METHODOLOGICAL STUDIES APPLYING NEW TECHNIQUES FOR EVALUATION OF ARTERIAL FUNCTION

Margareta Ring

Karolinska Institutet

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Methodological studies applying new techniques for evaluation of arterial function

THESIS FOR DOCTORAL DEGREE (Ph.D.)

By

Margareta Ring

Principal Supervisor:
Professor Kenneth Caidahl
Karolinska Institutet
Department of Molecular Medicine and Surgery

Cosupervisor:
Associate Professor Maria J Eriksson
Karolinska Institutet
Department of Molecular Medicine and Surgery

Opponent:
Professor Eva Nylander
Linköping University
Department of Medical and Health Sciences
Division of Cardiovascular Medicine

Examination Board:
Associate Professor Åsa Rydén-Ahlgren
Lund University
Department of Clinical Sciences, Malmö
Clinical Physiology and Nuclear Medicine Unit

Associate Professor Kerstin Jensen-Urstad
Karolinska Institutet
Department of Clinical Science and Education,
Södersjukhuset

Associate Professor Per Lindqvist
Umeå University
Department of Public Health and Clinical Medicine

To Putte, Johan and Julia

ABSTRACT

Atherosclerotic cardiovascular disease is the most common cause of death in the Western world today. Atherosclerosis in the arterial wall is related to aging and the inflammatory and calcification processes, and is influenced by risk factors such as smoking, male gender, diabetes, hypertension, obesity, stress, inactivity, hyperlipidemia, and genetic factors. Adequate diagnostic tools are needed for early detection and evaluation of treatment effects. The general aim of this thesis was to evaluate new and more established noninvasive techniques for measurement of arterial structure and function.

Methods: Study I measured carotid intima–media thickness (cIMT) in ultrasound recordings from 99 participants without known cardiovascular disease and compared the results between two semiautomated techniques: AMS and GE. In Study II aortic pulse wave velocity (PWVao) and aortic augmentation index (AIxao) were measured with Arteriograph and SphygmoCor in 63 (20–69 years old) healthy participants and compared the results between the newer technique and the more established technique. Study III evaluated the effects of vitamin D, parathyroid hormone, and calcium levels on vascular structure and function measured by different noninvasive techniques in 48 patients with mild primary hyperparathyroidism and 48 controls. In Study IV we investigated the effects of gender, insulin resistance, and low-intensity physical activity on vascular stiffness, structure, and function in 201 participants.

Results: The cIMT measured by GE was slightly but significantly higher than by AMS, but these correlated well in subjects without known cardiovascular disease (Study I). The Arteriograph gave higher PWVao values than the SphygmoCor, especially in women, while the correlation for AIxao between the methods was excellent. Arterial stiffness measured with both methods was related to age, cIMT and serum cholesterol level (Study II). PWVao, AIxao, cIMT, and radial artery IMT were related to systolic blood pressure but not to vitamin D level. (Study III). Greater arterial stiffness in women was indicated by AIxao, which was related to systemic vascular resistance and exercise capacity. However, a 4-month program of low-intensity physical activity did not improve vascular variables, and there were no gender differences in the responses to the exercise intervention (Study IV).

Conclusion: In follow-up studies and when evaluating treatment effects, it seems favourable not to change the techniques used to measure cIMT and to estimate stiffness. Neither vitamin D level nor low-intensity physical activity training influenced vascular morphology and function as evaluated by the applied noninvasive techniques.

SAMMANFATTNING

Introduktion: Hjärt- och kärlsjukdom orsakad av åderförkalkning är den vanligaste dödsorsaken i västvärlden idag. Åderförkalkning är relaterad till åldrande och inflammation i artärväggen och påverkas av riskfaktorer som rökning, manligt kön, diabetes, högt blodtryck, fetma, stress, inaktivitet, höga blodfetter och genetiska faktorer. Pålitliga metoder behövs för att tidigt upptäcka förändringar i kärlväggen. Den övergripande målsättningen med studien var att utvärdera nya och mer etablerade icke-invasiva tekniker för mätning av artärers struktur och funktion.

Metod: I studie I jämförde vi två halv-automatiska tekniker, AMS och GE, för mätning av halspulsåderns väggtjocklek (cIMT) på 99 deltagare utan känd hjärt-och kärl sjukdom. I studie II jämfördes pulsvågens hastighet i aorta (PWVao) och pulsvågsförstärkningen i aorta (AIxao) med Arteriograph och SphygmoCor på 63 friska personer mellan 20-69 år. I studie III utvärderades eventuella effekter av D vitamin, parathormon och calcium nivåer på artärernas struktur och funktion mätt som PWVao, AIxao, cIMT, gråskala i halspulsåderns vägg (cIM-GSM) och väggtjockleken i handledsartären (IMTrad) på 48 patienter med mild primär hyperparatyroidism. I studie IV undersökte vi effekten av fysisk aktivitet med stavgång, insulin resistans och kön på artärernas struktur och funktion hos 201 deltagare.

Resultat: GE gav något högre (signifikant) värde på cIMT än AMS, men metoderna visade bra korrelation med varandra (studie I). Arteriograph metoden mätte högre PWVao värden än SphygmoCor, speciellt bland kvinnor. Metoderna korrelerade väl beträffande AIxao. Artärstyvheten med de båda metoderna korrelerade till ålder, cIMT och serum kolesterol (studie II). PWVao, AIXao, cIMT och IMTrad korrelerade till systoliskt blodtryck men inte till D vitamin nivå (studie III). AIxao var högre hos kvinnor jämfört med män och korrelerade till kärlresistans och arbetskapacitet. Vi fann ingen signifikant effekt på artärernas funktion eller struktur eller någon skillnad mellan könen efter 4 månaders stavgång (studie IV).

Sammanfattning: Vid uppföljningsstudier och vid utvärdering av läkemedelseffekter är det en fördel att inte växla metoder för mätning av cIMT och artärstyvhet. Varken D vitamin eller låg fysisk aktivitet med stavgång påverkade artärers struktur eller funktion mätt med icke-invasiva tekniker.

LIST OF SCIENTIFIC PAPERS

Ring M, Eriksson M.J, Jogestrand T, Caidahl K. Ultrasound measurements of carotid intima-media thickness by two semi-automated analysis systems. *Submitted manuscript*.

Ring M, Eriksson M.J, Zierath J.R, Caidahl K. Arterial stiffness estimation in healthy subjects: A validation of oscillometric (Arteriograph) and tonometric (SphygmoCor) techniques. Hypertens Res. 2014 Jul 24. doi: 10.1038/hr.2014.115. [Epub ahead of print].

Ring M, Farahnak P, Gustavsson T, Nilsson I-L, Eriksson M.J, Caidahl K. Arterial structure and function in mild primary hyperparathyroidism is not directly related to parathyroid hormone, calcium, or vitamin D. PLoS One. 2012;7(7):e39519. doi: 10.1371/ journal.pone. 0039519. Epub 2012 Jul 16.

Ring M, Eriksson M.J, Fritz T, Nyberg G, Östenson CG, Krook A, Zierath J.R, Caidahl K. Influence of physical activity and gender on arterial function in type 2 diabetes, normal and impaired glucose tolerance. *Manuscript*.

Contents

1 LIST OF ABBREVIATIONS

$1,25(OH)_2D$	1,25-dihydroxyvitamin D
25(OH)D	25-hydroxyvitamin D
AG	Arteriograph
ao	Aortic
AMS	Artery Measurement System
Apo	Apolipoprotein
AIx	Augmentation index
BMI	Body mass index
BSA	Body surface area
BP	Blood pressure
cSBP	Central systolic blood pressure
c	Carotid
CO	Cardiac output
Ca++	Ionized calcium
CCA	Common carotid artery
CV	Cardiovascular
DBP	Diastolic blood pressure
DVP	Digital volume pulse
ECG	Electrocardiogram
ECM	Extracellular matrix
GE	General Electric or ‘GE IMT analysis package’
GFR	Glomerular filtration rate
GSM	Gray-scale median
HR	Heart rate
hs-CRP	High-sensitivity C-reactive protein
IGF-1	Insulin-like growth factor 1
IGT	Impaired glucose tolerance
IM	Intima–media
IMT	Intima–media thickness
IMTrad	Intima-media thickness of the radial artery
LD	Lumen diameter

LDrad	Lumen diameter of the radial artery
LDL	Low-density lipoprotein
LV	Left ventricular
LVOT	Left ventriculara outflow tract
MAP	Mean arterial pressure
NGSP	National Glycohemoglobin Standardization Program
NGT	Normal glucose tolerance
NO	Nitric oxide
NOS	Nitric oxide synthase
OxLDL	Oxidized LDL
pHPT	Primary hyperparathyroidism
PP	Pulse pressure
PTH	Parathyroid hormone
PTX	Parathyroid adenomectomy
PWA	Pulse wave analysis
PWV	Pulse wave velocity
RI	Reflection index
ROI	Region of interest
ROS	Reactive oxygen species
SC	SphygmoCor
SBP	Systolic blood pressure
SD	Standard deviation
SI	Stiffness index
St-PWVao	Standardized aortic pulse wave velocity
SV	Stroke volume
T2DM	Type 2 diabetes mellitus
TG	Triglyceride
VTI	Velocity time integral
VWF	Von Willebrand factor

2 INTRODUCTION

2.1 BACKGROUND

Atherosclerotic cardiovascular (CV) diseases such as myocardial infarction, angina pectoris, stroke, and peripheral artery diseases are the most common causes of death in the world today.[1-3] In Sweden, 42% of all deaths are caused by CV diseases. The atherosclerotic process is progressive and may begin early in life.[4-6] Cigarette smoking, male gender, diabetes mellitus, hypertension, obesity, stress, inactivity, elevated cholesterol level, and genetic factors have been identified as risk factors associated with adverse outcomes.[7-10] The atherosclerotic process in the arterial wall reflects both normal aging and, importantly, the risk factors mentioned above.

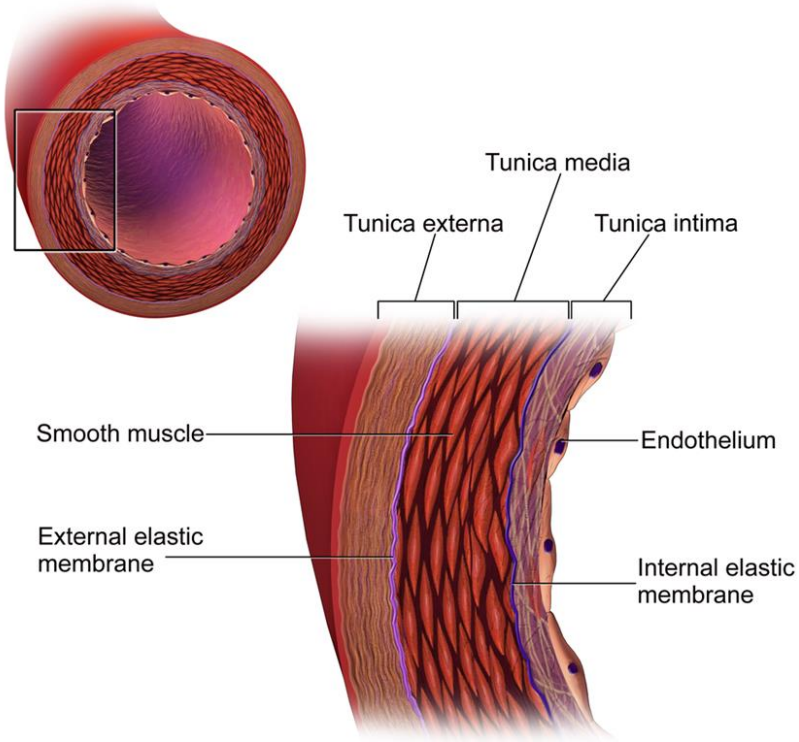


Figure 1. Schematic illustration of the normal arterial wall showing the tunica intima, tunica media, and the outer wall layer tunica externa/adventitia. *This illustration by Bruce Blaus is used with permission from the Wikimedia OTRS system, http://en.wikipedia.org/ wiki/File:Blausen_0055 _Artery WallStructure.png*

2.2 THE ARTERIAL WALL STRUCTURE AND FUNCTION

The arterial wall comprises the tunica intima, tunica media, and tunica externa/adventitia (**Figure 1**). The tunica intima is composed of endothelial cells, extracellular matrix (ECM),[11] and the internal elastic membrane. Nitric oxide (NO) is produced in the endothelial cells from L-arginine by activity of the enzyme NO synthase (NOS)[12] and relaxes smooth muscle cells. [13, 14] The tunica media comprises smooth muscle cells, which contract and maintain the tone of the arterial wall. The tunica media also contains ECM comprised of proteoglycans, elastin, and collagen.[15] The outer layer, the tunica adventitia, consists mainly of collagen and the vasa vasorum, which supplies the arterial wall with blood. Collagen and elastin provide structural integrity and elasticity, and are regulated by matrix metalloproteases.[16] The normal process of aging reduces elastin content and increases collagen content, causing an imbalance between the elastin/collagen distribution and fracture of elastin lamellae, a process that leads to the loss of elasticity in the elastic arterial wall and increased arterial diameter and intima–media thickness (IMT).[17, 18] These changes of dilatation and decreased distensibility lead to further structural changes[19] caused by local inflammation, infiltration of vascular smooth muscle cells and macrophages, fibrosis, focal necrosis of media smooth muscle cells, and calcification.[18] Through these processes, the IMT triples between ages 20 and 90 years.[18] Thus, arterial stiffening has a multifactorial origin in the three layers of the arterial wall and from extrinsic influences (**Figure 2**).[16]

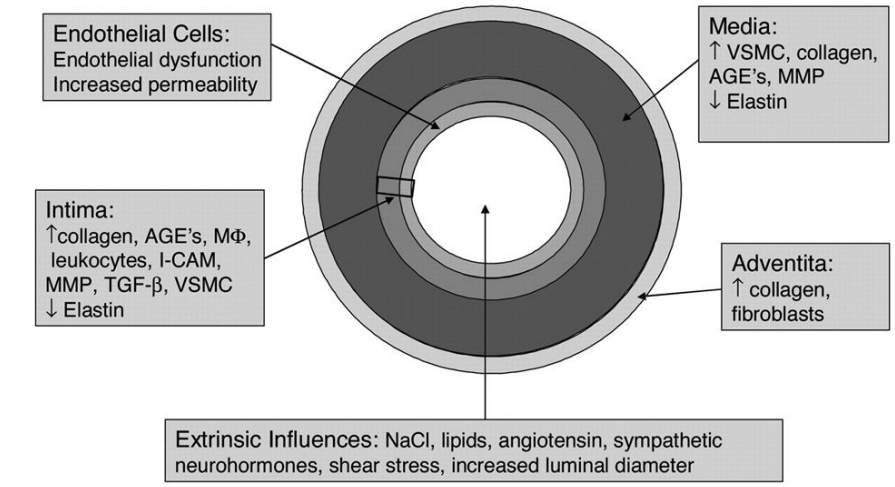


Figure 2. Multiple causes of arterial stiffness and their locations. *Used with permission from Wolters Kluwer Health.*[16]

Table 1. Vascular variables

Shear stress	The frictional force per unit area resulting from blood flow. Shear stress = (4 x blood viscocity x blood flow)/ (π x radius^3). [20]
Carotid intima–media thickness (cIMT), mm	The thickness of the intima and media of the carotid artery, measured by ultrasound.[21]
Carotid intima media gray-scale median (cIM-GSM)	The degree of whiteness (echogenicity) or blackness (echolucency) in the intima and media of the common carotid artery wall.[22, 23]
Arterial stiffness	Arterial stiffness can be measured e.g. in terms of PWV or carotid distensibility (see below), [24] and is influenced by normal aging and atherosclerosis.[4-6]
Aortic pulse wave velocity (PWVao), m/s	PWV = distance / TT. PWVao is considered a reference measure of arterial stiffness. Transit time (TT) is time for the pulse wave from the upper aorta to the femoral artery and corresponding distance.[25]
Augmentation index (AIx), %	AIx = (P2 – P1) / PP; where P1 is the systolic and P2 the reflected pulse wave peaks.[25]
Pulse pressure (PP), mmHg	The difference between systolic and diastolic blood pressure.
Stiffness index (SI), m/s	SI = subject's height / time between the first systolic wave and the reflected wave; influenced by stiffness in small arteries in the finger, and by large artery stiffness.[26, 27]
Reflection index (RI), %	RI = reflected wave / first systolic wave. The RI is a measure of vascular tone of small arteries.[26, 27]
Arterial compliance (AC), μm^3/mmHg	AC = ΔV / ΔP,[28] expressing change in blood volume (ΔV) due to a given change in arterial blood pressure (ΔP).
Strain, %	Strain = 100 × (diameter in systole – diameter in diastole) / diameter in diastole.[17]
Carotid arterial stiffness, βCCA	Calculated as ln(systolic blood pressure / diastolic blood pressure) / CCA strain.[17]
Carotid distensibility, (CD)	CD coefficient = 2ΔD / (D x PP); where D is end-diastolic carotid diameter, ΔD absolute systolic change. [24, 29]

Different variables used to describe vascular function are shown in **Table 1**. Arterial stiffness depends on the compliance of the arterial wall and is recognized as a major factor associated with CV events.[30, 31] Stiff arteries cause increased blood pressure and a faster pulse wave velocity (PWV) and reflection of the pulse wave from arterial bifurcations and the peripheral arterial tree.

In addition to transporting oxygen and nutrients to the cells, an important arterial function is reduction of the oscillation pressure, caused by the left ventricular (LV) contraction and ejection of stroke volume (SV), so that the capillary blood flow is almost continuous. The dampening of the pressure is enabled by the viscoelastic properties of the arterial wall.[32] Arterial stiffness can be evaluated from the velocity of the arterial pulse waves; i.e. PWV. Arterial pulse waves are generated during LV contraction. The ejected SV forms the early pulse wave, and the second pulse wave is generated by the returning pulse wave from the periphery. The difference between the first and reflected pulse waves is called augmentation. Stiffer arteries increase the augmentation and augmentation index (AIx), which is calculated from the relationship between augmentation and pulse pressure (PP). Stiffness and reflection of the pulse wave can also be measured in small arteries by the stiffness index (SI) and reflection index (RI). Structural changes in the arterial wall can be measured locally by ultrasound techniques in terms of IMT, echogenicity of the intima–media complex, and functional changes such as arterial stiffness. In addition to its important effect on IMT, age is a major factor affecting arterial stiffness.[18] At a younger age, the greater arterial compliance leads to low pulse pressure and the reflected wave reaches the heart in diastole, which increases coronary blood flow. The aging process in the arteries leads to increased PWVao (**Figure 3**), and early wave reflection followed by increased systolic blood pressure (SBP) and decreased diastolic blood pressure (DBP). The left ventricle responds to the increased afterload with hypertrophy and increased oxygen demand, and consequently an increased risk of development of myocardial ischemia and LV failure.[19, 33]

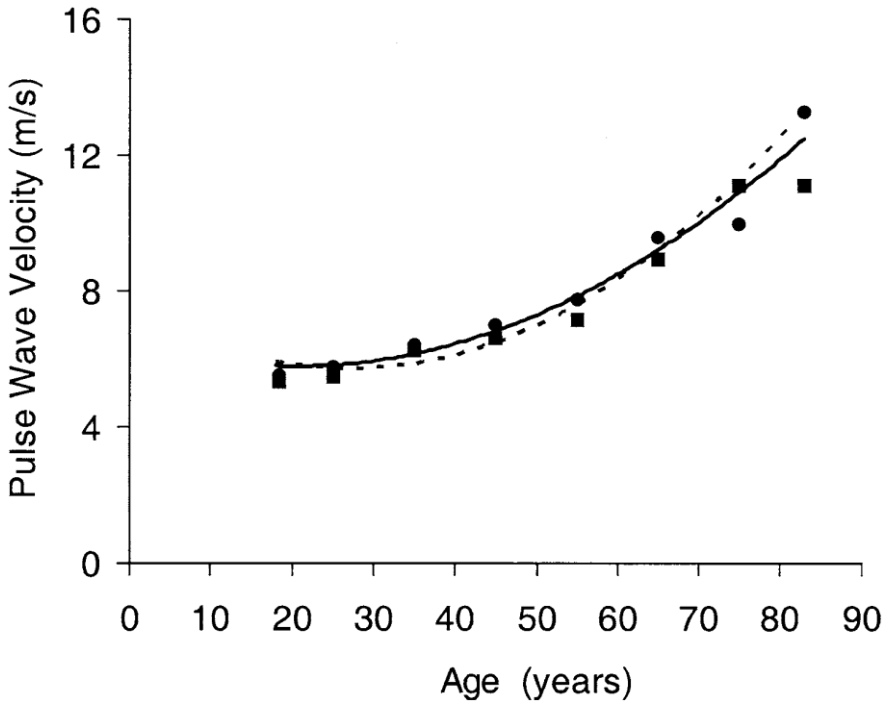


Figure 3. Reference values for pulse wave velocity (PWV) measured using the SphygmoCor in 998 healthy subjects aged 20–90 years. *This illustration by Carmel M. McEniery is used with permission from the Elsevier system, Journal of the American College of Cardiology, 2005.* [34]

2.3 FACTORS INFLUENCING ARTERIAL FUNCTION

2.3.1 Atherosclerosis, lipids, and inflammation

The atherosclerosis process causes an increase in IMT, which gradually develops as plaque that is often found in the outer walls of vessel bifurcations.[20] Chronic inflammation is an important factor in the atherosclerosis process.[35] Proinflammatory cytokines are low molecular weight proteins comprising >100 secreted factors that are important for the inflammatory and immune responses.[36] Cytokines can alter endothelial function in the early stages of arteriosclerosis.[36] Low-density lipoprotein (LDL) contributes to the inflammatory process at an early stage of the atherosclerotic process.[5] LDL in the tunica intima combines with proteoglycans and undergoes oxidation to oxidized LDL (oxLDL). OxLDL is inflammatory by its ability to activate T cells, monocytes/macrophages, and endothelial cells in humans.[37-39]

2.3.2 Hypertension, shear stress, and oxidative stress

Shear stress is caused by friction between blood flow and endothelial cells. Vessel regions that are exposed to laminar blood flow and greater shear stress remain relatively disease free.[20] Disturbed flow, such as flow turbulence in the artery, causes low shear stress. The magnitude of shear stress can be estimated by including blood flow, radius of the vessel wall, and blood viscosity in the calculation. Low shear stress <4 dyne/cm^2 occurs frequently at atherosclerotic sites[20] and correlates with increased carotid IMT (cIMT) in healthy males.[40, 41] Stimuli such as increased blood pressure can cause turbulence in the arterial tree branches and altered shear stress, which affects NO production in endothelial cells. NOS uncoupling contributes to age-related endothelial dysfunction, increased vascular stiffness, and atherosclerosis, which in turn lead to increased PWV.[18] Oxidative stress is another factor that influences the progress of atherosclerosis. Reactive oxygen species (ROS) are produced through cell metabolism. Oxidative stress is caused by an imbalance between ROS and antioxidant defenses. This imbalance induces endothelial dysfunction and is believed to contribute to the progression of atherosclerosis.[42] Dyslipidemia, oxidative stress, and inflammation are important CV risk factors that influence the atherosclerosis process and are associated with the echolucency of the carotid intima–media complex.[43]

2.3.3 Vitamin D deficiency, parathyroid hormone, and calcium

Low vitamin D (25-hydroxyvitamin D, 25(OH)D) and high circulating parathyroid hormone (PTH) and calcium levels are associated with increased CV risk in the general population. [44-47] Increased CV mortality is associated with high parathyroid hormone (PTH) level in the general population and in patients with coronary disease.[48, 49] Other recent studies have not found a relationship between low 25(OH)D level and all-cause mortality in elderly men.[50] Thus, there are still unresolved issues concerning the importance of vitamin D as a key factor for vascular integrity.

The possible association of vitamin D deficiency with CV risk is intriguing and has attracted considerable interest.[44-47, 51, 52] Low vitamin D level and high levels of PTH and calcium are characteristic of primary hyperparathyroidism (pHPT),[53] which may provide a suitable model for examining the relationships of these circulating factors with CV function. pHPT is a common endocrine disorder caused by a single adenoma in 80–85% of patients or by multiple adenomas in 15–20% of patients. Premature death due to cardiovascular or malignant disease

has been noted in a long-term study.[54] Increased cIMT,[55, 56] elevated SBP, higher prevalence of metabolic syndrome,[57] and increased PWV[58] are CV risk factors associated with pHPT. Nowadays, pHPT is often diagnosed early without classical symptoms and is defined as mild pHPT.

PTH is secreted as a polypeptide by the parathyroid cells of the parathyroid gland. It increases the concentration of calcium in the blood by acting upon the PTH receptors 1 and 2. PTH regulates the serum calcium level by increasing the release of calcium from the bones. Calcium is the most essential mineral in the human body, and most of the body's calcium is stored in the skeleton. The blood calcium level is regulated by PTH, calcitonin, and cholecalciferol (1,25-dihydroxyvitamin D_3, $1,25(OH)_2D$). High serum calcium level is associated with increased mortality[59] and is a risk factor for myocardial infarction and carotid plaque thickness, suggesting that extracellular calcium level is important in the atherosclerotic process.[60, 61] The body exposure to sunlight increases the synthesis of cholesterol to vitamin D_3. Vitamin D_3 can also be found in some foods such as fatty fish, liver, and dairy products. Vitamin D_3 is converted to 25(OH)D in the liver and to its biologically active form $1,25(OH)_2D$ in the kidney. Vitamin D deficiency is common in patients with pHPT[53] and is associated with CV disease in the general population.[62] The exact causal link between vitamin D and pHPT is unclear, although one possible explanation is that chronic vitamin D deficiency stimulates the parathyroid glands and leads to adenoma growth.[63] The expert guidelines state that the 25(OH)D level should be assessed if pHPT is suspected and that a serum level <50 nmol/L should be corrected.[64]

2.3.4 Glucose metabolism and physical activity

Diabetes is an increasing global problem, and 1–2% of the European population exhibit diabetes. Type 2 diabetes mellitus (T2DM) is the most common type of diabetes; it represents about 80% of diabetes cases in European countries, and is associated with increased CV mortality. [65, 66] T2DM is characterized by disturbances in insulin action and insulin secretion, and usually occurs in middle-aged people and in those who are overweight and physically inactive.[67, 68] Insulin is synthesized in and secreted by the β cells within the islets of Langerhans in the pancreas. The pathogenesis involves a combination of insufficient insulin secretion and resistance to the metabolic effects of insulin in various tissues such as skeletal muscle, liver, and

fat cells.[69] Physical activity has been reported to reduce the CV risk in patients with T2DM.[70] Increased intensity and volume of physical activity have been reported to be associated with reduced mortality risk in healthy middle-aged men and women,[71] and low physical activity levels are an important risk factor for all-cause mortality.[72] The inflammatory mechanisms play a crucial role in the pathogenesis of T2DM.[73] It has been proposed that regular physical activity can improve the function of the aging endothelium by modulating oxidative stress and inflammatory processes.[74] However, a recent report on obese individuals with T2DM randomized to lifestyle intervention with reduced caloric intake and increased physical activity did not indicate a reduced incidence of CV events.[75]

2.4 ULTRASONIC IMAGING OF ARTERIAL STRUCTURE AND FUNCTION

The use of ultrasonography to measure the IMT of the arterial wall was first reported in 1986.[76] The ultrasound technique for imaging tissues is well established, and the arterial wall can be measured locally in different vascular territories. Briefly, the ultrasound transducer comprises piezoelectric crystals that vibrate when exposed to electrical impulses and generate sound waves. When the sound waves reach the interfaces where the acoustic impedance differs (e.g., between blood and the vessel wall), sound waves are reflected back to the transducer, which also functions as a receiver. The depth the signal reaches before it is reflected back is proportional to the return time, and an image can be generated in which the pixel intensity is proportional to the amount of returning sound waves at a given moment. The typical frequency for carotid transducers is 8–12 MHz, and ultrahigh-frequency transducers of 30–70 MHz may allow extreme resolution in small arteries.

2.4.1 Intima–media thickness (IMT) in the common carotid artery

The cIMT can be measured manually or with a semiautomated system and analysis software. The cIMT is usually measured from a 10-mm-long segment proximal to the carotid bulb and according to the leading edge principle (**Figure 4**).[21] An increased cIMT represents an early stage of atherosclerosis,[77] and measurements of cIMT are used in early studies of atherosclerosis.[76] Increased cIMT is associated with morbidity caused by CV events such as stroke, myocardial infarction, and heart failure [78-81]. Therefore, measurement of cIMT is suggested as a complement to conventional CV risk factor analysis.[82]

Increased cIMT has also been reported in patients with pHPT.[55, 56] Attenuation of the progression of the increase in cIMT has been found after aerobic exercise training in T2DM patients.[83]

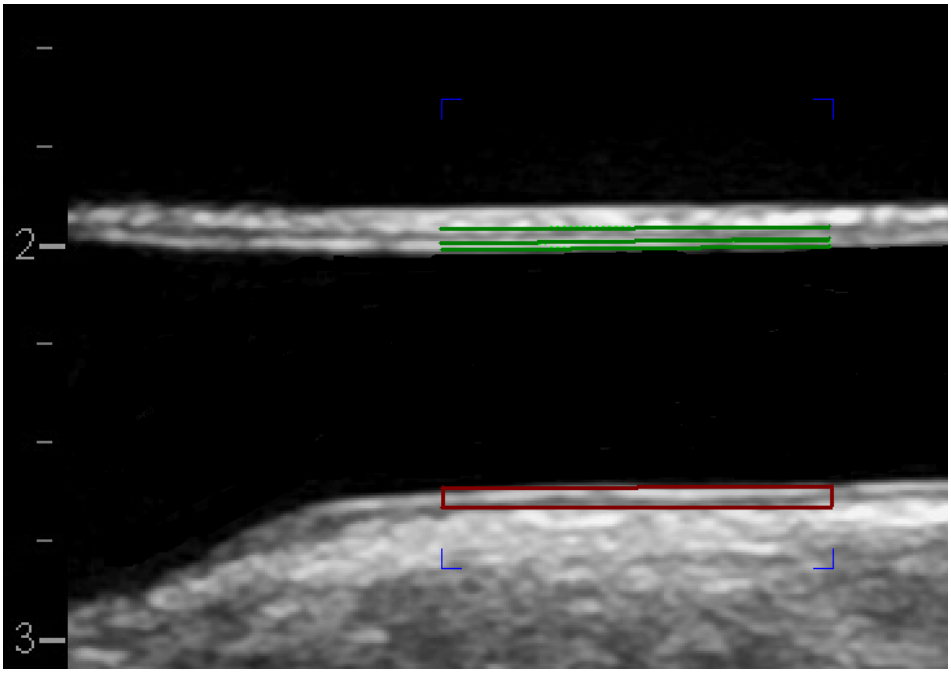


Figure 4. 2D image of the right common carotid artery obtained by ultrasound. Black color represents the blood. The red box in the far wall represents the IMT. The blue lines indicate the length of the region of interest (10 mm) and the green lines the intima–media in the near wall (not used in our studies).

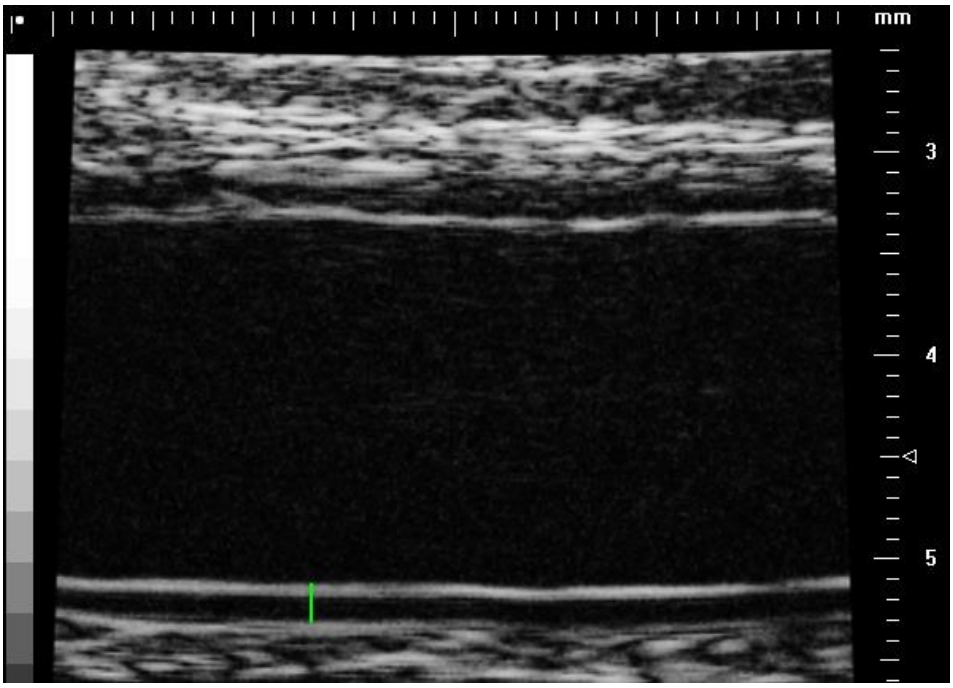


Figure 5. 2D image of the right radial artery obtained by high-frequency ultrasound. The black color represents the blood. The green mark represents the IMT.

2.4.2 Intima–media thickness (IMT) in the radial artery

High-resolution ultrasound enabling the study of small peripheral arteries with frequencies around 30-70 MHz has been introduced recently.[84] The radial artery IMT (IMTrad) can be measured by high-resolution ultrasound according to the leading edge principle,[21] and thickness of the intimal layer of the radial artery is associated with hypertension[85] and CV events.[86, 87] An example of radial artery measurement of the IMT in the far wall is shown in **Figure 5**.

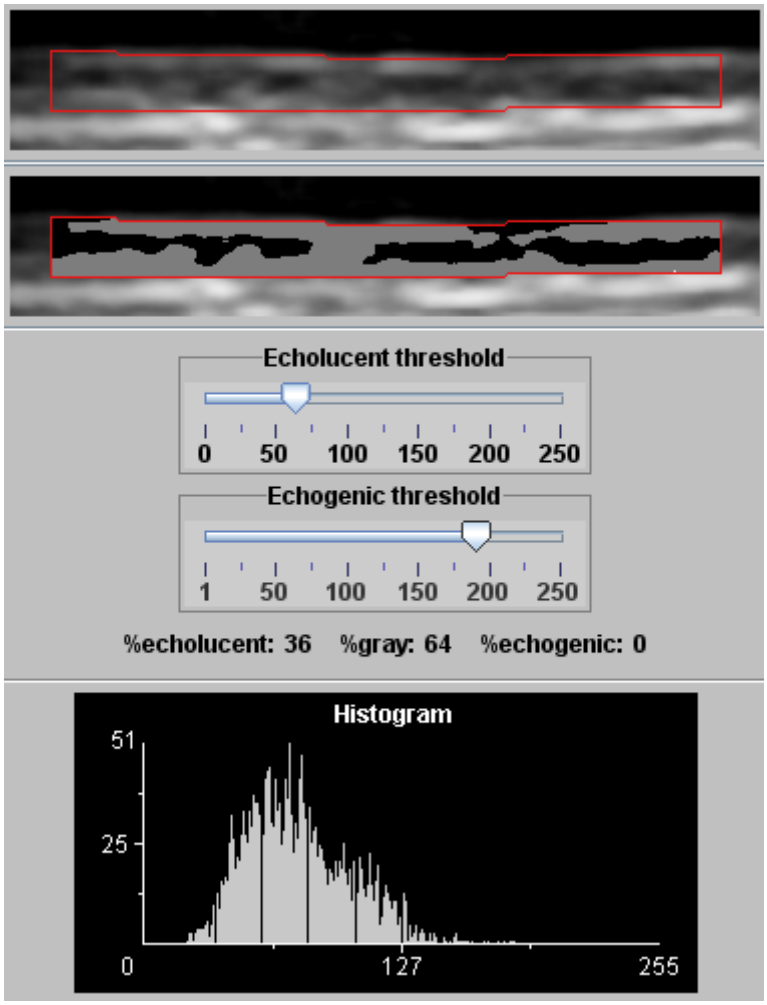


Figure 6. Echogenicity measurement (cIM-GSM) in the intima–media of the far wall of the CCA. The histogram (grey scale on horizontal axis and number of pixels on vertical axis) shows the amount of echolucent and gray structure in the intima–media; no echogenic structure is detected in this image. The IM-GSM is measured from a scale of 256 pixels, ranging from 0 (black) to 255 (white), and the values are shown as median gray. The adventitia is used as the reference for white and the blood as the reference for black. A whiter IM-GSM shows more echogenic tissue, whereas a darker IM-GSM shows a more echolucent tissue. The echolucency of the carotid intima–media is related to CV risk factors such as dyslipidemia and inflammation, [43] and to older age, high body mass index (BMI), hypertension, and low HDL cholesterol level. [88]

2.4.3 Echogenicity in the common carotid artery

A new method that evaluates the echogenicity of the intima–media has been developed for assessment of plaque in the common carotid artery (CCA).[23] The echogenicity is expressed as the carotid intima–media gray-scale median (cIM-GSM) and is measured in the intima–media complex of the far arterial wall (**Figure 6**).

2.4.4 Arterial stiffness in the common carotid artery (CCA)

Arterial stiffness can be measured locally in the CCA. The diameter changes in systole and diastole can be measured in M-mode. In an M-mode image, the time of events and the distances can be measured (**Figure 7**). The relative diameter changes with pulsations in the CCA is described as strain and is expressed as a percentage. Carotid stiffness βCCA, as defined in **Table 1**, is calculated in a modified equation based on the observed linear relationship between the natural logarithm (ln) of relative pressure and strain.[17]

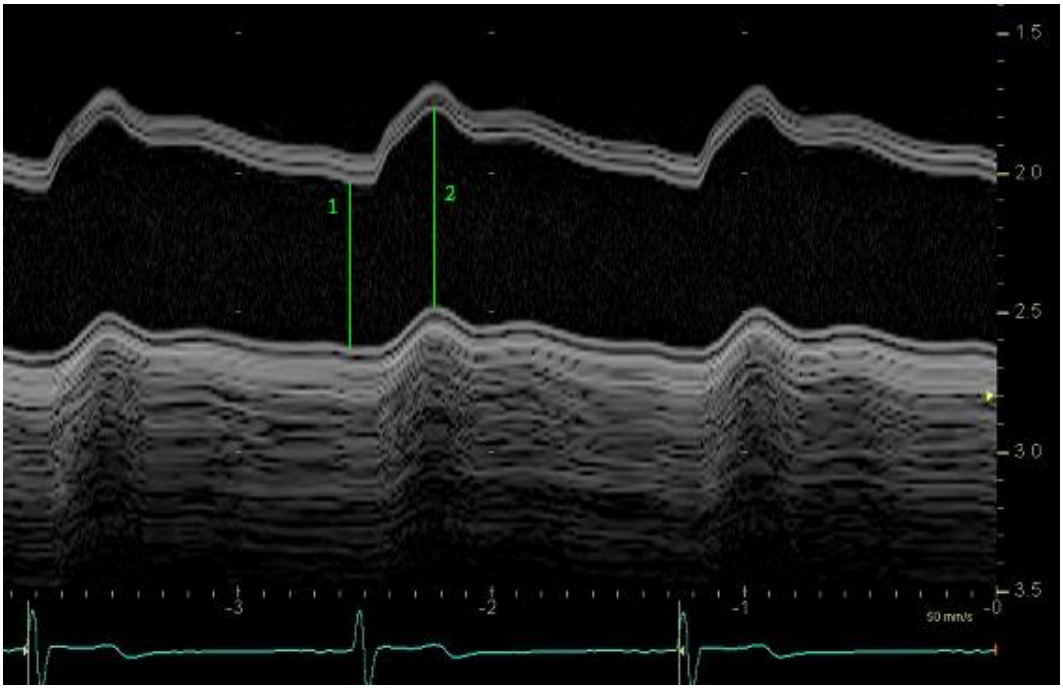


Figure 7. M-mode image of the right common carotid artery. Cyclic changes in the diameter between systole and diastole are seen. The green lines show the minimal lumen diameter at the R-wave of the ECG (1) and the maximal lumen diameter at the peak of the T-wave of the ECG (2).

2.5 ARTERIAL STIFFNESS MEASURED BY NONINVASIVE PULSE WAVE METHODS

Around 1860, Marey designed the Sphygmograph, a recorder of radial artery pulse (described in *La circulation du sang*, 1881). The Sphygmograph device recorded the radial pulse wave and could print the pulse wave with a mechanical transmission to a rotating cylinder (**Figure 8**). The Sphygmograph was used in early studies of artery-dilating drugs such as nitroglycerine.[89, 90] In 1896, Riva-Rocci proposed a method for noninvasive measurement of SBP.[91] In 1905, Korotkoff introduced the auscultatory noninvasive blood pressure method we use today. With a cuff placed on the upper arm and a stethoscope over the brachial artery, SBP and DBP are obtained by listening to the distinctive arterial sounds.[92] Pulse wave recordings with the photoplethysmographic method were introduced in 1937 by Hertzman, and contour analysis of the digital volume pulse (DVP) was initiated by Dillon and Hertzman in 1941.[27]

The arterial stiffness expressed as PWVao and aortic AIx (AIxao), and central SBP (cSBP) can be measured noninvasively by different technologies. Examples of such are the 'SphygmoCor', which uses applanation tonometry with a connected electrocardiogram, and the 'Arteriograph', which uses an oscillometric method with the brachial cuff connected to a high-fidelity pressure sensor. The SI and RI can be measured by 'Pulse Trace', which uses photoplethysmography.
See more detailed description of Sphygmocor, Arteriograph and Pulse Treace below.

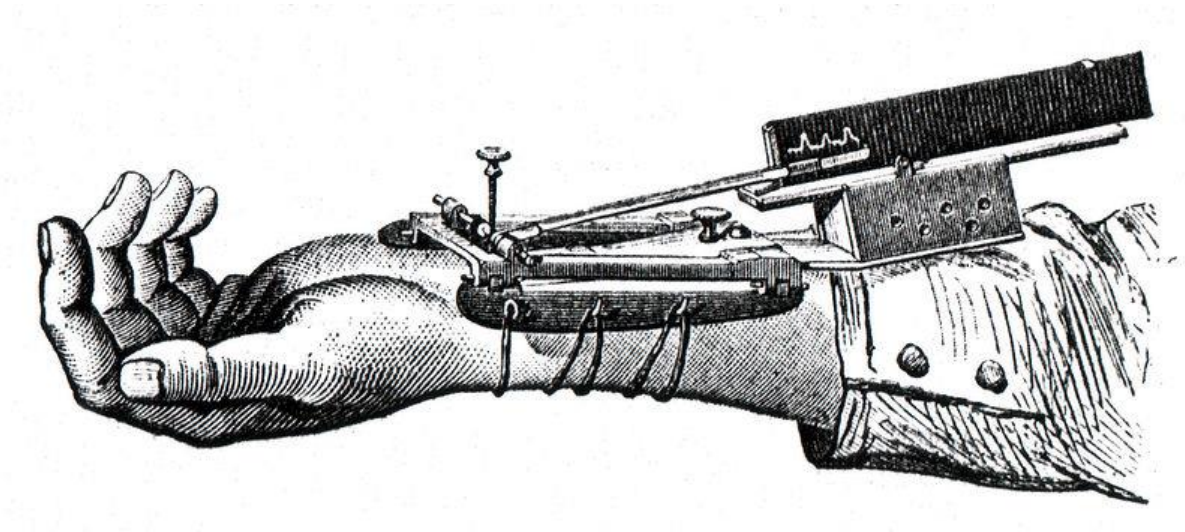


Figure 8. Illustration of the Sphygmograph by Marey. The pressure to the artery was regulated with a screw. The registration unit recorded the blood pressure wave forms. *This image is in the public domain due to its age.*

The speed of the pulse wave, PWV, is a measure of arterial stiffness, and the pulse wave travels faster with decreased distensibility of the arterial wall. As mentioned previously, the PWVao increases slowly with age, more so in older people, whereas the increase in AIxao is more pronounced at younger ages, suggesting that AIxao is a more sensitive marker of arterial stiffening and risk in the young and that PWVao is a better measure in older individuals.[34]

The AIx is affected by the speed of the pulse wave propagation, LV function, the Windkessel effect, body height (length of aorta), gender, heart rate, and attenuation of the pressure wave along the arterial tree.[93, 94] The estimation of cSBP is generated from the radial artery wave form using a validated transfer function.[95,96] At a young age, the cSBP is lower than the peripheral blood pressure; however, at an older age, the cSBP increases because of the stiffer aortic walls with more collagen and less elastin.[97]

The principle of the tonometric and oscillometric techniques is the generation of two systolic peaks. First, there is an early systolic pressure peak (P1) created by the ejection of the blood volume from the left ventricle into the aorta. The pressure wave is transmitted to the lower part of the body, and the reflected wave from the peripheral arteries generates the late systolic peak (P2). A third peak (P3) is generated when the returning wave meets the closed aortic valve.

2.5.1 Tonometric method

In the 1990s, O'Rourke introduced the tonometric SphygmoCor technique (**Figure 9**) to study the augmentation of the returning pulse wave, and later PWV.[98] The principle of the SphygmoCor is pulse recording using a sensitive pressure sensor, 0.5 mm in diameter. The pulse wave is recorded by compression of the artery against the bone, with the sensor gently pressed downward enough to flatten the artery. The tonometric method determines the PWVao from pulse wave recordings obtained at two sites—the carotid and femoral arteries—and the electrocardiogram (ECG) provides a time reference. Measures of pulse waves obtained in the carotid and femoral arteries are shown in **Figure 10**.

The AIxao (%) is defined as the difference between the second (P2) and the first (P1) peaks of the central aortic wave form and is expressed as a percentage of PP. The radial augmentation index (AIxrad, %) is calculated as $100 \times (P2 - DBP) / (P1 - DBP)$. The central or aortic SBP is calculated from the P2 of the radial pulse wave (**Figure 11**).

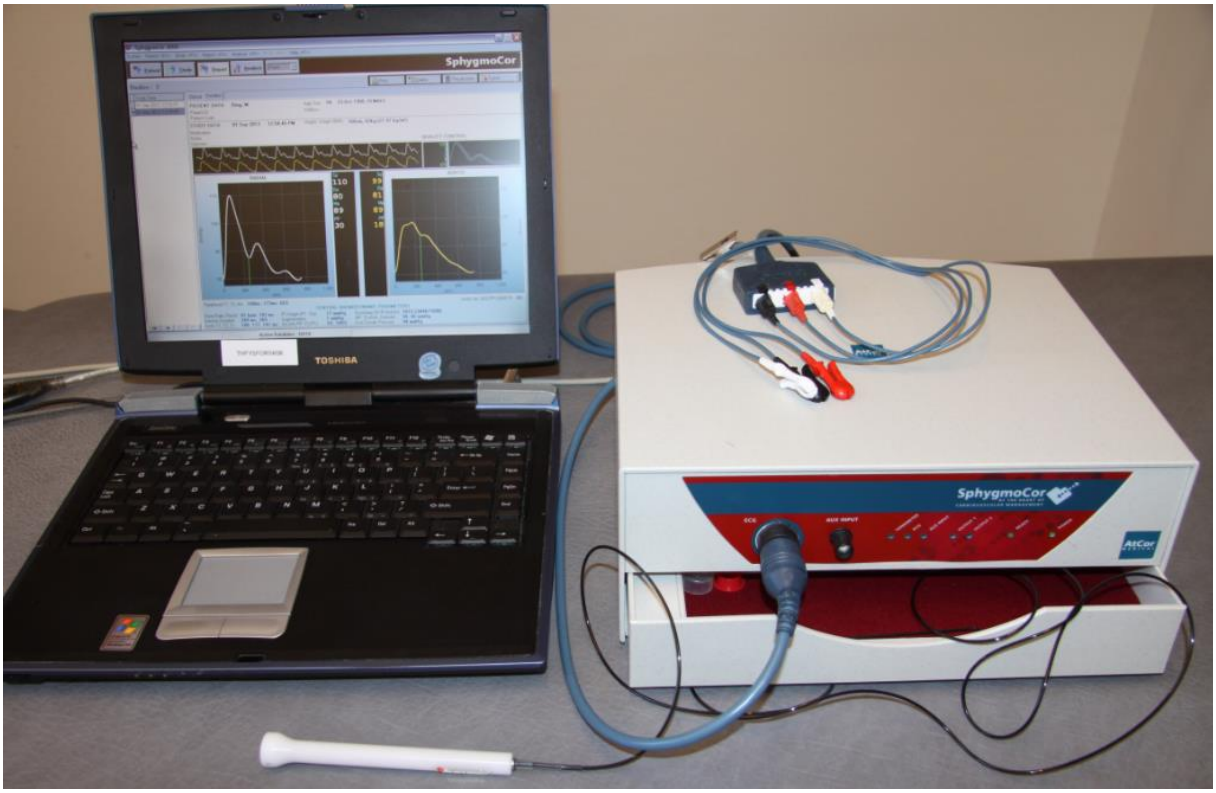


Figure 9. SphygmoCor equipment connected to a computer with the tonometer in front.

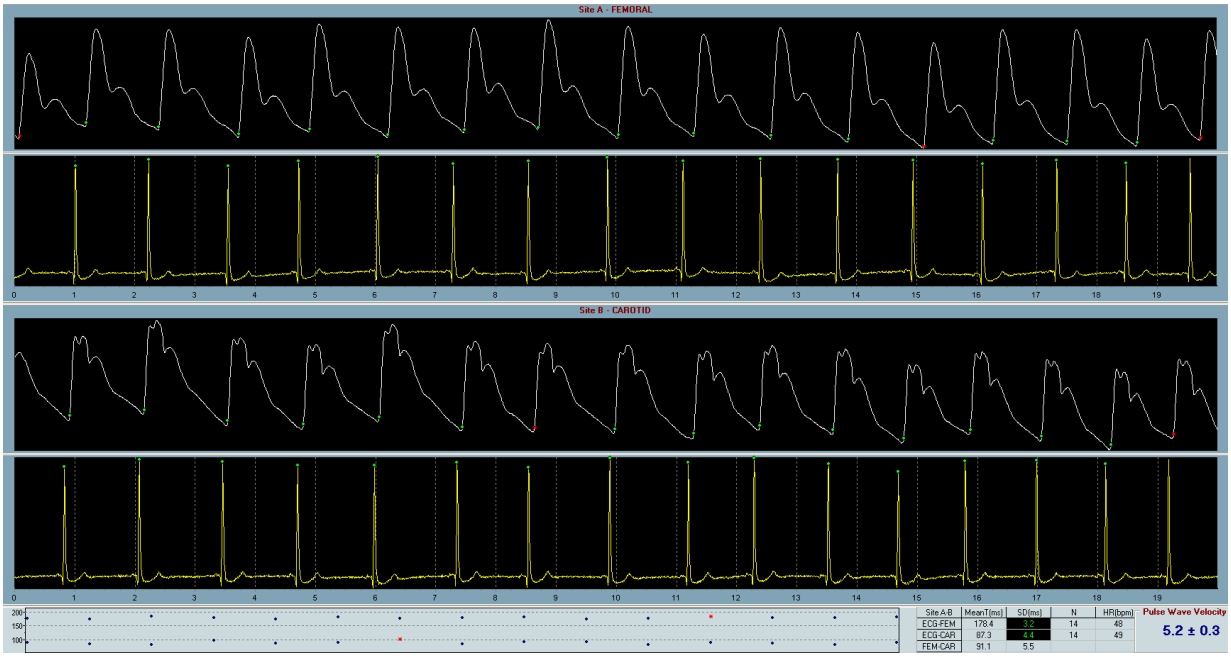


Figure 10. Pulse waves in the carotid and femoral arteries obtained by the SphygmoCor.

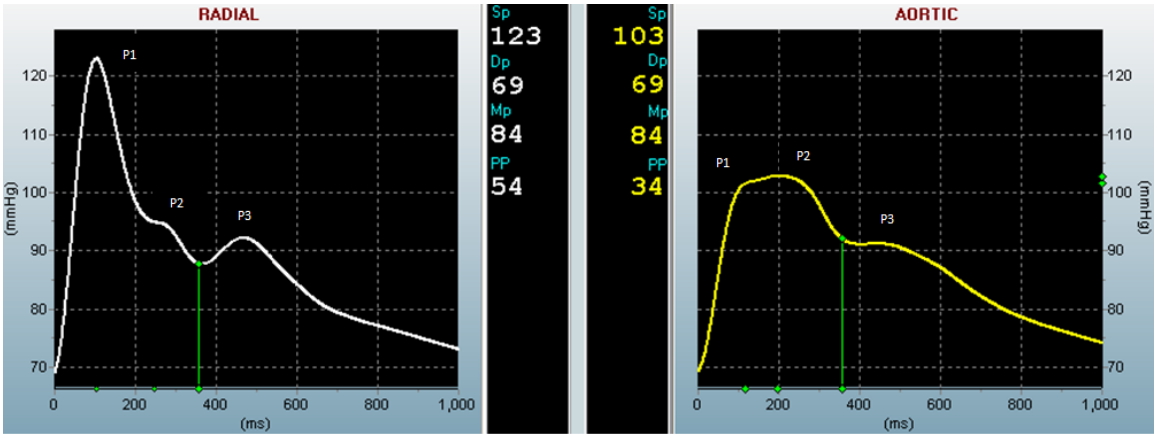


Figure 11. Pulse waves in the radial artery and aorta obtained by the SphygmoCor. The early systolic pressure wave (P1), the late (reflected) systolic pressure wave (P2), and the diastolic (reflected) pressure wave (P3) are shown.

2.5.2 Oscillometric method

The Arteriograph (TensioMed Kft., Budapest, Hungary) is a new technique invented around 2005 by Dr. Miklós Illyés (**Figure 12**).

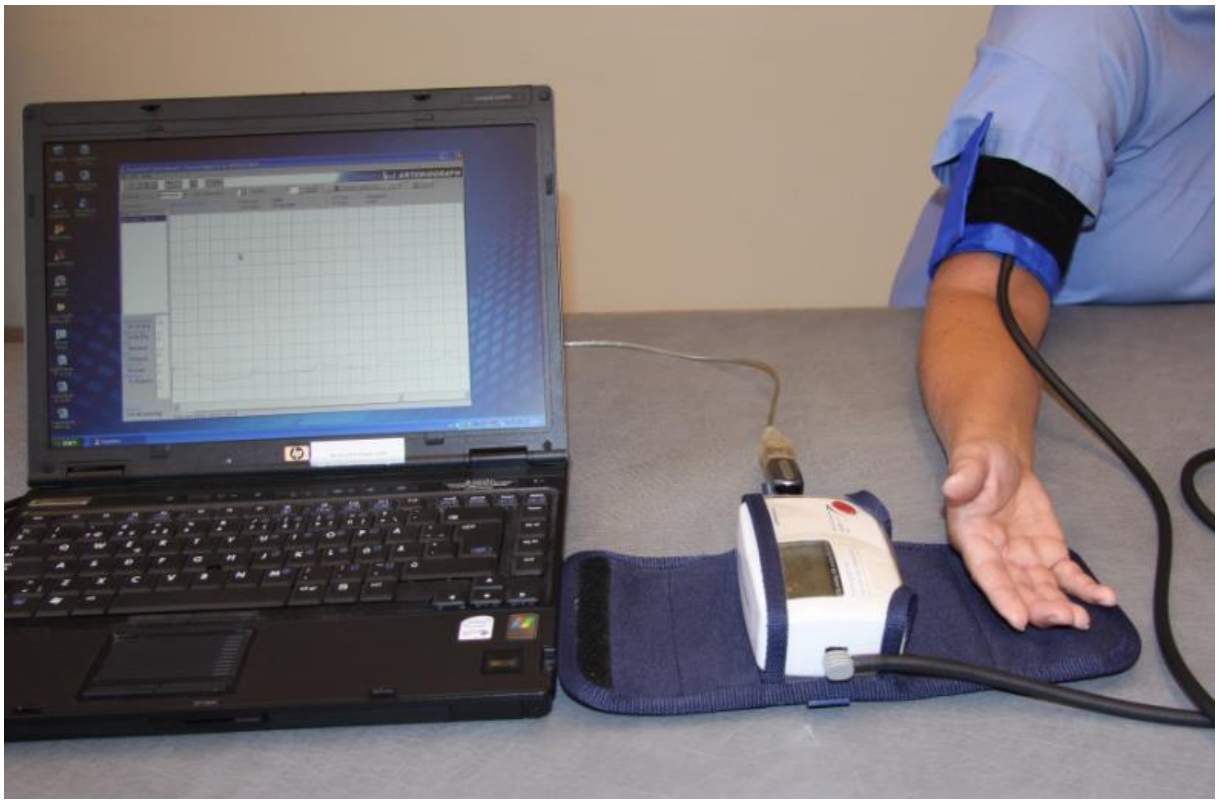


Figure 12. Arteriograph with the cuff on the arm connected to the receptor which is connected to the computer via an infrared communication. The oscillometric method estimates PWV, Aix and cSBP from the pulse curve obtained during cuff occlusion of the brachial artery.

The blood pressure is measured at the first inflation of the cuff. Thereafter, volume and pressure changes from the cuff are detected when the cuff is inflated to a pressure of 35 mmHg above the SBP. The weak pressure and volume variations in the cuff are received by a pressure receptor and transferred via an IR port to the computer, which calculates AIx, PWV, and cSBP. Pulse waves from a patient with normal blood pressure are shown in **Figure 13A** and a patient with hypertension in **Figure 13B**.

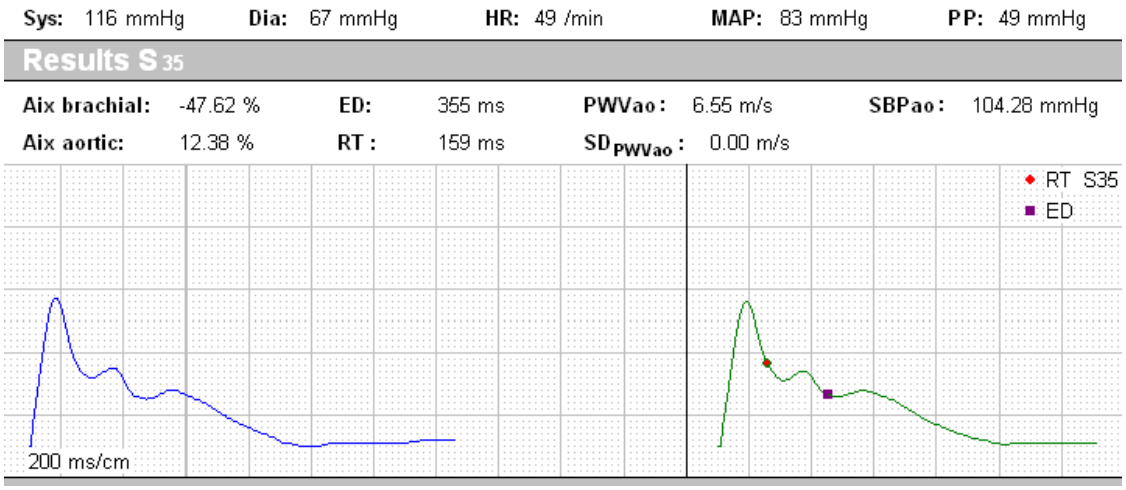


Figure 13A. Measurements by the Arteriograph in a patient with normal blood pressure.

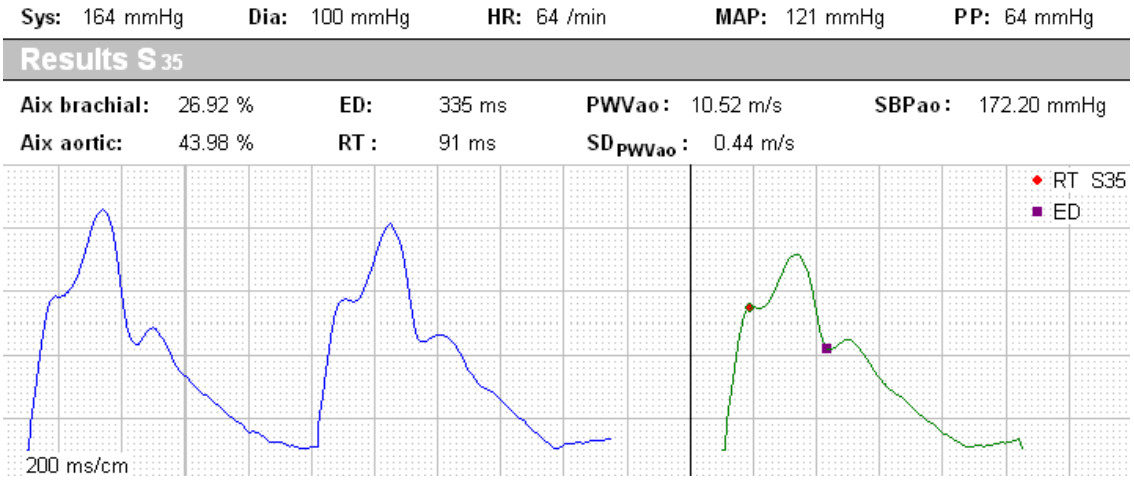


Figure 13B. Measurements by the Arteriograph in a patient with hypertension.

2.5.3 Photoplethysmographic method

The photoplethysmographic technique by Pulse Trace (**Figure 14**), which measures DVP, is based on the transmission of infrared light, usually at 940 nm, through the finger pulp, where the amount of light transmitted through the finger is proportional to the blood volume.[26, 27] The infrared light provides pulsatile signals. The finger clip includes an infrared light-emitting diode and a photodiode. The photodiode measures the emitted infrared light through the fingertip.

The first peak of the pulse wave is caused by the ejection of the blood volume from the left ventricle to the finger, where it is reflected back to the aorta and then to the finger, creating a second peak (**Figure 14**). Earlier studies have shown a relationship between RI and age,[99] and RI and blood pressure.[100] However, PWV and measures by photoplethysmography seem to correlate weakly with each other.[100, 101]

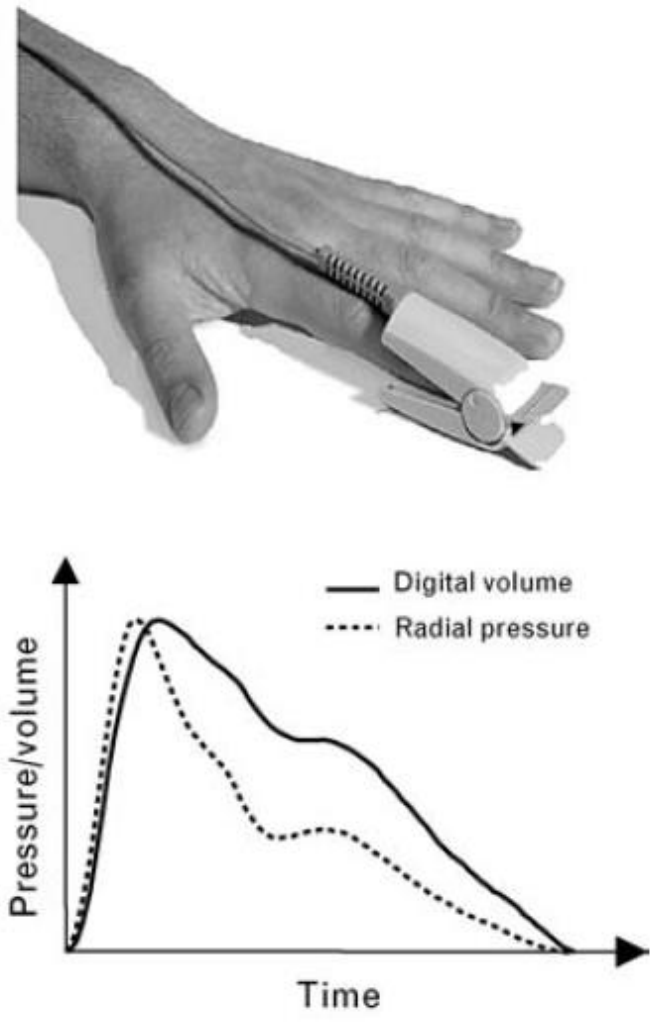


Figure 14. Photoplethysmograph with a light-emitting diode and sensor within a finger clip. *This illustration by Sandrine C. Millasseau[27] is used with permission from the Journal of Hypertension, 2006.*

3 AIMS

The general aim of this thesis is to evaluate both new and more established noninvasive techniques for the measurement of arterial structure and function.

Specific aims:

- To study two semiautomated techniques for measurement of the cIMT (Study I).
- To compare two noninvasive techniques for recording of pulse waves and measurement of PWV and AIx and their relationship to the cIMT in healthy people (Study II).
- To study whether vascular structure and function are influenced by PTH, calcium, and vitamin D levels in patients with mild pHPT without CV risk factors before and after parathyroidectomy in comparison with healthy subjects (Study III).
- To study the influence of gender and physical activity on vascular structure and function in people with normal or impaired glucose tolerance or T2DM (Study IV).

4 METHODS

4.1 MATERIALS AND STUDY DESIGN

All studies were approved by the Regional Ethics Review Board of the Karolinska Institutet, Stockholm, Sweden, and all participants gave their written informed consent.

Participants in Studies I–III were examined after an overnight fast and in Study IV after a 4-hour fast. Height and weight were measured immediately before the noninvasive investigations. A schematic overview of the four Studies, with the study titles and numbers of participants included in the studies, is given in **Figure 15**.

Study I

Forty-eight patients with mild pHPT without know CV disease and 51 healthy controls were included. The patients were recruited consecutively from referrals for parathyroid surgery caused by mild pHPT at the Karolinska University Hospital in Stockholm, Sweden, from January 2006 to November 2008; the patients were recruited primarily for Study III. A healthy control group of people matched by age, gender, and geographic area was randomly selected from the population registry of the city of Stockholm.

Study II

Sixty-three healthy nonsmoking participants, aged 20–69 years (42 women) were included in this study. They were selected from the population registry of the city of Stockholm or recruited by local advertisement at the Karolinska Institutet.

Study III

In this prospective case–control study, 410 patients (319 women) with pHPT who had been accepted for parathyroid adenomectomy (PTX) were evaluated consecutively for possible participation between January 2006 and November 2008. The inclusion criteria were age >18 but <70 years; BMI <28 kg/m^2; calcium <3.0 mmol/L; no diagnosed hypertension, diabetes mellitus, or renal diseases; no current smoking; and no medication affecting the CV system.

Fifty-three patients with mild pHPT were included consecutively in the study. Five patients were excluded: one patient because of a later finding of familial hypocalciuric hypercalcemia, two men because they were taking lipid-lowering medication, one woman who regretted her decision to undergo PTX, and one man because of the loss of carotid image due to storage failure.

A healthy control group, matched by age, gender, and geographic area, was randomly selected from the population registry of the city of Stockholm. They received a mailed invitation to participate in the study and were recruited if they had normal calcium and PTH levels, and fulfilled the inclusion criteria listed above. Two subjects were excluded from participation because of hypertension and high PTH level. Finally, 48 pHPT patients (13 men, 35 women) and 48 healthy controls (13 men, 35 women) were included in the study.

Baseline vascular values (PWVao, AIxao, IMTrad, cIMT, and cIM-GSM) in the pHPT patients were measured and compared with those in healthy controls. The vascular effects of normalizing PTH, calcium, and vitamin D after PTX were studied in the pHPT patients.

Study IV

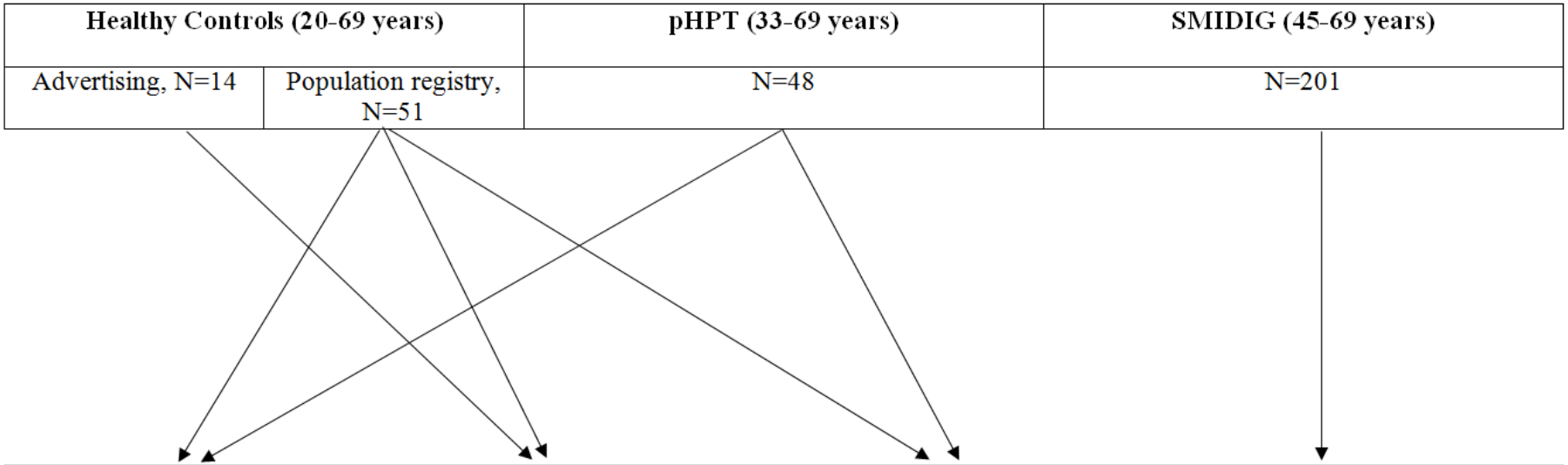
Two hundred twelve participants were included in a randomized controlled study performed at the primary health care center at Gustavsberg, Stockholm County, Sweden, and at the Department of Clinical Physiology, Karolinska University Hospital, Stockholm, during the years 2006 to 2008.

The inclusion criteria were age 45–69 years, BMI >25 kg/m^2, and HbA1c level for those with T2DM between 7.4% and 9.3% (National Glycohemoglobin Standardization Program (NGSP) standard). The exclusion criteria were insulin treatment, SBP >160 mmHg or DBP >100 mmHg, symptoms of angina pectoris, physical impairment, or atrial fibrillation on the baseline ECG. Based on the results of an oral glucose tolerance test (OGTT), the subjects were classified as having normal glucose tolerance (NGT), impaired glucose tolerance (IGT), or T2DM. Blood glucose concentration was measured 2 hours after the OGTT, which involved ingestion of 75 g of glucose in water solution. NGT was defined as a blood glucose concentration <8.9 mmol/L, IGT as glucose concentration between 8.9 and 12.1 mmol/L, and T2DM as glucose concentration ≥12.2 mmol/L.

The subjects were randomized into an intervention group or a control group at their first study visit. The subjects in the intervention group were instructed to increase their level of physical activity by walking with poles (Nordic walking) 5 hours/week for 4 months from May to September. The physical activity was self-reported. The participants in the control group were told not to change their level of physical activity. All subjects were also instructed not to change their eating habits. Of the initial 212 patients, 201 (108 women) were included in the study. Three subjects were excluded from participation because of high blood pressure, two were excluded because of other diseases, and six were excluded for personal reasons.

At baseline and after 4 months, all participants performed an exercise test on a Rodby cycle ergometer RE 820/830 (Rodby Innovation AB, Hagby, Vänge, Sweden). The initial work load was set at 50 W and was increased by 10 W/min. Baseline measures of vascular structure and function (PWVao, AIxao, SI, RI, cIMT, cIM-GSM, and IMTrad) were compared between gender and diagnosis groups (NGT, IGT, or T2DM), and the vascular effects of 4 months of Nordic walking were studied.

Participants



Study I	Study II	Study III	Study IV
Ultrasound measurements of carotid 'intima-media' thickness by two 'semi-automated' analysis systems. 99 healthy controls and pHPT patients Methods: Ultrasound.	Arterial stiffness estimation in healthy subjects: A validation of oscillometric (Arteriograph) and tonometric (SphygmoCor) techniques. 63 healthy controls (49 from population registry) Methods: SphygmoCor, Arteriograph and ultrasound.	Arterial structure and function in mild primary hyperparathyroidism is not directly related to parathyroid hormone, calcium, or vitamin D. 48 pHPT patients 48 matched healthy controls Methods: SphygmoCor, ultrasound, 'high frequency ultrasound'.	Influence of physical activity and gender on arterial function in NGT, IGT and T2DM. 121 NGT 33 IGT 47 T2DM Methods: Arteriograph, pulse trace, ultrasound, 'high frequency' ultrasound.

Figure 15. Schematic overview of the four manuscripts, with study name and number of participants included in the studies.

4.2 INTIMA–MEDIA THICKNESS (IMT)

4.2.1 Common carotid artery (CCA)

In Studies I–IV, the CCA was examined and the best images from the posterior or lateral position, 1–2 cm proximal to the carotid bulb were used in measurements. Two-dimensional (2D) images of the CCA were acquired using a 7L transducer (8 MHz) (Studies I–III) or a 12L transducer (4.9/10.7 MHz) (Studies II and IV) (Vivid 7, GE Vingmed Ultrasound AS, Horten, Norway). Both the right and left CCAs were assessed in Studies I and III, but only the right CCA was examined in Studies II and IV. To provide optimal image quality, a depth of 20–30 mm, a frame rate of about 19 frames/s, and a log gain compensation of 63 dB were used. The gain settings were adjusted to obtain symmetrical brightness in the image. At least three images at the time of the ECG R-wave were stored digitally on magneto optical discs and on an Image Vault 5.0 server system (GE Vingmed Ultrasound AS).

Two validated semiautomated analysis programs were used to measure cIMT: IMT Analysis Package from GE (GE Vingmed Ultrasound AS), and Artery Measurement Software (AMS), developed by the Department of Signals and Systems at Chalmers University of Technology in collaboration with the physiology group at the Wallenberg Laboratory (www.wlab.gu.se), Gothenburg University, Gothenburg, Sweden. Both systems identified the borders of the carotid intima–media automatically. A 10-mm-long segment of the region of interest (ROI) proximal to the carotid bulb was placed manually for detection of the carotid intima–media. The cIMT in the far wall was defined as the distance from the leading edge of the lumen–intima interface to the leading edge of the media–adventitia.[21] Both systems measured the average cIMT within the ROI for each image. The mean cIMT was calculated as the mean value of 3–6 images. In Studies I and III, three images from the left CCA and three images from the right CCA were measured, and a mean value for the cIMT was calculated from the six images. In Studies II and IV, the cIMT was measured in three images of the far wall of the right CCA. An image of the CCA and cIMT measurements is shown in **Figure 4**.

4.2.2 Radial artery

The IMT was measured in the radial artery using a recently introduced high-resolution ultrasound technique. The IMTrad was measured in Studies III and IV (**Figure 5**). 2D images were recorded with a 55 MHz transducer (Vevo 770, VisualSonics, Toronto, Canada) from the

right radial artery in a longitudinal projection 1–2 cm proximal to the fold separating the palm of the hand from the forearm. The IMTrad and lumen diameter (LDrad) were measured according to the leading edge principle.[21] IMTrad and LDrad were measured manually with calipers using VisualSonics software (Vevo 770). Three sets of IMTrad measurements were taken from each of three representative images and are presented as the mean value from nine measurements corresponding to end diastole, the smallest lumen diameter. LD_{rad} is presented as the mean value taken from three representative beats.

4.3 ECHOGENICITY

The echogenicity of the intima–media complex in the far wall of the CCA was investigated in Studies III and IV. The echogenicity is represented as the cIM-GSM and was measured using AMS. The cIM-GSM was measured in the far wall of the CCA in the same image and ROI as that used to measure cIMT using a scale of 256 grey levels ranging from 0 (black) to 255 (white). The adventitia was used as the reference for white and the blood as the reference for black; the measured values are shown as median gray. After the automatic measurement of cIMT, a calibration for the black and white pixels for cIM-GSM was chosen. The calibration boxes were placed manually along the CCA within the ROI (first the black box and then the white box), and the cIM-GSM was measured automatically. An example of the measurement of cIM-GSM is shown in **Figure 6**.

4.4 ARTERIAL STIFFNESS

4.4.1 Ultrasound

In Study II, the right CCA was examined from the best image of the posterior or lateral position, 1–1.5 cm proximal to the carotid bulb. M-mode images of the CCA were acquired using a 7L transducer (8 MHz) or a 12L transducer (4.9/10.7 MHz) (GE Vingmed Ultrasound AS). Measurements of the minimum lumen diameter (at the R-wave on the ECG) and maximum lumen diameter (at the peak of the T-wave on the ECG) were taken, and a mean value of three images was calculated (**Figure 7**).

4.4.2 SphygmoCor

A high-fidelity tonometer (SPT-301B, Millar Instruments, Houston, Texas, USA) and the SphygmoCor equipment (version 7.01, AtCor Medical, Sydney, Australia) were used to

investigate measures of arterial stiffness and central blood pressures in Studies II and III. The PWV and AIx were calculated from noninvasively recorded pulse waves with the tonometer pressed gently to the artery of interest. The tonometer was connected to the SphygmoCor equipment. The arterial pulse waves were processed by the system software, and the corresponding aortic pressure wave form and cSBP were generated from the radial artery wave form using a validated transfer function.[95,96] The radial pulse was calibrated against the brachial blood pressure (Omron M7, Healthcare Co., Ltd., Kyoto, Japan) measured in the right arm just before the examination and are presented as the mean value of two measurements. The participants were studied in the supine position after a rest of about 60 min.

The ECG-gated carotid and femoral artery pressure wave forms were recorded with the SphygmoCor device to obtain the PWVao. A tape measure was used to measure the distance from the carotid site to the jugulum and from the jugulum to the femoral site. The aortic path length was estimated as the subtracted surface length by subtracting the carotid–jugulum length from the jugulum–femoral length ($L_{subtracted}$). The PWVao (m/s) was calculated as $L_{subtracted}$ divided by the difference in time between the beginning of the carotid pulse wave and the beginning of the femoral pulse wave. $L_{subtracted}$ has shown good agreement with invasive measurements.[102, 103]

The time difference (ΔT) or the transit time between the carotid pulse wave and femoral pulse wave was measured. A computerized algorithm, the 'intersecting tangent' algorithm, for PWV calculation was used and was preset in the software.[104] Other algorithms built into the software for PWV calculation were 'maximum dP/dt', 'maximum 2nd derivate',[104] and 'height percent'. The ECG R-wave was used as a reference for timing. The 'intersecting tangent' and 'maximum 2nd derivate' algorithms are shown in **Figure 16**.

In a study from 2010, a European collaboration gathered data from 13 different centers to establish normal and reference values for PWV_{ao} in healthy subjects.[105] The standardized formula for PWVao (St-PWVao) was calculated as $0.8 \times L_{direct} / \Delta T$, m/s. L_{direct} leads to an overestimation of the 'real' PWVao, and the scaling factor (0.8) was used to convert PWV_{ao} to the 'real' or standardized PWVao. The formula for calculation of the direct distance (L_{direct}) was $0.45 \times L_{subtracted} + 0.21 \times height + 0.08$.[106]

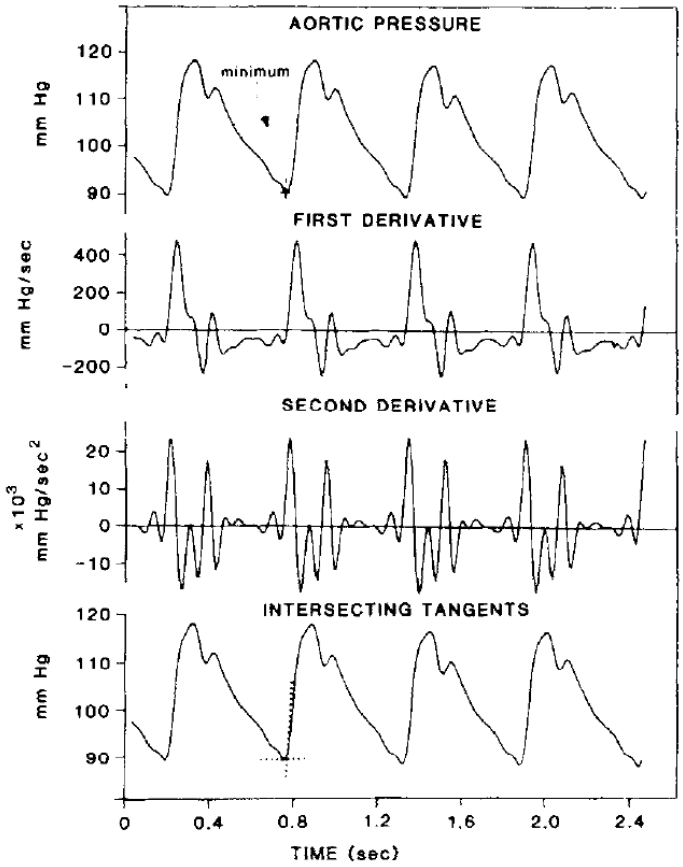


Figure 16. Four methods for determination of the characteristic points are illustrated: the minimum, the maximum first derivative, the maximum second derivative, and the intersection tangents methods.
Used with permission from the American Heart Journal, Y. Christopher Chiu[104]

The AIx was defined as the difference between the second (P2) and the first (P1) peaks of the central aortic wave form and is expressed as a percentage of PP. The PWV recordings were chosen from the wave forms with the smallest SD, and AIx from wave forms within the limits of the current quality control settings with a quality index >0.85 based on pulse height and diastolic variation.

4.4.3 Arteriograph

Studies II and IV used the relatively new method, the Arteriograph (TensioMed Software v.1.9.9.2, Budapest, Hungary), which is based on an occlusion technique. The aortic distance was measured from the jugulum to the symphysis with a tape measure. The cuff was placed on the upper arm and connected to the Arteriograph device. The blood pressure in the upper arm was measured at the initial cuff inflation and, thereafter, the pulse wave configuration was recorded at an inflation of 35 mmHg above the SBP. The weak pressure and volume variations

in the cuff were received by a pressure receptor and transferred via an IR port to the computer. The algorithm measuring blood pressure in the AG device has been validated.[107]

The surface distance was measured from the jugulum to symphysis, and the RT was measured as the difference in time between the peaks of first (P1) and reflected systolic wave (P2). The pressure wave (P1) is transmitted to the lower part of the body and then reflected from the periphery, which is assumed to be around the aortic bifurcation, and generates the late systolic peak (P2). The PWVao was then calculated as the (jugulum – symphysis) distance (m) divided by RT/2 (s). AIxao was calculated as: 100 × (P2 – P1) / PP. PWVao and AIxao are presented as mean values from two recordings. The two recordings with the smallest SD were chosen, and in these recordings calculations were made from every heartbeat during a period of 8 s. The estimation of cSBP was based on the relationship between the brachial pulse and the aortic pulse.[108]

4.4.4 Photoplethysmograph

The DVP was studied using the Pulse Trace technique (B 2.00, Micro Medical Gillingham, Kent, UK) in Study IV. The DVP was measured after at least 30 min of rest in the supine position. The SI (m/s) is a measure of large artery stiffness and the RI (%) is a measure of vascular tone in small arteries. The SI was calculated as the subject's height divided by the distance between the first systolic peak and the reflected peak. The RI was calculated by the formula: 100 x reflected peak / early systolic peak (%). The pulse waves were recorded for 10 s, and the RI and SI were derived from the pulse wave form and are presented as the mean values from three recordings measured from the right index finger.

4.5 LABORATORY METHODS

All blood samples were obtained in the fasting state. All biochemical measures were analyzed at the Laboratory of Clinical Chemistry at Karolinska University Hospital, Stockholm. Plasma glucose concentration was measured by a HemoCue B-Glucose analyzer in Study IV. In Study III, renal function was estimated by calculating the glomerular filtration rate (GFR) according to the Cockroft–Gault formula: GFR = (140 – age in yr) × (weight in kg / creatinine concentration) × (1.23 in men or 1.04 in women). Vitamin D deficiency was defined as a 25(OH)D concentration <50 nmol/L.

4.6 BODY MASS INDEX AND BODY SURFACE AREA

BMI in Studies I–IV was calculated by dividing weight (kg) by the square of height (m^2). Body surface area (BSA) in Study III was calculated as: $0.007184 \times (\text{weight, kg})^{0.425} \times (\text{height, cm})^{0.725}$.

4.7 SYSTEMIC VASCULAR RESISTANCE

In Studies II and IV, the systemic vascular resistance (SVR, mmHg × min/L) was calculated as mean arterial blood pressure (MAP) in mmHg divided by cardiac output (CO) in L/min. Using transthoracic echocardiography with an M4S transducer and Vivid 7 (GE Vingmed Ultrasound AS, Horten, Norway), the LV outflow tract (LVOT) diameter was measured in systole from the parasternal long axis view. The velocity–time integral (VTI) was measured from a pulsed Doppler blood flow recording in LVOT from the apical view. SV was calculated according to the formula $SV = \pi \times (\text{LVOT diameter} / 2)^2 \times \text{LVOT VTI}$. CO was calculated by multiplying SV by heart rate (HR), and MAP was calculated as $DBP + 1/3 \times (SBP - DBP)$. Pulsed Doppler for VTI was recorded at the end of the investigation and and blood pressure measured directly after.

4.8 STATISTICS

Statistical analyses in Studies I–IV were performed using Statistica (StatSoft, Inc. version 9.0., Tulsa, OK, USA). PASW Statistics v18 (PASW Inc., Chicago, IL, USA) was also used in Study III, and SAS 9.3 (SAS Institute Inc., Cary, NC, USA) in Study IV. All data are expressed as mean and SD. The statistical tests were two-tailed and $p<0.05$ was considered significant. Spearman's rank correlation coefficient, *r*, was computed to assess the relationships between variables. The Mann–Whitney *U* test for unpaired data was used to compare between genders and between patients and controls. The Wilcoxon signed-rank sum test was used to test differences between methods and for intraindividual analyses. Univariate and stepwise multiple linear regression analyses were used to evaluate relationships and to adjust for confounders. Results from the multiple regression analyses are presented as standardized beta and adjusted R^2. Intraclass correlation coefficient (ICC) was used to evaluate the strength of agreement between the methods in Study I. An ICC > 0.75 is considered good reliability, and ICC > 0.90 is often required for clinical applications to ensure the valid interpretation of findings.

The Bland–Altman test was used to evaluate the variability between methods and intra- and interobserver variability. The coefficient of variation (CV%), defined as the SD of the absolute differences between measurements divided by the mean of two measurements, was used to evaluate variability.

In Study IV, to analyze the baseline variables in the data, a two-way factorial ANOVA was used. In the ANOVA model, the effects of the factors gender and diagnosis group (three levels), and the interaction between gender and diagnosis group were analyzed. When the Levene's test of homogeneity of variances between groups indicated large variations of the variance across groups, a mixed-model analysis considering inhomogeneity of variances was used instead. If the distribution of the outcome variable was too skewed to the right, the variable was transformed with a log transformation based on the natural logarithm.

To analyze the differences between 4-month and baseline variables, a three-way factorial ANOVA was used. The ANOVA model included analysis of: (1) the effects of the factors gender and diagnosis group (two levels), and their intervention; (2) the interactions between gender and diagnosis group, between gender and intervention, and between diagnosis and intervention; and (3) the three-factor interaction between intervention, diagnosis, and gender. When the Levene's test of homogeneity of variances between groups indicated problems with the variance, a cross-group mixed-model analysis considering inhomogeneity of variances was used instead.

5 RESULTS

5.1 STUDY I

In this study, we compared two semiautomated techniques, GE and AMS, for the measurement of cIMT. The mean SBP was 120±13 mmHg, and the mean DBP 76±8 mmHg. The mean age of the study population was 54.4±8.9 years. Women (71%) were significantly older than men (56.1±8.3 years versus 50.2±9.0 years, $p<0.01$).

The cIMTmean (mean of left+right cIMT) measured by GE and AMS correlated strongly with each other (r=0.90, $p<0.001$). Slightly significantly higher values were measured by GE (0.72±0.12 mm) than by AMS (0.69±0.12 mm, $p<0.001$) (Table 1, Paper I). Comparisons between the cIMT measured by GE and AMS are shown in Bland–Altman and correlation plots (Paper I, Figure 2A–2C). The mean difference in cIMT between GE and AMS was 0.03 mm. The CV% for intraobserver variability for each method was evaluated in 30 subjects. The CV% for cIMTmean (left+right) was 1.0% for GE and 2.2% for AMS. The difference in cIMTmean (left+right) between the GE and AMS software and the limits of agreement for measurements performed by one reader on two separate occasions are shown in Bland–Altman plots in Paper I (Figure 3A and 3B). For AMS, one observer measured the cIMTmean (left+right) in the same image with both adjusted and unadjusted edge detection of the CCA far wall in 30 subjects (not shown in Paper I). The CV% for the variability between the adjusted and unadjusted cIMT measures was 3%. The relationships are shown in a Bland–Altman plot; the unadjusted values were slightly higher (0.71±0.12) compared with the adjusted values (0.69±0.12; $p<0.01$) (**Figure 17**).

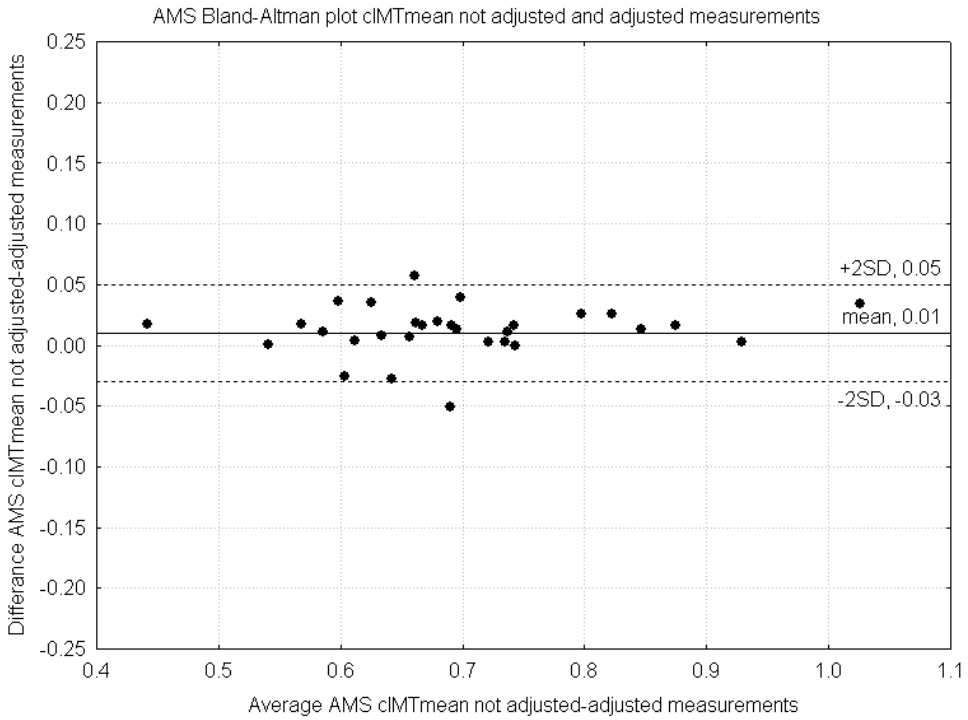


Figure 17. Bland–Altman plot comparing the common carotid intima–media thickness (cIMT) in mm, cIMTmean (left+right), not adjusted versus adjusted edge detection in 30 subjects by AMS.

5.2 STUDY II

Study II compared two noninvasive techniques: the well-established tonometric SphygmoCor technique as a reference, and the recently introduced oscillometric Arteriograph technique. AIx and PWV were measured in healthy subjects, and the associations between these values and carotid ultrasound measures and traditional CV risk indicators were evaluated.

Arterial stiffness was measured in 63 healthy subjects with a mean age of 48.0±14.9 years (range 20–69 years). The women (51±14 years) were significantly older than the men (41±15 years, $p<0.01$). PWVao was higher when measured by the Arteriograph (8.00±2.16 m/s) than by the SphygmoCor (6.87±1.47 m/s) ($p<0.001$) but did not differ significantly from St-PWVao measured by the SphygmoCor (7.68±1.58 m/s). PWVao measured by the Arteriograph correlated with PWVao (r=0.54) and St-PWVao (r=0.59) measured by the SphygmoCor ($p<0.001$). For values below the median (7.4 m/s), St-PWVao measured by the SphygmoCor (6.78±0.83 m/s) was significantly higher than PWVao measured by the Arteriograph (6.37±0.54 m/s) ($p<0.01$). PWVao by SphygmoCor and Arteriograph did not differ significantly below median. For values above the median (7.4 m/s), PWVao was higher when measured by the

Arteriograph (9.62±1.94 m/s) compared with St-PWVao measured by the SphygmoCor (8.58±1.66 m/s). The Bland–Altman and correlation plots are shown in **Figure 18A** and **18B**.

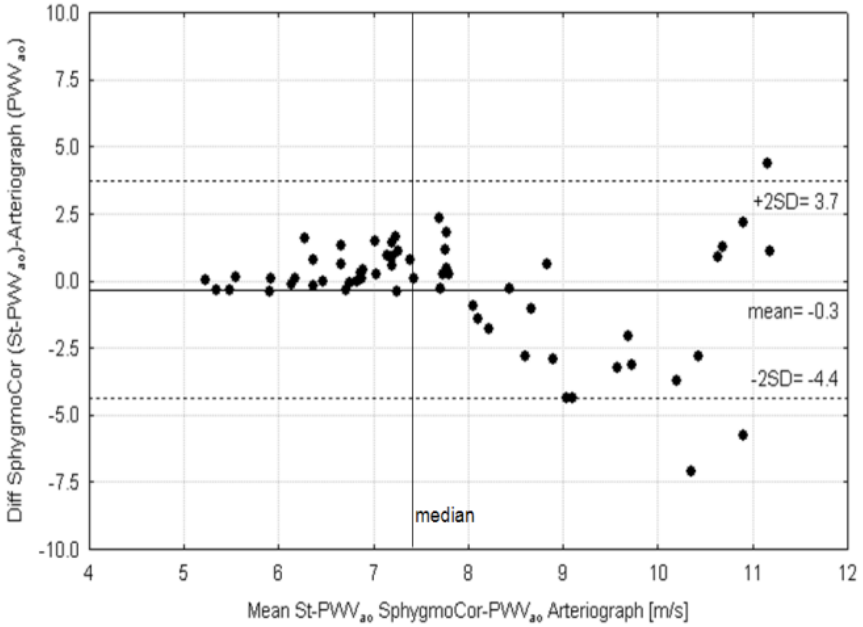


Figure 18A. Bland–Altman plot testing the agreement between standardized PWVao measured by the SphygmoCor (St-PWVao SC) and PWVao measured by the Arteriograph (PWVao AG). *Used with permission from Nature Publishing Group.*

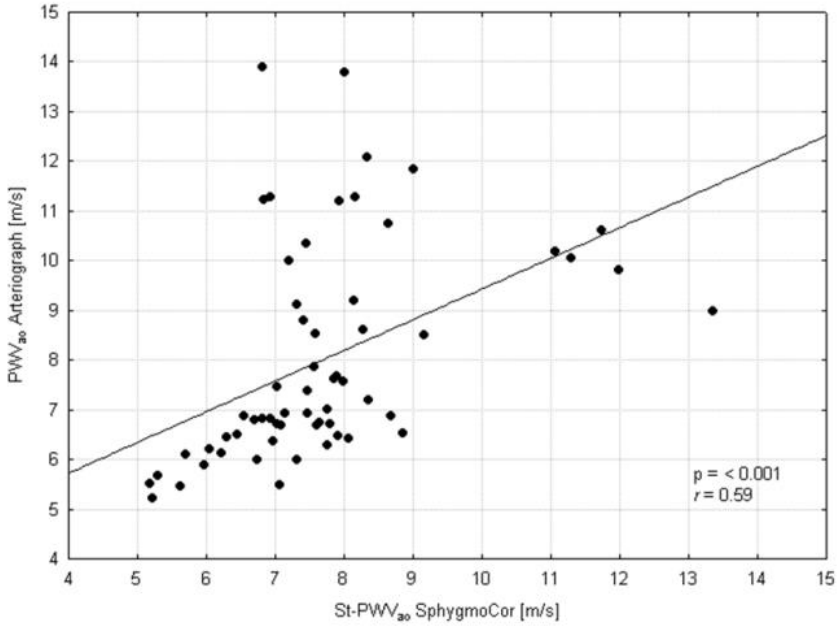


Figure 18B. Correlation between standardized PWVao measured by the SphygmoCor (St-PWVao SC) and PWVao measured by the Arteriograph (PWVao AG). *Used with permission from Nature Publishing Group.*

PWVao differed significantly when measured by the SphygmoCor and the Arteriograph in women ($p<0.001$), but not in men ($p=0.40$). The Bland–Altman and correlation plots are shown in **Figure 19A** and **19B**.

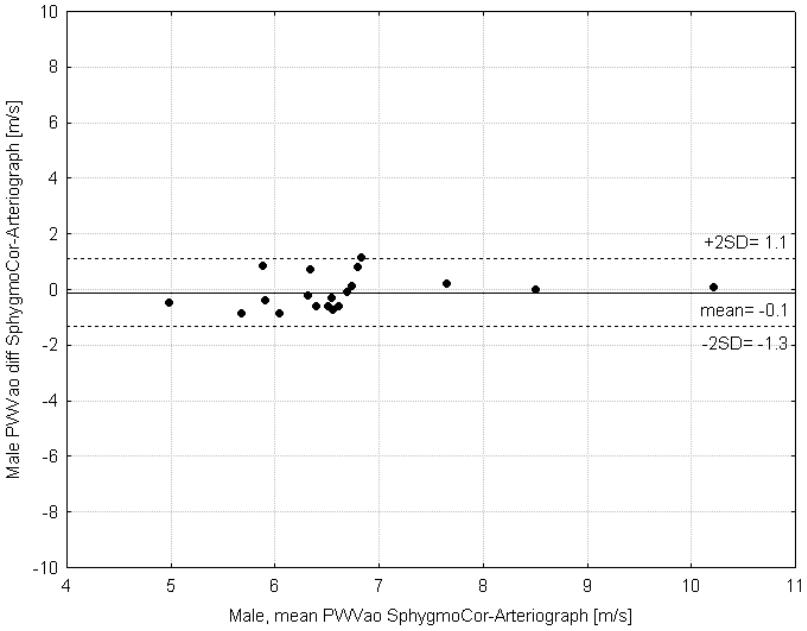


Figure 19A. Bland–Altman plot illustrating PWVao measurements by the SphygmoCor (SC) and the Arteriograph (AG) in 21 males. *Used with permission from Nature Publishing Group.*

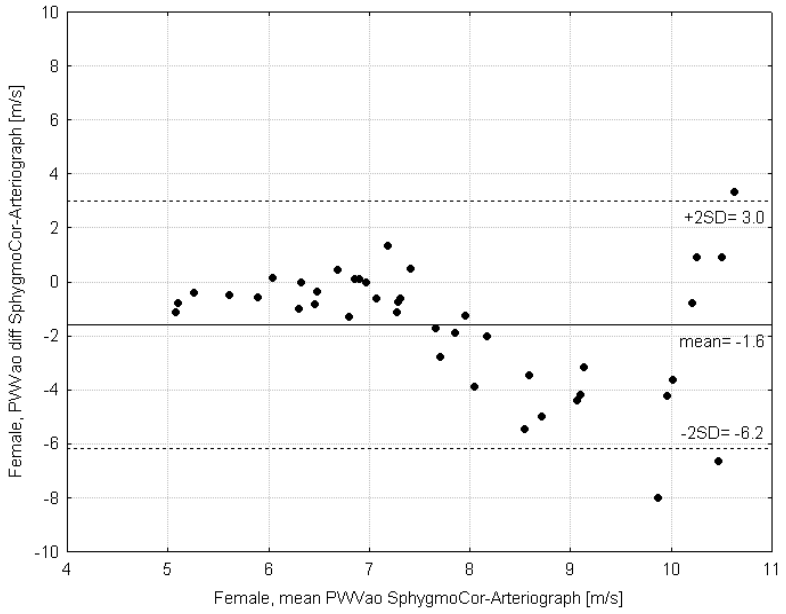


Figure 19B. Bland–Altman plot illustrating PWVao measurements by the SphygmoCor (SC) and the Arteriograph (AG) in 42 females. *Used with permission from Nature Publishing Group.*

AIxao measured by the Arteriograph (27.5±14.5%) was higher than AIxao measured by the SphygmoCor (20.5±17.4%) ($p<0.001$), but these values correlated closely ($r=0.97$, $p<0.001$).

cSBP was significantly higher when estimated by the Arteriograph (112±17 mmHg) than by the SphygmoCor (106±15 mmHg) ($p<0.001$), but these values correlated significantly ($r=0.88$, $p<0.001$). St-PWVao, PWVao, and AIxao by measured by the SphygmoCor, and PWVao and AIxao measured by the Arteriograph correlated significantly with serum cholesterol concentration and cIMTmean ($p<0.001$ for each).

This study showed that arterial stiffness indices measured by the Arteriograph and the SphygmoCor correlate with vascular risk markers in healthy subjects. AIxao measured by the Arteriograph and the SphygmoCor were closely correlated, but the Arteriograph gave higher values. PWVao measured by the Arteriograph corresponded overall with St-PWVao by the SphygmoCor, but SphygmoCor gave significantly higher values in the lower range (submedian), where nonstandardized PWVao by Sphygmocor did not differ from Arteriograph. Our results imply the need to apply the same technique in repeated studies.

5.3 STUDY III

In this prospective case–control study (see Paper III), we examined the effects of PTH, calcium, and vitamin D levels on arterial structure and function in patients with mild pHPT without known CV disease. All patients with pHPT were studied at baseline and again 1 year after PTX. The values for the patients were compared with those from healthy age- and gender-matched controls. No significant differences were observed between pHPT patients and controls at the baseline for AIxao (28.6±12.2% versus 27.7±12.8%), IMTrad (0.27±0.06 mm versus 0.26±0.05 mm), cIMT (0.69±0.11 mm versus 0.68±0.14 mm), or cIM-GSM (82.3±17.2 versus 86.5±15.3). PWVao was slightly higher in patients (8.68±1.50 m/s versus 8.13±1.55 m/s, $p<0.05$) and did not decrease after PTX (**Table 2**).

SBP and calcium and PTH concentrations were higher in patients compared with controls at the baseline and decreased after PTX in the patients. Vitamin D concentration was lower in patients and increased after PTX in the patients. SBP correlated significantly with AIxao ($r=0.39$), PWVao ($r=0.50$), cIMT ($r=0.44$) (all $p<0.001$), and IMTrad ($r=0.26$, $p<0.05$). PWVao correlated weakly with the concentrations of plasma PTH ($r=0.29$, $p<0.01$) and ionized calcium ($r=0.22$, $p<0.05$), but these relationships were no longer significant after adjustment for age and SBP.

Table 2. Augmentation index and ultrasound measurements in healthy controls and patients with mild primary hyperparathyroidism before and after parathyroidectomy.

Variable	Controls	PHPT		PHPT baseline	
		Baseline	Follow-up	vs. Controls	vs. Follow-up
	n=48 (35 F)	n=48 (35 F)	n=48 (35 F)	p-value	p-value
AIx, %	27.7±12.8	28.6±12.2	29.8±11.9[a]	0.881	0.333
PWV_{ao}, m/s	8.13±1.55[b]	8.68±1.50[c]	8.61±1.37[c]	0.027	0.095
Ao SBP, mmHg	109.8±15.3	114.4±17.3	114.8±15.8[a]	0.205	0.473
Ao DBP, mmHg	75.0±9.7	78.1±11.3	78.0±8.7[a]	0.097	0.181
IMT_{rad}, mean, mm	0.255±0.053[a]	0.271±0.060[c]	0.271±0.046[b]	0.340	0.623
LD_{rad}, mean, mm	1.87±0.35[a]	1.79±0.30[c]	1.90±0.38[b]	0.199	0.020
IMT_{cca}, mean, mm	0.680±0.135	0.688±0.113	0.702±0.119[a]	0.354	0.172
LD_{cca}, mean, mm	5.94±0.62	6.03±0.45	6.03±0.47[a]	0.229	0.197
IM-GSM, mean	86.5±15.3	82.3±17.2	81.8±15.9[a]	0.091	0.941

[a]n=47, [b]n=43–45, [c]n=36–41. Values are shown as mean±SD. F= female, AIx=augmentation index, PWV_{ao}=aortic pulse wave velocity, Ao SBP=aortic systolic blood pressure, Ao DBP=aortic diastolic blood pressure, IMT_{rad}=intima–media thickness in the right radial artery, LD_{rad}=lumen diameter in the right radial artery, IMT_{cca}=intima–media thickness in both the left and right common carotid arteries, LD_{cca}=lumen diameter in both the left and right common carotid arteries, IM-GSM=intima–media gray-scale median in both the left and right common carotid arteries. *Used with permission from PLoS ONE in accordance with the Creative Commons Attribution License.*

AIxao, IMTrad, and cIMT did not correlate with the concentrations of vitamin D, PTH, or ionized calcium. cIM-GSM correlated inversely with body weight (r=–0.31, $p<0.01$) and PTH level (r=–0.24, $p<0.05$). The interobserver variability for cIM-GSM, and IMTrad is shown by Bland–Altman plots in **Figures 20** and **21**.

In summary, we found no indices of arterial structure or function that were related to high calcium or PTH level or low vitamin D level in patients with mild pHPT without CV risk factors. Nor did we observe any changes 1 year after PTX. However, we found somewhat

increased arterial stiffness, as indicated by higher PWV in patients, which relates partly to blood pressure.

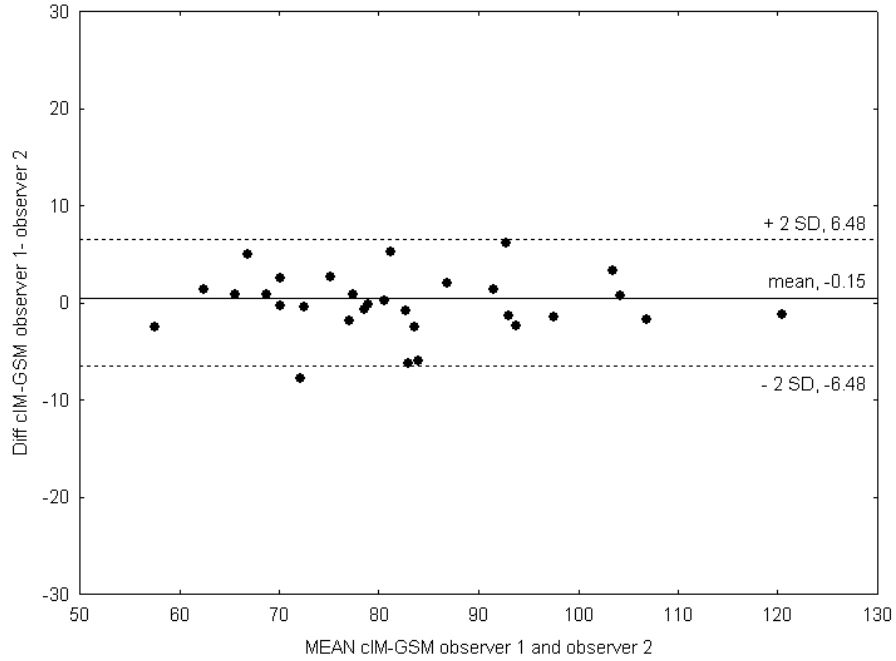


Figure 20. Bland–Altman plot of interobserver variability of intima–media gray-scale in the carotid artery. *Used with permission from PLoS ONE in accordance with the Creative Commons Attribution License.*

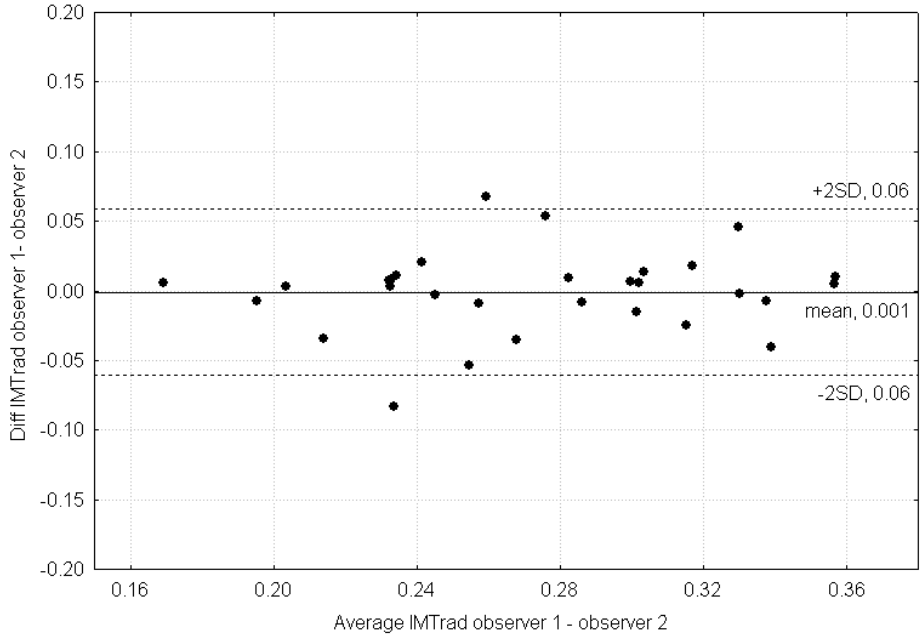


Figure 21. Bland–Altman plot of interobserver variability of intima–media thickness in the radial artery. *Used with permission from PLoS ONE in accordance with the Creative Commons Attribution License*

5.4 STUDY IV

In Study IV, we investigated whether low-intensity exercise training by Nordic walking (walking with poles) over 4 months improved CV function in middle-aged men and women with NGT, IGT, or T2DM. Because the sample sizes were small, the two latter groups were grouped together in the analysis.

Two hundred one participants were included in a randomized (intervention or control) study: 121 had NGT, 33 IGT, and 47 T2DM. The intervention group was instructed to increase their weekly level of physical activity by 5 hours of Nordic walking for 4 months, and controls were instructed not to change their physical activity. PWVao, AIxao, SI, RI, and cIMT were measured at the baseline and after 4 months, and the new methods were used to measure IMTrad and cIMT-GSM at the same times.

In women compared to men, PWVao was higher, and SI and RI were lower in NGT, while RI was lower in IGT. The IMTrad was lower in women than in men with NGT and T2DM. None of the vascular variables differed significantly between the intervention group and the control group after 4 months of physical activity in the NGT and in the combined 'IGT + type 2 diabetes' groups. Vascular variables showed no gender difference in response to intervention over 4 months.

6 DISCUSSION

The World Health Organization (WHO) considers CV diseases as the number one cause of mortality from the global perspective.[1] Degeneration of the arterial wall caused by atherosclerosis leads to increased IMT and arterial stiffness, the earliest stage and marker of CV disease. Data collected in epidemiological studies evaluating these markers of atherosclerosis have contributed to knowledge about CV risk factors, monitoring the progress of atherosclerosis, and planning intervention strategies. The development of computer-based methods for measurement of IMT and stiffness has been essential for research and clinical purposes by minimizing variability and user dependency and enabling standardization of methods. The validation of new modalities against gold standard methods is also crucial for being able to interpret the results obtained with different techniques. The present work focused on comparisons between new and more established noninvasive techniques for evaluation of arterial structure and function.

We studied healthy subjects and patients with expected, minor alterations in the vasculature. The ultrasound techniques designed for vascular studies are easy to apply, and the examination can be completed in a short time. However, these methods are user dependent when obtaining images and especially in terms of measurement methodology. In addition to cIMT, we studied the IMT of the radial artery with a new high-frequency ultrasound.[84] We also assessed the value of a recently introduced method for assessment of echogenicity, cIM-GSM, of the intima–media of the CCA wall. The echolucency of the cIM has been found to be related to CV risk factors such as dyslipidemia, oxidative stress, and inflammation[43] and to triglyceride (TG) level,[109] as well as to obesity and insulin resistance.[110]

Arterial stiffness, measured as PWVao and AIxao, is an important marker of CV risk and is an independent predictor of all-cause CV morbidity and mortality,[30] coronary artery disease,[111, 112] and hypertension.[113] Detection of early vascular aging is becoming more important in everyday clinical practice, and noninvasive techniques to measure arterial stiffness are warranted. New modalities for measurement of arterial stiffness must be validated against established methods. In our study, we compared the more established SphygmoCor, an applanation tonometer technique, with a relatively new method, the Arteriograph, which uses an oscillometric technology for measuring PWVao, AIxao, and cSBP.

6.1 INTIMA–MEDIA THICKNESS (IMT)

cIMT is a strong predictor of stroke and myocardial infarction.[78, 114-116] Measurement of cIMT for detection of subclinical atherosclerosis is currently recommended by several guidelines[116, 117] for assessing CV disease risk. In the clinic, the use of semiautomated edge-detection software for the measurement of cIMT has been found to be timesaving, reproducible, and user independent, and to decrease the interobserver bias when compared with manual tracings.[118, 119] The main objective in Study I was to compare two semiautomated software packages, GE and AMS, for measuring cIMT. We studied 99 participants without known CV diseases and found that cIMTmean measured by GE was slightly, but significantly, higher than that measured by AMS. However, cIMT correlated highly between the two methods despite the fact that the participants lacked overt CV disease and had a rather normal and narrow range of cIMT. Because increased IMT is a marker for atherosclerosis,[77] it may provide an opportunity for early intervention and for monitoring atherogenic disease progression.[82] In Study II of 63 healthy persons, we found that cIMT correlated well with PWVao and AIxao, two other markers of CV risk.[30, 112, 113, 120]

Several ultrasound imaging systems and software packages for edge detection are commercially available for IMT assessment in research and clinical use, and it is important to compare these software packages. Manual and semiautomated measurements have been compared for the GE system[121] and have shown no differences between the methods. For AMS, higher cIMT values were obtained by the semiautomated method compared with the manual method.[122] This could be explained by the operator, who tends to manually set the mark closer to the top of the intensity curve of the lumen–intima interface, which gives a thinner IMT compared with automated measurements.[122]

We measured the same images for both GE and AMS in Study I and the edge-detection lines were not changed manually to reduce bias by the manual settings. Our study identified a standardization problem that has not been previously reported regarding differences in cIMT measurements between two semiautomated systems, GE and AMS. We found that the measurements of cIMTmean were slightly higher by the GE method compared with the AMS method. Our finding stresses the importance of using only one type of measurement program within a study and when evaluating progression of vascular changes or effects of treatment.

The cIMT has been studied in patients with mild pHPT with conflicting results. pHPT patients with CV risk factors have been found to have an increased cIMT.[56, 123] In contrast, studies have shown no differences in cIMT between patients with pHPT and healthy controls, nor improvement after parathyroidectomy.[124, 125] We studied the relationships between vascular effects and vitamin D, calcium, and PTH levels in patients with mild pHPT and healthy controls in Study III. Vitamin D deficiency is associated with CV disease in the general population,[62] and is common in patients with pHPT.[53] In our study, 77% of the patients and 20% of the controls had 25(OH)D values <50 nmol/L. However, vitamin D, calcium, or PTH level were not significantly related to cIMT in patients or controls in our study.

The new high-frequency ultrasound technique was used to assess IMTrad in Studies III and IV. IMTrad is associated with coronary heart disease[87] and hypertension[85] but to our knowledge, it has not been studied in patients with mild pHPT or in studies of the effects of physical activity on people with T2DM. However, in our study we found no correlation between IMTrad and the vitamin D, calcium, or PTH levels.

Four months of Nordic walking may be an insufficient duration to affect the arterial wall structure in terms of cIMT and IMTrad. Decreased progression of cIMT in T2DM patients has been reported after 6 months of brisk walking[126] and supervised aerobic training.[83] Our results showing no differences in cIMT after the physical activity intervention are consistent with those of previous studies that showed no decrease in cIMT after physical exercise of different duration and type (aerobic or resistance training).[127 128] Statin treatment has been proposed to slow the progression of cIMT.[129] In our study, 50% of the T2DM subjects were under statin treatment, which might have influenced our results. However, our results are consistent with those of previous studies,[127, 128] although inconsistent results have also been reported.[126] cIMT and IMTrad correlated with plasma glucose and HbA1c levels, which implies that IGT and T2DM may affect the arterial wall, despite the lack of vascular changes in the current study.

6.2 ECHOGENICITY

We added a new variable—cIM-GSM—to the established measurement of cIMT in the quantitative assessment of the echogenicity in the intima–media of the carotid artery in Studies III and IV. Echogenicity has been measured in both the carotid intima–media and carotid

plaques, and has been found to be related to CV risk factors.[23, 43, 130] People with the metabolic syndrome have been shown to have a more echolucent carotid intima–media complex compared with those without this syndrome.[23] In patients with mild pHPT, cIM-GSM correlated negatively with body weight and PTH level but not with vitamin D or serum calcium level. We found no differences in cIM-GSM between pHPT patients and aged-matched healthy controls at baseline or 1 year after parathyroidectomy.

We also studied effects of a 4-month program of Nordic walking on echogenicity in people with NGT, IGT, or T2DM. cIM-GSM did not differ between men and women at the baseline or after intervention in any of the groups. cIM-GSM correlated negatively with weight, BMI, and TG and HbA1c levels. A similar negative correlation between cIM-GSM and TG level has been described in patients with recent myocardial infarction.[109] In elderly men, echolucency was found to be related to obesity and insulin resistance.[110] There is increasing evidence that cIM-GSM is associated with CV risk factors in different populations, however further studies are needed to establish its role among the vascular indices.

6.3 ARTERIAL STIFFNESS

Arterial stiffness, measured as PWVao and AIxao, is an important marker of CV risk and is an independent predictor of all-cause CV morbidity and mortality,[30] coronary artery disease,[111, 112] and hypertension.[113] There are a number of techniques and modalities available for measuring arterial stiffness.[131-134] We compared the SphygmoCor, which is based on an applanation tonometer technique and used as the gold standard, with the new Arteriograph, which uses an oscillometric technique, in the measurement of PWVao, AIxao, and cSBP. Previous studies have compared the SphygmoCor and Arteriograph techniques in patients with CV diseases,[131-133, 135] but not in healthy individuals. We studied 63 healthy subjects with a wide age range (20–69 years) and found similar differences between the two techniques in the upper range (supramedian) of PWVao, as found in previous studies of populations with CV disease. [131, 133] In the lower range of PWVao values (below a median of 6.9 m/s), the SphygmoCor showed essentially similar values as the Arteriograph. Applying the St-PWVao made average results, over the whole range of values, more equal to Arteriographic values. However, a submedian difference with higher SphygmoCor values appeared, while the supramedian scatter remained. Our results, therefore, did not show any advantage by applying standardized PWVao when using SphygmoCor. When the data were analyzed by gender, it became evident that the

difference in agreement and large spread in values above the median occurred only in women. In men, the agreement between the methods was excellent. This discrepancy between the methods has been previously reported, but not separately according to gender.[131] The reasons why the methods showed greater discrepancy in women need to be studied further.

The estimation of transit time affects the accuracy and precision of regional PWV. The 'intersecting tangent' is the most common computerized algorithm; it accurately allows the determination of the PWV and is proposed to be favorable in the SphygmoCor and is also preset in the software.[104] In a recent report, the algorithms used for estimating pulse transit time by the SphygmoCor have been questioned[136] because of discrepancies for higher PWV values. As a result, the authors suggested a new algorithm, a 'diastole-patching' method, which showed the greatest agreement in comparison with other algorithms used by the SphygmoCor.

AIx, a measure of the vascular function influenced by peripheral arteries, was higher when measured by the Arteriograph than by the SphygmoCor in our study; however, the values correlated well, which is consistent with earlier results.[131, 135] Interestingly, in a report in young males, the authors proposed that AIx is related to cardiac motion more than to arterial stiffness and endothelial function.[93] Further studies are needed to assess whether the relationship between the central pulse wave form and cardiac motion remains over a broader age range, in both genders, and in patients with CV disease.[94]

Comparison of cSBP measured by the two methods showed that higher values were obtained by the Arteriograph than by the SphygmoCor. It is known that the SphygmoCor gives lower cSBP than the Arteriograph because of the algorithm used for calculation of MAP. In a study comparing intravascular central SBP with SphygmoCor-generated central SBP in patients with coronary heart disease, the SphygmoCor-generated pressures were underestimated by 2-23 mmHg. [137] Recently, a new mathematic formula for the calculation of MAP was tested and gave a higher cSBP for the SphygmoCor.[133] However, the results of another study showed more errors using MAP and DBP.[138] AtCor Medical, the manufacturer of the SphygmoCor, recommends the use of SBP and DBP. In our studies, we used SBP and DBP to calibrate the radial wave form.

In Studies III and IV, we investigated whether various factors such as the PTH, calcium, and vitamin D concentrations and reduced glucose tolerance are related to arterial stiffness and

whether physical exercise training affects measures of arterial stiffness. We found no difference in AIxao between pHPT patients and healthy controls at the baseline and 1 year after parathyroidectomy. PWVao was slightly higher in pHPT patients at the baseline but did not decrease after surgery. Previous studies have reported that AIxao increases in patients with mild pHPT,[139, 140] in contrast to our results. However, people with CV risk factors were included in those studies. Increased PWVao was found in pHPT patients both with and without hypertension.[141] In contrast, no difference in PWVao was shown between patients with pHPT and controls.[124] In our study, after careful exclusion of patients with CV risk factors, we found no differences between patients and controls at the baseline apart from a slightly higher, but within the normal range, SBP and PWVao in the patients. PWVao correlated with serum calcium and PTH levels but not with vitamin D level.

In Study IV, we used the Arteriograph to measure PWVao and AIxao, and photoplethysmography to assess the SI and RI. Arterial stiffness in CCA has been reported to be higher in women than in men with insulin-dependent diabetes.[142] In the present study, PWVao, AIxao, SI, and RI did not change with 4 months of Nordic walking, despite we previously demonstrated improved physical capacity and reduced body weight among the participants. [143] There were also no differences in the effects on vascular variables between men and women after the 4 months of increased physical activity in the intervention group. Therefore, longer time and /or more intense physical activity seem required to obtain beneficial vascular effcts that can be demonstrated with the methods we applied.

There is a need for preventive measures in the population, to avoid serious events resulting from the multitude of cardiovascular risk factors noted also in the young. [144, 145] In addition to active reduction of risk factors, physical inactivity being one, pursued efforts are warranted to find and validate noninvasive measures suitable to detect cardiovascular disease at an early stage and to follow and analyze results of corrective efforts.

7 CONCLUSIONS

The research findings reported in this thesis show the following.

- There was a small but significant systematic difference between the cIMT values measured by the semiautomated edge-detection methods AMS and GE.
- The Arteriograph obtained higher PWVao values compared with the SphygmoCor, but values below median did not differ. Use of the standardized PWVao removed the overall difference between SphygmoCor and Arteriograph values, but gave significantly higher submedian values than Arteriograph, while it did not reduce the supramedian scatter. Our results do therefore not support the use of standardized PWVao. The Arteriograph showed higher AIxao and cSBP values compared with the SphygmoCor, but Arteriograph and Sphygmocor correlated well for AIxao. PWVao and AIxao measured by the SphygmoCor and Arteriograph correlated with serum cholesterol level and cIMT. This finding verifies the validity of pulse tracings as risk estimates, even in a healthy population without known CV disease.
- Patients with mild pHPT without CV risk factors showed normal arterial function despite having high calcium and PTH, and low vitamin D levels. Of the vascular variables, only PWVao was slightly higher in patients than in healthy controls. Neither PWVao nor any of the other vascular variables measured with established or new methods showed any improvement 1 year after parathyroidectomy.
- Arterial structure and function was unchanged with intervention in terms of Nordic Walking, without differences by gender or diagnosis. Longer time or higher intensity than four months of low-intensity physical activity seems required to alter vascular function irrespective of glucose tolerance.

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