and women (r=0.89, p<0.001), though strongest for those in the oldest tertile (youngest: r=0.75, middle: r=0.83, oldest: r=0.85). For the same reasons as outlined in *Chapter 5*, and for uniformity, brachial PP was used in all our main analyses. Nevertheless, and for completeness, we have also estimated the reference intervals for DC_{fem} calculated with local femoral PP. (Table 6.10 and Figure 6.11)

Measurement of diameter and distension

Only external (diastolic) femoral diameter and distension data obtained by means of echotracking was included (either pure echotracking or related techniques).
Different types of ultrasound systems were used across centres:

(1) the ART.LAB system (*n*=466; advanced version of WTS; ESAOTE, Maastricht, The Netherlands);
(2) the Wall Track System (*n*=2,577; WTS, ESAOTE, Maastricht, The Netherlands[158]);
(3) the Vivid-7 US system, with Echopac post-processing, which has been validated against the WTS.[159] (*n*=2,026; GE Vingmed Ultrasound, Horten, Norway)

The exact anatomical location of the measurement of femoral artery diameter and distension differed across centres:

(1) 1 cm proximal to the femoral artery bifurcation (*n*=1,305);

(2) between 1 and 2 cm proximal to the femoral artery bifurcation (*n*=2,226);

(3) 2 cm proximal to the femoral artery bifurcation (*n*=1,538)

Therefore, prior to the calculation of DC_{fem}, we standardized all femoral artery diameter and distension values obtained with different echotracking systems and anatomical locations (Table 6.2). To this aim, original femoral artery diameter and distension values were rescaled to the same metric as used in *Chapter 5* (for uniformity reasons), i.e. with the ART.LAB system and centred at 1 cm proximal to the femoral bifurcation.

Measurement of local pulse pressure

Different techniques to estimate local pulse pressure include:

(1) femoral artery tonometry calibrated using brachial DBP and MAP obtained through:

(1a) maximal oscillometry, or

(1b) the area under the curve (AUC) of brachial artery tonometry waveforms.

(2) femoral artery distension calibrated using brachial DBP and MAP obtained through:

(2a) the 40%-rule, i.e. MAP = DBP + 0.4.(SBP-DBP), or

(2b) the area under the curve (AUC) of brachial artery distension waveforms.[127]

Similar to the calibration of diameter and distension values, original femoral PP values were calibrated to the reference technique. (Table 6.3)

Table 6.2 Calibration factors for femoral diameter and distension values as obtained with different measurement devices and locations.

	Femoral diameter			Femoral distension		
	β	95% CI	p	β	95% CI	p
Echotracking system (Reference=Art.Lab*) (n=466)	-	-	-	-	-	-
Wall Track system (n=2,577)	1.666	1.531; 1.801	<0.001	0.070	0.056; 0.084	<0.001
Vivid-7 (n=2,026)	0.599	0.457; 0.741	<0.001	0.187	0.172; 0.202	<0.001
Anatomical location (reference= 1 cm**) (n=1,305)	-	-	-	-	-	-
1-2 cm (n=2,226)	-1.795	-2.005; -1.585	<0.001	0.012	-0.009; 0.033	0.256
2 cm (n=1,538)	-0.995	-1.226; -.0.764	<0.001	0.047	0.024; 0.070	<0.001

Regression coefficients β represent the mean difference in femoral artery diameter (in mm) or distension (in mm) when using each of the echotracking systems and/or anatomical locations vs. the reference (as indicated above) at mean levels of age, sex, MAP, total-HDL cholesterol ratio, BMI, smoking, diabetes, history of CVD, and use of BP- and/or lipid-lowering medication in the total reference population (n =5,069).

* In contrast to the Wall-track system and Vivid-7, which select a single M-line, ART.LAB takes measures over an arterial width of > 10mm, comprising multiple M-lines, which may yield considerably more precise results.

**Anatomical location is expressed as distance (in cm) proximal to the femoral bifurcation.

These regression coefficients can be used to rescale diameter (by subtracting 1.666 or 0.599 mm) and distension (by subtracting 0.070 or 0.187 mm) values obtained by, respectively, the Wall Track System (WTS) or the Vivid-7 systems, to those as obtained by the ART.LAB system (i.e. the values presented in the paper). In addition, the appendix (p203) contains reference tables calibrated to each specific device.

Table 6.3 Calibration factors for local femoral pulse pressure values as obtained with different methods

	Femoral artery pulse pressure		
	β	95% CI	p
Reference method	-	-	-
Femoral distension + 40%-rule	-11.6	-13.9; -9.3	<0.001
Femoral tonometry + brachial tonometry	1.1	0.4; 1.8	0.003
Femoral tonometry + maximal oscillometry	0.8	0.0; 1.5	0.046

Regression coefficients β represent the mean difference in local femoral pulse pressure (in mm Hg) when this was calculated by each of techniques listed vs. the reference one (i.e. femoral distension + brachial distension), at the mean levels of age, sex, MAP, heart rate, total-HDL cholesterol ratio, BMI, smoking, diabetes history of CVD, and use of BP- and/or lipid-lowering medication in the total reference population (n=5,069). On the basis of these regression coefficients femoral PP values obtained by, for instance, femoral tonometry + brachial tonometry can be re-scaled to values as obtained by femoral distension + brachial distension by subtracting 1.1 mm Hg.

6.3.1 Statistical analyses

6.3.1.1 *Multiple imputation of missing values*

A total of 124 individuals (2.4% of the total reference population) had missing values for one (2.2%) or more (0.2%) of the variables of interest. The percentage of missing values per variable varied from 0.02% (current smoking) to 2.2% (HDL cholesterol). We used multiple imputation chained equations to impute those values rather than perform complete case analyses.

6.3.1.2 Data analyses

Methods used to define age-and sex-specific reference intervals for DC_{fem} and to examine the association with risk factors were identical to those described in detail in *Chapter 5*.

Table 6.4 Risk factors and clinical characteristics of the total, healthy and reference sub-populations in *men*.

	Total Reference population	Healthy sub-population	Sub-population without CVD		Sub-population with CVD
			without treatment[a]	with treatment[a]	
n	2,510	634	1,803	391	316
Femoral diameter (mm)	8.8 ± 1.4	8.8 ± 1.3	8.8 ± 1.3	8.9 ± 1.5	8.5 ± 1.8
Femoral distension (mm)	0.20 ± 0.11	0.24 ± 0.11	0.23 ± 0.11	0.16 ± 0.11	0.10 ± 0.05
PP (mm Hg)	55.0 ± 11.6	50.6 ± 7.9	53.0 ± 9.7	58.4 ± 13.0	62.4 ± 15.3
DC_{fem} (10^{-3} kPa^{-1})	6.8 ± 4.3	8.4 ± 4.2	7.7 ± 4.2	5.2 ± 3.9	3.1 ± 2.3
Age [years (range)]	50 (39-60)	42 (36-49)	45 (37-52)	56 (48-66)	67 (63-73)
Body mass index (kg/m^2)	26.1 ± 3.7	24.0 ± 2.7	25.5 ± 3.5	28.1 ± 3.6	27.4 ± 3.6
SBP (mm Hg)	131.1 ± 16.7	120.6 ± 10.7	127.7 ± 14.8	139 ± 16.9	140.9 ± 19.3
DBP (mm Hg)	76.1 ± 10.6	69.9 ± 8.5	74.6 ± 10.5	80.7 ± 9.7	78.5 ± 10
MAP (mm Hg)	98.1 ± 12.1	90.1 ± 8.6	95.8 ± 11.5	104 ± 11.4	103.5 ± 12.4
Hypertension [n (%)]	760 (30)	-	391 (22)	199 (51)	170 (54)
Total cholesterol (mmol/L)	5.4 ± 1.0	5.0 ± 0.7	5.4 ± 1.0	5.5 ± 0.9	5.3 ± 1.0
LDL cholesterol (mmol/L)	3.4 ± 0.9	3.0 ± 0.7	3.4 ± 0.9	3.3 ± 0.9	3.3 ± 0.8
HDL cholesterol (mmol/L)	1.4 ± 0.4	1.6 ± 0.3	1.4 ± 0.4	1.2 ± 0.3	1.2 ± 0.3
Total-to-HDL cholesterol	4.3 ± 1.5	3.3 ± 0.7	4.1 ± 1.3	4.7 ± 1.3	4.8 ± 2.3
Triglycerides (mmol/L)	1.7 (0.9-1.9)	1.2 (0.7-1.4)	1.6 (0.9-1.8)	2 (1.2-2.3)	1.7 (1.1-2)
Fasting glucose (mmol/L)	5.5 ± 1.1	5.1 ± 0.5	5.3 ± 0.8	5.6 ± 1.3	6.6 ± 1.6
Diabetes [n (%)]	304 (12)	-	62 (3)	81 (21)	161 (51)
Current smoking [n (%)]	538 (21)	-	442 (25)	71 (18)	25 (8)
BP-lowering drugs [n (%)]	492 (20)	-	-	296 (76)	196 (62)
Lipid-lowering drugs [n (%)]	288 (11)	-	-	168 (43)	120 (38)
Glucose-lowering drugs [n (%)]	104 (4)	-	-	51 (13)	53 (17)
History of CVD [n (%)]	316 (13)	-	-	-	316 (100)

Data are presented as means ± SD, medians [interquartile ranges] or numbers (percentages), as appropriate. [a]BP-, lipid- and/or glucose-lowering treatment. P-values were obtained from a one-way ANOVA on the last three sub-populations.

Table 6.5 Risk factors and clinical characteristics of the total, healthy and reference sub-populations in *women*.

	Total Reference population	Healthy sub-population	Sub-population without CVD		Sub-population with CVD
			without treatment[a]	with treatment[a]	
n	2,559	855	1,948	384	227
Femoral diameter (mm)	7.5 ± 1.6	7.4 ± 1.6	7.5 ± 1.6	7.4 ± 1.3	7.7 ± 1.3
Femoral distension (mm)	0.20 ± 0.11	0.23 ± 0.11	0.22 ± 0.11	0.17 ± 0.10	0.10 ± 0.05
PP (mm Hg)	53.7 ± 13.6	47.8 ± 8.0	50.5 ± 10.9	60.6 ± 14.7	70.0 ± 16.4
DC_{fem} (10^{-3} kPa^{-1})	8.6 ± 5.6	11.0 ± 6.0	9.8 ± 5.6	6.2 ± 4.3	3.3 ± 2.2
Age [years (range)]	48 (38-56)	41 (36-48)	44 (37-51)	57 (49-66)	68 (63-73)
Body mass index (kg/m^2)	25.2 ± 4.2	23.1 ± 2.8	24.5 ± 3.9	27.4 ± 4.4	27.4 ± 4
SBP (mm Hg)	127.6 ± 19	118 ± 10.6	123.1 ± 15.9	138.7 ± 19.8	147.3 ± 21.9
DBP (mm Hg)	73.9 ± 10.4	70.2 ± 8.4	72.6 ± 10	78.1 ± 11.1	77.3 ± 9.6
MAP (mm Hg)	95.4 ± 12.8	89.3 ± 8.5	92.8 ± 11.5	102.4 ± 13.4	105.3 ± 13.5
Hypertension [n (%)]	606 (24)	-	290 (15)	176 (46)	140 (62)
Total cholesterol (mmol/L)	5.5 ± 1.0	5.0 ± 0.7	5.4 ± 1.0	5.8 ± 1.0	5.9 ± 1.1
LDL cholesterol (mmol/L)	3.3 ± 1.0	2.7 ± 0.7	3.1 ± 0.9	3.5 ± 0.9	3.7 ± 1.0
HDL cholesterol (mmol/L)	1.7 ± 0.4	1.8 ± 0.4	1.7 ± 0.4	1.6 ± 0.4	1.5 ± 0.4
Total-to-HDL cholesterol	3.5 ± 1.2	2.8 ± 0.6	3.4 ± 1.1	3.9 ± 1.2	4.1 ± 1.4
Triglycerides (mmol/L)	1.3 (0.8-1.6)	1.0 (0.6-1.2)	1.2 (0.7-1.4)	1.6 (1-2)	1.6 (1-1.9)
Fasting glucose (mmol/L)	5.2 ± 1	4.8 ± 0.5	5.0 ± 0.7	5.6 ± 1.3	6.5 ± 1.7
Diabetes [n (%)]	215 (8)	-	33 (2)	72 (19)	110 (48)
Current smoking [n (%)]	463 (18)	-	392 (20)	56 (15)	15 (7)
BP-lowering drugs [n (%)]	418 (16)	-	-	311 (81)	107 (47)
Lipid-lowering drugs [n (%)]	176 (7)	-	-	123 (32)	53 (23)
Glucose-lowering drugs [n (%)]	58 (2)	-	-	37 (10)	21 (9)
History of CVD [n (%)]	227 (9)	-	-	-	227 (100)

Data are presented as means ± SD, medians [interquartile ranges] or numbers (percentages), as appropriate. [a]BP-, lipid- and/or glucose-lowering treatment. P-values were obtained from a one-way ANOVA on the last three sub-populations.

6.4 Results

Table 6.4 and Table 6.5 show the participants' characteristics of the healthy and reference sub-populations, in men and women, respectively. In the total reference population, women had on average a more favourable CV-RF profile than men, which was reflected by a higher fraction of women allocated to the 'healthy' subpopulation (men: 25%, women: 33%). In addition, both in men and women, CV-RFs were more unfavourable from the sub-populations with treatment and/or with prior CVD compared to those from the population without treatment and CVD (p-values for trend were <0.001 for all comparisons).

6.4.1 Age- and sex-specific reference intervals for DC_{fem} in the healthy sub-population

The best fitting fractional polynomial (FP) powers (*p, q*) for the mean DC_{fem} curves were *p*=2 *q*=2 for men and *p*=3 *q*=3 for women and for the standard deviation (SD) DC_{fem} curves were *p*=-1 and *p*=3 for men and women. Accordingly, the equations derived on the basis of the estimated coefficients were,

for *men*:

- Mean DC_{fem} (in $10^{-3}.kPa^{-1}$) =5.604 + 0.779 x $(age/10)^2$-0.411 x $(age/10)^2$ x ln (age/10)
- SD DC_{fem} (in $10^{-3}.kPa^{-1}$) = 2.829 + 3.677 x $(age/10)^{-1}$

and, for *women*:

- Mean DC_{fem} (in $10^{-3}.kPa^{-1}$)= 9.44 + 0.163 x $(age/10)^3$ - 0.092 x $(age/10)^3$ x ln(age/10)
- SD DC_{fem} (in $10^{-3}.kPa^{-1}$) = 5.984 - 0.005 x $(age/10)^3$

The estimated Z-scores had a mean value of 0 and a SD of 1 and, when plotted against age, were randomly distributed above and below 0 (Figure 6.2), indicating good model fit and no residual dependency on age.

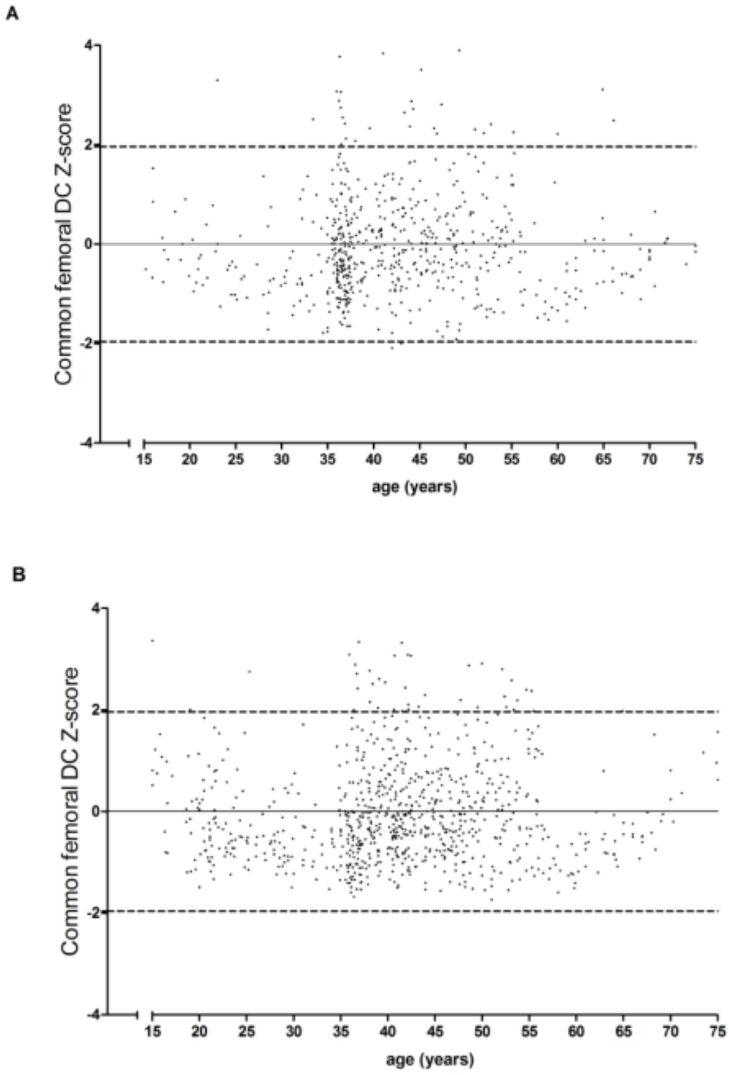


Figure 6.2 Scatter plot of DC_{fem} Z-scores by age, showing the mean (horizontal line) and +/- 1.96 SD (dotted lines), from the fitted model for DC_{fem} data for men (**A**) and women (**B**)

Sex-specific percentile lines superimposed on the raw data are shown in Figure 6.3 and the respective levels of DC_{fem} by age category are presented in Table 6.6.

Table 6.6 Age- and sex-specific percentiles of DC_{fem} (in $10^{-3}.kPa^{-1}$) in the healthy sub-population.

		percentiles						
	Age (years)	2.5th	10th	25th	50th	75th	90th	97.5th
Men (n=634)	20	-1.6*	1.6	4.5	7.6	10.7	13.6	16.7
	30	0.6	3.4	5.8	8.6	11.3	13.7	16.5
	40	1.6	4.2	6.5	9.0	11.5	13.8	16.3
	50	1.6	4.0	6.2	8.6	11.0	13.1	15.5
	60	0.4	2.8	4.9	7.2	9.5	11.6	13.9
	70	-1.9*	0.3	2.4	4.6	6.9	8.9	11.2
Women (n=855)	20	-1.4*	2.6	6.3	10.2	14.2	17.9	21.9
	30	-0.4*	3.6	7.2	11.1	15.0	18.6	22.6
	40	0.6	4.4	7.9	11.7	15.5	19.0	22.9
	50	0.7	4.4	7.7	11.3	14.9	18.2	21.9
	60	-0.8*	2.6	5.7	9.0	12.4	15.4	18.8
	70	-4.8*	-1.8*	0.9	3.9	6.9	9.6	12.6

*Negative values of DC_{fem} estimated for the 2.5^{th} percentile are not realistic, but are likely artefacts derived from a model accounting for high variability across age categories.

In addition, the appendix (p203) contains reference tables (Table 0.4-0.5) calibrated to devices other than the reference method (Art.lab)

Mean values of DC_{fem} were slightly lower in men than in women at any age (p<0.001). The relationship with age was also different between sexes, with DC_{fem} levels in women plateauing until the age of 50, when they exhibited a sudden steep drop from the age of 60 onwards, in contrast to the more gradual decrease in men, starting at the age of 50. However, both in 'healthy' men and women, DC_{fem} levels were only weakly dependent on age, as illustrated by the fact that the most optimal equation based on FPs explained only 8.1% (women) to 8.6% (men) of the variation in DC_{fem} across age.

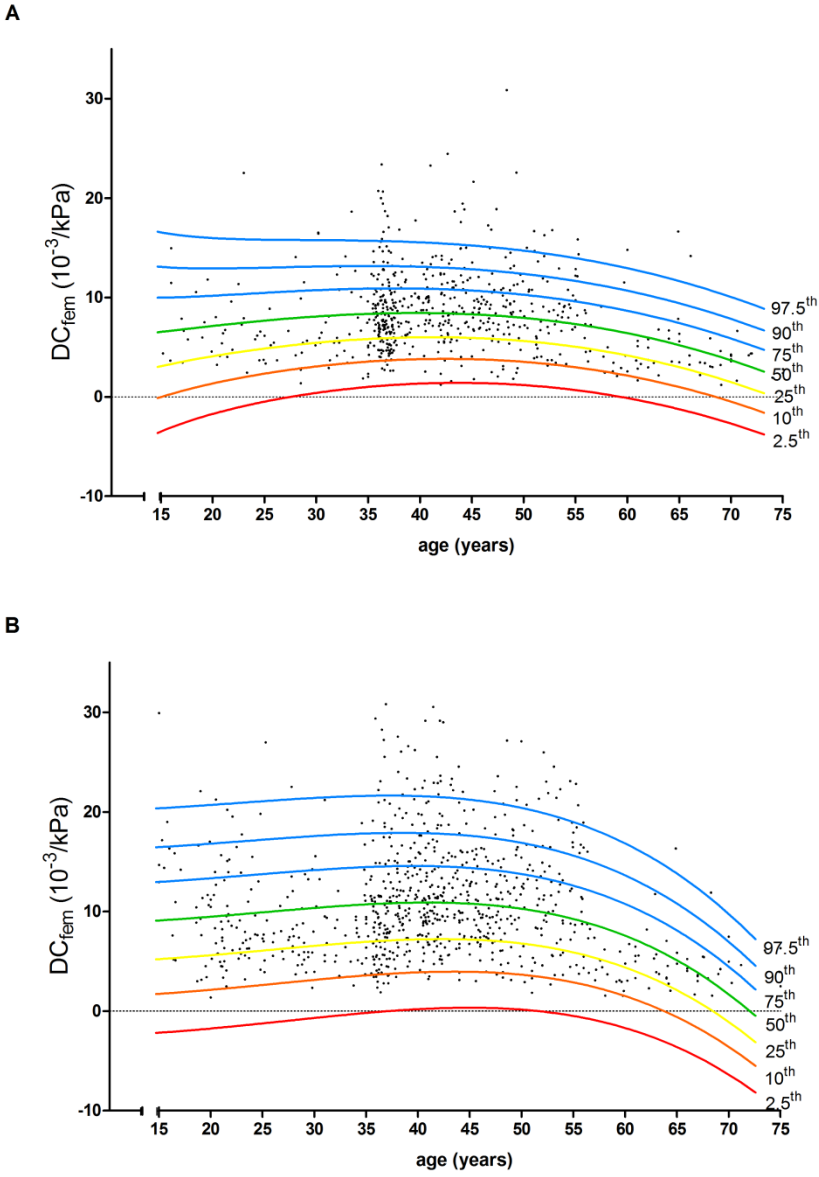


Figure 6.3 Age-specific percentiles of DC_{fem} in the healthy sub-population. ***A***, men; ***B***, women.

6.4.2 Age sex-specific reference intervals for femoral PWV in the healthy sub-population

To enable comparison with carotid-femoral PWV metrics, DC_{fem} was converted to femoral PWV (in m/s) through the Bramwell-Hill equation.[69]

The best fitting FPs' powers (*p*, *q* …) for the $mean_{PWV}$ curves were *p*=3 *q*=3 for both men and women and for the SD_{PWV} curves *p*=3 for both men and women. Accordingly, the equations derived on the basis of the estimated coefficients were,

for men:

- $Mean_{PWV}$ (in m/s) = 12.58 – 0.100 x $(age/10)^3$ + 0.057 x $(age/10)^3$ x ln(age/10)
- SD_{PWV} (in m/s) = 2.48 + 0.004 x $(age/10)^3$

and, for women:

- $Mean_{PWV}$ (in m/s) = 11.55 – 0.125 x $(age/10)^3$ + 0.072 x $(age/10)^3$ x ln(age/10)
- SD_{PWV} (in m/s) = 2.55 + 0.004 x $(age/10)^3$

Sex-specific percentile lines superimposed on the raw data are shown in Figure 6.4 and the respective levels of femoral PWV by age category are presented in Table 6.7.

Table 6.7 Age- and sex-specific percentiles of femoral PWV (in m/s) in the healthy sub-population.

		percentiles						
	Age (years)	2.5th	10th	25th	50th	75th	90th	97.5th
Men (n=634)	20	7.2	8.9	10.4	12.1	13.8	15.3	17.0
	30	6.5	8.3	9.8	11.6	13.3	14.9	16.6
	40	5.9	7.7	9.4	11.2	13.1	14.7	16.6
	50	5.7	7.7	9.6	11.5	13.5	15.4	17.4
	60	6.5	8.8	10.8	13.0	15.3	17.3	19.6
	70	8.8	11.4	13.7	16.3	18.9	21.3	23.9
Women (n=855)	20	5.9	7.6	9.2	10.9	12.7	14.3	16.0
	30	5.1	6.9	8.5	10.3	12.1	13.7	15.5
	40	4.4	6.3	8.1	9.9	11.8	13.5	15.4
	50	4.4	6.5	8.4	10.4	12.5	14.3	16.4
	60	5.7	8.0	10.1	12.4	14.7	16.8	19.1
	70	9.0	11.7	14.1	16.7	19.4	21.8	24.4

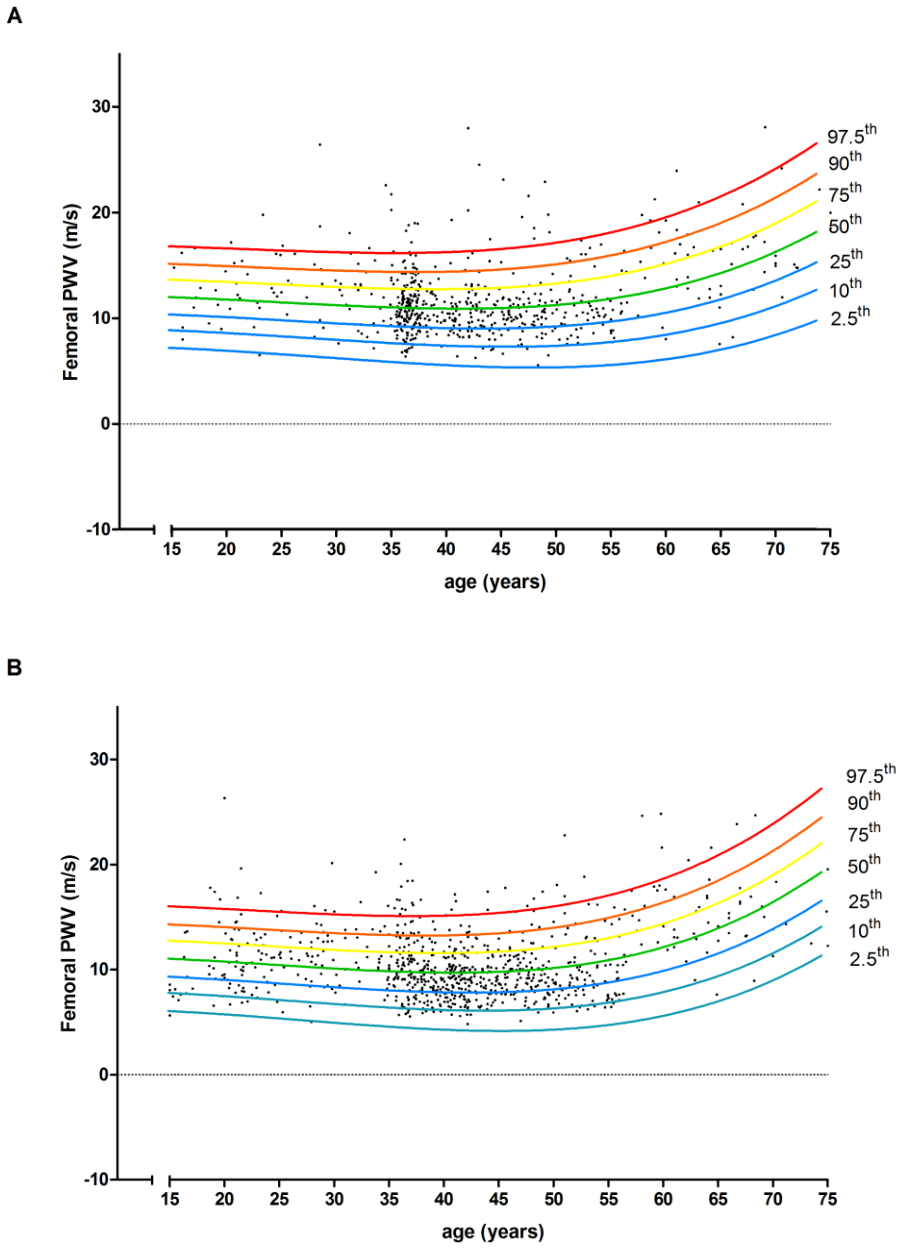


Figure 6.4 Age-specific percentiles of femoral PWV in the healthy sub-population. ***A***, men; ***B***, women.

6.4.3 Associations of cardiovascular risk factors with DC_{fem} percentiles derived from the healthy sub-population

In the sub-population without prior CVD and treatment (Table 6.8 and Table 6.9, Figure 6.5), and both in men and women, lower DC_{fem} Z-scores (i.e. negative deviation from the healthy population mean) were strongly associated with BMI, followed by total-to-HDL cholesterol ratio and MAP. Diabetes was also negatively associated with DC_{fem} Z-scores, in men only, and smoking even showed a positive association (in men). The seemingly favorable effect of smoking was true for all 3 age tertiles, not exhibiting any age-dependency [standardized betas in men, for T1: 0.11 (p=0.002); for T2: 0.05 (p=0.13); for T3: 0.10 (p=0.06)] (data not in table). In the sub-population without prior CVD but under BP-, lipid- and/or glucose-lowering treatment (Table 6.8 and Table 6.9, Figure 6.6), BMI, MAP and total-to-HDL cholesterol ratio showed a significant (negative) association in men. In the sub-population with prior CVD (Table 6.8 and Table 6.9, Figure 6.7), the associations with BMI and MAP were maintained (in men only), also showing an association with lipid-lowering medication.

Comparisons by sex showed that in the sub-population without prior CVD and treatment (Figure 6.5) MAP was more negatively associated with DC_{fem} Z-scores in men compared to women. Furthermore, the effects of smoking (positively related only in men), diabetes (negatively related only in men), and total-to-HDL cholesterol ratio (less negatively related in men) also differed between sexes. However, in the other subpopulations (Figure 6.6 and Figure 6.7), only the influence of MAP remained significantly different between men and women.

Table 6.8 Relation between known cardiovascular risk factors and DC_{fem} Z-scores in the reference sub-populations in *men*

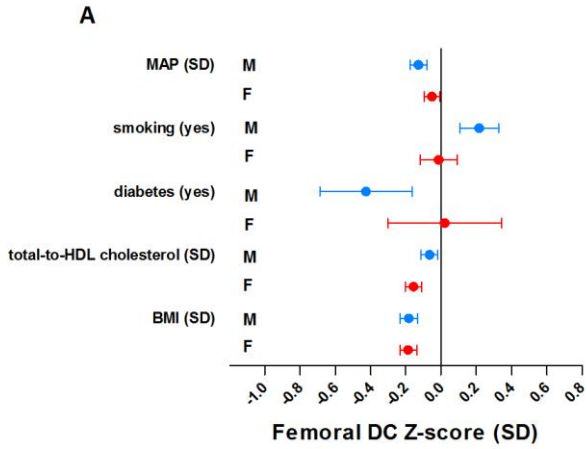
		Sub-population without CVD						Sub-population with CVD (n =316)		
		without treatment[a] (n =1,803)			with treatment[a] (n =391)					
Risk factor	Model	ß	95%CI	P-value	ß	95%CI	P-value	ß	95%CI	P-value
Mean arterial pressure (10 mmHg)	1	-0.209	-0.251; -0.167	<0.001	-0.159	-0.244; -0.074	<0.001	-0.152	-0.225; -0.080	<0.001
	2	-	-	-	-	-	-	-	-	-
	3	-0.118	-0.163; -0.074	<0.001	-0.092	-0.176; -0.007	0.033	-0.141	-0.210; -0.072	<0.001
Current smoking (yes)	1	0.240	0.125; 0.355	<0.001	0.043	-0.213; 0.299	0.741	-0.270	-0.609; 0.068	0.117
	2	0.220	0.108; 0.332	<0.001	0.031	-0.220; 0.283	0.807	-0.343	-0.674; -0.012	0.042
	3	0.217	0.107; 0.327	<0.001	0.000	-0.238; 0.238	0.998	-0.257	-0.574; 0.059	0.110
Diabetes (yes)	1	-0.701	-0.972; -0.43	<0.001	-0.478	-0.717; -0.24	<0.001	-0.218	-0.400; -0.036	0.019
	2	-0.548	-0.815; -0.282	<0.001	-0.413	-0.653; -0.174	<0.001	-0.202	-0.380; -0.025	0.026
	3	-0.426	-0.687; -0.165	0.001	-0.108	-0.405; 0.190	0.478	-0.099	-0.291; 0.094	0.313
Total-to-HDL cholesterol ratio (unit)	1	-0.132	-0.171; -0.094	<0.001	-0.138	-0.211; -0.065	<0.001	-0.024	-0.064; 0.017	0.246
	2	-0.092	-0.130; -0.053	<0.001	-0.137	-0.209; -0.065	<0.001	-0.023	-0.063; 0.016	0.242
	3	-0.054	-0.094; -0.014	0.008	-0.111	-0.181; -0.041	0.002	-0.013	-0.051; 0.026	0.518
Body mass index (kg/m^2)	1	-0.081	-0.095; -0.068	<0.001	-0.062	-0.089; -0.036	<0.001	-0.075	-0.099; -0.052	<0.001
	2	-0.065	-0.079; -0.050	<0.001	-0.055	-0.082; -0.028	<0.001	-0.070	-0.093; -0.046	<0.001
	3	-0.056	-0.071; -0.041	<0.001	-0.036	-0.062; -0.010	0.008	-0.059	-0.083; -0.034	<0.001
Use of BP-lowering medication (yes)	1	-	-	-	-	-	-	-0.181	-0.369; 0.007	0.059
	2	-	-	-	-	-	-	-0.160	-0.344; 0.023	0.087
	3	-	-	-	-	-	-	0.027	-0.164; 0.217	0.782
Use of lipid-lowering medication (yes)	1	-	-	-	-	-	-	-0.352	-0.537; -0.167	<0.001
	2	-	-	-	-	-	-	-0.373	-0.553; -0.193	<0.001
	3	-	-	-	-	-	-	-0.323	-0.518; -0.128	0.001
Use of glucose-lowering medication (yes)	1	-	-	-	-	-	-	-0.271	-0.514; -0.027	0.030
	2	-	-	-	-	-	-	-0.243	-0.481; -0.005	0.045
	3	-	-	-	-	-	-	0.076	-0.187; 0.339	0.570

The regression coefficient ß represents the change in DC_{fem} (in SD from the healthy population mean among individuals of the same age and sex) per unit increase in each risk factor. Model 1: unadjusted. Model 2: adjusted for MAP. Model 3: adjusted for MAP and all other risk factors. [a]BP-, lipid- and glucose-lowering treatment

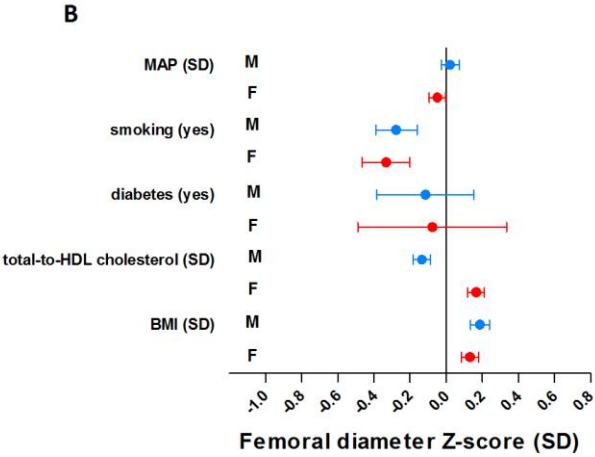
Table 6.9 Relation between known cardiovascular risk factors and DC_{fem} Z-scores in the reference sub-populations in *women*

		Sub-population without CVD						Sub-population with CVD (n =316)		
		without treatment[a] (n =1,803)			with treatment[a] (n =391)					
Risk factor	Model	ß	95%CI	P-value	ß	95%CI	P-value	ß	95%CI	P-value
Mean arterial pressure (10 mmHg)	1	-0.100	-0.137; -0.062	<0.001	-0.050	-0.116; 0.015	0.132	0.069	-0.048; 0.185	0.246
	2	-	-	-	-	-	-	-	-	-
	3	-0.043	-0.081; -0.005	0.026	-0.034	-0.101; 0.034	0.327	0.076	-0.044; 0.197	0.212
Current smoking (yes)	1	-0.009	-0.116; 0.099	0.873	-0.027	-0.277; 0.222	0.830	-0.757	-1.384; -0.130	0.018
	2	-0.043	-0.150; 0.065	0.435	-0.059	-0.311; 0.193	0.647	-0.717	-1.352; -0.083	0.027
	3	-0.013	-0.118; 0.092	0.804	-0.079	-0.329; 0.171	0.534	-0.646	-1.295; 0.004	0.051
Diabetes (yes)	1	-0.264	-0.597; 0.07	0.122	0.128	-0.097; 0.353	0.265	-0.167	-0.481; 0.148	0.298
	2	-0.188	-0.521; 0.145	0.268	0.153	-0.074; 0.380	0.185	-0.206	-0.525; 0.113	0.205
	3	0.022	-0.301; 0.344	0.895	0.276	0.001; 0.551	0.049	-0.081	-0.429; 0.267	0.647
Total-to-HDL cholesterol ratio (unit)	1	-0.192	-0.23; -0.155	<0.001	-0.054	-0.126; 0.017	0.135	-0.103	-0.214; 0.009	0.071
	2	-0.187	-0.224; -0.149	<0.001	-0.054	-0.125; 0.017	0.137	-0.107	-0.219; 0.004	0.060
	3	-0.135	-0.174; -0.096	<0.001	-0.027	-0.101; 0.047	0.475	-0.074	-0.191; 0.043	0.215
Body mass index (kg/m^2)	1	-0.063	-0.073; -0.052	<0.001	-0.027	-0.047; -0.007	0.008	-0.032	-0.071; 0.007	0.108
	2	-0.059	-0.071; -0.048	<0.001	-0.025	-0.045; -0.005	0.015	-0.037	-0.077; 0.002	0.063
	3	-0.046	-0.058; -0.034	<0.001	-0.020	-0.041; 0.001	0.062	-0.029	-0.071; 0.014	0.186
Use of BP-lowering medication (yes)	1	-	-	-	-	-	-	-0.199	-0.514; 0.116	0.215
	2	-	-	-	-	-	-	-0.221	-0.537; 0.096	0.171
	3	-	-	-	-	-	-	-0.018	-0.368; 0.332	0.920
Use of lipid-lowering medication (yes)	1	-	-	-	-	-	-	-0.298	-0.669; 0.072	0.114
	2	-	-	-	-	-	-	-0.293	-0.664; 0.077	0.120
	3	-	-	-	-	-	-	-0.282	-0.675; 0.110	0.158
Use of glucose-lowering medication (yes)	1	-	-	-	-	-	-	-0.261	-0.804; 0.283	0.345
	2	-	-	-	-	-	-	-0.301	-0.847; 0.245	0.279
	3	-	-	-	-	-	-	-0.012	-0.611; 0.588	0.970

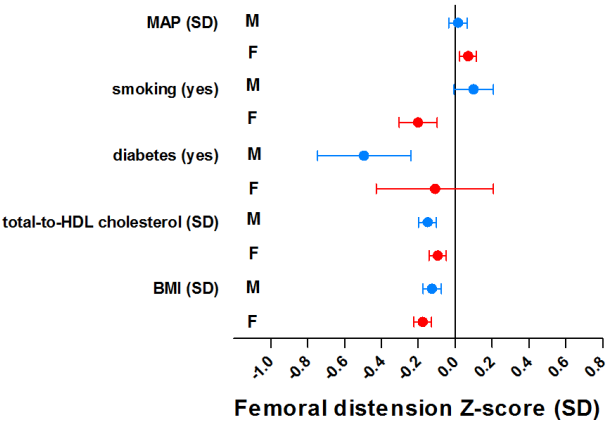
The regression coefficient ß represents the change in DC_{fem} (in SD from the healthy population mean among individuals of the same age and sex) per unit increase in each risk factor. Model 1: unadjusted. Model 2: adjusted for MAP. Model 3: adjusted for MAP and all other risk factors. [a]BP-, lipid- and glucose-lowering treatment



	standardized β (95% CI)	*p*-value for sex interaction
MAP (SD) M	-0.13 (-0.18; -0.08)	0.011
MAP (SD) F	-0.05 (-0.10; -0.01)	
smoking (yes) M	0.22 (0.11; 0.33)	0.003
smoking (yes) F	-0.01 (-0.12; 0.09)	
diabetes (yes) M	-0.43 (-0.69; -0.17)	0.037
diabetes (yes) F	0.02 (-0.30; 0.34)	
total-to-HDL cholesterol (SD) M	-0.07 (-0.11; -0.02)	0.005
total-to-HDL cholesterol (SD) F	-0.16 (-0.20; -0.11)	
BMI (SD) M	-0.18 (-0.23; -0.13)	0.300
BMI (SD) F	-0.19 (-0.23; -0.14)	



	standardized β (95% CI)	*p*-value for sex interaction
MAP (SD) M	0.02 (-0.03; 0.07)	0.037
MAP (SD) F	-0.05 (-0.10; -0.00)	
smoking (yes) M	-0.27 (-0.39; -0.16)	0.528
smoking (yes) F	-0.33 (-0.47; -0.20)	
diabetes (yes) M	-0.11 (-0.39; 0.16)	0.881
diabetes (yes) F	-0.08 (-0.49; 0.33)	
total-to-HDL cholesterol (SD) M	-0.14 (-0.18; -0.09)	<0.001
total-to-HDL cholesterol (SD) F	0.17 (0.12; 0.21)	
BMI (SD) M	0.19 (0.14; 0.24)	0.136
BMI (SD) F	0.13 (0.08; 0.18)	



	standardized β (95% CI)	*p*-value for sex interaction
MAP (SD) M	0.02 (-0.03; 0.06)	0.135
MAP (SD) F	0.07 (0.02; 0.12)	
smoking (yes) M	0.10 (-0.00; 0.21)	<0.001
smoking (yes) F	-0.20 (-0.30; -0.10)	
diabetes (yes) M	-0.49 (-0.75; -0.24)	0.066
diabetes (yes) F	-0.11 (-0.43; 0.21)	
total-to-HDL cholesterol (SD) M	-0.15 (-0.20; -0.10)	0.167
total-to-HDL cholesterol (SD) F	-0.09 (-0.14; -0.05)	
BMI (SD) M	-0.13 (-0.18; -0.07)	0.512
BMI (SD) F	-0.18 (-0.22; -0.13)	

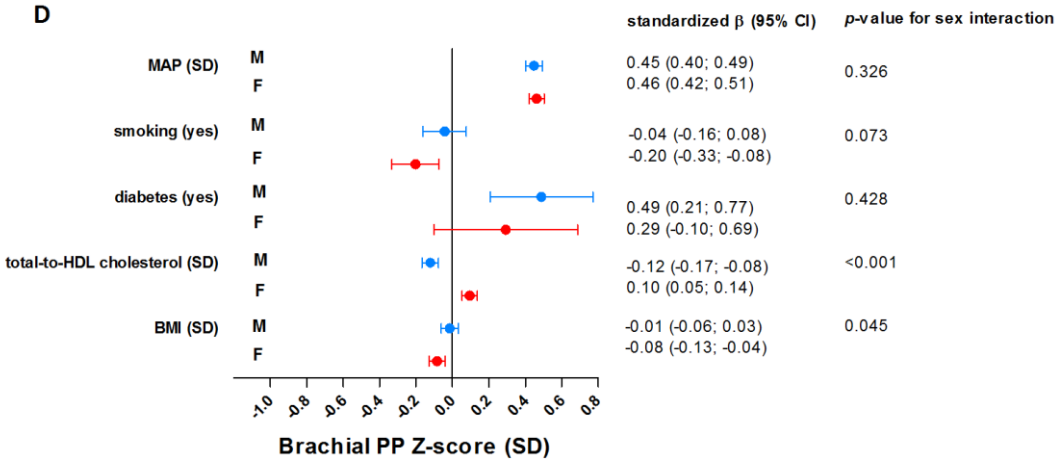
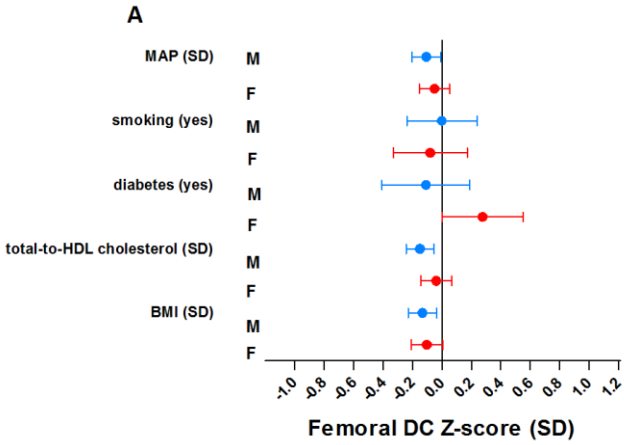
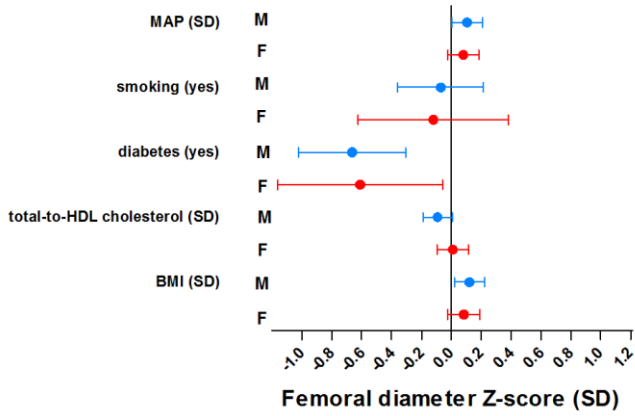


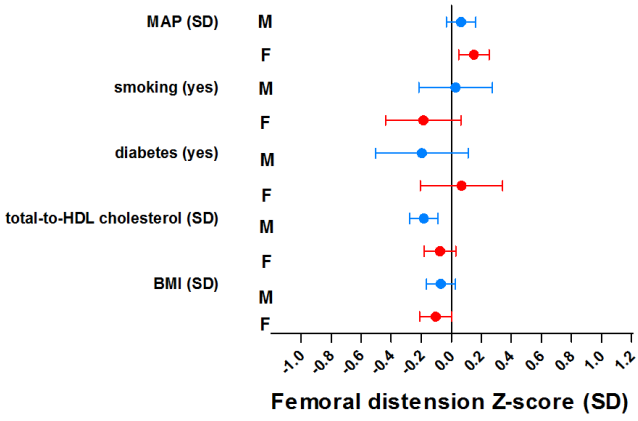
Figure 6.5 Associations between CV-RFs and DC_{fem} (***A***), femoral diameter (***B***), femoral distension (***C***) and brachial PP (***D***) Z-scores: ***reference sub-population without CVD or treatment***. Point estimates and 95% confidence intervals represent the increase in the Z-scores (in SD from the healthy population mean) per SD increase (or for presence vs. absence) in risk factor resulting from a multivariable regression model including all risk factors, stratified by sex (male (M) and female (F), respectively). *BMI*, body mass index. ; *MAP*, mean arterial pressure. SD's in men and women respectively: 3.5 and 3.9 kg/m² for *BMI*, 11.5 and 11.5 mm Hg for *MAP*, 1.3 and 1.1 for *total-to-HDL cholesterol ratio*. The SD equations in men and women, respectively, were $2.829 + 3.677 \times (age/10)^{-1}$ and $5.984 - 0.005 \times (age/10)^3$ for DC_{fem} (in 10^{-3}*kPa^{-1}), $1.018 + 0.002 \times (Age/10)^3$ and $3.502 - 1.492 \times \ln(Age/10)$ for *femoral diameter* (in mm), $0.137 - 0.001 \times Age$ and $0.110 - 0.001 \times (Age/10)^3$ for *femoral distension* (in mm), and $7.566 + 3.309 \times (Age/10)^{-2}$ and $5.835 - 0.046 \times Age$ for *brachial PP* (in mm Hg).



	standardized β (95% CI)	*p*-value for sex interaction
MAP (SD) M	-0.11 (-0.20; -0.01)	0.290
MAP (SD) F	-0.05 (-0.15; 0.05)	
smoking (yes) M	0.00 (-0.24; 024)	0.654
smoking (yes) F	-0.08 (-0.33; 0.17)	
diabetes (yes) M	-0.11 (-0.41; 0.19)	0.063
diabetes (yes) F	0.28 (0.00; 0.55)	
total-to-HDL cholesterol (SD) M	-0.15 (-0.24; -0.06)	0.107
total-to-HDL cholesterol (SD) F	-0.04 (-0.14; 0.07)	
BMI (SD) M	-0.130 (-0.23; -0.04)	0.354
BMI (SD) F	-0.100 (-0.21; 0.01)	



	standardized β (95% CI)	*p*-value for sex interaction
MAP (SD) M	0.11 (0.04; 0.21)	0.996
MAP (SD) F	0.08 (-0.02; 0.19)	
smoking (yes) M	-0.07 (-0.36; 0.22)	0.861
smoking (yes) F	-0.12 (-0.62; 0.38)	
diabetes (yes) M	-0.66 (-1.02; -0.31)	0.868
diabetes (yes) F	-0.61 (-1.16; -0.06)	
total-to-HDL cholesterol (SD) M	-0.09 (-0.19; 0.01)	0.276
total-to-HDL cholesterol (SD) F	0.01 (-0.10; 0.12)	
BMI (SD) M	0.12 (0.02; 0.22)	0.855
BMI (SD) F	0.08 (-0.02; 0.19)	



	standardized β (95% CI)	*p*-value for sex interaction
MAP (SD) M	0.06 (-0.03; 0.16)	0.463
MAP (SD) F	0.15 (0.05; 0.25)	
smoking (yes) M	0.03 (-0.22; 0.27)	0.231
smoking (yes) F	-0.19 (-0.43; 0.06)	
diabetes (yes) M	-0.20 (-0.50; 0.11)	0.210
diabetes (yes) F	0.07 (-0.21; 0.34)	
total-to-HDL cholesterol (SD) M	-0.18 (-0.28; -0.09)	0.097
total-to-HDL cholesterol (SD) F	-0.08 (-0.18; 0.03)	
BMI (SD) M	-0.07 (-0.17; 0.03)	0.951
BMI (SD) F	-0.11 (-0.21; 0.00)	

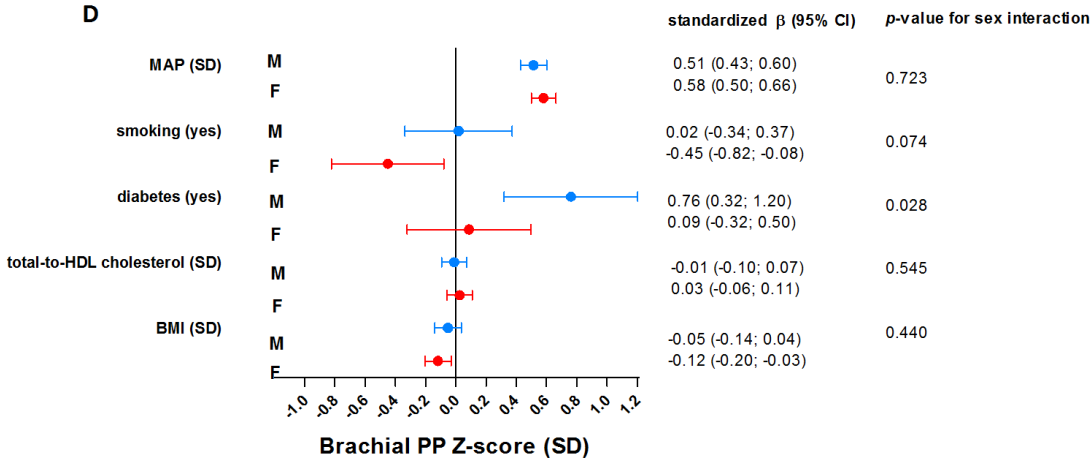
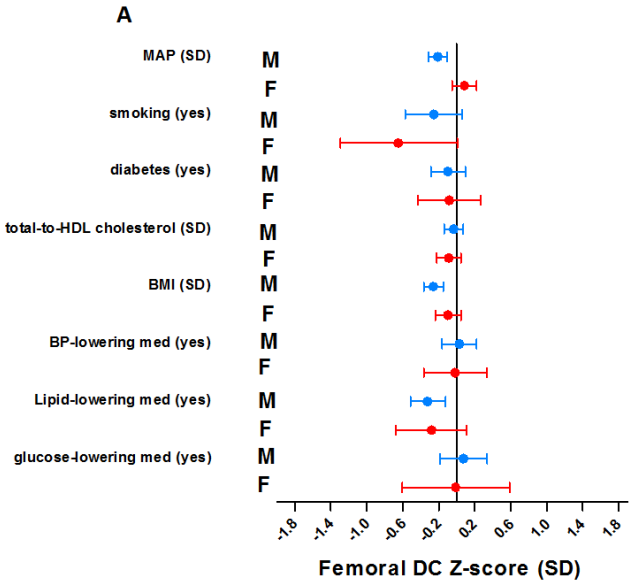
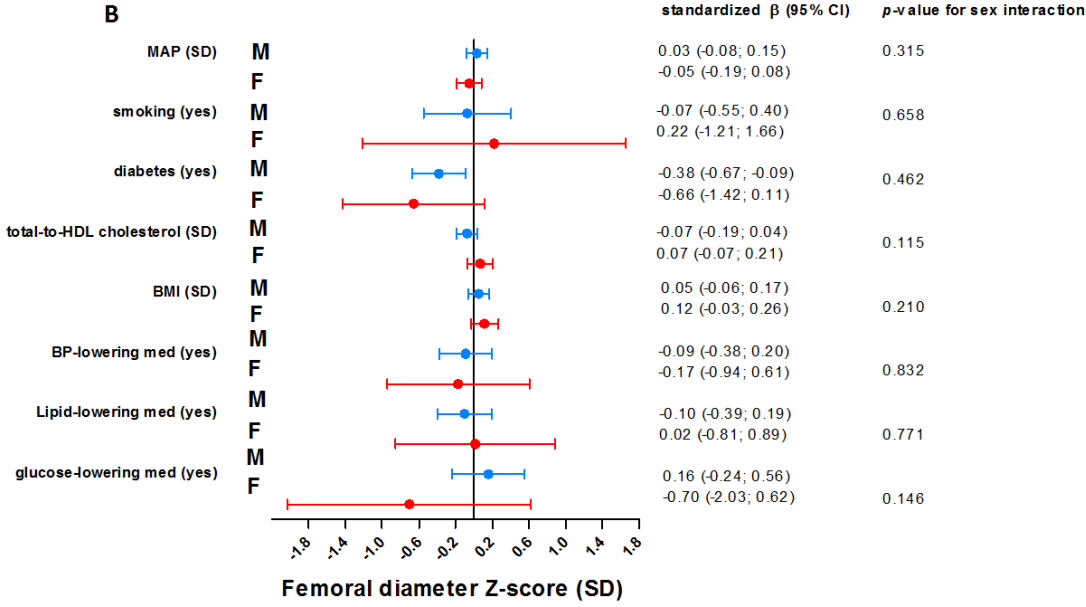
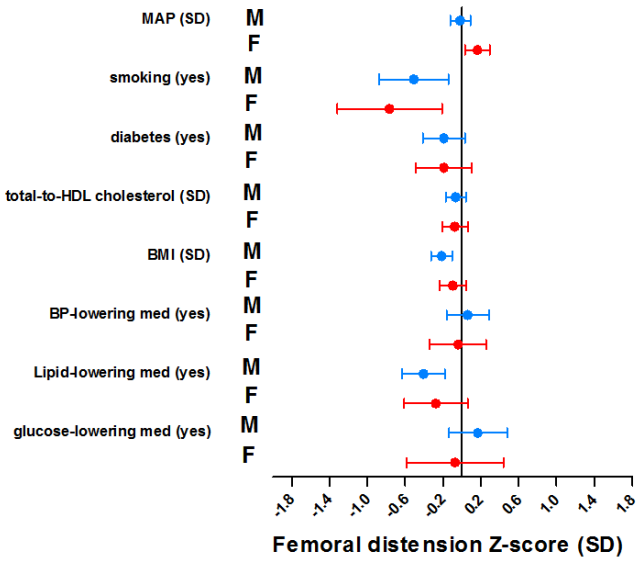


Figure 6.6 Associations between CV-RFs and DC_{fem} (***A***), femoral diameter (***B***), femoral distension (***C***) and brachial PP (***D***) Z-scores: ***reference sub-population without CVD with BP-, lipid- and/or glucose-lowering treatment***. Point estimates and 95% confidence intervals represent the increase in the Z-scores (in SD from the healthy population mean) per SD increase (or for presence vs. absence) in risk factor resulting from a multivariable regression model including all risk factors, stratified by sex (male (M) and female (F), respectively). *BMI*, body mass index; *MAP*, mean arterial pressure. SD's in men and women respectively: 3.6 and 4.4 kg/m² for *BMI*, 11.4 and 13.4 mm Hg for *MAP*, 1.3 and 1.2 for *total-to-HDL cholesterol ratio*. The SD equations in men and women, respectively, were $2.829 + 3.677 \times (age/10)^{-1}$ and $5.984 - 0.005 \times (age/10)^3$ for DC_{fem} (in 10^{-3}*kPa^{-1}), $1.018 + 0.002 \times (Age/10)^3$ and $3.502 - 1.492 \times \ln(Age/10)$ for *femoral diameter* (in mm), $0.137 - 0.001 \times Age$ and $0.110 - 0.001 \times (Age/10)^3$ for *femoral distension* (in mm), and $7.566 + 3.309 \times (Age/10)^{-2}$ and $5.835 - 0.046 \times Age$ for *brachial PP* (in mm Hg).


A
standardized β (95% CI)
p-value for sex interaction
MAP (SD)
M
F
-0.21 (-0.32; -0.11)
0.09 (-0.05; 0.22)
0.001
smoking (yes)
M
F
-0.26 (-0.57; 0.06)
-0.65 (-1.26; 0.00)
0.247
diabetes (yes)
M
F
-0.10 (-0.29; 0.09)
-0.08 (-0.43; 0.27)
0.924
total-to-HDL cholesterol (SD)
M
F
-0.04 (-0.14; 0.07)
-0.09 (-0.22; 0.05)
0.257
BMI (SD)
M
F
-0.26 (-0.37; -0.15)
-0.10 (-0.24; 0.05)
0.204
BP-lowering med (yes)
M
F
0.03 (-0.16; 0.21)
-0.02 (-0.37; 0.33)
0.813
Lipid-lowering med (yes)
M
F
-0.32 (-0.52; -0.13)
-0.28 (-0.68; 0.11)
0.842
glucose-lowering med (yes)
M
F
0.08 (-0.19; 0.34)
-0.01 (-0.61; 0.59)
0.770
-1.8
-1.4
-1.0
-0.6
-0.2
0.2
0.6
1.0
1.4
1.8
Femoral DC Z-score (SD)


B
standardized β (95% CI)
p-value for sex interaction
MAP (SD)
M
F
0.03 (-0.08; 0.15)
-0.05 (-0.19; 0.08)
0.315
smoking (yes)
M
F
-0.07 (-0.55; 0.40)
0.22 (-1.21; 1.66)
0.658
diabetes (yes)
M
F
-0.38 (-0.67; -0.09)
-0.66 (-1.42; 0.11)
0.462
total-to-HDL cholesterol (SD)
M
F
-0.07 (-0.19; 0.04)
0.07 (-0.07; 0.21)
0.115
BMI (SD)
M
F
0.05 (-0.06; 0.17)
0.12 (-0.03; 0.26)
0.210
BP-lowering med (yes)
M
F
-0.09 (-0.38; 0.20)
-0.17 (-0.94; 0.61)
0.832
Lipid-lowering med (yes)
M
F
-0.10 (-0.39; 0.19)
0.02 (-0.81; 0.89)
0.771
glucose-lowering med (yes)
M
F
0.16 (-0.24; 0.56)
-0.70 (-2.03; 0.62)
0.146
-1.8
-1.4
-1.0
-0.6
-0.2
0.2
0.6
1.0
1.4
1.8
Femoral diameter Z-score (SD)

C


standardized β (95% CI)
p-value for sex interaction
MAP (SD)
M
F
-0.02 (-0.12; 0.09)
0.16 (0.03; 0.29)
0.031
smoking (yes)
M
F
-0.51 (-0.88; -0.14)
-0.77 (-1.32; -0.21)
0.437
diabetes (yes)
M
F
-0.19 (-0.42; 0.03)
-0.19 (-0.49; 0.11)
0.999
total-to-HDL cholesterol (SD)
M
F
-0.07 (-0.17; 0.04)
-0.08 (-0.21; 0.06)
0.581
BMI (SD)
M
F
-0.22 (-0.33; -0.11)
-0.10 (-0.24; 0.04)
0.189
BP-lowering med (yes)
M
F
0.06 (-0.16; 0.28)
-0.04 (-0.34; 0.26)
0.583
Lipid-lowering med (yes)
M
F
-0.41 (-0.64; -0.18)
-0.28 (-0.61; 0.06)
0.509
glucose-lowering med (yes)
M
F
0.17 (-0.14; 0.48)
-0.07 (-0.59; 0.44)
0.409
-1.8
-1.4
-1.0
-0.6
-0.2
0.2
0.6
1.0
1.4
1.8
Femoral distension Z-score (SD)

D

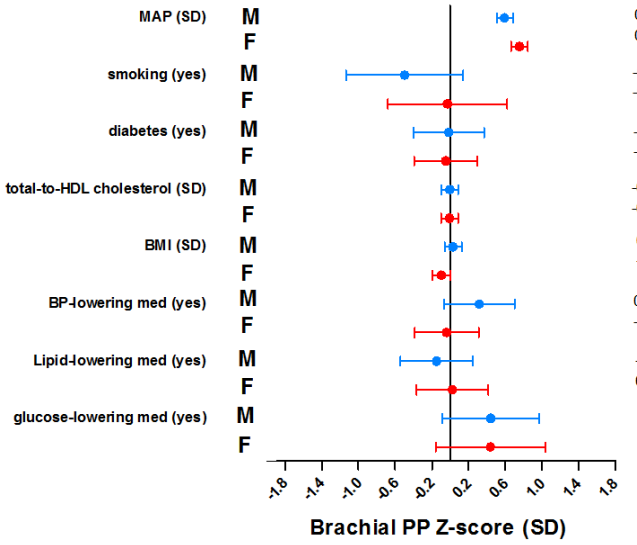

standardized β (95% CI)
p-value for sex interaction
MAP (SD)
M
F
0.59 (0.51; 0.68)
0.76 (0.66; 0.85)
0.601
smoking (yes)
M
F
-0.50 (-1.14; 0.14)
-0.03 (-0.68; 0.62)
0.335
diabetes (yes)
M
F
-0.02 (-0.40; 0.37)
-0.05 (-0.40; 0.30)
0.901
total-to-HDL cholesterol (SD)
M
F
-0.00 (-0.09; 0.09)
-0.00 (-0.10; 0.09)
0.953
BMI (SD)
M
F
0.03 (-0.06; 0.12)
-0.10 (-0.19; 0.00)
0.088
BP-lowering med (yes)
M
F
0.32 (-0.07; 0.70)
-0.04 (-0.39; 0.31)
0.195
Lipid-lowering med (yes)
M
F
-0.15 (-0.54; 0.24)
0.02 (-0.37; 0.41)
0.564
glucose-lowering med (yes)
M
F
0.44 (-0.09; 0.97)
0.44 (-0.16; 1.04)
0.993
-1.8
-1.4
-1.0
-0.6
-0.2
0.2
0.6
1.0
1.4
1.8
Brachial PP Z-score (SD)

Figure 6.7 Associations between CV-RFs and DC_{fem} (***A***), femoral diameter (***B***), femoral distension (***C***) and brachial PP (***D***) Z-scores: ***reference sub-population with CVD***. Point estimates and 95% confidence intervals represent the increase in the Z-scores (in SD from the healthy population mean) per SD increase (or for presence vs. absence) in risk factor resulting from a multivariable regression model including all risk factors, stratified by sex (male (M) and female (F), respectively). *BMI*, body mass index; *BP*, blood pressure; *med*, medication; *MAP*, mean arterial pressure. SD's in men and women respectively: 3.6 and 3.9 kg/m² for *BMI*, 12.4 and 11.5 mm Hg for *MAP*, 2.3 and 1.4 for *total-to-HDL cholesterol ratio*. The SD equations in men and women, respectively, were $2.829 + 3.677 \times (age/10)^{-1}$ and $5.984 - 0.005 \times (age/10)^{3}$ for DC_{fem} (in $10^{-3}*kPa^{-1}$), $1.018 + 0.002 \times (Age/10)^{3}$ and $3.502 - 1.492 \times \ln(Age/10)$ for *femoral diameter* (in mm), $0.137 - 0.001 \times Age$ and $0.110 - 0.001 \times (Age/10)^{3}$ for *femoral distension* (in mm), and $7.566 + 3.309 \times (Age/10)^{-2}$ and $5.835 - 0.046 \times Age$ for *brachial PP* (in mm Hg).

6.4.4 Additional analyses

6.4.4.1 Reference intervals for femoral artery diameter, distension, and brachial PP

The equations derived from FP analyses on the individual components of DC_{fem}, i.e. femoral artery diameter, femoral artery distension and brachial PP and the sex-specific percentile lines according to age superimposed on the raw data are provided (Figure 6.8, Figure 6.9 and Figure 6.10 for diameter, distension and PP, respectively). In the healthy sub-population, the age-related decrease in DC_{fem} reflected a drop in distension, no change in diameter, and a rise in PP. Of these three components, distension seemed to be the major driver behind the reduction in DC_{fem}, while the effect of PP was limited.

Age- and sex-specific reference intervals for femoral diameter in the healthy sub-population

The best fitting FPs' powers (*p, q*) for the $mean_{diameter}$ curves were *p*=-2 q=0.5 for men and *p*=2 *q*=2 for women and for the $SD_{diameter}$ curves were *p*=3 for men and *p*=0 women. Accordingly, the equations derived on the basis of the estimated coefficients were, for men:

- $Mean_{diameter}$ (in mm) = 8.319 - 2.892 x $(Age/10)^{-2}$ + 0.370 x $(Age/10)^{0.5}$
- $SD_{diameter}$ (in mm) = 1.018 + 0.002 x $(Age/10)^{3}$

and, for women:

- $Mean_{diameter}$ (in mm) = 8.79 - 0.28 x $(Age/10)^2$ + 0.14 x $(Age/10)^2$ x ln(Age/10)
- $SD_{diameter}$ (in mm) = 3.502 - 1.492 x ln(Age/10)

Age- and sex-specific reference intervals for femoral distension in the healthy sub-population

The best fitting FPs' powers (*p,q*) for the $mean_{distension}$ curves were *p*=2, q=3 for men and *p*=3 *q*=3 for women and for the $SD_{distension}$ curves were *p*=1 for men and *p*=3 women. Accordingly, the equations derived on the basis of the estimated coefficients were, for men:

- $Mean_{distension}$ (mm) = 0.184 + 0.012 x $(Age/10)^2$ - 0.002 x $(Age/10)^3$
- $SD_{distension}$ (in mm) = 0.137 - 0.001 x Age

and, for women:

- $Mean_{distension}$ (in mm) = 0.197 + 0.004 x $(Age/10)^3$ - 0.002 x $(Age/10)^3$ x ln(Age/10)
- $SD_{distension}$ (in mm) = 0.110 - 0.001 x $(Age/10)^3$

Age- and sex-specific reference intervals for brachial PP in the healthy sub-population

The best fitting FPs' powers (*p*) for the $mean_{PP}$ curves were *p*=1 *q*=2 for men and *p*=-1 *q*=-0.5 for women and for the SD_{PP} curves were *p*=-2 for men and *p*=1 women. Accordingly, the equations derived on the basis of the estimated coefficients were, for men:

- $Mean_{PP}$ (in mm Hg) = 62.626 - 7.124 x (Age/10) + 0.027 x $(Age/10)^2$
- SD_{PP} (in mm Hg) = 7.566 + 3.309 x $(Age/10)^{-2}$

and, for women:

- $Mean_{PP}$ (in mm Hg) = 89.136 + 97.779 x $(Age/10)^{-1}$ - 132.336 x $(Age/10)^{-0.5}$
- SD_{PP} (in mm Hg) = 5.835 - 0.046 x Age

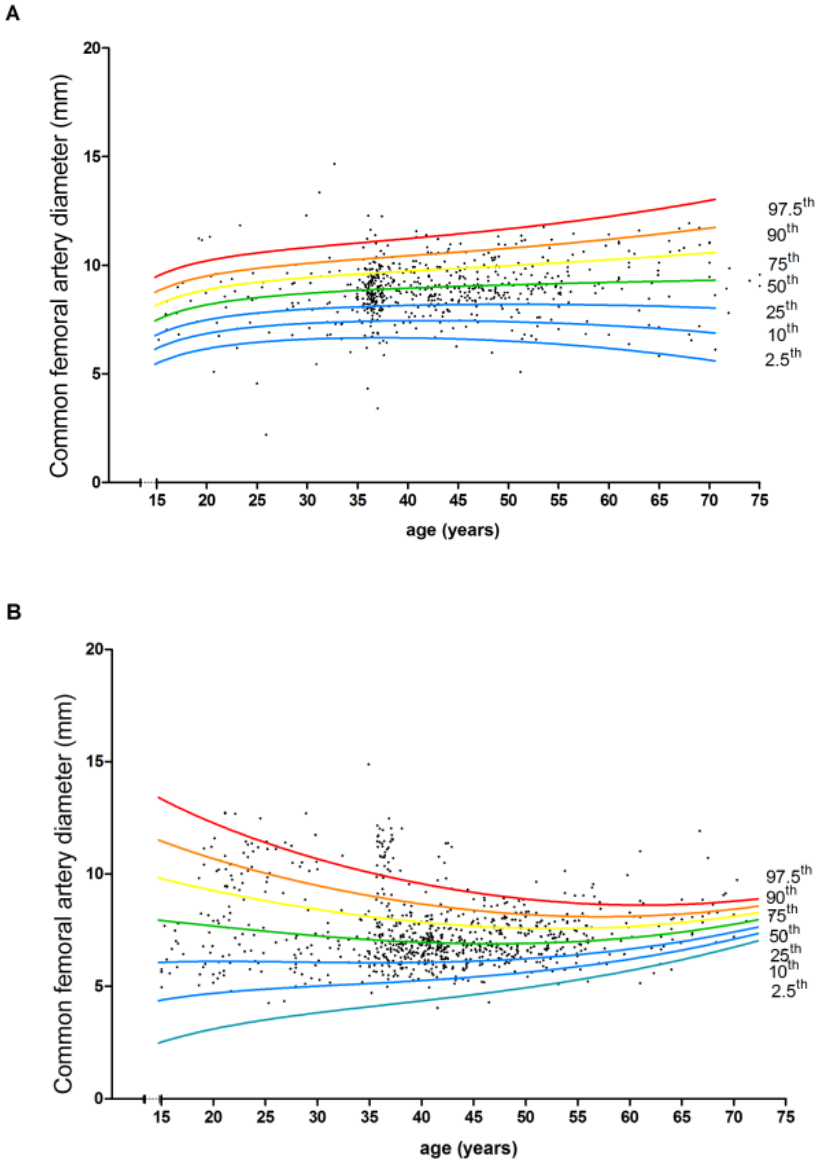


Figure 6.8 Age-specific percentiles of *femoral diameter* in the healthy sub-population. ***A***, men; ***B***, women.

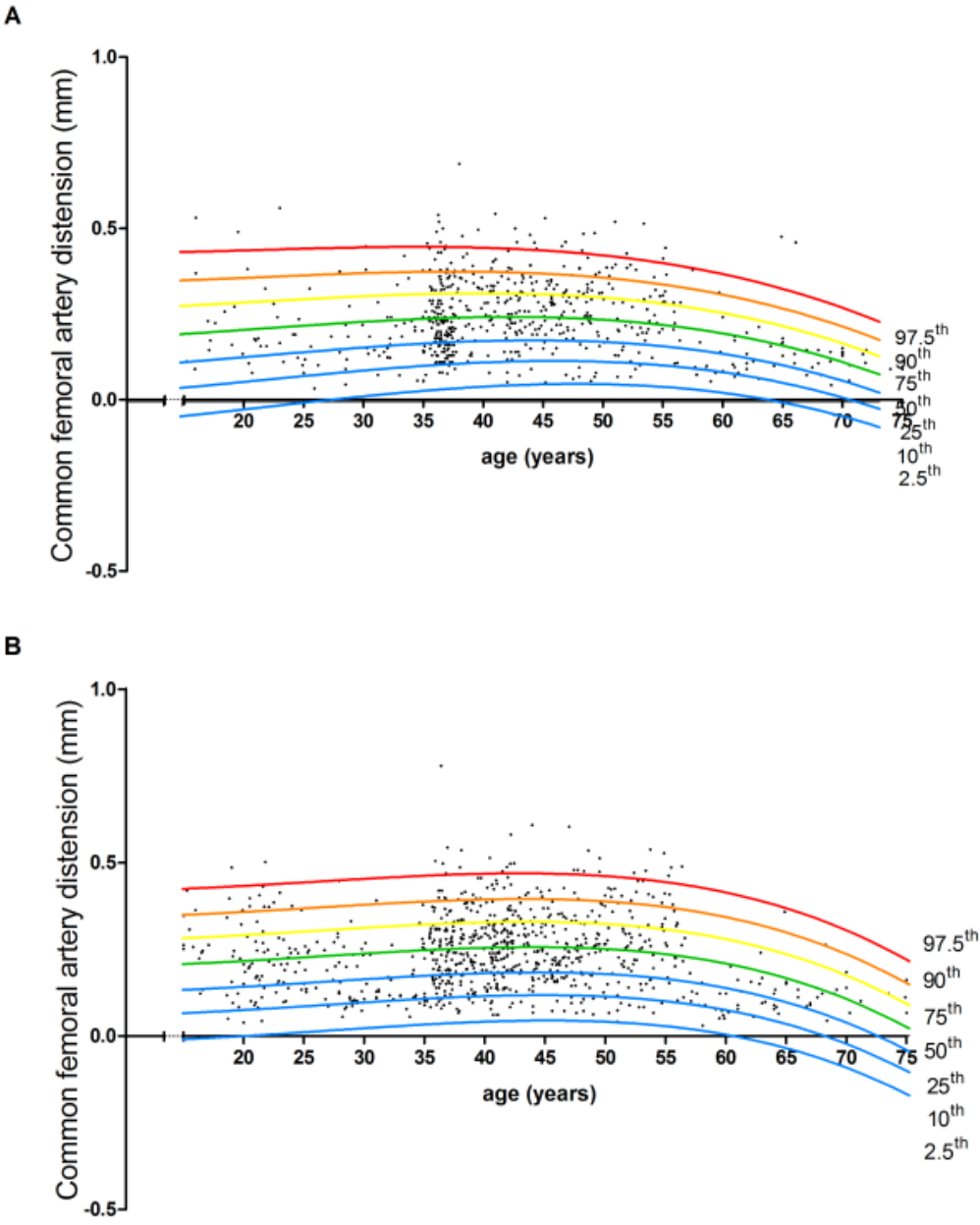


Figure 6.9 Age-specific percentiles of *femoral distension* in the healthy sub-population. ***A***, men; ***B***, women.

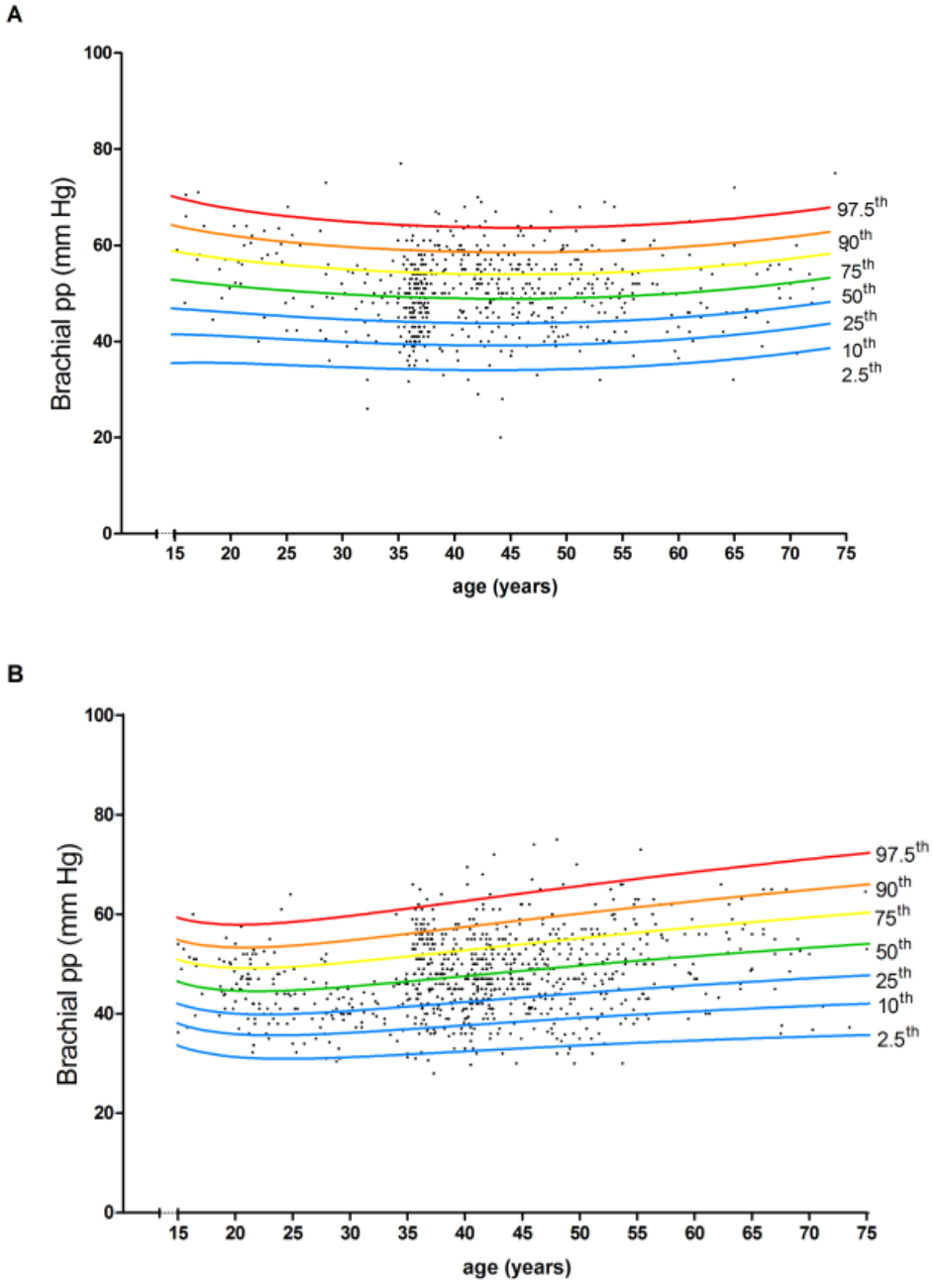


Figure 6.10 Age-specific percentiles of *brachial pulse pressure* in the healthy sub-population. ***A***, men; ***B***, women.

6.4.4.2 *Associations of cardiovascular risk factors with percentiles of components of DC_{fem} (i.e. diameter, distension, PP)*

In the sub-population without prior CVD and treatment (Figure 6.5), the negative association between MAP and DC_{fem} Z-score seemed to be mainly driven by the strong *positive* association of MAP and brachial PP. The *negative* association of both BMI and total-to-HDL ratio with DC_{fem} Z-score could be explained by a *negative* association with femoral artery distension, and the *positive* association of BMI with femoral artery diameter. The seemingly favorable effect of smoking in men (*positive* association with DC_{fem}) was driven by a *negative* association between smoking and femoral artery diameter while distension remained unaltered. This effect, however, is not observed in subjects on medication (Figure 6.6) or with a history of CVD. (Figure 6.7)

6.4.4.3 *Reference intervals for DC_{fem} calculated with local femoral artery PP and the association with CV-RFs*

Reference intervals were additionally established for DC_{fem} calculated with local femoral artery PP. Accordingly, the equations derived on the basis of the estimated coefficients were, for *men*:

- *Mean* DC_{fem} (in 10^{-3}.kPa^{-1}) = $7.506 + 0.411 \times (age/10)^2 - 0.246 \times (age/10)^2 \times \ln(age/10)$
- *SD* DC_{fem} (in 10^{-3}.kPa^{-1}) = $0.232 + 6.884 \times (age/10)^{-0,5}$

or, women:

- *Mean* DC_{fem} (in 10^{-3}.kPa^{-1}) = $9.325 + 0.119 \times (age/10)^3 - 0.070 \times (age/10)^3 \times \ln(age/10)$
- *SD* DC_{fem} (in 10^{-3}.kPa^{-1}) = $3.931 + 5.114 \times (age/10)^{-1}$

Sex-specific percentile lines according to age superimposed on the raw data (Figure 6.11), the respective levels of DC_{fem} by age category (Table 6.10) and the associations with CV-RFs (Table 6.11 and Table 6.12).

Since local femoral artery PP was on average higher than brachial PP (P50 men: 56.4 mmHg *vs.* 54 mmHg; P50 women: 54.5 mmHg *vs.* 52 mmHg), calculating DC_{fem} using local femoral artery PP resulted in slightly lower absolute values of DC_{fem} (P50 men: 5.7 *vs.* 6.1 10^{-3}.kPa^{-1}; P50 women: 6.7 *vs.* 7.6 10^{-3}.kPa^{-1}).

Table 6.10 Age- and sex-specific percentiles of DC_{fem} (in 10^{-3}.kPa^{-1}) calculated using local PP in the healthy sub-population.

		Percentiles						
	Age (years)	2.5th	10th	25th	50th	75th	90th	97.5th
Men (n=588)	20	-1.5*	2.0	5.1	8.5	11.9	15.0	18.5
	30	0.5	3.4	6.0	8.8	11.6	14.2	17.0
	40	1.4	3.9	6.1	8.6	11.1	13.3	15.8
	50	1.3	3.6	5.6	7.8	10.0	12.1	14.3
	60	0.4	2.4	4.3	6.3	8.4	10.2	12.3
	70	-1.5*	0.4	2.1	4.0	5.9	7.7	9.6
Women (n=721)	20	-2.8*	1.6	5.5	9.9	14.2	18.2	22.6
	30	-0.6*	3.3	6.7	10.5	14.2	17.7	21.5
	40	0.5	4.1	7.2	10.7	14.2	17.4	20.9
	50	0.4	3.8	6.8	10.1	13.4	16.4	19.8
	60	-1.5*	1.8	4.7	7.9	11.1	14.0	17.3
	70	-5.8*	-2.6*	0.2	3.3	6.5	9.3	12.5

*Negative values of DC_{fem} estimated for the 2.5th percentile are not realistic, but are likely artefacts resulting from a model accounting for high variability across age categories.

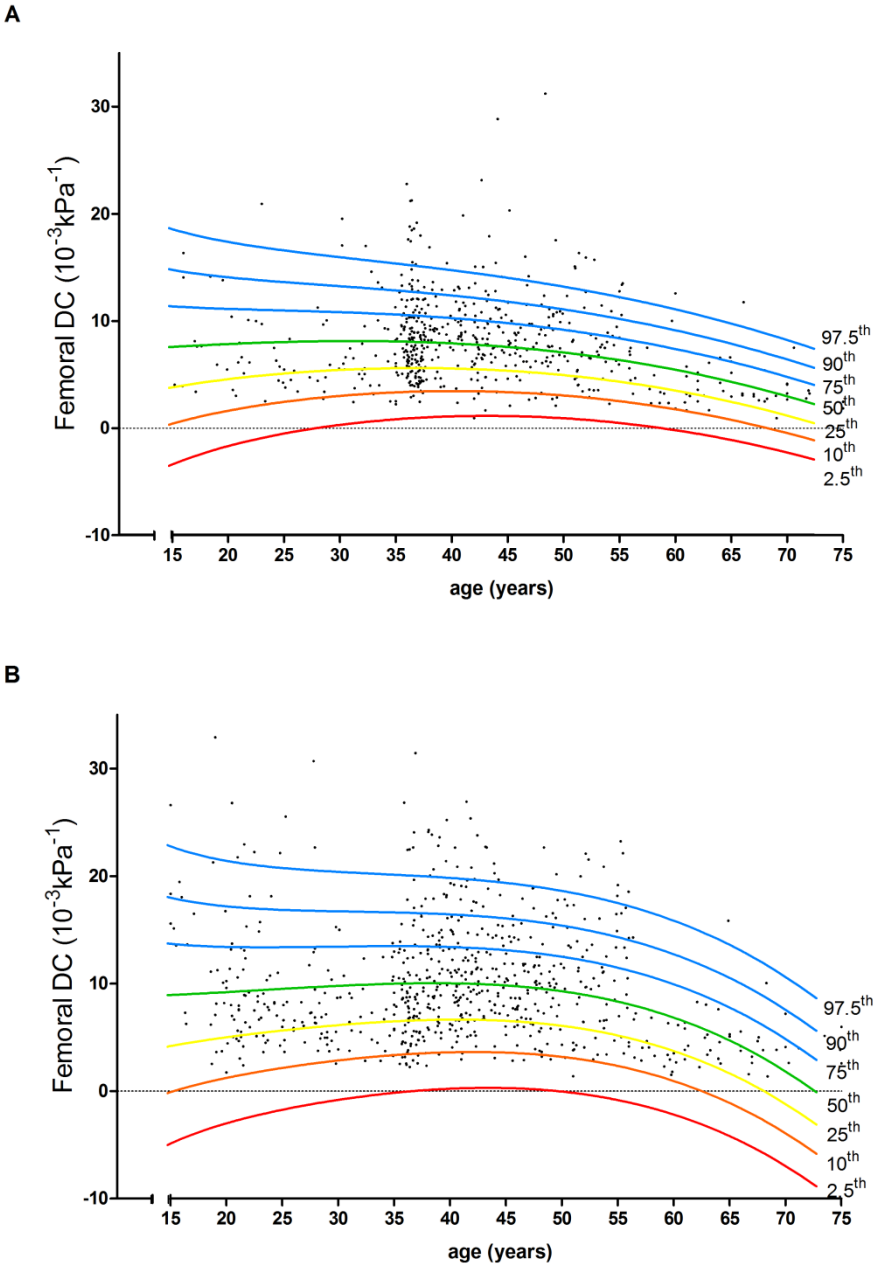


Figure 6.11 Sex-specific percentiles of DC_{fem} calculated using local PP according to age in the healthy sub-population. ***A***, men; ***B***, women.

Table 6.11 Relation between known cardiovascular risk factors and DC_{fem} Z-scores in the reference sub-populations in *men*

		Sub-population without CVD						Sub-population with CVD (n =316)		
		without treatment[a] (n =1,803)			with treatment[a] (n =391)					
Risk factor	Model	ß	95%CI	P-value	ß	95%CI	P-value	ß	95%CI	P-value
Mean arterial pressure (10 mmHg)	1	-0.209	-0.251; -0.167	<0.001	-0.159	-0.244; -0.074	<0.001	-0.152	-0.225; -0.080	<0.001
	2	-	-	-	-	-	-	-	-	-
	3	-0.118	-0.163; -0.074	<0.001	-0.092	-0.176; -0.007	0.033	-0.141	-0.210; -0.072	<0.001
Current smoking (yes)	1	0.240	0.125; 0.355	<0.001	0.043	-0.213; 0.299	0.741	-0.270	-0.609; 0.068	0.117
	2	0.220	0.108; 0.332	<0.001	0.031	-0.220; 0.283	0.807	-0.343	-0.674; -0.012	0.042
	3	0.217	0.107; 0.327	<0.001	0.000	-0.238; 0.238	0.998	-0.257	-0.574; 0.059	0.110
Diabetes (yes)	1	-0.701	-0.972; -0.43	<0.001	-0.478	-0.717; -0.24	<0.001	-0.218	-0.400; -0.036	0.019
	2	-0.548	-0.815; -0.282	<0.001	-0.413	-0.653; -0.174	<0.001	-0.202	-0.380; -0.025	0.026
	3	-0.426	-0.687; -0.165	0.001	-0.108	-0.405; 0.190	0.478	-0.099	-0.291; 0.094	0.313
Total-to-HDL cholesterol ratio (unit)	1	-0.132	-0.171; -0.094	<0.001	-0.138	-0.211; -0.065	<0.001	-0.024	-0.064; 0.017	0.246
	2	-0.092	-0.130; -0.053	<0.001	-0.137	-0.209; -0.065	<0.001	-0.023	-0.063; 0.016	0.242
	3	-0.054	-0.094; -0.014	0.008	-0.111	-0.181; -0.041	0.002	-0.013	-0.051; 0.026	0.518
Body mass index (kg/m2)	1	-0.081	-0.095; -0.068	<0.001	-0.062	-0.089; -0.036	<0.001	-0.075	-0.099; -0.052	<0.001
	2	-0.065	-0.079; -0.050	<0.001	-0.055	-0.082; -0.028	<0.001	-0.070	-0.093; -0.046	<0.001
	3	-0.056	-0.071; -0.041	<0.001	-0.036	-0.062; -0.010	0.008	-0.059	-0.083; -0.034	<0.001
Use of BP-lowering medication (yes)	1	-	-	-	-	-	-	-0.181	-0.369; 0.007	0.059
	2	-	-	-	-	-	-	-0.160	-0.344; 0.023	0.087
	3	-	-	-	-	-	-	0.027	-0.164; 0.217	0.782
Use of lipid-lowering medication (yes)	1	-	-	-	-	-	-	-0.352	-0.537; -0.167	<0.001
	2	-	-	-	-	-	-	-0.373	-0.553; -0.193	<0.001
	3	-	-	-	-	-	-	-0.323	-0.518; -0.128	0.001
Use of glucose-lowering medication (yes)	1	-	-	-	-	-	-	-0.271	-0.514; -0.027	0.030
	2	-	-	-	-	-	-	-0.243	-0.481; -0.005	0.045
	3	-	-	-	-	-	-	0.076	-0.187; 0.339	0.570

The regression coefficient ß represents the change in DC_{fem} (in SD from the healthy population mean among individuals of the same age and sex) per unit increase in each risk factor. Model 1: unadjusted. Model 2: adjusted for MAP. Model 3: adjusted for MAP and all other risk factors. [a]BP-, lipid- and glucose-lowering treatment

Table 6.12 Relation between known cardiovascular risk factors and DC_{fem} Z-scores in the reference sub-populations in *women*

		Sub-population without CVD						Sub-population with CVD (n =316)		
		without treatment[a] (n =1,803)			with treatment[a] (n =391)					
Risk factor	Model	ß	95%CI	P-value	ß	95%CI	P-value	ß	95%CI	P-value
Mean arterial pressure (10 mmHg)	1	-0.100	-0.137; -0.062	<0.001	-0.050	-0.116; 0.015	0.132	0.069	-0.048; 0.185	0.246
	2	-	-	-	-	-	-	-	-	-
	3	-0.043	-0.081; -0.005	0.026	-0.034	-0.101; 0.034	0.327	0.076	-0.044; 0.197	0.212
Current smoking (yes)	1	-0.009	-0.116; 0.099	0.873	-0.027	-0.277; 0.222	0.830	-0.757	-1.384; -0.130	0.018
	2	-0.043	-0.150; 0.065	0.435	-0.059	-0.311; 0.193	0.647	-0.717	-1.352; -0.083	0.027
	3	-0.013	-0.118; 0.092	0.804	-0.079	-0.329; 0.171	0.534	-0.646	-1.295; 0.004	0.051
Diabetes (yes)	1	-0.264	-0.597; 0.07	0.122	0.128	-0.097; 0.353	0.265	-0.167	-0.481; 0.148	0.298
	2	-0.188	-0.521; 0.145	0.268	0.153	-0.074; 0.380	0.185	-0.206	-0.525; 0.113	0.205
	3	0.022	-0.301; 0.344	0.895	0.276	0.001; 0.551	0.049	-0.081	-0.429; 0.267	0.647
Total-to-HDL cholesterol ratio (unit)	1	-0.192	-0.23; -0.155	<0.001	-0.054	-0.126; 0.017	0.135	-0.103	-0.214; 0.009	0.071
	2	-0.187	-0.224; -0.149	<0.001	-0.054	-0.125; 0.017	0.137	-0.107	-0.219; 0.004	0.060
	3	-0.135	-0.174; -0.096	<0.001	-0.027	-0.101; 0.047	0.475	-0.074	-0.191; 0.043	0.215
Body mass index (kg/m2)	1	-0.063	-0.073; -0.052	<0.001	-0.027	-0.047; -0.007	0.008	-0.032	-0.071; 0.007	0.108
	2	-0.059	-0.071; -0.048	<0.001	-0.025	-0.045; -0.005	0.015	-0.037	-0.077; 0.002	0.063
	3	-0.046	-0.058; -0.034	<0.001	-0.020	-0.041; 0.001	0.062	-0.029	-0.071; 0.014	0.186
Use of BP-lowering medication (yes)	1	-	-	-	-	-	-	-0.199	-0.514; 0.116	0.215
	2	-	-	-	-	-	-	-0.221	-0.537; 0.096	0.171
	3	-	-	-	-	-	-	-0.018	-0.368; 0.332	0.920
Use of lipid-lowering medication (yes)	1	-	-	-	-	-	-	-0.298	-0.669; 0.072	0.114
	2	-	-	-	-	-	-	-0.293	-0.664; 0.077	0.120
	3	-	-	-	-	-	-	-0.282	-0.675; 0.110	0.158
Use of glucose-lowering medication (yes)	1	-	-	-	-	-	-	-0.261	-0.804; 0.283	0.345
	2	-	-	-	-	-	-	-0.301	-0.847; 0.245	0.279
	3	-	-	-	-	-	-	-0.012	-0.611; 0.588	0.970

The regression coefficient ß represents the change in DC_{fem} (in SD from the healthy population mean among individuals of the same age and sex) per unit increase in each risk factor. Model 1: unadjusted. Model 2: adjusted for MAP. Model 3: adjusted for MAP and all other risk factors. [a]BP-, lipid- and glucose-lowering treatment

6.5 Discussion

In the present study, we estimated age- and sex-specific percentiles of femoral artery stiffness, based on individual-level data obtained through echotracking from 1,489 healthy individuals not exhibiting any of the classical CV-RFs. As such, we have provided a framework for "healthy femoral artery ageing", indicating what normal changes in stiffness may be expected when a person ages. In addition, we also examined the association of CV-RFs with deviations from these normal curves, helping interpretation of femoral artery stiffness values obtained both in research and clinical settings.

Epidemiological studies on femoral artery stiffness are limited in number and often restricted to specific subpopulations. However, those few studies reporting *normal* values do show consistent results. In the FLEMENGHO[182] cohort, femoral artery functional properties (i.e. stiffness and buffering capacity) changed very little with age, despite a wide age range. This was also seen in the middle-aged healthy subjects from the ASKLEPIOS study[183], and is further supported by a lack of change in brachial artery stiffness over time, as described earlier.[60] In (sub-)populations at increased cardiovascular risk, such as the (pre-) diabetic subjects of the Hoorn study or obese participants of AGHALS[184,111] and FLEMENGHO[185], femoral artery stiffness was increased compared to normal subjects.

Largely drawing on the same datasets, this study confirms the findings of the previously described epidemiological studies, i.e., in healthy individuals, femoral artery stiffness remains constant over many years, only to increase significantly between the 6th and 8th decade, in both men and women.

CV-RFs related with metabolic syndrome (BMI, MAP and dyslipidaemia) show associations with greater femoral artery stiffness. However, we cannot exclude the possibility that compression by perivascular fat tissue (or excep-

tionally, by the operator) is the main driver behind the strong association between femoral stiffness and BMI. Smoking showed a paradoxically negative relationship with femoral stiffness in men. However, it is possible that the chronic effects of *current* smoking were outweighed by the acute effects of *withdrawal* from smoking, which has an immediate impact on the sympathetic nervous system activity.[174] Indeed, all protocols institute abstinence from smoking at the time of measurement, ranging in time from at least 3h[182] till 6h[183,186] in advance. In addition, the cross-sectional assessment of smoking (i.e. a yes/no question, not taking into account the quitters) may have further confounded these results.

To illustrate the absolute impact of the negative relationships between the other CV-RFs and DC_{fem}, the change in CV-RF level associated with a theoretical drop in DC_{fem} of 2.36 10^{-3}.kPa^{-1} (which predicted incident cardiovascular events in the Hoorn Study[87]) was calculated. For BMI, this equates to a rise of about 7 points (+6.9 kg/m² in men, +6.7 kg/m² in women). For MAP, a rise of 27 mmHg (in men) or 42 mmHg (in women) would be needed, and for total-HDL cholesterol ratio this means a rise of 4.3 (in men) or 2.2 (in women). In terms of percentiles of DC_{fem}, such a decrease (-2.36 10^{-3}.kPa^{-1}) corresponds with going from P50 to P25 (in men, age 50) or from P50 to P33 (in women, age 50). However, we must be cautious when interpreting such effects, since we are dealing with different populations here. Longitudinal data will be necessary to show what percentile levels are unfavourable and eligible to serve as thresholds for intervention.

6.5.1 Comparison between carotid and femoral artery stiffness

Percentile curves reveal a different relationship with ageing between femoral and carotid artery stiffness. In contrast to the long plateau phase and

steep rise at old age for femoral artery stiffness, carotid artery stiffness increases rapidly in early adulthood, followed by a slower rise later in life (*Chapter 5*). Associations with CV-RFs were more similar. However, MAP was a stronger determinant for carotid artery stiffness, while BMI was the most important factor for femoral artery stiffness. Smoking showed a similar favorable effect at both locations, although the mechanism may be different (i.e. for the carotid artery: through increased distension, for the femoral artery: through a reduced diameter). Diabetes was linked with carotid artery stiffness in men and women, while only in men it showed a relationship with femoral artery stiffness.

Comparing determinants of carotid and femoral artery diameter, a clear (positive) influence of MAP was seen on the carotid, but not on femoral artery diameter. Both carotid and femoral artery diameters strongly correlated with BMI, while the relationship with smoking was opposite (positive for carotid artery, negative for femoral artery diameter)

Although we cannot exclude the possibility that these discrepancies are caused by differences in study population between *Chapter 5* (carotid artery) and *Chapter 6* (femoral artery), results of carotid artery stiffness did not materially change when restricted to subjects also available in *Chapter 6*.

6.5.2 Limitations

This study has several limitations, the most important of which is its cross-sectional design and noise introduced by calibration between centres. In addition, femoral artery reference values show huge scatter, with models in men and women only explaining a marginal proportion of the variation. A possible contributor to this large variability on the population level is the seemingly random variability in the same individual over time. Indeed, Hof-

stra *et al*,[187] performing serial measurements on the same individual, observed large variation in femoral artery distension throughout the measurement period, which could not be explained by changes in diastolic or pulse pressure. Femoral artery stiffness also shows no linear dependency on diastolic pressure[188] (in contrast to carotid artery stiffness), suggesting a more autonomous, spontaneous behaviour. A possible explanation is that elastic properties of a muscular artery are under the influence of vasoactive substances (e.g. angiotensin, noradrenaline, atrial natriuretic factor), and the central nervous system, producing a permanently changing vascular tone. In addition, both intra-[188] and inter-observer[111] variability are always larger for the femoral compared to the carotid artery,[189] although this may also reflect the difficulty to obtain high quality images of the anatomically more curved femoral artery. To illustrate, of all data sent to us 521 subjects could not be included in the total database because of missing femoral artery diameter/distension data. Since this sample of excluded subjects was significantly more obese (BMI 28.0 kg/m² vs 25.7 kg/m²), had a higher MAP (103.9 mmHg vs 96.7 mmHg), had a worse lipid profile (total-to-HDL ratio of 4.4 vs 3.9), contained relatively more diabetic individuals (17.3% vs 10.2%) and slightly more smokers (20.7% vs 19.7%), it is likely that the impact of these CV-RFs on DC_{fem} is underestimated in the present study.

6.5.3 Conclusion

Reference values for femoral artery stiffness have been established. In young and middle-aged men and women, *normal* femoral artery stiffness does not change substantially with increasing age up to the 6th decade. Our data confirm that CV-RFs related to early metabolic disease are associated with increased femoral artery stiffness.

Chapter 7 Macro- and microcirculation in normal-tension glaucoma

Adapted from:

Bossuyt J, Vandekerckhove G, Van de Velde S, De Backer TLM, Azermai M, Stevens A, Kestelyn P, Raemdonck T, Segers P, Vanmolkot F, Van Bortel LM.

Vascular dysregulation in normal-tension glaucoma is not reflected by alterations in the micro- or macrocirculation at rest. A case-control study. (Submitted to the Journal of Glaucoma)

7.1 Abstract

Aims: In normal-tension glaucoma (NTG), optic nerve damage occurs despite a normal intraocular pressure. Studies implicating arterial stiffness in the pathophysiology of NTG have produced conflicting results. Our aim was to investigate whether NTG is associated with alterations in arterial structure or function.

Methods: Cardiovascular measurements included peripheral (Omron M6) and central (Sphygmocor) blood pressures, wave reflection, arterial stiffness measures [Pulse wave velocity (PWV), Sphygmocor and Esaote AU5 Wall track system], Intima-media thickness (IMT), cardiac output (Esaote AU5) and total peripheral resistance index (TPRI). Symptoms of vascular dysregulation were assessed using a questionnaire.

Results: 30 patients with NTG (mean age 65y, range 46-79) and 33 healthy subjects (mean age 67y, range 42-79) matched for age and sex were recruited. There were no statistically significant differences in arterial structure and function, for any of the measured arterial segments; for NTG versus controls, respectively: blood pressure 126±15 / 77±8 mmHg vs. 127±16 / 76±7 mmHg, p=0.81; aortic PWV 9.8±2.1 m/s vs. 10.1±1.9 m/s, p=0.60; TPRI 1833±609 vs. 1779±602 dyne.s/cm5/m², p=0.79; carotid IMT 0.65±0.14 mm vs. 0.68±0.13 mm; p=0.39. Questionnaire reports revealed an increased prevalence of cold extremities in the NTG group (73% vs. 21%, p<0.001) suggesting vascular dysregulation is present in most NTG patients

Conclusion: NTG is not associated with altered arterial stiffness, IMT, TPRI, cardiac output, peripheral or central hemodynamics. Although the majority of NTG patients do exhibit symptoms of vascular dysregulation, in the present study this did not translate into alterations in the micro- or macrocirculation at rest.

7.2 Introduction

As described in *Chapter 1*, Normal-tension glaucoma (NTG) is associated with vascular dysregulation, although the exact role of this alteration in the pathophysiology of NTG remains to be identified.[124] Historically, NTG has been linked with low arterial blood pressure, either diurnally[190,191], or at night only[192,193]. Many studies, however, did not find an association between NTG and low blood pressure[194–203] or did not show overdipping[97, 105–107]. Focusing on more integrative measures of vascular health did not solve these discrepancies. Augmentation index (a measure of wave reflections) in NTG patients was found increased by Mroczkowska et al,[200] but unaltered by Graham et al[202]. Pulse wave velocity (a measure of arterial stiffness) in NTG was found increased in one study[207] while not different from controls in other studies.[197,208]

Still, not all hemodynamic variables have been investigated in NTG, such as muscular artery properties and total peripheral resistance. However, compliance of a muscular (the brachial) artery was found decreased in migraine patients,[186] whose condition might share a common etiology with NTG.[209] Similarly, total peripheral resistance may be an interesting parameter to examine in NTG, as it can be altered in case of systemic microvascular abnormalities.[210]

Since many of the proposed systemic factors are treatable, it is of clinical importance that they are identified and described. Therefore, the aim of this study was to gain more insight into the function of the systemic micro- and macrocirculation in NTG, by comparing NTG patients with healthy age- and sex-matched controls. To this aim, non-invasive measurements of arterial structure and function were performed: diameter, intima media thickness and stiffness of elastic (carotid) and more muscular (femoral) arteries; aortic stiffness (carotid-to-femoral pulse wave velocity); total peripheral resistance and peripheral and central hemodynamics.

7.3 Materials and Methods

A description of the methods (hemodynamic measurements) and study population characteristics is provided in chapter 2. Since the ophthalmic investigations were only included in the study to confirm or exclude the diagnosis of NTG, they were not discussed in the methods section of this thesis (Chapter 2).

7.3.1 Study design

Control and NTG subjects underwent the following ophthalmic examinations: (1) Visual acuity assessment; (2) Slit-lamp examination; (3) Goldmann applanation tonometry; (4) Fundoscopy; (5) Haag-Streit Octopus perimeter (30.2); (6) Spectral Domain Optical Coherence Tomography: nerve fiber layer thickness and (7) Central corneal thickness measurement. Following ophthalmic examination, all participants underwent a screening visit and a study visit. At screening, a fasted blood sample was drawn [to determine total cholesterol, low-density lipoproteins (LDL), high density lipoproteins (HDL), creatinine, glucose and triglycerides], brachial blood pressure was measured and a questionnaire was completed (medical history, life-style habits, medication use, signs of vascular dysregulation, Table 7.2). The study visit included all hemodynamic measurements. Subjects who were on vasoactive drugs were asked to stop treatment 3 days before study visit.

Table 7.1 Baseline characteristics of the study population.

Variable	NTG (n=30)	Control (n=33)	p-value
Age, y	65 ± 8	67 ± 8	0.46
Male, n (%)	7 (23)	8 (24)	0.93
BMI, kg/m^2	25.8 ± 3.5	26.3 ± 3.6	0.59
Biochemical parameters			
Total cholesterol, mg/dl	201 ± 34	215 ± 30	0.08
HDL-cholesterol, mg/dl	69 ± 16	75 ± 21	0.18
LDL-cholesterol, mg/dl	111 ± 28	120 ± 32	0.25
Triglycerides, mg/dl	94 ± 31	98 ± 40	0.72
Creatinin, mg/dl	0.82 ± 0.13	0.85 ± 0.19	0.61
Glucose, mg/dl	94 ± 12	92 ± 10	0.51
Lifestyle variables			
Active smoking, n (%)	0 (0)	1 (3)	0.84
Regular alcohol use, n (%)	8 (27)	9 (27)	0.23
Medication use			
Lipid-lowering drugs, n (%)	8 (27)	7 (21)	0.62
Antihypertensive drugs, n (%)	12 (40)	14 (42)	0.85

Data are mean±SD or frequency (percentage).

7.4 Results

Of all screened NTG subjects (n=32), two participants were excluded because of type II diabetes mellitus and history of CVD, respectively. Baseline characteristics of subjects are summarized in Table 7.1. There were no significant differences between NTG and control subjects for age, sex, BMI, lifestyle habits or any of the biochemical variables. Survey data (Table 7.2) revealed that significantly more NTG patients suffered from cold hand and/or feet (73% *vs*. 27%, p<0.001). There were also trends towards an increased prevalence of migraine (p=0.17), fibromyalgia (p=0.14), and sleep apnea (p=0.26) in the NTG group. None of the cardiovascular parameters were different between NTG and control subjects (Table 7.3). Femoral IMT was borderline significant (p=0.05), and lower in the NTG subjects. However, when this parameter (IMT) was corrected for differences in arterial diameter, this near statistical significance disappeared (CSWA, p=0.21).

Table 7.2 Results of the study questionnaire.

Variable	NTG (n=30)	Control (n=33)	p-value
Co-morbidities			
History of hypertension (n, %)	8 (27)	10 (30)	0.75
Respiratory disease (n, %)	3 (10)	2 (6)	0.57
Hypothyroidism (n, %)	2 (7)	2 (6)	0.92
Hyperthyroidism (n, %)	1 (3)	1 (3)	1.00
Rheumatoid arthritis (n, %)	1 (3)	3 (9)	0.36
Sleep apnea (n, %)	3 (10)	1 (3)	0.26
Fibromyalgia (n, %)	2 (7)	0 (0)	0.14
Allergy (n, %)	9 (30)	6 (18)	0.28
Symptoms of vascular dysregulation			
History of hypotension (n, %)	4 (13)	2 (6)	0.33
History of migraine (n, %)	10 (33)	6 (18)	0.17
Cold extremities (n, %)	22 (73)	9 (27)	<0.001
Reduced thirst sensation (n, %)	5 (17)	5 (15)	0.87

Table 7.3 Hemodynamic measurements

Variable	NTG (n=30)	Control (n=33)	p-value
Hemodynamics			
Peripheral			
SBP, mm Hg	126 ± 15	127 ± 16	0.81
DBP, mm Hg	77 ± 8	76 ± 7	0.60
PP, mm Hg	49 ± 9	51 ± 11	0.49
MAP, mm Hg	96 ± 10	96 ± 10	0.98
Central			
cSBP, mm Hg	122 ± 16	125 ± 17	0.49
PP amplification	1.11 ± 0.17	1.06 ± 0.14	0.19
RM, %	69 ± 6	71 ± 7	0.41
AIx, %	128 ± 20	130 ± 18	0.55
Cardiac			
HR, beats/min	63 ± 8	65 ± 8	0.32
SI, ml/m²	41 ± 9	39 ± 10	0.62
CI, l/min/m²	2.4 ± 0.6	2.4 ± 0.6	1.00
TPRI, dyne.s/cm^5/m²	1833 ± 609	1779 ± 602	0.79
Vascular properties			
Femoral artery			
Diameter, mm	8.61 ± 1.45	8.44 ± 1.00	0.59
IMT, mm	0.71 ± 0.18	0.83 ± 0.26	0.05
CSWA, mm²	17.9 ± 5.4	19.8 ± 6.3	0.21
CC, mm²/kPa	0.99 ± 0.49	0.97 ± 0.68	0.90
DC, 10^{-3}/kPa	17.6 ± 9.3	18.3 ± 14.1	0.84
Carotid artery			
Diameter, mm	6.92 ± 0.64	7.16 ± 0.85	0.21
IMT, mm	0.65 ± 0.14	0.68 ± 0.13	0.39
CSWA, mm²	12.8 ± 3.3	13.9 ± 3.7	0.26
CC, mm²/kPa	0.78 ± 0.26	0.80 ± 0.34	0.78
DC, 10^{-3}/kPa	21.3 ± 8.7	20.5 ± 9.3	0.74
Aorta			
PWV, m/s	9.8 ± 2.1	10.1 ± 1.9	0.60

Data are mean±SD; IMT = intima-media thickness; WCSA = Wall Cross-Sectional Area; CC = cross-sectional compliance coefficient; DC = distensibility coefficient; SBP = systolic blood pressure; DBP = diastolic blood pressure; MAP = mean arterial pressure; PP = pulse pressure; PWV = pulse wave velocity; AIx = augmentation index; RM = reflection magnitude; HR = heart rate; CI = cardiac index; SVI = stroke volume index; TPRI = total peripheral resistance index.

7.5 Discussion

A comprehensive assessment of the macro- and microcirculation at rest did not reveal any difference between NTG patients and age- and sex-matched healthy controls. This finding confirms those of others who observed no difference in blood pressure and/or waveform parameters, and pulse wave velocity.[202, 208] In addition, we showed that muscular artery stiffness, reflection magnitude and total peripheral resistance, which to our knowledge constitute a blind spot in NTG research, were also not different from the controls. However, questionnaire reports do suggest vascular dysregulation is present in the majority of NTG patients, and is not restricted to the eye. To summarize, despite arguments for a systemic involvement, no systemic differences in cardiovascular structure and function were found at rest.

There are several possible explanations for this paradox:

(1) Vascular dysregulation represents a defective response to a certain *stressor*, while all cardiovascular parameters were measured at *rest*. As symptoms of vascular dysregulation occur only episodically (e.g. at night, after cold exposure, etc.), provocative tests may be needed to unmask alterations in cardiovascular function. Indeed, Su et al. observed no differences in brachial artery blood flow at baseline, but an impaired response following ischemia in NTG patients.[211] Similarly, Nicolela et al. found no difference in plasma endothelin-1 levels at baseline, but a significantly higher endothelin-1 concentration in glaucoma patients after cold exposure.[212]

(2) Although it is evident to consider improper cardiovascular function as a direct cause of inadequate ocular blood flow, the pathophysiology of NTG may involve defects in other organ systems as well. Indeed, glaucoma is a multi-factorial disease, having an immunological, endocrine and neurological component, which may make it difficult to isolate a single (cardiovascular) profile.[120,213–216]

(3) This is a cross-sectional study. Therefore, we cannot exclude the possibility that cardiovascular alterations were present long before diagnosis, but were in the

meantime influenced by other factors, such as lifestyle changes, medication, course of disease, etc. To illustrate, glaucoma patients often recall having low blood pressure in youth,[217] but this effect may disappear with ageing.

Table 7.4. Associations with NTG tested in literature and/or in this study

	Literature		This study
	Association with NTG	**No association with NTG**	
Primary vascular dysregulation			
Female sex	218–221		+ +
Cold extremities	212,222–224		+ +
History of Migraine	225,226	227	+
Reduced thirst sensation	NA	NA	-
Alterations in the macrocirculation			
Carotid intima-media thickening	200		-
Increased augmentation index	200	202	-
Increased reflection magnitude	NA	NA	-
Elastic artery stiffening		197	-
Muscular artery stiffening	NA	NA	-
Increased central pressure		202	-
Alterations in the microcirculation			
Total peripheral resistance	NA	NA	-

References are shown for associations described in literature between NTG and symptoms of PVD, and alterations in macro-or microcirculation ($p<0.05$). 'NA' indicates not described in literature. Associations with NTG in the present study are indicated with symbols: + + significant association with NTG ($p<0.05$); + trend ($p>0.05$); - no association.

Table 7.4 provides an overview of literature data and associations with NTG tested in the present study. From this Table, it is clear that the vast majority of studies associated NTG with signs of vascular dysregulation, but not consistently with vascular alterations at rest, while no literature data exists on muscular artery stiffness, total peripheral resistance and reflection magnitude.

7.5.1 Strengths and limitations

The strength of this study is that the influence of confounders is limited by matching subjects for age and gender, which was successful and resulted in similar levels of biochemical (e.g. cholesterol, fasting glucose, etc.) and physical (e.g. height, weight, etc.) variables between the case and control group. However, this study also has some limitations. First, as already mentioned, this study suffers from its cross-sectional design. Second, due to low prevalence of NTG, the sample size was small. However, to detect a difference in cross-sectional compliance of 20%, as was found in migraine patients,[186] this sample size was deemed adequate (power 80%, α=0.05).

7.5.2 Conclusion

To conclude, our data show no alterations of the micro- or macrocirculation in NTG at rest, despite a history of clinical symptoms of systemic vascular dysregulation. In particular, vascular dysregulation did not lead to statistically significant alterations in vascular tone as evidenced by no differences in function of the muscular femoral artery, total peripheral resistance, mean arterial pressure and measures of wave reflection. Provocative tests may be needed to reveal alterations in cardiovascular function in NTG patients.

Chapter 8 Conclusion

8.1 Main findings

The main findings of this thesis will be approached from the perspective of the five study objectives (Table 1.5), which will be answered or commented on.

Study objective n°1

= to investigate the impact of body *side* and *size* on carotid-femoral PWV.

The main finding was that the human body is not symmetrical at the level of the arteries. In particular, a significant difference in length exists between the left *vs.* right aortic-femoral path (the latter being longer). This difference is in part, but not fully compensated by the also slightly longer right *vs.* left aortic-carotid path. As a result, the total travelled path between carotid and femoral is slightly longer on the right side of the body. The total difference, however, still falls within the margins of error of cf-PWV assessment and might be less important for a single measurement, but can add to other inaccuracies. In addition, the distance between carotid and femoral artery as measured with a tape may be significantly affected by body contours.

Since all sources of noise should be avoided as much as possible, it is important 1) to measure on one side of the body, i.e. not 'crossing over' from left carotid to right femoral, or right carotid to left femoral 2) not switching between left/right side on serial measurement periods on the same subject and 3) to use additional tools, such as an anthropometer, if measurement in a straight line using a tape is not possible.

Overall, these findings do not hamper the clinical applicability of cf-PWV, but rather stress the importance of standardizing operating procedures.

Study objective n°2

= to investigate the left-right distribution of atherosclerosis.

The main finding was that the geometrical asymmetry that was suggested earlier (in the MRI study) was reflected by an asymmetry in atherosclerosis distribution. In a large population sample, this was seen at the femoral artery, where atherosclerosis was more prevalent on the right side (expressed by higher IMT values and increased plaque presence), consistent with the anatomical asymmetry. At the carotid artery, the distribution was equal, reflecting the more symmetrical anatomy (at the measurement location).

With regard to the clinical implications of this finding, it needs to be shown whether *clinical* atherosclerosis (i.e. symptomatic lesions, such as stenosis or peripheral artery disease) is also more prevalent on the right femoral artery. In addition, although femoral IMT and plaques are predictive for atherosclerosis elsewhere, a relationship with clinical events (outcome data) is currently lacking. Outcome studies are also needed to indicate whether the predictive value of atherosclerosis of the femoral artery will differ between right and left side.

Furthermore, it needs to be stressed that although *on average* there was no difference in atherosclerosis prevalence between left and right carotid artery, this is certainly not the case on an *individual* level. Indeed, a single person may show a substantial difference between left and right IMT values, or may have (a) plaque(s) unilaterally. What our results show is that on a *population* level*,* lesions are eventually equally distributed between left and right carotid artery, while on a *population* level, the right femoral artery may on average be more frequently affected than the left.

Overall, findings in this and other studies underscore the local character of atherosclerosis and might suggest to measure IMT and plaque always bilaterally, since a significant number of cases would be missed when considering one side of the body only.

Study objective n°3

= to establish normal values for carotid artery stiffness.

The main finding was that, in a healthy population, carotid artery stiffness increases already in early life (adolescence), plateauing near old age. The relationship with age is somewhat steeper in women than men, suggesting a more rapid decline of carotid artery elasticity in women. Including also subjects with one or more CV-RFs in the analysis showed that increased carotid artery stiffening is associated with (in decreasing order of importance) high levels of MAP, the presence of diabetes, unfavorable lipid profile and increased BMI.

Study objective n°4

= to establish normal values for femoral artery stiffness.

The main finding was that, in a healthy population, femoral artery stiffness changes little with age, only to increase significantly at about the age of 60. This rise in stiffness is steeper and less gradual in women than men, suggesting a more sudden onset of femoral artery stiffening in women. Including also subjects with one or more CV-RFs in the analysis showed that increased femoral artery stiffening is associated with (in decreasing order of importance) high levels of BMI, MAP and unfavorable lipid profile. Presence of diabetes also showed a significant association, but in men only.

Comparing femoral with carotid stiffness showed that the relationship with age and other risk factors is different. In contrast to the rise in carotid artery stiffness early in life, femoral artery stiffening occurs only near old age. Also the determinants (or at least their hierarchy) are different. While BMI is the most important continuous factor influencing femoral artery stiffness, MAP has a relatively stronger impact on carotid artery stiffness.

Overall, it needs to be shown with longitudinal studies what the CV risk is of being in a certain percentile of carotid or femoral artery stiffness, and of the transition from one percentile into another. In addition, the predictive value of femoral artery

stiffness is based on one single study,[87] and should be investigated more thoroughly.

Study objective n°5

= to examine cardiovascular structure and function in normal-tension glaucoma.

The main finding was that no direct alterations in macro- or microcirculation could be demonstrated in NTG patients, when measured at rest, although they do suffer more frequently from signs and symptoms of vascular dysregulation.

Overall, this finding suggests that measurements done at rest may not be sufficient to reveal vascular dysregulation in NTG, and functional tests may be necessary.

8.2 Future perspectives

Cf-PWV is the gold standard measure of arterial stiffness, mainly reflecting aortic stiffness. Reference values already exist,[106] and operator guidelines have been tested and validated. However, although cf-PWV may theoretically be 'ready' for implementation in clinical practice, there are still some critical obstacles that need to be removed. When applying the 'criteria for evaluation of novel markers of cardiovascular risk' (Table 8.1), published in *Circulation*,[228] Laurent *et al.* have demonstrated that cf-PWV still fails on two out of the six criteria:

1) The verdict is still out on whether a cf-PWV-guided therapy will improve outcome in a randomized controlled trial. The ultimate test to demonstrate this (i.e. participants receiving either a cf-PWV-based treatment or a conventional blood pressure-based treatment, both groups followed prospectively in time), is now being launched in France, under the name of the SPARTE (the Statégie de Prévention Cardiovasculaire Basée sur la Rigidité Arterielle) study.[229]

2) The cost-effectiveness of implementing cf-PWV measurements in routine clinical practice needs to be evaluated.

Table 8.1

Phases of Evaluation of a Novel Risk Marker, adapted from Hlatky *et al.* [228]
1) **Proof of concept:** Do novel marker levels differ between subjects with and without outcome?
2) **Prospective validation**: Does the novel marker predict development of future outcomes in a prospective cohort or nested case-cohort/case-cohort study?
3) **Incremental value**: Does the novel marker add predictive information to established, standard risk markers?
4) **Clinical utility:** Does the novel risk marker change predicted risk sufficiently to change recommended therapy?
5) **Clinical outcomes:** Does use of the novel risk marker improve clinical outcomes, especially when tested in a randomized clinical trial?
6) **Cost-effectiveness:** Does use of the marker improve clinical outcomes sufficiently to justify the additional costs of testing and treatment.

However, when the same criteria are applied to other measures of vascular TOD, it is clear cf-PWV is still in pole-position (Table 8.2). Cf-PWV is the only measure that has been shown to significantly alter risk classification,[81] which is a critical checkpoint. This is not the case for carotid IMT. Although carotid IMT has added predictive value, its net reclassification improvement (NRI) is not significant in the general population,[32] casting doubt on its clinical utility for primary prevention. With regard to the other measures discussed in this thesis (i.e. femoral IMT, femoral DC and carotid DC), the potential to change predicted risk has not yet been sufficiently evaluated.

The clinical value of measuring parameters of vascular TOD will need to be further evaluated in future studies. One remaining challenge is whether it will be possible for general practitioners to adopt the more time-consuming and cumbersome

Table 8.2 Criteria for evaluation of novel markers of cardiovascular risk applied to measures of vascular TOD.

Criterion	Arterial stiffness			Arterial wall thickness	
	Cf-PWV	Carotid DC	Femoral DC	Carotid IMT	Femoral IMT
Proof of concept	✓[85]	✓[86]	✓[98]	✓[27]	✓[43]
Prospective validation	✓[95]	✓[87]	✓[87]	✓[230]	?
Incremental value	✓[231]	✓[87]	✓[87]	✓[232]	?
Clinical utility	✓[81]	?	?	✗[32]	?
Clinical outcomes	?	?	?	?	?
Cost-effectiveness	?	?	?	✓[233]	?

Symbols indicate positive (✓), negative (✗) or absent (?) evidence from literature.

techniques into their daily clinical practice. In contrast to the conventional blood pressure measurement, the types of vascular TOD discussed in this thesis require more time and effort, both from physician and patient. Attempts to facilitate measurements (e.g. cuff-based methods to quantify aortic stiffness) have come at the cost of providing more questionable results. Examples include devices such as the Arteriograph[234] or Mobil-o-graph,[235] which claim to measure aortic stiffness by capturing signals in the upper arm, but have been called into question.[236] On the other side of the spectrum, we find measurement of cf-PWV using the Sphygmocor device, which is far more time-consuming (requiring subjects to undress, a skilled operator to locate carotid and femoral arteries, setting up an ECG) but may also yield the most valid measure of a subjects' aortic stiffness (close relationship with invasive aortic PWV).[237] Finding a balance between the validity and ease of use is a challenge all parameters and devices must face, but often struggle with. However, reaching this balance will be crucial for becoming and staying routinely implemented into daily clinical practice. In the coming years, it will be interesting to see which parameters and/or devices will be able to make the leap from research to clinical practice.

Samenvatting

De rode draad in dit proefschrift is onderzoek naar arteriële structuur en functie. Concreet is het doel van deze thesis om cardiovasculaire (CV) risico stratificatie te verbeteren, door meetmethodes van vasculaire orgaanschade (arteriële stijfheid, verdikking van de slagaderwand) te helpen de overstap te maken naar de dagelijkse klinische praktijk. Dit doel wordt benaderd vanuit verschillende oogpunten, waaronder de methodologische en pathofysiologische apsecten van arteriële structuur en functie.

De tekst bestaat uit 8 hoofdstukken, waarvan het eerste een algemene inleiding geeft en de probleemstelling aankaart (*hoofdstuk 1*), en het laatste deze vragen tracht te beantwoorden en concludeert (*hoofdstuk 8*). Tussenin bevinden zich een beschrijving van de gebruikte methoden (*hoofdstuk 2*), en de resultaten van vijf specifieke onderzoeken, die overeenkomen met de vijf studie objectieven (*hoofdstukken 3-7*).

Hoofdstuk 1: Inleiding

In *Hoofdstuk 1* wordt de onderliggende drijfveer om onderzoek te doen in het cardiovasculaire veld blootgelegd: het feit dat cardiovasculaire ziektes tot op vandaag nog steeds de primaire doodsoorzaak zijn, zowel lokaal (in België) als wereldwijd. Cardiovasculaire ziektes worden gedefinieerd en de huidige methodes om het risico op cardiovasculaire sterfte te bepalen (d.i. risicostratificatie met behulp van risicofactoren) worden beschreven. Vervolgens maken we kennis met integrerende parameters, zoals 'Systematic COronary Risk Evaluation (SCORE)' en 'Framingham Risk Score (FRS)', die een totaalbeeld geven van iemands risico profiel. Van de beperkingen van deze scores wordt dan overgegaan op 'eind-orgaanschade', als een relatief nieuw hulpmiddel voor risicostratificatie. Er wordt dieper ingegaan op "verdikking van de slagaderwand" en "arteriële stijfheid" als types van vasculaire orgaanschade. Deze worden verder gedefinieerd en hun predictieve waarde wordt beoordeeld. De inleiding wordt besloten met de

probleemstelling en doelen: ondanks hun additieve predictieve waarde worden de besproken parameters van vasculaire schade nauwelijks gemeten in de dagdagelijkse klinische praktijk. Het primaire doel van dit onderzoek is daarom om hun klinische toepasbaarheid te verhogen door enkele hindernissen uit de weg te ruimen die momenteel de weg naar de kliniek nog versperren. Concreet betekent dit 1) voor regionale (carotidofemorale) arteriele stijfheid: het verfijnen van de huidige meetprocedures door na te gaan wat het effect is van lichaamszijde en lichaamsvormen; 2) voor verdikking van de slagaderwand: het testen van huidige meetprocedures m.b.t. verschillen tussen rechter en linker lichaamszijde; 3) en 4) voor lokale stijfheid: het opstellen van referentiewaarden, voor respectievelijk stijfheid van de halsslagader (carotis) en dijbeenslagader (femoralis); 5) een toepassing van het bovenstaande, door te onderzoeken of metingen van arteriële structuur en functie van nut kunnen zijn bij mensen met normale-druk glaucoom.

Hoofdstuk 2: Methodes

In *Hoofdstuk 2* worden alle gebruikte methodes en populaties opgelijst. Concreet worden volgende zaken in detail beschreven; de gestandaardizeerde meetomgeving, manieren om brachiale en lokale (femoralis en carotis) bloeddruk te meten, methodes voor de bepaling van lokale diameter en distensie, het meten van regionale (carotidofemorale) stijfheid, het kwantificeren van pulsgolfreflecties, het berekenen van de totale perifere weerstand aan de hand van cardiale output en de gemiddelde bloeddruk, het meten van preklinische atherosclerose (IMT en plaques), en het bepalen van de arteriële padlengtes met behulp van MRI. Verder wordt ook een korte beschrijving van de gebruikte populaties gegeven (MRI-vrijwilligers, deelnemers aan de Asklepios studie en normale-druk glaucoom patiënten).

Hoofdstuk 3-7: Resultaten

In *Hoofdstuk 3* worden de resultaten van de MRI studie beschreven, waarin de arteriële padlengte van rechtercarotis tot rechterfemoralis vergeleken wordt met hetzelfde traject aan de linkerkant van het lichaam. Eveneens werd berekend wat

de rechtstreekse afstand, “in vogelvlucht”, tussen deze punten is, om metingen met een antropometer te simuleren. Deze analyse leerde ons dat er wel degelijk een verschil is in arteriële padlengte tussen beide lichaamszijden, maar dat dit verschil over het algemeen binnen de foutenmarge van de methode zelf (het meten van carotidofemorale pulsgolfsnelheid, cf-PWV) valt. Het belang van het meten van de afstand in rechte lijn, eventueel met behulp van een antropometer, werd wel aangetoond. Na toepassing van de 80%-regel blijkt deze afstand het dichtst aan te leunen bij de ‘echte’ arteriële padlengte.

In *Hoofdstuk 4* wordt een stap verder gegaan in het bekijken van bilaterale verschillen in gepaarde bloedvaten. De hypothese wordt getest dat een bilateraal asymmetrische geometrie op populatieniveau tot een scheve verdeling van atherosclerose zou leiden. Concreet verwachten we vooral links-rechts verschillen in atherosclerose op het niveau van de femoralis, aangezien de asymmetrie hier meer uitgesproken is, en dit bloedvat een meer gebogen traject volgt. Ter hoogte van de carotis zou de minder uitgesproken asymmetrie, gebufferd door het meer rechte verloop van deze bloedvaten ter hoogte van de meetlocatie, tot een meer evenredige links-rechts distributie van atherosclerose moeten leiden. Deze hypothese blijkt te kloppen op basis van resultaten van de Asklepios-studie, waarin de distributie van preklinische atherosclerose perfect symmetrisch is ter hoogte van de carotis (linker- en rechterkant evenveel plaques en IMT), maar significant verschillend is ter hoogte van de femoralis (meer plaques en hogere IMT waarden t.h.v. de rechter femoralis). Wat de klinische gevolgen van deze resultaten betreft, is voorzichtigheid geboden. Aangezien enkel gekeken wordt naar *preklinische* atherosclerose in gezonde (symptoomvrije) mensen, zal in de toekomst moeten onderzocht worden of hetzelfde patroon ook gevonden wordt voor *klinische* atherosclerose. Verder zal ook uit longitudinale (outcome) studies moeten blijken of er een verschil is in predictieve waarde tussen atherosclerose op de rechter *vs.* linker femoralis. Wat echter wel uit deze resultaten kan besloten worden is dat (1) deze de huidige richtlijnen bevestigen voor het meten van preklinische atherosclerose ter hoogte van de carotis (nl. data van linker-en

rechterlichaamszijde kan samengevoegd worden), en (2) het uitgesproken lokaal karakter van atherosclerose nog maar eens bevestigd wordt, geïllustreerd door de scheve verdeling ter hoogte van de femoralis. Deze laatste bevinding suggereert echter niet om enkel de rechterfemoralis te meten, maar onderstreept eerder het belang om steeds overal te meten, aangezien anders atherosclerose kan gemist worden. Het identificeren van de specifieke geometrieën die de rechter femoralis vermoedelijk meer vatbaar maken voor atherosclerose is een interessante piste voor verder mechanistisch onderzoek.

Hoofdstuk 5 en *Hoofdstuk 6* handelen over het opzetten van referentiewaarden voor vaatstijfheid van respectievelijk de arteria carotis (halsslagader) en arteria femoralis (dijbeenslagader). In deze projecten worden percentielen opgesteld van femoralis-en carotisdistensibiliteit (d.i. het omgekeerde van stijfheid), bekomen in gezonde proefpersonen, uit een verzameling van Europese studies. Deze curves tonen aan dat, in gezonde personen, de stijfheid van de femoralis constant blijft gedurende vele jaren, en slechts significant toeneemt rond de leeftijd van 60 jaar. In schril contrast hiermee neemt de stijfheid van de carotis al toe in jongvolwassenen, met een meer gematigde stijging bij ouderen. Uit een analyse van de invloed van cardiovasculaire risicofactoren blijkt BMI het meest bij te dragen tot verhoogde femoralisstijfheid, terwijl de gemiddelde arteriële druk de belangrijkste continue factor is voor carotisstijfheid. Of de invloed van BMI een louter mechanisch effect is (compressie door verhoogde (vet)massa), dan wel een intrinsieke verhoging van de arteriële stijfheid in obese individuen, kan op basis van deze resultaten niet worden besloten. Studies die focussen op de elasticiteit van het perivasculaire weefsel, en hun eventuele verband met zwaarlijvigheid, kunnen hier mogelijks een antwoord op bieden.

In *Hoofdstuk 7* wordt onderzocht of normale-druk glaucoom gepaard gaat met veranderingen in micro- en/of macrocirculatie. Concreet worden patiënten met normale-druk glaucoom en gematchte controles uitvoerig cardiovasculair geprofileerd, met onder andere metingen van lokale en regionale stijfheid,

pulsgolfreflecties, arteriële verdikking, hartfunctie en totale perifere weerstand. Resultaten van deze tests, aangevuld met persoonlijke klinische informatie uit een vragenlijst, leert ons dat hoewel er duidelijk aanwijzingen zijn voor vasculaire dysregulatie in normale-druk glaucoom, dit niet vertaald wordt in één of meerdere veranderingen in cardiovasculaire parameters in rust. Studies met dynamische functietesten lijken daarom aangewezen.

Hoofdstuk 8: Besluit

In *Hoofdstuk 8* worden de vraagstellingen van in *Hoofdstuk 1* opnieuw aangehaald en beantwoord. In het algemeen heeft dit onderzoek bijgedragen tot de klinische toepasbaarheid van het meten van vasculaire orgaanschade, d.m.v. het valideren van huidige standaardisatieprocedures en het opzetten van referentiewaarden. Dit is echter slechts een deel van de puzzel, die nog niet voltooid is. Daarom wordt ook een aanzet gegeven naar toekomstperspectieven. Wanneer alle besproken parameters objectief worden getoetst, blijkt dat er, afhankelijk van de parameter, toch nog één of meerdere essentiële stap(pen) moeten genomen worden om te voldoen aan de minimale voorwaarden voor klinische toepassing als biomarker. Deze analyse toont dat, van alle parameters, regionale carotidofemorale stijfheid (cf-PWV) het verst staat op vlak van klinische toepasbaarheid. Echter moet nog steeds zwart op wit (via een gerandomiseerd onderzoek met controlegroep) aangetoond worden dat een therapie gebaseerd op cf-PWV-reductie significant beter is voor de zorg van de patiënt, en dat invoering van deze procedure kosteneffectief is. Beide stappen zijn essentieel om de poort naar het dokterskabinet open te breken. Tenslotte wordt ook erkend dat het gebruiksgemak van bv. cf-PWV nog niet optimaal is, en ook dit een mogelijk struikelblok kan vormen naar klinische implementatie toe. Hoewel dit probleem niet werd aangekaart in dit proefschrift, vormt het toch een belangrijke factor. Het juiste evenwicht vinden tussen gebruiksgemak en validiteit vormt één van de uitdagingen voor de toekomst.

Summary

The central theme of this PhD project is research around arterial structure and function. In particular, the primary goal of this thesis is to improve CV risk stratification by enhancing the clinical applicability of methods to measure vascular target organ damage (i.e. arterial stiffness and wall thickening). This objective will be approached from a broad perspective, involving methodological and pathophysiological aspects of arterial structure and function.

The manuscript is organized into eight chapters, the first of which provides a general introduction and problem statement (*Chapter 1*), and the last answers and concludes these questions (*Chapter 8*). Descriptions of the methods (*Chapter 2*) and results from five studies, corresponding with five specific study objectives (*Chapters 3-7*) are included in between.

Chapter 1: Introduction

Chapter 1 stresses the importance of doing cardiovascular research, by showing that cardiovascular disease (CVD) is still the main cause of death, globally as well as on a local (Belgian) level. Different types of CVD are described and methods to perform risk stratification are given, starting with an overview of the classical risk factors for CVD and moving on to the more integrated parameters, such as the systematic COronary risk evaluation (SCORE) and the Framingham risk score (FRS). From the limitations of today's systems, the concept of target organ damage (TOD) is introduced as a relatively new tool and aid in risk stratification. Two specific types of vascular TOD are then described in more detail, i.e. 'arterial stiffness' and 'arterial wall thickening'. The introduction is concluded with the problem statement and aims of the thesis: despite their added predictive value, beyond classical risk factors, the measures of vascular TOD that were described in this thesis are only marginally implemented into daily clinical practice. Therefore, the primary aim of this thesis is to bring those parameters to the clinic by removing some of the obstacles hampering their clinical applicability. In particular, five specific study

objectives can be distinguished: 1) testing and fine-tuning of consensus guidelines to measure carotid-to-femoral stiffness by investigating the influence of body side and body contours; 2) checking for differences in atherosclerosis prevalence between left and right body side; 3) and 4) establishing reference values for local carotid and femoral stiffness respectively; and 5) an application of all of the above, investigating the utility of arterial structure and function measurements in patients with normal-tension glaucoma.

Chapter 2: Methods

Chapter 2 provides an overview of all the methods and populations used in this thesis. This includes a detailed description of the following procedures and/or environments: the standardized measurement conditions, methods to quantify brachial and local (carotid and femoral) blood pressure, ways to asses arterial diameter and distension, measurement of regional (carotid-to-femoral) stiffness, estimation of wave reflections, calculation of total peripheral resistance by determining cardiac output and mean arterial pressure, measurement of preclinical atherosclerosis (intima-media thickness and plaques) and calculation of arterial path lengths using magnetic resonance imaging (MRI). A separate paragraph describes the populations worked with in this thesis (volunteers eligible for MRI, Asklepios study participants and normal-tension glaucoma patients).

Chapters 3-7: Results

Chapter 3 reports the results of the 'MRI study', in which the intra-arterial distance between right carotid and right femoral artery is compared with the same trajectory on the left side of the body. In addition, the direct distance is calculated on both sides of the body, simulating the superficial distance measured with an anthropometer. An analysis of the results shows that although there is a small difference between left and right intra-arterial path length, this still remains within the margins of error of the method itself. More emphasized is the importance of obtaining a straight line (if necessary using an anthropometer), since this distance more

closely approximates the real travelled distance (after application of the 80-percent rule).

Chapter 4 delves deeper into bilateral differences between paired arteries. In this chapter, we examine the hypothesis that the anatomical bilateral asymmetry suggested in *Chapter 3* will translate into a different distribution of atherosclerosis prevalence. In particular, we anticipated to see potential left-right differences at the level of the femoral artery (given its pronounced asymmetry and curved trajectory), while the less asymmetrical and more buffered course of the carotid artery is expected to result in a more equal distribution of atherosclerosis. Data analyzed from the Asklepios study are in line with this hypothesis, showing an almost identical prevalence of (asymptomatic) atherosclerosis at right vs left carotid artery, in contrast to a different distribution between the femoral arteries. However, we must be cautious with drawing strong conclusions from these findings. It remains to be investigated whether the distribution of symptomatic, clinical atherosclerosis follows the same pattern, and what the clinical implications of a different distribution are in terms of outcome prediction. Nevertheless, these results do allow us to draw the following conclusions: 1) there is no substantial difference between left and right carotid IMT or plaque prevalence, so data from studies measuring on the left carotid can be pooled with data from the right side, 2) this is another example of the strong local character of atherosclerosis, as illustrated by the different distribution between left and right femoral artery. The latter does not immediately suggests measuring exclusively the right femoral artery and ignoring the left side, but rather stresses the importance of always measuring at all sites, in order not to miss any lesions. Furthermore, the identification of specific geometries rendering the right femoral artery more vulnerable to atherosclerosis than its left counterpart might be interesting for further mechanistic research.

Chapter 5 and *Chapter 6* describe results from the 'reference values projects', i.e. the assessment of age- and sex-specific normal values for carotid artery and femoral artery stiffness respectively. In these two studies, percentile curves of femoral

and carotid distensibility coefficients (i.e. the inverse of stiffness) are established, based on pooled data from various European cohorts. These curves show that, in apparently healthy subjects, stiffness of the femoral artery remains relatively constant during lifespan, only increasing significantly near the 6th decade. In contrast, the evolution of carotid artery stiffness is characterized by an early rise, starting already in adolescence, with a subsequently more gentle increase near old age. Looking at associations with CV risk factors in the total population, increased body mass index (BMI) showed the strongest correlation with femoral artery stiffening, while mean arterial pressure was the most important factor influencing carotid artery stiffness. Whether the association with BMI reflects intrinsic stiffening of the arterial wall, rather than a consequence of mechanical constraints in obese subjects (i.e. compression of the femoral artery by adipose tissue), cannot be concluded from our data. In the future, studies measuring strain of the perivascular tissue and its relationship with obesity may provide an answer to this question.

C*hapter 7* reports the results of the 'Normal-tension glaucoma (NTG) study', investigating whether NTG is associated with alterations in the micro- and/or macrocirculation. In particular, cardiovascular structure and function of NTG patients was compared to age- and sex-matched healthy controls, including measurements of local and regional stiffness, wave reflections, arterial wall thickening, cardiac function and total peripheral resistance. Results of these tests, complemented with information from a study questionnaire, show that although there are clear indications for systemic vascular dysregulation in NTG (based on the questionnaire), these were not translated into one or more alterations in cardiovascular parameters at rest.

Chapter 8: Conclusion

In *Chapter 8* the research questions that were proposed in *Chapter 1* are recalled and answered. In general, this thesis has contributed to the clinical applicability of measures of vascular organ damage, by validating current operator procedures and establishing reference values. However, this corresponds to only a small piece of

the puzzle, which is not complete. Therefore, future perspectives are discussed. By testing all discussed parameters to objective criteria, we recognize that (depending on the parameter) one or more crucial steps still need to be taken before fulfilling all criteria a biomarker must meet to become implemented into routine clinical practice. This analysis also shows that, of all parameters, regional carotid-to-femoral stiffness has made the most progress in recent years. However, it still needs to be demonstrated that therapy based on arterial stiffness reduction will eventually improve patient care, and whether this is cost-effective. Both of these steps are crucial for opening the gate to the doctor's office. Another possible stumbling block, i.e. the methodological "ease of use" (or lack thereof) is also mentioned. Although this issue was not addressed in this thesis, we do recognize it will be important to improve the ease of use of current techniques, without the expense of losing validity. Striking the balance between ease of use and valid results will be one of the challenges for the future.

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Appendix: Device-specific reference tables

Table 0.1 reference values for DC_{car} (in $10^{-3}.kPa^{-1}$) from table 5.6, calibrated to the **Wall Track System.**

		percentiles						
	Age (years)	2.5th	10th	25th	50th	75th	90th	97.5th
Men (n=1,724)	20	16.1	23.0	29.1	35.9	42.7	48.8	55.7
	30	10.3	15.9	20.9	26.4	31.9	37.0	42.6
	40	7.4	12.1	16.3	20.9	25.5	29.7	34.4
	50	6.1	10.1	13.7	17.6	21.6	25.1	29.1
	60	5.7	9.1	12.2	15.5	18.9	22.0	25.4
	70	5.6	8.6	11.2	14.1	17.0	19.6	22.6
Women (n=1,877)	20	17.7	26.2	33.8	42.1	50.5	58.0	66.5
	30	11.6	17.7	23.1	29.0	35.0	40.4	46.5
	40	7.7	12.5	16.9	21.6	26.4	30.7	35.5
	50	5.6	9.7	13.4	17.4	21.5	25.2	29.3
	60	4.9	8.5	11.8	15.3	18.9	22.1	25.8
	70	5.2	8.5	11.5	14.7	17.9	20.9	24.1

Table 0.2 reference values for DC_{fcar} (in $10^{-3}.kPa^{-1}$) from table 5.6, calibrated to the **Vivid7 system.**

		percentiles						
	Age (years)	2.5th	10th	25th	50th	75th	90th	97.5th
Men (n=1,724)	20	21.8	29.7	36.8	44.6	52.4	59.4	67.3
	30	16.0	22.4	28.2	34.5	40.8	46.6	53.0
	40	13.1	18.5	23.3	28.6	33.9	38.7	44.0
	50	11.9	16.5	20.6	25.0	29.5	33.6	38.1
	60	11.6	15.5	18.9	22.8	26.6	30.1	33.9
	70	11.7	15.0	18.0	21.2	24.5	27.5	30.8
Women (n=1,877)	20	24.9	34.6	43.3	52.8	62.3	71.0	80.6
	30	18.5	25.6	31.9	38.8	45.7	52.0	59.0
	40	14.5	20.2	25.3	31.0	36.6	41.7	47.4
	50	12.2	17.1	21.5	26.4	31.2	35.6	40.5
	60	10.9	15.2	19.2	23.5	27.8	31.7	36.1
	70	10.0	14.0	17.6	21.5	25.4	29.0	33.0

Table 0.3 reference values for DC_{car} (in $10^{-3}.kPa^{-1}$) from table 5.6, calibrated to the **Carotid studio system.**

		percentiles						
	Age (years)	2.5th	10th	25th	50th	75th	90th	97.5th
Men (n=1,724)	20	20.1	28.0	35.0	42.8	50.5	57.6	65.4
	30	14.0	20.4	26.1	32.4	38.6	44.3	50.7
	40	11.0	16.3	21.1	26.3	31.5	36.3	41.6
	50	9.8	14.3	18.3	22.7	27.1	31.1	35.6
	60	9.4	13.2	16.6	20.4	24.2	27.6	31.4
	70	9.5	12.7	15.6	18.8	22.0	25.0	28.2
Women (n=1,877)	20	22.8	32.5	41.2	50.8	60.3	69.0	78.7
	30	16.3	23.3	29.5	36.4	43.2	49.4	56.4
	40	12.0	17.6	22.6	28.1	33.6	38.6	44.2
	50	9.6	14.3	18.6	23.3	28.0	32.3	37.1
	60	8.6	12.9	16.7	20.8	25.0	28.8	33.0
	70	8.9	12.7	16.1	19.9	23.7	27.1	31.0

Table 0.4 reference values for DC_{fem} (in $10^{-3}.kPa^{-1}$) from table 6.6, calibrated to the **Wall Track System.**

		percentiles						
	Age (years)	2.5th	10th	25th	50th	75th	90th	97.5th
Men (n=634)	20	1.5	4.1	6.4	8.9	11.5	13.8	16.3
	30	2.4	4.8	7.0	9.4	11.8	13.9	16.3
	40	3.1	5.3	7.3	9.6	11.8	13.8	16.1
	50	3.2	5.3	7.1	9.2	11.3	13.2	15.3
	60	2.5	4.4	6.2	8.1	10.0	11.8	13.7
	70	0.8	2.6	4.2	6.0	7.7	9.3	11.1
Women (n=855)	20	1.4	4.7	7.7	10.9	14.2	17.1	20.4
	30	2.2	5.5	8.4	11.6	14.8	17.8	21.0
	40	2.9	6.1	8.9	12.1	15.2	18.1	21.2
	50	2.9	5.9	8.7	11.7	14.7	17.4	20.5
	60	1.5	4.3	6.9	9.7	12.5	15.1	17.9
	70	-2.1	0.5	2.8	5.3	7.9	10.2	12.8

Table 0.5 reference values for DC_{fem} (in $10^{-3}.kPa^{-1}$) from table 6.6, calibrated to the **Vivid7 system.**

		percentiles						
	Age (years)	2.5th	10th	25th	50th	75th	90th	97.5th
Men (n=634)	20	5.7	9.1	12.1	15.4	18.8	21.8	25.2
	30	6.1	9.2	12.1	15.2	18.3	21.1	24.3
	40	6.2	9.1	11.8	14.7	17.6	20.2	23.1
	50	6.0	8.7	11.1	13.8	16.5	18.9	21.7
	60	5.4	7.9	10.1	12.6	15.0	17.3	19.8
	70	4.3	6.5	8.6	10.8	13.1	15.1	17.4
Women (n=855)	20	4.0	8.7	13.0	17.7	22.4	26.7	31.4
	30	5.6	10.1	14.1	18.6	23.0	27.1	31.6
	40	6.8	11.1	14.9	19.1	23.3	27.1	31.4
	50	6.9	10.9	14.5	18.5	22.4	26.0	30.0
	60	4.9	8.7	12.1	15.8	19.5	22.9	26.6
	70	-0.2	3.3	6.5	9.9	13.4	16.6	20.1

Curriculum Vitae

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EDUCATION

DOCTOR IN MEDICAL SCIENCES, Ghent University
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BACHELOR IN BIOCHEMISTRY AND BIOTECHNOLOGY, Ghent University
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TRAINING

COURSES ORGANIZED BY THE DOCTORAL SCHOOLS

Clinical studies: study design, implementation and reporting
Advanced Academic English: Conference Skills - Presentation Skills
Effective Graphical Displays

COURSES ORGANIZED BY GHENT UNIVERSITY HOSPITAL

Statistical analyses: SPSS for starters
Statistical analyses: SPSS for experts
Good Clinical Practices in clinical research

PUBLICATIONS

Non-invasive assessment of carotid-femoral pulse wave velocity: the influence of body side and body contours. J Hypertens 2013; 31:946–951.

Asymmetry in prevalence of femoral but not carotid atherosclerosis. J Hypertens 2014;32(7):1429–1434.

ABSTRACTS

European Society of Hypertension meeting 2014, Athens:
- Reference intervals for femoral artery stiffness, obtained from 5,069 subjects. (oral)
- Macro- and microcirculation in normal-tension glaucoma. A case-control study (poster)

Physphar 2014, Maastricht:
- The influence of pregnancy on maternal hemodynamics and cardiovascular function (poster)

Artery Research meeting 2013, London
- Distribution of atherosclerosis between left and right body side: carotid symmetry, femoral asymmetry. (poster)
- Arterial stiffness and wave reflections decrease during pregnancy. (poster)

Belgian hypertension committee 2013, Ghent:
- The effect of body side on atherosclerotic plaque distribution at the carotid and femoral arteries. (poster)
- The Influence of pregnancy on arterial stiffness and wave reflections.

(oral)

European Society of Hypertension meeting 2013, Milan:

The influence of pregnancy on maternal hemodynamics and cardiovascular function (poster)

Asymmetry in femoral but not carotid atherosclerosis prevalence. (oral)

Artery Research meeting 2012, Vienna:

Non-invasive assessment of carotid-femoral pulse wave velocity. Does the measurement side matter? (poster)

Belgian hypertension committee 2012, Lausanne:

Non-invasive assessment of carotid-femoral pulse wave velocity. Some practical considerations. (poster)

European Society of Hypertension meeting 2012, London:

Real travelled aortic path length for PWV is not substantially different between left and right measurement locations (poster)

Belgian hypertension committee 2012, Genval:

Normal values for femoral artery stiffness (poster)

AWARDS | Poster prize Alberto Ferrari – European Society of Hypertension meeting 2013

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