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HELENA TAIVAINEN

CAROTID ARTERY LONGITUDINAL WALL MOTION IN THE ASSESSMENT OF PRECLINICAL ARTERIOSCLEROSIS

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ABSTRACT

The vascular disease process starts already in childhood or adolescence. There is a problem in detecting the pathological process in time because manifestations of the disease process only appear decades later when permanent structural alterations have already taken place. The Vascular Biomechanics Research Group in Kuopio University Hospital has developed a new non-invasive ultrasound imaging analysis software for measuring the biomechanical characteristics and especially the longitudinal motion of the carotid artery wall. This analysis software opens new possibilities with which to investigate the subclinical vascular changes that precede the later manifesting cardiovascular diseases. The magnitude of longitudinal motion within the vascular wall is similar to that of radial motion. Measuring the longitudinal motion is challenging for technical reasons and it is only recently with advances in modern ultrasound equipment with a high frame has it been possible to gather detailed information about arterial structure. This method has been under development by other research groups. In this dissertation, the new in-house method for analysing longitudinal motion developed in the Vascular Biomechanics Research Group was applied for the first time in clinical research; this represents a notable step forward in validating the new method.

Even arteriosclerosis and atherosclerosis are distinct concepts that express their own features, these concepts are still interdependent. In the arteriosclerotic process, vascular wall stiffening is the central change whereas in the atherosclerotic process, lipid accumulation in the vascular wall, inflammation and plaque formation are observed. When this thesis was started, the association of the longitudinal motion between these two subtypes of preclinical vascular diseases among young and healthy adults was unclear.

The subjects of this thesis were from the on-going national multicentred study of atherosclerosis precursors in Finnish children and young adults - The Cardiovascular Risk in the Young Finns Study. Kuopio centre data is from the year 2007, when the 27-year follow-up was conducted. Total of 465 subjects were evaluated in whom

vascular examinations had been conducted and 292 successful longitudinal motion analysis had been performed. The cross-sectional substudies consisted of 281-292 participants aged between 30- to 45 years. Analysing the ultrasound-video data from the common carotid arteries produced new knowledge of the biomechanical characteristics of the carotid arteries. No corresponding study had previously been performed in such a large, well-characterized and homogenous study population of this age where the longitudinal motion of common carotid artery wall had been evaluated as a marker of vascular health.

The results revealed that the longitudinal motion occurred only partly in the same direction as the blood flow. The main part of the motion was directed backwards against the blood flow. Elastic arteries displayed a larger backward motion and a greater total amplitude of the longitudinal motion than stiffer arteries. Instead, stiffening of the arteries was associated with a minor longitudinal total amplitude and especially with the restriction of its backward oriented component.

In this dissertation, the peak-to-peak and retrograde amplitudes of the longitudinal motion were indirectly associated with blood pressure and body mass index. Retrograde longitudinal motion was also inversely correlated with total cholesterol and triglycerides. It was found that the forward oriented component of longitudinal motion increased when diastolic blood pressure, total cholesterol, LDL and triglyceride levels were elevated, and body weight increased. Furthermore, the backward component of longitudinal motion and the total amplitude decreased and antegrade oriented increased as the number of risk factors increased. The magnitude of correlation coefficients between parameters of CALM and risk factors was comparable with those for traditional measures, intima-media thickness and distensibility. In the third substudy, the presence of metabolic syndrome and insulin resistance caused disturbances in the longitudinal motion. Especially, hypertension, obesity and hyperinsulinemia were associated with reduced total peak-to-peak and retrograde amplitudes and increased antegrade amplitudes. This finding indicates that longitudinal motion reacts also to the disruption glucose metabolism.

Measuring the longitudinal motion is a rather new method with which to investigate vascular health at the level of the vasculature and this dissertation confirms the concept emerging from a few previous studies. As a new finding, carotid stiffness parameters were associated with the longitudinal motion and the presence of the metabolic syndrome and insulin resistance as well as compensatory hyperinsulinemia caused longitudinal motion disturbances in the common carotid artery. The longitudinal motion of the common carotid artery reflects the status of vascular health and it was found to represent the changes in the state of the vascular bed, especially in the subclinical phase of arteriosclerosis but also significant associations with primary atherosclerotic risk factors were found. The longitudinal motion of the common carotid artery is a rather new phenomenon, and the results confirm the concept that measuring the longitudinal motion is a feasible method for evaluating vascular health.

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Medical Subject Headings: Carotid Arteries/diagnostic imaging; Ultrasonography; Arteriosclerosis; Atherosclerosis; Cardiometabolic Risk Factors; Vascular Stiffness; Endothelium, Vascular/physiopathology; Carotid Intima-Media Thickness; Carotid-Femoral Pulse Wave Velocity; Cardiovascular Diseases; Metabolic Syndrome; Insulin Resistance; Hyperinsulinism; Hypertension; Cross-Sectional Studies; Middle Aged; Young Adult; Finland

Keywords: arterial stiffness, arteriosclerosis, atherosclerosis, cardiovascular risk factors, common carotid artery, longitudinal motion, ultrasound imaging

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TIIVISTELMÄ

Valtimonkovettumatautiprosessi alkaa nykytiedon mukaan jo lapsuudessa ja nuoruudessa. Ongelmana on ollut, että sairaudet todetaan vasta, kun ne ovat jo ehtineet tehdä pysyviä muutoksia verisuonten seinämiin. Kuopion yliopistollisen sairaalan Vascular Biomechanics -tutkimusryhmässä on kehitetty uusi ei-kajoava analyysiohjelmisto verisuonten biomekaanisten ominaisuuksien ja erityisesti kaulavaltimon seinämän pitkittäisliikkeen mittaamiseksi ultraäänikuvista. Analyysiohjelmisto avaa uusia mahdollisuuksia tutkia varhaisvaiheen valtimomuutoksia, jotka ennakoivat myöhemmin kehittyviä sydän- ja verisuonisairauksia. Itse valtimon seinämässä ilmenee poikittaissuuntaisen liikkeen lisäksi suuruusluokaltaan yhtä laajaa liikettä pitkittäissuunnassa. Pitkittäisliikkeen tarkka mittaaminen ja kuvausmetodin kehitys on ollut mahdollista vasta viime vuosina, kun nykyaikaisilla ultraäänikuvantamislaitteilla on alettu saada hyvin yksityiskohtaista kuvaa valtimon seinämien rakenteesta ja tallenteita on voitu kerätä riittävän nopealla videokuvauksella. Tässä väitöskirjatyössä uutta analyysiohjelmistoa sovellettiin ensimmäistä kertaa kliinisessä tutkimuksessa ja samalla kyseessä oli merkittävä askel myös uuden menetelmän kliinisessä validoimisessa.

Arterioskleroosi ja ateroskleroosi eivät ole synonyymeja, vaan ne käsitetään erillisiksi sairausprosesseiksi, joilla on omat tyypilliset piirteensä, vaikka ne ovat myös keskinäisriippuvaisia. Arterioskleroosissa verisuonen seinämän jäykistyminen on keskeinen piirre, kun taas ateroskleroosissa keskeisenä voidaan pitää veren rasvayhdisteiden kertymistä suonen seinämään, tulehdusprosessia ja plakin muodostumista verisuonen sisäkerrokseen. Tutkimuksen alussa kaulavaltion pitkittäisliikkeen yhteys näiden kahden sairausprosessin varhaisvaiheeseen on perusterveillä nuorilla aikuisilla epäselvä.

Tutkimushankkeen aineisto pohjautui kansallisessa monikeskustutkimuksessa (Lasten sepelvaltimotaudin riskitekijät LASERI – The Cardiovascular Risk in Young Finns Study) 27-vuotisseurantakäynnillä vuonna 2007 kerättyyn materiaaliin ja

tutkimusjoukkona toimi Itä-Suomen alueella asuvat koehenkilöt (n=281-292), jotka tutkimushetkellä olivat iältään 30-45 -vuotiaita. Valtimoiden biomekaanisista ominaisuuksista saatiin entistä tarkempaa tietoa koehenkilöiden kaulavaltimoiden ultraäänikuvausarkistoa analysoimalla. Vastaavaa tutkimusta, jossa valtimon seinämän pitkittäisliikettä käytettäisiin verisuonen terveyden mittarina, ei ole aiemmin tehty yhtä laajassa ja hyvin karakterisoidussa kliinisessä aineistossa.

Väitöskirjatyössä havaittiin, että pitkittäisliike tapahtuu vain osittain verivirran suuntaisesti. Pääosa pitkittäisliikkeestä suuntautui yllättäen verivirtaa vastaan taaksepäin. Tämä taaksepäin kulkeva liike ja pitkittäisliikkeen kokonaislaajuus olivat sitä suurempia, mitä joustavammaksi tutkittavien suonet oli todettu. Sen sijaan valtimoiden jäykistymiseen viittaavat löydökset olivat yhteydessä pienempään pitkittäissuuntaiseen liikelaajuuteen ja erityisesti taaksepäin suuntautuvan liikkeen rajoittumiseen.

Väitöskirjatyössä havaittiin pitkittäisliikkeen kokonaisamplitudin ja taaksepäin suuntautuvan liikkeen korreloivan käänteisesti kohonneeseen verenpaineeseen ja painoindeksiin. Valtimon seinämän taaksepäin suuntautuva liike korreloi käänteisesti myös veren kokonaiskolesteroli- ja triglyseriditasojen kanssa. Työssä havaittiin myös, että eteenpäin suuntautuva liike suureni diastolisen verenpaineen, triglyseriditasojen ja kokonaiskolesterolin noustessa ja painon lisääntyessä. Riskitekijöiden kasautuminen aiheutti myös pitkittäisliikkeen liikehäiriötä: eteenpäin suuntautuva liike kasvoi ja taaksepäin suuntautuva liike pieneni. Kolmannessa osatyössä havaittiin, että metabolinen oireyhtymä ja insuliiniresistenssi ovat yhteydessä pitkittäisliikkeen liikehäiriöön. Erityisesti kohonnut verenpaine, ylipaino ja hyperinsulinemia olivat yhteydessä pienentyneeseen pitkittäisliikkeen kokonaisamplitudiin ja pienempään taaksepäin suuntautuvaan liikkeeseen sekä eteenpäin suuntautuvan liikkeen suurenemiseen.

Kaulavaltimon pitkittäisliikkeen mittaaminen on melko uusi menetelmä verisuoniterveyden tutkimiseen verisuonen tasolta ja saadut tulokset vahvistivat sitä käsitystä, joka muutamasta aiemmasta tutkimuksesta on saatu. Uutena löydöksenä pitkittäisliikkeen havaittiin assosioituvan etenkin verisuonen seinämää kuvaaviin perinteisiin jäykkyysparametreihin. Lisäksi uutena löydöksenä havaittiin metabolisen oireyhtymän aiheuttavan pitkittäisliikkeen liikehäiriötä, samoin kuin siihen liittyvän insuliiniresistenssin ja kompensatorisen hyperinsulinemian. Kaulavaltimon seinämän pitkittäisliike ilmentää verisuonen seinämän terveyttä, ja tulokset viittaavat pitkittäisliikkeen heijastavan erityisesti arterioskleroosin varhaisvaiheen muutoksia, mutta myös merkittäviä yhteyksiä ensisijaisiin ateroskleroosin riskitekijöihin havaittiin. Kyse on vielä vähän tutkitusta ilmiöstä ja tutkimus vahvistaa käsitystä, että kaulavaltimon seinämän pitkittäisliikkeen mittaaminen on käyttökelpoinen valtimoterveyden arvioinnissa.

Yleinen suomalainen ontologia: kaulavaltimot; ultraäänitutkimus; sydän- ja verisuonitaudit; ateroskleroosi; valtimonkovettumistauti; riskitekijät; insuliiniresistenssi; hyperinsulinismi; kohonnut verenpaine; metabolinen oireyhtymä

Avainsanat: kaulavaltimon seinämän pitkittäisliike, sydän- ja verisuonisairauksien riskitekijät, valtimonkovettumatauti, valtimojäykkyys, ultraäänikuvantaminen, yhteinen kaulavaltimo

”Koska ennaltaehkäisy on inhimillistä ja kannattavaa”

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Kuopio, December 2020
Helena Taivainen

”Koska ennaltaehkäisy on inhimillistä ja kannattavaa”

LIST OF ORIGINAL PUBLICATIONS

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II S. Helena Taivainen, Heikki Yli-Ollila, Markus Juonala, Mika Kähönen, Olli T. Raitakari, Tiina M. Laitinen ja Tomi P. Laitinen. Influence of cardiovascular risk factors on longitudinal motion of the common carotid artery wall. Atherosclerosis 272: 54-59, 2018.

III S. Helena Taivainen, Tiina M. Laitinen, Heikki Yli-Ollila, Markus Juonala, Mika Kähönen, Olli T. Raitakari ja Tomi P. Laitinen. Carotid artery longitudinal wall motion alterations associated with metabolic syndrome and insulin resistance. Clinical Physiology and Functional Imaging, In press.

CONTENTS

ABBREVIATION

ampl	Amplitude
ante	Antegrade direction
AO	Longitudinal motion of adventitia layer
AOampl	Peak-to-peak amplitude of the longitudinal motion of adventitial layer
AOante	Antegrade amplitude of the longitudinal motion of adventitia layer
AOdev	Average deviation of the longitudinal motion of adventitia layer from its initial location
AOretro	Retrograde amplitude of the longitudinal motion of adventitia layer
BMI	Body mass index
CALM	Carotid artery longitudinal wall motion
Cdist	Carotid artery distensibility
CIMT	Carotid intima-media thickness
CV	Coefficient of variation
dev	Deviation
ECG	Electrocardiograph
Eγ	Young`s elastic modulus
FMD	Flow-mediated dilatation of brachial artery
HDL	High-density lipoprotein
HOMA-IR	Homeostasis model assess-ment of insulin resistance
IA	Longitudinal motion between intima-media and adventitia layers
IAampl	Peak-to-peak amplitude of the longitudinal motion between intima-media and adventitia layers
IAante	Antegrade amplitude of the longitudinal motion between intima-media and adventitia layers
IAdev	Average deviation of the longitudinal motion between intima-media and adventitia layers
IAretro	Retrograde amplitude of the longitudinal motion between intima-media and adventitia layers
IMT	Intima-media thickness

IO	Longitudinal motion of intima-media layer
IOampl	Peak-to-peak amplitude of the longitudinal motion of the intima-media complex
IOante	Antegrade amplitude of the longitudinal motion of intima-media complex
IOdev	Average deviation of the longitudinal motion of intima-media complex from its initial location
IOretro	Retrograde amplitude of the longitudinal motion of intima-media complex
LDL	Low-density lipoprotein
MetS	Metabolic syndrome
NO	Nitric oxide
NS	Nonsignificant
PP	Pulse pressure
PWV	Pulse wave velocity
RAlength	Length of the hysteresis curve formed by plotting the diameter change graph and the longitudinal motion against each other
retro	Retrograde direction
ROI	Region of interest
SD	Standard deviation

1 INTRODUCTION

Cardiovascular diseases and their complications are a considerable burden for health care systems as well as causing suffering to the patients; they are still the most common global causes of death (Murray, Lopez 1997, Lopez, Mathers et al. 2006, Joshi, Jan et al. 2008, World Health Organization 2020). The disease process often begins in childhood and adolescence (Strong, Malcom et al. 1999, Raitakari, Juonala et al. 2003), but disruptions are not detected in the cardiovascular system until late middle age or old age, when the disease process has made permanent alterations in vascular bed and target-tissue damages are evident. Prevention of cardiovascular diseases in its early phase would be advantageous in relieving individual suffering and lowering the financial burden on the communal purse.

It would be beneficial to pinpoint at risk individuals early to allocate the effective and needed interventions to prevent the cardiovascular disease process. Prevention and lifestyle modifications have been shown to delay and inhibit already initiated pathological process, and even reverse it (Ornish, Scherwitz et al. 1998, Shai, Spence et al. 2010, Pahkala, Hietalampi et al. 2013). One problem in recognizing individuals at risk is the lack of suitable measuring methods as it is challenging to detect subclinical changes in the vascular beds. However, several non-invasive techniques have been developed and used to identify alterations in arterial structure and function. The intima media thickness of the artery wall (IMT) has been exploited to find the early and advanced atherosclerotic changes (Raitakari, Juonala et al. 2003, Lorenz, Markus et al. 2007, Stein, Korcarz et al. 2008, Perk, De Backer et al. 2012, Touboul, Hennerici et al. 2012). Pulse wave velocity (PWV) and distensibility (Cdist) are used to estimate the stiffness of the artery wall (O'Rourke, M. F., Staessen et al. 2002, Pannier, Avolio et al. 2002, Oliver, Webb 2003, Laurent, Cockcroft et al. 2006, Calabia, Torguet et al. 2011). It is possible to estimate endothelial function with brachial flow-mediated dilatation (FMD) (Celermajer, Sorensen et al. 1992, Corretti, Anderson et al. 2002). These conventional measurements function as surrogate markers that attempt to detect the alterations at the level of the target tissues before the obvious manifestations of cardiovascular diseases. However, the cardiovascular disease process is a multiform phenomenon, and no single method has been shown to provide all-encompassing information with which to assess the health status of an individual´s arteries. For example, although these conventional measurements are widely used in epidemiological studies, measuring carotid IMT is not recommended in clinical practise to evaluate an individual`s risk for the first cardiovascular disease event, or to prognosticate the course of the cardiovascular disease process (Lorenz, Polak et al. 2012, Touboul, Hennerici et al. 2012, Goff, Lloyd-Jones et al. 2014). In some cases, IMT can be recommended when clinically indicated (previous transient ischemic attack (TIA) or cerebrovascular disease, presence of carotid bruit) (Williams, Mancia et al. 2018). PWV is considered as impractical and also not recommended for clinical routine practice (Williams, Mancia et al. 2018). A better understanding of the

pathophysiology and improved methods for finding individuals at elevated cardiovascular disease risk are urgently needed.

The longitudinal motion of the carotid artery (CALM) is a rather new focus of interest as it has been shown to illustrate vascular function. Earlier this phenomenon was considered as insignificant, but decades later, the amplitude of the longitudinal motion of the common carotid artery has been reported to be of the same magnitude as the diameter change occurring in the common carotid artery in healthy individuals (Persson, Rydén Ahlgren et al. 2003, Cinthio, Rydén Ahlgren et al. 2006). This longitudinal motion of the common carotid artery has found to be bidirectional, where the inner part of the vessel, called the intima media region, first moves along the blood flow and then turns back (Cinthio, Rydén Ahlgren et al. 2006, Au, Ditor et al. 2016). The motion in the inner part of the vessel wall also has been shown to be larger than that in the outer part of the vessel wall (Persson, Rydén Ahlgren et al. 2003, Cinthio, Rydén Ahlgren et al. 2006). The typical longitudinal motion waveform for an individual has been reported to remain stable over months (Rydén Ahlgren, Cinthio, Persson et al. 2012). However, there are variations in the waveform between individuals (Cinthio, Rydén Ahlgren et al. 2006, Tat, Au et al. 2015) and cardiovascular risk factors seem to influence the longitudinal motion of the common carotid artery, e.g. effects on amplitudes and direction have been found (Svedlund, Gan 2011a, Zahnd, Vray et al. 2012, Tat, Psaromiligkos et al. 2016a). The longitudinal motion as a phenomenon has been suggested to be a potential new indicator of arterial health (Svedlund, Eklund et al. 2011, Svedlund, Gan 2011a, Zahnd, Boussel et al. 2011, Rizi, Au et al. 2020) although the driving force for this phenomenon still remains unclear. Rather few investigators have examined the association between the cardiovascular risk and longitudinal motion, especially the associations between longitudinal motion and other surrogate markers of vascular diseases are poorly understood. There had been no overall consensus of associations between cardiovascular risk and longitudinal motion as a phenomenon until this year, when the first expert consensus -review of CALM was prepared (Rizi, Au et al. 2020).

Measuring the longitudinal motion is non-invasive but challenging to perform and a special technology is needed for the ultrasound image analysis. In this thesis, a motion tracking analysis software, which had been earlier being developed in our laboratory (Yli-Ollila, Laitinen et al. 2013, Yli-Ollila, Laitinen et al. 2014), was applied for the first time in clinical research. This is a notable step forward in the validation of this new method.

Arteriosclerosis and atherosclerosis are distinct but interdependent pathological processes. These terms are sometimes thought of as synonyms, but they have their own distinctive features. In the arteriosclerotic process, vascular wall stiffening is the fundamental change whereas in atherosclerosis, inflammation and plaque formation are the key characteristics. The term arteriosclerosis is also utilised as an umbrella concept and its subtypes are different arteriosclerotic states (like arteriolosclerosis, Mönkenberg´s sclerosis and hyaline arteriosclerosis) and atherosclerosis. In this thesis, atherosclerosis and vascular stiffening arteriosclerosis are considered as two

distinct concepts and the relationship of carotid wall longitudinal motion to arteriosclerosis and atherosclerosis among young adults will be examined.
We have measured the longitudinal motion in a large, well defined study population (The Cardiovascular Risk in Young Finns Study) and made new findings and gathered information regarding the longitudinal motion phenomenon. Associations with conventional cardiovascular measurements and cardiovascular risk factors in early phase of disease process as well as the effect of metabolic syndrome (MetS) on longitudinal motion phenomenon have been evaluated. Corresponding study had previously not been performed in such a large, well-characterized and homogenous study population of this age where the longitudinal motion of common carotid artery wall had been evaluated as a marker of vascular health. The aim of the study was to determine, whether a longitudinal motion analysis could provide new findings and information to help detect early pathological alterations in the vascular bed and consider if these findings could serve as a new reliable indicator of vascular health.

2 REVIEW OF THE LITERATURE

2.1 ARTERIAL PHYSIOLOGY AND BIOMECHANICS

2.1.1 Basics of blood circulatory system

The function of the arteries is to transport blood under relatively high and descending pressure from the heart towards the capillary beds, where the transfer of oxygen, nutrients, hormones, electrolytes, carbon dioxide and other metabolic breakdown products, cells of the immune system and many other substances occurs between the blood and the interstitial fluid (Hall 2016). The need for oxygen homeostasis has been the driving force in the evolution of a functioning circulatory system (Semenza 2007). Major diseases and mortality involve changes in vascularization and oxygen delivery to tissues (Semenza 2007).

The two main blood circulatory systems are the systemic and pulmonary systems; the third is a portal blood system, which connects two capillary systems with another and does not depend on the central pumping of the heart (Stevens, Lowe 2005, Hall 2016). In the systemic circulation, oxygenated blood is transferred from the left ventricle of the heart via arteries to all body tissues except the lungs under high pressure. In addition, small bronchial arteries originating from the systemic circulation supply oxygenated blood to the supporting tissues of the lungs. In the pulmonary circulation, deoxygenated blood with a high carbon dioxide content is transferred from the right ventricle to the pulmonary arterial system with its lower blood pressure because of the shorter distances that the blood needs to be transported than in the systemic circulation. Venules and veins carry blood back from capillary beds to the right chamber of the heart from the systemic circulation and to the left chamber from the pulmonary circulation at relatively low blood pressure. Veins are also a major reservoir of extra blood.

The principle of a three-layered structure of blood vessel is found throughout the circulatory system, but the specific features of the layers vary considerably in the different vessel types, especially in the media-layer and extracellular matrix, reflecting their distinct functional roles (Young, Lowe et al. 2006). The exception is found in capillaries, where just one layer of endothelial cells supported by pericytes is found, with the two other layers, tunica media and adventitia being absent (Young, Lowe et al. 2006, Semenza 2007).

2.1.2 Basics of the arterial system

The arterial system consists of three types of arteries: elastic arteries, muscular arteries, and arterioles (Young, Lowe et al. 2006). The cyclical contraction of the heart ventricles produces a pulsatile blood flow first into the elastic, also named conducting, arteries including the major distribution arteries like the aorta,

brachiocephalic trunk (the innominate), common carotid arteries, subclavian arteries, iliac arteries and most of the large pulmonary arteries (Moore, Dalley 1999, Young, Lowe et al. 2006). These arteries have a large diameter and a high proportion of elastic tissue in the walls enabling the expansion and recoil of arterial walls in a response to the pulsatile blood flow. This helps to maintain the blood pressure between heart contractions and smoothens out the systolic pressure wave. Figure 1 illustrates the aortic arch and its main branches.

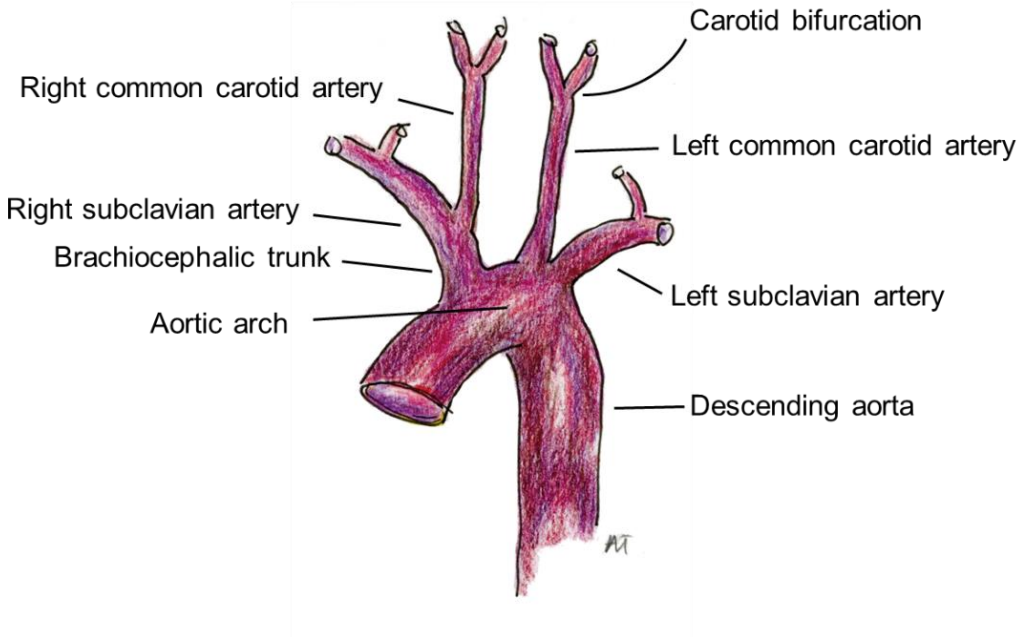

Figure 1. The aortic arch and its branches. The location of the common carotid arteries and the carotid bifurcation where the common carotid artery divides into the internal and external carotid arteries.

Distal to the large arteries, the artery walls become proportionally more muscular and artery walls lose most of their elastic sheets. Already in the distal aorta, there are changes in the collagen-to-elastin ratios (Safar, M. E., Levy et al. 2003). The muscular arteries, such as radial, femoral, renal, coronary, and cerebral arteries, are the main distributing branches of arterial tree. In muscular arteries, elastic tissue is mainly present as two well-defined elastic sheets, an internal elastic lamina and an external elastic lamina (Young, Lowe et al. 2006). The prominent tunica media is composed almost entirely of circularly arranged smooth muscle fibres which are highly contractile with a few elastic fibres between them (Stevens, Lowe 2005, Young, Lowe et al. 2006). By regulating the diameter of these distributing vessels, the body is able to regulate the blood flow and thus ensure the oxygen supply to various organs and tissues and also actively alter the propagation of the blood flow velocity (Safar, M.

E., Levy et al. 2003). This is principally under the control of the autonomous nervous system, especially sympathetic, and adrenal medullary hormones, endothelium-derived vasoactive substances, and cellular interactions (Hall 2016).

The size of the arteries gradually decreases from large elastic arteries to muscular arteries when they branch within tissues until they form arterioles, the smallest type of arteries (Safar, Michel E. 2007). The tonus of the smooth muscle in the arteriolar wall regulates the blood pressure in the arterial system (Hall 2016). When the tonus increases, blood pressure rises; thus, arterioles are also major reflection sites for the pulse wave (Safar, M. E., Levy et al. 2003).

2.1.3 **The structure and function of elastic artery**

In an elastic artery, such as the common carotid artery, the tunica intima is composed of a single layer of flattened polygonal endothelial cells, connected to each other by junctional complexes, and supported by a layer of collagenous tissue rich in elastin (Stevens, Lowe 2005, Young, Lowe et al. 2006) (Figure 1 and 2). Elastin is present in the form of both fibres and as a discontinuous sheet. The thin subendothelial supporting tissue contains also myointimal cells; these are contractile cells with some of the features of smooth muscle cells, but they also share some of the features of fibroblasts, like the capability to synthetize elastin and collagen. These myointimal cells can also have similar phagocytic properties as macrophages have.

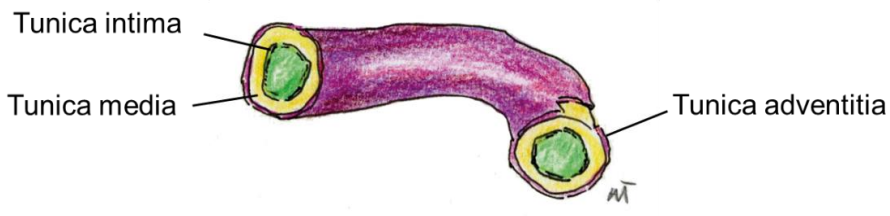

Figure 2. An elastic artery and its three-layered structure: the innermost layer is the tunica intima, the thickest is the tunica media; finally, the tunica adventitia is located in the outer layer.

Tunica media is the middle and the thickest layer of the elastic artery wall (Figure 2). It is highly elastic and consists of concentric fenestrated sheets of elastin separated by collagenous tissue and smooth muscle fibres (Stevens, Lowe 2005, Young, Lowe et al. 2006). The structure of medial elastin influences arterial wall mechanical properties and determines arterial function (Avolio, Jones et al. 1998). The high elastin content in the elastic artery allows it to expand during systole and recoil during diastole. The smooth muscle content in the media-layer grows from aorta in the direction of muscular arteries (Young, Lowe et al. 2006). The tunica adventitia is located on the outside of the tunica media (Figure 2). This is a collagenous structure, with loose connective tissue; it contains vasa vasorum, small

arterioles, that penetrate also to the outer half of the media, as well as the autonomic nerve fibres (Young, Lowe et al. 2006, Kumar, Abbas et al. 2015c). Vasa vasorum transports oxygen and nutrients to the outer portion of the media in the large arteries.

The common carotid artery is illustrated in Figure 1 and 2. It is located near the skin and for this reason, it can be non-invasively accessed, e.g. in ultrasound studies. Pathological alterations in the common carotid artery such as arterial wall thickening have been shown to correlate with the presence of coronary atherosclerosis (Mancini, Dahlöf et al. 2004) and carotid artery stenosis is considered as a marker of systemic atherosclerosis (Jusufovic, Skagen et al. 2019). Local carotid arterial stiffening has also been related to the presence and severity of cardiovascular disease (Giannattasio, Capra et al. 2007). The carotid artery is considered to be the first vessel commonly involved in the atherosclerotic process and its evaluation is thought to provide valuable information about both arterial aging and the atherosclerotic risk definition (Maloberti, Meani et al. 2015).

2.1.4 **The role of endothelium**

The endothelial cells are multifunctional cells forming a monolayer called the endothelium which is the interface between the vascular wall and blood flow; these cells function as a central regulator of vascular homeostasis (Hayoz, Mazzolai 2007). In response to chemical and physical signals, endothelial cells produce multiple autocrine and paracrine vasoactive substances.

For example, endothelial cells maintain the appropriate balance between anticoagulative and coagulative process by synthesizing and secreting molecules which minimise pathological thrombus formation (Kumar, Abbas et al. 2015b). Endothelial cells synthesize molecules that promote the protective thrombus formation if there is vascular damage and bleeding, e.g. von Willebrand factor (Factor VIII). In haemostasis, local neurohumoral, endothelium-derived factors such as endothelin cause a local vasoconstriction, which inhibits bleeding. Endothelial cells also control the passage of fluids and macromolecules between blood and the surrounding tissues (Pober, Min et al. 2009).

The endothelium modulates the vascular tone by secreting vasoactive substances that influence the vasoreactivity of the underlying smooth muscle cells by relaxing them with e.g. nitric oxide (NO), prostacyclin, endothelium-derived hyperpolarizing factors and C-type natriuretic peptide, or contracting them with e.g. endothelin-1 and thromboxane (Veerasamy, Bagnall et al. 2015). Hence it is the constriction and relaxation of smooth muscle cells which maintains the balance between oxygen supply and demand in downstream tissues and ensures organ perfusion. Endothelial cells synthesize the constituents of the basement membrane collagen and proteoglycans and act as a permeability barrier. The permeability of the endothelium depends on its location in the vascular tree (Young, Lowe et al. 2006).

The endothelium suppresses acute inflammatory reactions and participates in immune surveillance (Pober, Min et al. 2009). Endothelium mediates acute

inflammatory reactions by expressing cell adhesion molecules and producing bioactive molecules e.g. interleukins 1,6, and 8 and chemokines, growth factors, vasoactive substances that act as vasoconstrictors or vasodilators, procoagulants and anticoagulant factors and variety of other endothelium-derived factors (Pober, Cotran 1990, Kumar, Abbas et al. 2015a). Endothelium also mediates the proliferation and neo-vascularization of smooth muscle cells (Vita, Keaney 2002, Veerasamy, Bagnall et al. 2015).

In times of endothelial activation, the endothelium adapts to changes in its environment and this activation is defined as the acquisition of a new endothelial function that benefits the host (Pober, Min et al. 2009). There are many inducers e.g. cytokines, bacterial and lipid products, viruses, hypoxia, hemodynamic stress and advanced glycation end-products; these trigger the endothelial cells to respond by adjusting their constitutive functions and by expressing inducible properties e.g. increased expression of adhesion molecules and procoagulants and altered expression of cytokines, chemokines and growth factors (Kumar, Abbas et al. 2015a). In the normal endothelial function, there is a balance between activation mechanisms.

2.1.5 Endothelial dysfunction

When there is endothelial dysfunction, the normal balance of endothelial cell responses for different stimuli has been disturbed (Trepels, Zeiher et al. 2006, Hayoz, Mazzolai 2007). In many pro-inflammatory and pro-thrombogenic conditions, as well as in conditions where normal laminar shear stress has been altered by either abnormal low or bidirectional blood flow, one encounters conditions of increased endothelial activation (Pober, Cotran 1990, Trepels, Zeiher et al. 2006, Hayoz, Mazzolai 2007, Incalza, D'Oria et al. 2018). Activation of the endothelium precedes dysfunction, but when the recovery mechanisms fail to maintain the normal endothelial functions, then the onset of endothelial dysfunction is seen (Pober, Min et al. 2009). The principal cause of endothelial dysfunction is usually an injury or death of endothelial cells (Pober, Min et al. 2009).

Endothelial dysfunction has a role in the early stages of atherogenesis (Quyyumi 1998, Kinlay, Libby et al. 2001, Vita, Keaney 2002, Hayoz, Mazzolai 2007) and preceedes the development of the morphological changes (Veerasamy, Bagnall et al. 2015). A close relationship with peripheral artery and coronary artery dysfunction has been found, pointing to the concept of a systemic endothelial dysfunction, although the effects in different sites of the vascular bed may differ (Kuvin, Patel et al. 2001). Endothelial dysfunction seems to have a role also in the ultimate phase of vascular disease; it may contribute to the stages of the disease when patients develop clinical symptoms (Vita &Keaeney 2002) i.e. endothelial dysfunction is associated with an increased risk of cardiovascular disease events (Veerasamy, Bagnall et al. 2015).

In situations of increased endothelial activation and in endothelial dysfunction, the expression of cell-surface adhesion molecules is increased, promoting the recruitment and attachment of inflammatory cells (Pober, Min et al. 2009). Under inflammatory conditions, cytokines are secreted, and this induces the activation of endothelial cells (Incalza, D'Oria et al. 2018). Oxidative stress has a notable role in mediating the production of cytokines (Zhou, R., Yazdi et al. 2011, Bulua, Simon et al. 2011) and it also reduces the bioavailability of NO (Cai, Harrison 2000). Shear stress also seems to modulate the redox state of the endothelium (Hojo, Saito et al. 2002). In the endothelium, nitric oxide (NO) is a key factor maintaining vascular homeostasis (Incalza, D'Oria et al. 2018). NO regulates vascular tone acting directly to smooth muscle cells, but it has also a pleiotropic influence on many other factors in the endothelium, e.g. affecting the balance and mediating the action of vasoconstrictors as well as limiting platelet adhesion and aggregation, preventing the recruitment of leukocytes, inhibiting smooth muscle cell proliferation and the production of various tissue factors (Hayoz, Mazzolai 2007). When NO production becomes reduced or the degradation of NO is elevated, the signs of endothelial dysfunction appear. The cascade of NO degradation triggered by superoxide anions leads to the formation of toxic by-products and end products that contribute to dysfunction and even the death of endothelial cells (Incalza, D'Oria et al. 2018).

Traditional cardiovascular risk factors such as diabetes, hypercholesterolemia, hypertension, smoking, aging and family history, are all associated with endothelial dysfunction (Vita, Keaney 2002, Hayoz, Mazzolai 2007) but also other pathological conditions such as mental stress or specific drugs have been claimed to influence the molecular mechanisms regulating NO availability (Toda, Nakanishi-Toda 2011, Soultati, Mountzios et al. 2012). Physiological flow conditions seem to have a favourable effect on inflammation, switching off atherogenic genes and switching on their atheroprotective counterparts, at least under *in vitro* conditions (Hayoz, Mazzolai 2007). In contrast, unfavourable biomechanical forces can influence the structure and function of endothelial cells and at the level of complex transcriptional gene regulation, lead to the expression of genes that promote atherogenesis (Davies, Polacek et al. 1999, Pober, Min et al. 2009, Davies 2009). The presence of specific sites in arteries like bifurcations, branches and curvatures induces disturbances in the laminar blood flow; turbulence increases and shear stress decreases and it is believed that these sites are more vulnerable to pathological lesion formation (Ross 1999).

Improvement of endothelial function by physical exercise, smoking cessation, lipid-lowering medication, angiotensin-converting enzyme inhibitors have all been shown to reduce the cardiovascular risk (Vita, Keaney 2002). They also have been demonstrated to improve endothelium-dependent vasodilation in the peripheral and coronary vascular system (Vita, Keaney 2002). There is also evidence that antioxidants, like ascorbic acid, increase the bioavailability of NO (Tomasian, Keaney et al. 2000).

2.2 THE NATURAL COURSE OF ARTERIOSCLEROSIS

Arteriosclerosis is an umbrella concept for arterial stiffening; wall-thickening and loss of elasticity (Kumar, Abbas et al. 2015a). Elastin is a very inert substance and remains chemically unchanged for decades, but over the years it can suffer physical damage similar to other non-living materials (O'Rourke, M. 1995). Fragmentation of elastic lamellae can cause increased artery stiffness (Virmani, Avolio et al. 1991, O'Rourke, M. F., Kelly 1993) and increased cyclic stress may trigger fragmentation of elastin and calcification, especially in older patients (Safar, M. E., Levy et al. 2003). In addition, chemical modification of collagen, like the breakdown and glycation result in changes in arterial stiffness (Safar, M. E., Levy et al. 2003). Although elastic lamellae fragmentation is considered as a primary cause of arterial stiffening, fibrous remodelling appears to be the second event according to O´Rourke (1995), where the media-layer of elastic artery was shown to be disorganized and this disorganized area displayed evidence of mucoid degeneration and medionecrosis. This has been observed in both the elderly and in patients with long-standing hypertension (O'Rourke, M. 1995). The weakened wall areas predispose the individual to aortic dissection and aortic rupture associated with aortic dissection. Degeneration of the media layer of the aorta has been linked with increased stiffness and dilatation of aorta (O'Rourke, M. 1995). The central arteries have been shown to be more vulnerable to medial degeneration than peripheral muscular arteries, where expansion due to pulsatile blood flow is minor and smooth muscle cells and collagenous elements appear to protect elastin components (Boutouyrie, Laurent et al. 1992, O'Rourke, M. 1995). The small cerebral arteries contain regions where medial elastin fibres are poorly protected by the surrounding smooth muscle cells and elastin may stretch as much as in aorta or in the carotid arteries, predisposing the cerebral vessels to degeneration. This could explain the development of some aneurysms and their potential rupture (O'Rourke, M. 1995). When the walls of large arteries become stiffer and the cushioning function of the arteries decreases, central systolic arterial pressure increases, and diastolic arterial pressure decreases (Lakatta, Levy 2003). This leads also to an increase in the pulse pressure.

2.2.1 Classification of arteriosclerosis

Arteriosclerosis can be divided into several subtypes, arteriolosclerosis, Mönckeberg´s medial sclerosis and atherosclerosis (Kumar, Abbas et al. 2015a). This precise classification has originated from the different forms of vascular pathology and their clinical consequences. Different features are visible in microscope. In arteriolosclerosis, especially small arteries and arterioles are affected and it may cause ischemic injuries downstream (Kumar, Abbas et al. 2015a). Two anatomic variants, hyaline and hyperplastic variants have been identified. In the hyaline variant, there is a homogeneous hyaline thickening with an associated luminal narrowing. This occurs as a response to hypertension; there is an increased smooth muscle cell matrix synthesis as well as increased plasma protein leakage through the

injured endothelial cells. Hyaline arteriolosclerosis is often seen in older people, but patients with hypertension show a more generalized arteriolosclerosis which is more severe than in normotensives. The other variant, hyperplastic arteriolosclerosis, appears in patients with severe hypertension. Arterioles show concentric, laminated thickening with luminal narrowing. The numbers of smooth muscle cells increase, and the basal lamina is thickened. (Kumar, Abbas et al. 2015a).

In Mönckeberg´s medial sclerosis, the calcification of the muscular artery wall occurs and typically involves the internal elastic lamina. This type of calcification is typically clinically insignificant, since it does not encroach into the lumen of the vessel; it is encountered in patients older than 50 years. (Kumar, Abbas et al. 2015a).

Atherosclerosis is the most clinically significant pattern of arteriosclerosis. It is mostly a focal disease and affects coronary, cerebral, and peripheral vasculature (Safar, Michel, Frohlich 2007, Kumar, Abbas et al. 2015a). Significant morbidity and mortality are attributable to its major clinical manifestations including ischemic heart disease, ischemic stroke, and peripheral arterial disease. Ischemic heart disease is the leading global cause of premature adult mortality (Herrington, Lacey et al. 2016). In Europe, cardiovascular diseases are responsible for 3.9 million deaths every year, with 1.8 million deaths occurring in the European Union (Wilkins, Wickramasinghe et al. 2017). In 2015, there were just under 11.3 million new cases of cardiovascular diseases diagnosed in Europe.

Arteriosclerosis of large and muscular arteries and atherosclerosis may overlap with each other, and it is not easy to demonstrate causality (O'Rourke, M. 1995). The concept of arteriosclerosis and atherosclerosis are considered sometimes as synonyms nowadays, but still they can be divided, and the subtype atherosclerosis has its own features that differ from other arteriosclerotic changes (Table 1.)(Safar, Michel E. 2007).

Table 1. Feature differences between arteriosclerosis and atherosclerosis.

Feature	Arteriosclerosis	Atherosclerosis
Location	media and adventitia layers	intima layer
Distribution	diffuse	focal
Physiology	large artery stiffening	inflammation
Pathology	elastin decreases, collagen and Ca^{2+} increases	plaque formation
Geometry	dilatory, tortuous	occlusive
Hemodynamic	left ventricular workload increases, increase in pulse pressure	ischemia
Clinical manifestations	left ventricular hypertrophy, pulsating perfusion at tissue level, microvascular complications	potential plaque rupture and infarction, macrovascular complications

Modified from Safar ME, Frolich ED(eds): Atherosclerosis, large Arteries and Cardiovascular Risk, Adv Cardiol. Basel, Karger, 2007, vol 44, pp 1-18 (Safar, Michel E. 2007).

Arteriosclerosis is understood as stiffening and a dilatation of elastic and muscular arteries; it starts in the media layer and attenuates the cushioning function of vascular walls (O'Rourke, M. 1995). By increasing systolic pressure and pulse pressure, it disturbs the heart upstream and arteries in general, but this does not

influence the conducting function of arteries (O'Rourke, M. 1995). Nonetheless, the increase of pulse pressure and systolic blood pressure may evoke endothelial damage and initiate the atherosclerotic process in the vascular wall (O'Rourke, M. 1995, Frolich, Susic 2007). Atherosclerosis alters the morphology of the vascular wall and may influence the stiffness of an artery (Frolich, Susic 2007). Atherosclerosis starts primarily in the intima layer, is a focal phenomenon and in general is occlusive (Table 1.) (O'Rourke, M. 1995, Safar, Michel E. 2007). The arteriosclerotic process is demonstrated in Figure 3.

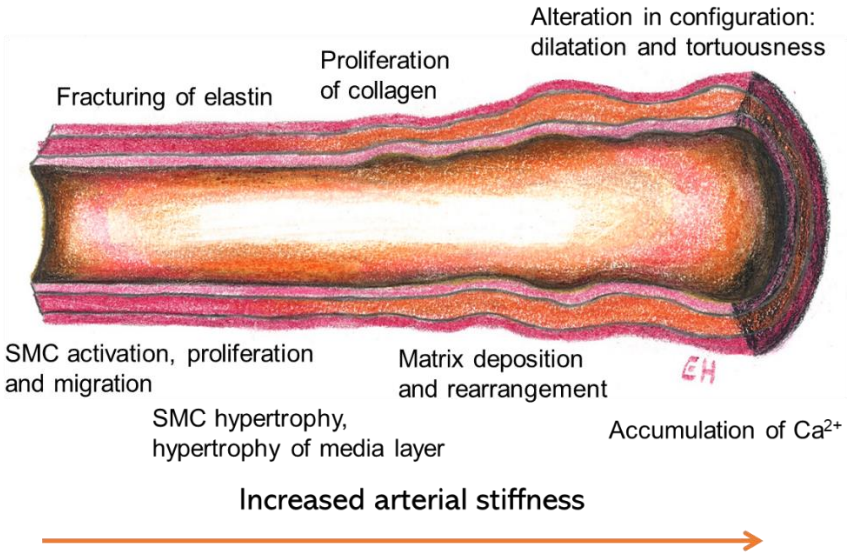

Figure 3. The arteriosclerotic process in an artery. SMC = smooth muscle cell.

2.2.2 **Atherosclerosis**

Atherosclerosis starts in the intima layer and endothelial dysfunction has a central role in initiating the disease process (Ross 1993, Ross 1999). In the response-to injury hypothesis postulated by Ross, each characteristic lesion of atherosclerosis represents a different stage in a chronic inflammatory process in the artery wall (Ross 1999). Normally endothelial cells resist the attachment of leukocytes but when exposed to irritating stimuli such as the presence of hypertension, dyslipidaemia or pro-inflammatory mediators, they start to express adhesion molecules to allow leukocytes to bind onto their surface (Libby, Ridker et al. 2011). At the site of an endothelial injury, inflammatory cells invade the vascular wall and produce proinflammatory factors, leading to both local and systemic inflammation (Trepels, Zeiher et al. 2006). At the same time, changes in the permeability of the endothelial surface and the composition of the extracellular matrix beneath the vascular wall lead a leakage of LDL-cholesterol into the intima layer, and the amount of influx depends on the plasma LDL-C levels (Tabas, Williams et al. 2007, Libby, Ridker et al. 2011).

LDL-particles that are protected against oxidation when in the bloodstream, are thought to become responsive to both enzymatic and non-enzymatic modifications when they reach the intima (Glass, Witztum 2001). Biochemically modified LDL-particles increase the levels of leukocyte-adhesion molecules and cytokines in the intima and these trigger monocyte migration into the vascular wall and then the monocytes differentiate into macrophages (Libby, Ridker et al. 2011, Hall 2016). These cells ingest and oxidise the lipid material and this process makes the macrophage to take on a foam-like appearance; they are called foam cells (Hall 2016). This accumulation of foam cells on the vascular wall creates a visible fatty streak, which traditionally has been viewed as a sign of an atherosclerotic process in its early stages (Hall 2016). The progression of atherosclerosis has been classified by the American Heart Association (AHA) (Stary, Chandler et al. 1994, Stary, Chandler et al. 1995) and is shown in Figure 4. As a response to injury, the inflammatory state or growth factors, medial smooth muscle cells become activated and they become transformed into migratory and secretory smooth muscle cells that migrate from media to intima and the proliferation of smooth muscle cells occurs as a response to certain mediators, like platelet growth factor (Faxon, Fuster et al. 2004, Libby, Ridker et al. 2011). Smooth muscle cells secrete extracellular matrix molecules including interstitial collagen and elastin that stabilize the plaques by forming a fibrous cap covering the plaque (Libby, Ridker et al. 2011, Kumar, Abbas et al. 2015a). The interaction between foam cells and T cells influences this phase of atheroma formation and leads to the presence of chronic inflammation within the vascular wall (Glass, Witztum 2001). Beneath the fibrotic core, foam cells may die and release lipids that accumulate in the extracellular space, and when there is an insufficient clearance of dead cells, extracellular lipids and cellular debris accumulate, forming the lipid-rich or necrotic core of the plaque (Libby, Ridker et al. 2011) that can also be calcified (Kumar, Abbas et al. 2015a). The clinical manifestations occur when the plaque grows leading to the stenosis of the lumen of the artery limiting blood flow leading to downstream tissue ischemia (Libby, Ridker et al. 2011). The growing plaque may also compress the underlying media which leads to its degeneration and causes a potential rupture (Kumar, Abbas et al. 2015c). Activated inflammatory cells may assist in the breakdown of extracellular matrix components such as collagen and elastin, leading to unstable plaques (Libby, Ridker et al. 2011, Kumar, Abbas et al. 2015a) or aneurysm formation (Faxon, Fuster et al. 2004). Rupture, ulceration, or erosion of the plaque leads to thrombosis which may partially or totally occlude the lumen of the artery (Kumar, Abbas et al. 2015c). Intraplaque haemorrhage may be caused by the rupture of the fibrous cap of the plaque or a vessel injury in the neovascular areas around the plaque may expand the size of the plaque and lead to plaque rupture. Atheroembolism may be traced to a ruptured plaque when atherosclerotic debris is released into the lumen.

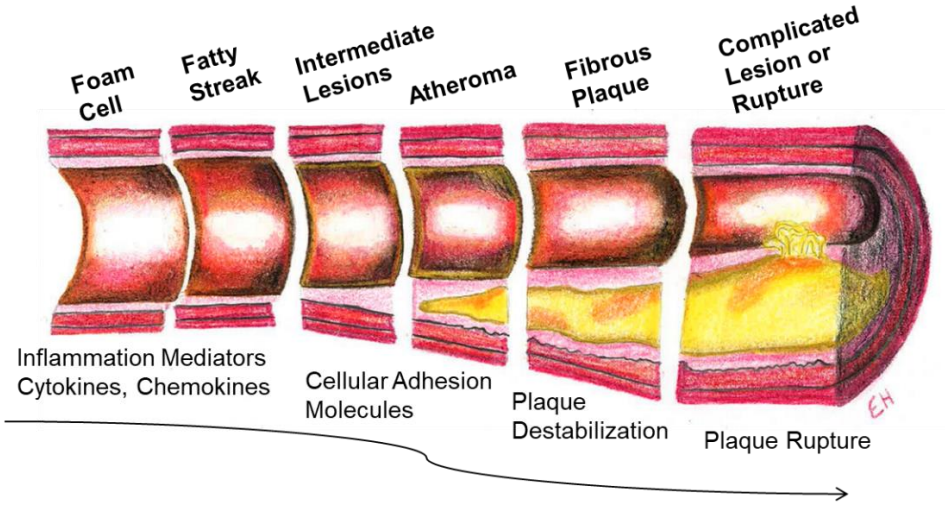

Figure 4. Progression of atherosclerotic changes in the vascular wall from the formation of foam cells to possible plaque rupture. Figure is redrawn and modified from the article by Koenig W. and Khuseyinova N. Biomarkers of Atherosclerotic Plaque Instability and Rupture (Koenig, Khuseyinova 2007).

2.3 RISK FACTORS FOR VASCULAR DISEASES

The risk factors of atherosclerosis can be divided into constitutional and modifiable risk factors (Kumar, Abbas et al. 2015c). These constitutional risk factors are family history, male gender, increasing age and genetic abnormalities; there are also modifiable risk factors e.g. hypertension, hyperlipidaemia, cigarette smoking, diabetes, inflammation.

2.3.1 Constitutional risk factors

In the normal aging process, vascular structural remodelling leads to vascular stiffness and advanced age is the major risk factor for vascular disease (Kumar, Abbas et al. 2015c). Vascular functional changes, like altered vascular tone due to reduced NO production, are also visible. Aging exerts a powerful influence on arterial stiffness, pulse wave reflection from peripheral arteries and pulse pressure, but considerable variability is seen with age; it seems to differ between different populations (Franklin, Gustin et al. 1997) and this variability is strongly influenced by other cardiovascular risk factors and concomitant vascular diseases (Safar, M. E., Levy et al. 2003). For example, in younger patients with hypertension, the mechanical factor represented by high blood pressure contributes considerably to the arterial stiffness in comparison with the situation in older patients where intrinsic alterations in the arterial wall play a more important role (Safar, M. E., Levy et al. 2003).

Reduced physical activity itself leads to non-favourable exaggerated age changes in vascular structure and function. Unfavourable aging refers to accelerated negative

changes in the vascular bed compared to the normal aging process (Lakatta, Levy 2003). Premenopausal women are relatively well protected against atherosclerosis when compared to age-matched men but after menopause, the incidence of atherosclerosis in women increases rapidly (Kumar, Abbas et al. 2015c). It is widely accepted that genetics also play an important role in the pathogenesis of atherosclerosis and multiple biomarkers have been developed (Tibaut, Caprnda et al. 2019). Monogenic disorders like familial hypercholesterolemia are strong predictors for cardiovascular diseases but there are multifactorial causes that explain a considerable proportion of the disease. In addition, epigenetics plays a role in the onset of cardiovascular disease process (Tibaut, Caprnda et al. 2019). In epigenetic alterations, environmental risk factors are found to induce changes in gene expression, in other words, the cell retains a memory of the past cellular states without changes in DNA sequence and these may be heritable (Khyzha, Alizada et al. 2017, Tibaut, Caprnda et al. 2019). For example, the exposure to environmental pollutants has been found to induce epigenetic changes which contribute to atherogenesis (Baccarelli, Ghosh 2012).

2.3.2 **Hypertension**

Hypertension is a risk factor for atherosclerosis through several mechanisms (Chobanian, Alexander 1996, Kannel 1996). It contributes to the pathogenesis of atherosclerosis and its complications (Dharmashankar, Widlansky 2010, Gkaliagkousi, Gavriilaki et al. 2015). Hypertension alters hemodynamic shearing forces and makes them even more severe predisposing to plaque rupture (Frolich, Susic 2007). Endothelial dysfunction is considered as an early step in the pathophysiology of essential hypertension (Dharmashankar, Widlansky 2010). Under conditions of oxidative stress, the bioavailability of NO decreases, leading to a disturbance in endothelial function (Gkaliagkousi, Douma et al. 2009, Gkaliagkousi, Gavriilaki et al. 2015). The excessive oxidative stress and inflammation promote endothelial dysfunction whereas a reduction in both of these processes has been shown to reverse it (Widlansky, Gokce et al. 2003). A defective endothelial L-arginine/NO pathway, decreased responsiveness to exogenous NO and a reduced availability of platelet NO characterize essential hypertension (Gkaliagkousi, Douma et al. 2009, Gkaliagkousi, Gavriilaki et al. 2015). This results in the dissipation of the protective properties of the endothelium and switches the vascular wall conditions to a pro-inflammatory, pro-atherogenic and pro-thrombogenic direction (Gkaliagkousi, Gavriilaki et al. 2015). In a cohort study of postmenopausal normotensive women, an impaired endothelial function was reported to elevate the risk for developing hypertension significantly during the follow-up time 0.5-6.9 years (Rossi, Chiurlia et al. 2004).

While it is widely accepted that hypertension is related to target organ damage, it also affects other organs, such as aorta, large arteries, and the descending arterial tree. An elevated systolic blood pressure level in midlife has been associated with an increased risk for Alzheimer disease 20-30 years later (Kivipelto, Helkala et al. 2001).

Systolic and diastolic pressure have been linked with the presence of arterial stiffness in children (Veijalainen, Tompuri et al. 2013). In prospective studies, elevated systolic blood pressure during childhood and a cumulative burden of systolic blood pressure since childhood have predicted the existence of arterial stiffness in young adults (Li, Chen et al. 2004).

2.3.3 Obesity

The prevalence of overweight and obese children and adolescence has increased all around the world in the recent decades (de Onis, Blossner et al. 2010). Overweight and obese youth have an increased risk to become overweight adults (Singh, Mulder et al. 2008). In the National FinTerveys 2017 survey, in the Finnish population of 18-64 years, 65% of women and 53% of men were overweight or obese (Body mass index (BMI) > 25) and 24% of women and 25% of men were obese (Koponen, Borodulin et al. 2019). Fundamentally, obesity results from an imbalance between energy intake and expenditure. Adopting a Westernized lifestyle including an excessive intake of energy and sedentary lifestyles are the natural causes for increases in obesity (Ford, Mokdad 2008). Youth obesity has been shown to be a strong independent risk factor of MetS in adulthood (Mattsson, Ronnemaa et al. 2008) and premature death in adulthood (de Onis, Blossner et al. 2010). Childhood overweightness or obesity has also been shown to be an independent risk factor for adult hypertension (Juhola, Oikonen et al. 2012). Adulthood carotid IMT as a marker of preclinical atherosclerosis has been associated with childhood BMI (Raitakari, Juonala et al. 2003).

Adiponectin is a bioactive product released from adipocytes; it is a cytokine with several properties, having a role in the regulation of cell growth and apoptosis and it has also a pathophysiological role by acting on glucose and lipid metabolism in the peripheral tissues (Orlando, Nava et al. 2019). Adiponectin has an important anti-atherogenic, anti-inflammatory and insulin-sensitizing properties. In obesity, in general adiponectin levels are low, and this absence of protective factors may explain the association with the obesity-related cardiovascular complications (Orlando, Nava et al. 2019). Interestingly, low adiponectin levels have been associated with hypertension, even after adjusting for BMI (Adamczak, Wiecek et al. 2003, Iwashima, Katsuya et al. 2004). According to Ohashi et al. (Ohashi, Ouchi et al. 2011) adiponectin seems to be an important linkage between adipose tissue and vascular walls, exerting beneficial effects on endothelial function and explaining the influence of obesity on blood pressure.

Abdominal obesity, especially visceral obesity has been associated with an increased risk for metabolic complications (Fox, Massaro et al. 2007). In obese children and adolescents, the amount of visceral fat is inversely associated with adiponectin levels (Orlando, Nava et al. 2019). Among adults, adiponectin levels have been shown to decrease in obese and in patients with type 2 diabetes and patients with cardiovascular diseases (Maury, Brichard 2010). Adiponectin has been speculated to influence the development of MetS, thus among young adults, high adiponectin levels have been shown to associate with a decreased incidence of MetS

(Juonala, Saarikoski et al. 2011). In contrast, low adiponectin levels in childhood have been associated with increased carotid IMT in adulthood, also when adjusted with adulthood adiponectin levels (Saarikoski, Juonala et al. 2017). Among young adults, low adiponectin levels are also related to increased carotid IMT and attenuated brachial FMD (Saarikoski, Huupponen et al. 2010).

Hypertension is more prevalent in obese subjects and this obese-related hypertension may be multifactorial in its nature (Kotsis, Jordan et al. 2018). In the Consensus Document of European Society of Hypertension Working Group, activation of the sympathetic nervous system has been thought to be crucial in the pathogenesis of hypertension in obese individuals from impaired function of baroreceptor sensitivity, increased levels angiotensin II and circulating free fatty acids, as well as in the actions of insulin and leptin. As in the case hyperinsulinemia (Nesto 2004), in obesity, there is increased primary sodium retention from renal tubules leading to an increase in the blood volume and the hypertensive state (Kotsis, Jordan et al. 2018).

2.3.4 Lipid risk factors

Hyperlipidaemia is a major risk factor for atherosclerosis, even in the absence of other known risk factors (Kumar, Abbas et al. 2015c). Hyperlipidaemia is very common in Western countries. For example, 48% North Americans have elevated cholesterol levels (WRITING GROUP MEMBERS, Benjamin et al. 2017). Various factors influence the plasma lipid levels, such as diet, physical activity, genetic factors, gender, and body weight (Persaud, Maguire et al. 2013, Pedersen, Saltin 2015, Karr 2017, Kotsis, Jordan et al. 2018). Dyslipidaemia has been shown to associate with endothelial dysfunction, also in childhood (Kosmeri, Siomou et al. 2019) and increased arterial stiffness (Wilkinson, Cockcroft 2007). Lipid factors play a significant role also in the later phases of disease process, e.g. the Mediterranean diet which is rich in alpha-linolenic acid has been found to decrease all-cause mortality in the secondary prevention of coronary heart disease by even as much as 70% in a prospective trial when only a dietary modification was applied (de Lorgeril, Renaud et al. 1994). Increased plasma free fatty acid levels are detected in subjects with obesity or type 2 diabetes (Ghosh, Gao et al. 2017).

Low-density lipoprotein

Low-density lipoprotein-cholesterol (LDL-C) and oxidized LDL cholesterol have numerous non-atheromatous and direct effects on the arterial wall which may lead to stiffening of the artery (Wilkinson, Cockcroft 2007). The presence of oxidized LDL-C leads to the formation of peroxynitrate and increased oxidative stress, and these both can directly cause elastin damage (Paik, Ramey et al. 1997, Wilkinson, Cockcroft 2007).

When the LDL-C level is raised in the circulation, the transport and retention of LDL-C is increased in the vascular wall (Lusis 2000). This accumulation of LDL in the vascular wall subendothelium is considered as a primary initiating event in

atherosclerosis (Lusis 2000). In the subendothelium, the modification of LDL-particles occurs e.g. via glycation, oxidation, interactions with proteoglycans, and modified LDL-particles in turn can trigger various proinflammatory reactions (Pentikäinen, Öörni et al. 2000). The oxidized LDL in the intima layer contributes to the initiation of an atherosclerotic fatty streak (Matsuura, Lopez 2004). Childhood LDL-C levels have shown to predict carotid IMT among young adults (Li, Chen et al. 2003, Raitakari, Juonala et al. 2003) and among young adults, baseline LDL-C levels have been directly associated with carotid IMT progression in a 6 year follow-up (Koskinen, Kähönen et al. 2009). Current LDL-C levels are a strong predictor for carotid IMT also in middle age (Davis, Dawson et al. 2001).

Familial hypercholesterolemia

In familial hypercholesterolemia, which is an inherited disease, a genetic mutation has occurred in the gene encoding for the LDL receptor; the disease is characterized by significantly elevated LDL-C -levels and the early appearance of atherosclerosis (Kumar, Abbas et al. 2015c). Patients with familial hypercholesterolemia and high LDL-C have shown a remarkable increase in cardiovascular disease as compared to subjects with normal LDL-C levels and no specific mutation affecting LDL-C metabolism (Wiegman 2018). Lifelong exposure to high LDL-C is associated with premature atherosclerosis although the risk for cardiovascular diseases seems to vary among familial hypercholesterolemia patients, also when genetics and LDL-C levels are identical. Additional traditional risk factors do not seem to be able to explain all of the variety of the manifestations and severities of clinical cardiovascular disease among these patients (Bianconi, Banach et al. 2020).

High-density lipoprotein

The high-density lipoprotein cholesterol (HDL-C) concentration is inversely associated with cardiovascular risk (Rader, Hovingh 2014, Barter, Kastelein et al. 2003). Low levels of HDL-C measured in childhood and adolescence have been shown to predict decreased carotid artery elasticity in adulthood (Juonala, Järvisalo et al. 2005) and among young adults, a low HDL-C level has independently predicted an increased arterial stiffness as measured in brachial-ankle-PWV (Li, Chen et al. 2004). HDL-C has been demonstrated to possess many atheroprotective properties; one of the most common is its ability to promote cholesterol efflux from cells, like macrophages, and the related complex physiological process of reverse cholesterol transport from tissues to liver (Rader, Alexander et al. 2009). The HDL-C possesses also anti-thrombogenic and anti-oxidant activity, it has a capacity to inhibit adhesion molecule expression in endothelial cells and it may also modulate endothelial function by stimulating NO production (Barter, Kastelein et al. 2003). In the rather new hypothesis about the function of HDL-C, it has been speculated that HDL-C itself is not atheroprotective but it has beneficial functions, which cannot simply be estimated by measuring HDL-C concentrations (Rader, Hovingh 2014). There seems to be a only a slight correlation between the HDL-C content and the capacity of HDL-

C to promote cholesterol efflux from cells in individuals, thus the HDL-C concentration does not predict accurately an individual´s capacity for cholesterol efflux from vascular wall macrophages.

Triglycerides

Triglycerides are lipid fractions that the body uses for energy storage; they are synthesized in the liver and derived from intestine uptake. Triglyceride particles are known to be atherogenic (Sarwar, Danesh et al. 2007) and gene alleles raising triglycerides have been found to display strong associations with cardiovascular endpoints (Dron, Hegele 2017). Also, in real-world analysis, elevated triglyceride levels have been detected and are commonly associated with an increased cardiovascular event risk (Toth, Fazio et al. 2020). However, hypertriglyceridemia often occurs simultaneously with low HDL-C and it cannot be excluded that the elevated triglycerides illustrate deeper causal metabolic disturbances (Dron, Hegele 2017). Triglycerides are carried in triglyceride-enriched lipoproteins like VLDL, VLDL remnants and intermediate-density lipoprotein. The associations found with triglycerides may also be reflections of the effects of both triglycerides and triglyceride-rich lipoproteins (Toth, Fazio et al. 2020). Plasma levels of triglycerides have been shown to correlate with an increased risk for cardiovascular events in patients with well-controlled LDL-levels on statin therapy (Miller, Cannon et al. 2008). Among young adults, serum triglycerides have been associated with decreased arterial elasticity (Li, Chen et al. 2004), but they may not be associated with IMT (Touboul, Labreuche et al. 2014).

Apolipoproteins

Apolipoproteins consist of lipids and protein components; apolipoprotein A-I (Apo-AI) is a major component of HDL-C (Libby, Ridker et al. 2011). Elevated levels of apoB and low apoA1 are risk factors for atherosclerosis and these markers have been used to predict the cardiovascular disease risk (Emerging Risk Factors Collaboration, Di Angelantonio et al. 2012). An elevation of apoB has been associated with increased arterial stiffness among young adults as measured by PWV (Koivistoinen, Hutri-Kähönen, Juonala, Kööbi et al. 2011).

2.3.5 Smoking

Both active and passive smoking are very well-known preventable risk factors for cardiovascular diseases, and they impact on all phases of the disease process (Ambrose, Barua 2004). The effect of cigarette smoke on the endothelial cells of vascular wall leads to a reduced bioavailability of NO and a loss of media smooth muscle cell function, an increase in the expression of adhesion molecules in the endothelial cells, the release of proinflammatory and proatherogenic cytokines and subsequently endothelial dysfunction. There is endothelial damage, an increase of oxidation of proatherogenic lipids, a decrease in the amount of HDL-C and

furthermore a shift to a procoagulant state occurs (Messner, Bernhard 2014). Smoking in childhood and adolescence predicts decreased carotid artery elasticity in adulthood (Juonala, Järvisalo et al. 2005). Smoking in childhood and adolescence, as well as smoking in adulthood, has been reported to increase the IMT in carotid artery (Raitakari, Juonala et al. 2003).

2.3.6 Impaired glucose metabolism

Insulin resistance

Insulin is an essential hormone that regulates cellular metabolism in many tissues in the body (Ormazabal, Nair et al. 2018). Hyperinsulinemia is the compensatory mechanism of pancreatic β-cells to combat insulin resistance in target tissues (Hall 2016). In insulin resistance, the target tissue response to insulin stimulation is decreased; the typical features for this state are defects in the uptake and oxidation of glucose and a decrease in glycogen synthesis as well as a decrease in the cell´s ability to inhibit lipid oxidation (Ormazabal, Nair et al. 2018). In adipose tissue, insulin resistance causes an inability to suppress lipolysis, leading to the release of free fatty acids and an increase in their influx into the skeletal muscles, liver, and other tissues. This influx of free fatty acids promotes their re-esterification which leads to insulin resistance in these tissues (Lewis, Carpentier et al. 2002, Samuel, Shulman 2012). Due to Eckel (Eckel, Grundy et al. 2005), the major contributor to the development of insulin resistance, is overabundance of free fatty acids.

Insulin exerts a direct influence on the renal tubules where it has a sodium retaining effect, and chronic hyperinsulinemia has been associated with vasoconstriction (Kotsis, Jordan et al. 2018). In addition, insulin resistance decreases NO synthesis and increases systemic and vascular inflammation that may induce endothelial dysfunction. An imbalance or disruption in the autonomic nervous system is found in patients with diabetes and the increases in sympathetic activity have been suggested to associate with hyperinsulinemia and hyperglycaemia (Nesto 2004). Insulin resistance is increased in non-diabetic overweight and obese individuals, but at the same time, it is possible that an overweight individual can be insulin-sensitive and a normal-weight person can be insulin-resistant (Abbasi, Brown et al. 2002).

Insulin resistance and/or compensatory hyperinsulinemia are strong predictors of type 2 diabetes (Abbasi, Brown et al. 2002). Compensatory hyperinsulinemia and/or insulin resistance have been associated with an increased risk of cardiovascular diseases (Laakso, Sarlund et al. 1991, Despres, Lamarche et al. 1996, Yip, Facchini et al. 1998, Nesto 2004, Grundy 2016). Insulin resistance seems to share similar pathological processes with cardiovascular diseases e.g. its silent character, even several years before reaching the diabetic threshold, the difficulty in early diagnosis and starting lifestyle and medical interventions, are of clinical relevance (Nesto 2004). The different forms of insulin resistance, both tissue specific, as in adipose tissue and endothelium, and cell-type specific insulin resistance as in macrophages, contribute

to the cardiovascular complications encountered in diabetes mellitus (Laakso, Kuusisto 2014). The presence of insulin resistance has been shown to predict the extent of coronary calcification in type 1 diabetes patients independently of their glucose-levels (Schauer, Snell-Bergeon et al. 2011).

The homeostatic model assessment (HOMA) of β-cell function and insulin resistance has been used for several decades, since 1985 (Wallace, Levy et al. 2004). This is based on fasting plasma insulin and glucose concentrations; it allows an assessment of insulin sensitivity and inherent β-cell function. Therefore, it can be applied to characterize the pathophysiology in individuals with impaired glucose tolerance. Insulin resistance measured by HOMA-IR (Homeostasis model assessment of insulin resistance) has been shown to correlate strongly with endothelial dysfunction in a study investigating non-diabetic patients (n=365) with suspected myocardial ischemia and referred to undergo a myocardial perfusion scintigram (Westergren, Svedlund et al. 2016). In addition, it showed independent prognostic value in patients without myocardial perfusion defects (Westergren, Svedlund et al. 2016). HOMA-IR was demonstrated to be a strong predictor of cardiovascular events in a meta-analysis of 65 studies (n=516 325) (Gast, Tjeerdema et al. 2012) and compared to glucose or insulin concentrations on their own, each standard deviation increase in HOMA-IR elevated the relative risk for cardiovascular diseases by more than the same increase in either of the two aforementioned parameters.

Impaired glucose tolerance

Impaired glucose tolerance, also referred to as prediabetes, is defined as a state where plasma glucose levels are above normal but do not reach the diabetic threshold and it is evaluated in terms of impaired fasting glucose or impaired glucose tolerance (Tabák, Herder et al. 2012, Cosentino, Grant et al. 2020). Impaired glucose tolerance is a high-risk state for developing diabetes, 5-10% prediabetic patients develop diabetes every year but the same amount will revert to normoglycemia (Tabák, Herder et al. 2012). The increased risk for coronary artery disease begins already in the state of hyperinsulinemia and impaired glucose tolerance and the risk increases along with elevated glucose levels (Nesto 2004, Emerging Risk Factors Collaboration, Sarwar et al. 2010, Cosentino, Grant et al. 2020). Chronic hyperglycaemia can trigger oxidative stress and inflict inflammatory responses that lead to cell damage (Ormazabal, Nair et al. 2018). The role of endothelial dysfunction in the development of atherosclerosis and arteriosclerosis has been described above.

Diabetes mellitus

Diabetes mellitus is present in a cluster of metabolic disorders that share the common feature of hyperglycaemia (Kumar, Abbas et al. 2015c). The generality of diabetes can be divided in two subtypes, type 1 diabetes where the pancreas does not produce insulin and type 2 diabetes where the insulin sensitivity of the body´s tissues has been reduced or the pancreas does not produce enough insulin to meet their

demands (Diabetes Canada Clinical Practice Guidelines Expert Committee, Punthakee et al. 2018). Gestational diabetes makes its first appearance during pregnancy and is characterized by glucose intolerance (Diabetes Canada Clinical Practice Guidelines Expert Committee, Punthakee et al. 2018). The prevalence of diabetes is rising alarmingly and according to International Diabetes Federation, in 2000, already 151 million adult were estimated to be living with diabetes worldwide; by the year 2009 that number had risen to 285 million (International Diabetes Federation 2019). The global estimate of International Diabetes Federation in 2019 is that 9.3% of adults aged 20-79 years, i.e. 463 million people are living with diabetes and 1.1 million children and adolescents under the age of 20 have type 1 diabetes. In Europe, approximately 60 million adults had type 2 diabetes in 2017 with half of them remaining undiagnosed (Cosentino, Grant et al. 2020).

Diabetes and the precursor symptoms of diabetes increase the risk for death from cardiovascular diseases and these two diseases share similar pathological processes (Nesto 2004). Many of the metabolic disturbances occurring in diabetes including hyperglycaemia, excess free fatty acid liberation and insulin resistance affect the synthesis or degeneration of NO and thus impact on endothelial cell function, but the influence of diabetes is not limited to endothelial cells – also the function of smooth muscle cells in the media layer is affected due to impaired function of the sympathetic nervous system (Creager, Lüscher et al. 2003). Furthermore, increased oxidative stress and the production of prothrombotic factors and disturbances in signal transduction and platelet function are encountered (Creager, Lüscher et al. 2003). According to Suzuki et al. (Suzuki, Poot et al. 2001), the presence of diabetes can accelerate the smooth muscle cell proliferation and the accumulation of these cells into atherosclerotic lesions and thus promote the further development of severe atherosclerotic lesions. In contrast, in the review of Nesto (Nesto 2004), it was speculated whether diabetes could affect the function of the smooth muscle cells; it was postulated that the diabetic state could inhibit the migration of smooth muscle cells to the plaque area leading to the presence of the unstable plaques found in diabetic patients, especially in "mild diabetes". In a large prospective meta-analysis, diabetes in general was found to approximately double the risk for coronary heart disease, ischaemic stroke, haemorrhagic stroke, and unclassified stroke as well as death from other vascular causes (Sarwar, Danesh et al. 2007). In the same meta-analysis, diabetes was found to be 30% more strongly related to fatal than non-fatal myocardial infarction than raised LDL-C, and the hazard ratio appeared to be greater in women, younger participants, non-smokers and individuals with lower-than-average blood pressure.

Metabolic syndrome

The metabolic syndrome (MetS) is a common metabolic disorder consisting of a cluster of metabolic risk factors for cardiovascular diseases and type 2 diabetes (Grundy 2016, Kassi, Pervanidou et al. 2011). MetS is a risk concept based on a risk factor cluster where insulin resistance has a central role, e.g. insulin resistance can

change the systemic lipid metabolism leading to the development of dyslipidaemia and the lipid triad: low HDL-C, high LDL-C, and the appearance of small density LDL-C (Ormazabal, Nair et al. 2018). Different versions of definition are reported, and new definitions are still being formulated (Eckel, Grundy et al. 2005). There is some confusion because of the different existing definitions, and the need for uniform criteria has been reported (Kassi, Pervanidou et al. 2011). Elevated blood pressure, atherogenic dyslipidaemia, central obesity, insulin resistance and hyperglycemia are key elements, representing the foundations for a proinflammatory and prothrombotic state (Grundy 2016). The driving forces for MetS are physical inactivity and obesity (Eckel, Grundy et al. 2005).

Most individuals can sustain normal glucose tolerance by compensatory hyperinsulinemia but hyperinsulinemic or insulin-resistant patients are at an increased risk in developing glucose intolerance, hypertriglyceridemia, low HDL-C levels, and essential hypertension (Reaven, G. M. 1988). At the end of 1980`s, Reaven proposed that this kind of risk cluster increased the risk for cardiovascular diseases, but because this association was not widely appreciated at that time, it was called "Syndrome X" to refer to the risk factors occurring in insulin-resistant patients (Kim, Reaven 2004). The knowledge of abnormalities associating with insulin resistance has now increased and the term metabolic syndrome has displaced the term "Syndrome X" although criticism has been raised against the terminology: "insulin resistant syndrome" would have illustrated the pathophysiological background of insulin-associated disturbances more accurately than MetS where the variety of criteria have been excessively large (Reaven, G. 2004). Although the more overweight individuals are, the more probably they will develop insulin resistance and increase their risk for cardiovascular diseases, but a considerable amount of people still remain insulin-sensitive despite being overweight or obese (Reaven, G. 2005). The presence of MetS in childhood and youth has been found to predict MetS in adulthood, subclinical atherosclerosis and type 2 diabetes but its assessment has not shown to better predictive value than the simpler measurement of BMI alone (Magnussen, Koskinen et al. 2010). One important consequence of MetS is still arterial stiffening, which is related the progression of diabetic complications (Prenner, Chirinos, 2015). Components of Mets have shown different associations with arterial stiffness parameters (Vágovicova, Seidlerová et al. 2015) and different cluster of MetS components have also shown to have different influence on arterial stiffness (Scuteri, Cunha et al. 2014).

There are various definitions for adults where different risk factors are weighted in different ways: in World Health Organization criteria (1998) define insulin resistance as type 2 diabetes or impaired fasting glucose plus two other risk factors (abdominal obesity, hypertriglyceridemia, low HLD-C, hypertension, microalbuminuria) (Kassi, Pervanidou et al. 2011). In 1999, the European Group for the Study of Insulin Resistance (EGIR) criteria included insulin resistance in non-diabetic patients plus two additional risk factors (waist circumference, hypertriglyceridemia, low HDL-C, hypertension or antihypertensive drugs, high

fasting glucose) (Kassi, Pervanidou et al. 2011). In 2001, National Cholesterol Education Program Adult Treatment Panel III (NCEP:ATPIII) proposed a new definition for MetS based on five measurements: waist circumference, hypertriglyceridemia, low HDL-C, hypertension and high fasting glucose (Kassi, Pervanidou et al. 2011) but hyperinsulinemia and microalbuminuria were removed from this definition. Subsequently, the American Association of Clinical Endocrinology criteria were published in 2003; these included impaired glucose tolerance plus two of the following: BMI over 25kg/m^2, hypertriglyceridemia or hypertension. In 2005, International Diabetes Federation criteria were introduced which emphasized central obesity as a key element in MetS: its diagnosis required the presence of central obesity as the necessary component plus two of the following: hypertriglyceridemia, low HDL-C, hypertension and high fasting glucose (Alberti, Zimmet et al. 2006). American Heart Association/National Heart, Lung, and Blood Institute (AHA/NHLBI) criteria from the year 2004 proposed any of three of the following parameters: waist circumference, hypertriglyceridemia, low HDL-C, hypertension and high fasting glucose (Kassi, Pervanidou et al. 2011). This was followed in 2009, by a joint statement from The International Diabetes Federation and AHA/NHLBI which introduced the harmonized criteria (Alberti, Eckel et al. 2009). In this definition, three abnormal findings of five were required: elevated waist circumference, hypertriglyceridemia, low HDL-C, hypertension, and elevated fasting glucose. The variety of definitions for the MetS reveals that the concept of MetS is not straightforward - it is still a controversial concept. Oda (Oda 2012) has raised the criticism that the criteria of MetS and threshold of different MetS components are not scientific enough and that there is no clear basis for excluding or including other cardiovascular risk factors like adiponectin, fatty liver or high-sensitivity CRP. In addition, Oda was critical of the proposal that measurements of waist circumference were superior to BMI; it was stated that this had not been scientifically established for defining obesity in the MetS criteria.

2.4 VASCULAR BIOMARKERS OF VASCULAR DISEASES

Several non-invasive techniques have been developed to characterize arterial properties. Safe, non-invasive techniques have gained acceptance in investigating vascular structure, function, and biomechanics also in non-symptomatic individuals of different ages e.g. to assess the individual´s cardiovascular risk (Oliver, Webb 2003, Slyper 2004, Engelen, Bossuyt et al. 2015).

2.4.1 Intima-media thickness

The ultrasound-measured common carotid artery intima-media -thickness (IMT) is a widely used method to assess structural changes in the arterial wall and atherosclerotic plaque disease, in other words, revealing a more advanced disease process rather than the early phase of the disease (Ter Avest, Stalenhoef et al. 2007). IMT measures the arterial wall thickening from the innermost interface between the

arterial lumen and the intima as far as the media-adventitia interface. IMT values correlate with several cardiovascular risk factors, also in the asymptomatic phase; IMT has been shown to associate with low HDL-C independently of LDL-C in a meta-analysis of 21 000 adult patients whereas no association was found with triglyceride levels (Touboul, Labreuche et al. 2014). Patients with familial hypercholesterolemia have shown evidence of increased IMT as compared controls (Masoura, Pitsavos et al. 2011). IMT has been found to increase already in obese children (Meyer, Kundt et al. 2006) and IMT measured in adulthood correlated with childhood cardiovascular risk factors such as LDL-C levels, systolic blood pressure, BMI and smoking habits as well as with the same risk factors when measured in adulthood (Raitakari, Juonala et al. 2003). Furthermore, the number of risk factors has been associated directly with IMT in young adulthood (Raitakari, Juonala et al. 2003). Cardiovascular risk factors are associated with increased IMT among young adults including advancing age, increases in blood pressure, LDL-C, triglycerides, HOMA-IR, low HDL-C, and smoking (Bhuiyan, Srinivasan et al. 2006). However, there is also evidence obtained from young adults that the number of cardiovascular risk factors correlates with increased IMT when endothelial dysfunction is present as measured by FMD, but subjects with enhanced endothelial function do not reveal any significant associations (Juonala, Viikari et al. 2004). It has been reported that exposure to a wide pulse pressure in adolescence may cause carotid IMT thickening in adulthood (Raitakari, Juonala et al. 2009). IMT has been shown to be a strong predictor of future vascular events (Lorenz, Markus et al. 2007).

2.4.2 Carotid artery stiffness

Carotid artery distensibility (Cdist) measures the ability of the artery to respond to the pulse pressure caused by cardiac contraction and relaxation (Juonala, Järvisalo et al. 2005). In this measure, the common carotid artery diameter is determined at end-diastole and at end-systole by ultrasound imaging as well as taking account of the systolic blood pressure and diastolic blood pressure values, and Cdist provides information about the relative change in arterial diameter with blood pressure. Cdist represents arterial elasticity and is commonly used for studying arterial stiffness (Oliver, Webb 2003). Among children with familial hypercholesterolemia Cdist has been found to be significantly reduced as compared to healthy controls (Aggoun, Bonnet et al. 2000); also, severely obese children have shown decreased Cdist when compared to controls (Tounian, Aggoun et al. 2001). Several risk factors identified in childhood and adolescence including high LDL-C, low HDL-C, elevated blood pressure, skinfold thickness and smoking were found to associate with reductions in Cdist in adulthood (Juonala, Järvisalo et al. 2005). It has been found to decrease in young adults with the metabolic syndrome (Mattsson, Rönnemaa et al. 2008). A 6-year follow-up study of young adults monitored baseline and 6-year changes in waist circumference, baseline insulin and the change in systolic blood pressure were independently associated with the 6-year decline in Cdist values (Koskinen, Magnussen et al. 2012). The decline in Cdist values has been found to predict

cardiovascular events in elderly people (Leone, Ducimetiere et al. 2008, Haluska, Jeffries et al. 2010) as well as predicting cardiovascular mortality and all-cause mortality (Yuan, Wang et al. 2016).

2.4.3 Brachial artery flow-mediated dilatation

Brachial flow-mediated dilatation (FMD) is considered as a marker of endothelial function i.e. revealing early changes in the vascular wall (Ter Avest, Stalenhoef et al. 2007). This non-invasive ultrasound technique was first described by Celemajer in 1992 (Celermajer, Sorensen et al. 1992, Corretti, Anderson et al. 2002) and it measures the response of the arterial endothelium to increased shear stress which was created by placing an occlusion cuff around the forearm for several minutes followed by its release (Raitakari, Celermajer 2000, Thijssen, Black et al. 2011).

Obese children have revealed impaired FMD as compared controls (Meyer, Kundt et al. 2006) and low serum adiponectin levels were shown to correlate with decreased brachial FMD in young adults (Saarikoski, Huupponen et al. 2010). Children with familial hypercholesterolemia have revealed also impaired FMD as compared to controls (Sorensen, Celermajer et al. 1994). When compared to normolipidemic controls, adult patients with familial hypercholesterolemia or familial combined hyperlipidaemia exhibited impaired FMD and treatment with statins was able to improve the arterial function as measured with FMD (Masoura, Pitsavos et al. 2011). In the Young Finns study investigating Finnish adults aged 24-39 year, FMD was not found to decrease in subjects with MetS (Mattsson, Rönnemaa et al. 2008). However, in the same study, MetS was associated with a higher carotid IMT in those participants with impaired FMD. Those subjects with MetS and enhanced FMD instead showed normal IMT values which were comparable with those of the average population. Nonetheless, the number of risk factors in young adults of 24-39 year was associated with increased IMT in those study participants with impaired FMD but not in those with enhanced FMD (Juonala, Viikari et al. 2004). Hence, systemic endothelial function is believed to modify the relationships between metabolic risk and atherosclerosis (Juonala, Viikari et al. 2004, Mattsson, Rönnemaa et al. 2008). Brachial FMD has also been shown to correlate well with coronary artery endothelial function (Anderson, Uehata et al. 1995) and the predictive power of a coronary artery disease risk factor model based on traditional risk factors of cardiovascular disease was improved when FMD and low-flow-mediated constriction were incorporated (Gori, Muxel et al. 2012).

High-risk patients with impaired FMD have revealed an increased risk for cardiovascular events (Gokce, Keaney et al. 2002, Chan, Mancini et al. 2003). This outcome was observed also in patients with overt coronary disease (Chan, Mancini et al. 2003) but furthermore, cardiovascular risk factors and their interaction were shown to decrease FMD in asymptomatic individuals (Celermajer, Sorensen et al. 1994).

2.4.4 **Pulse wave velocity**

Pulse wave velocity (PWV) is used to estimate the arterial stiffness and carotid-femoral PWV has been considered as the "golden standard" of arterial stiffness measurements (Laurent, Cockcroft et al. 2006). PWV measures the speed of a pressure wave as it travels along an artery with time and thus the stiffer the artery, the faster the pressure wave travels (Ter Avest, Stalenhoef et al. 2007). There are various techniques available to determine PWV; previously, methods were based on utilizing Doppler ultrasound or mechanoelectrical pulse transducers (Kööbi, Kähönen et al. 2003). The disadvantage of these methods was the requirement for knowledge of a precise distance measurement because even a slight inaccuracy could affect the PWV value and the positioning of the transducer needed to be exact (Kööbi, Kähönen et al. 2003, Laurent, Cockcroft et al. 2006). A newer method based on an impedance technique offers a convenient alternative also for the patient since there is no need to measure the femoral artery pulsating at the groin level or the popliteal artery. Kööbi et al. (Kööbi et al. 2003) conducted a validation study of a whole-body impedance cardiography method with complementary voltage sensing channels, demonstrating this to be a high repeatable and reproducible method. The reproducibility values of PWV measured by Doppler between aortic arch and popliteal artery and whole-body impedance were similar and changes in PWV measured with whole-body impedance cardiography and Doppler correlated strongly with each other, indicating that both of the methods are assessing the true PWV values.

Childhood elevated systolic blood pressure has independently predicted increased PWV in young adulthood (Aatola, Hutri-Kähönen et al. 2010, Li, Chen et al. 2004) as well as increased glucose levels in childhood. In addition, adulthood cardiovascular risk factors like increased systolic blood pressure, insulin and triglyceride levels were directly associated with increased PWV in adulthood (Aatola, Hutri-Kähönen et al. 2010). The cumulative burden of triglycerides from childhood as well as the duration of smoking years significantly associated with increased PWV in adulthood (Li, Chen et al. 2004). The number of cardiovascular risk factors identified in childhood including high LDL-C, low HDL-C, elevated systolic blood pressure, high BMI and smoking were directly associated with PWV in adulthood (Aatola, Hutri-Kähönen et al. 2010). The presence of MetS in childhood associated with increased PWV in adulthood and recovery from childhood MetS has been associated with decreased PWV in adulthood (Koivistoinen, Hutri-Kähönen, Juonala, Aatola et al. 2011). MetS and an increasing number of MetS components in young adults were claimed to be associated with a higher PWV (Koivistoinen, Aatola et al. 2010). Impaired glucose tolerance and type 2 diabetes was associated with a higher PWV in middle-aged population (Koivistoinen, Jula et al. 2011). PWV can be perceived as an index of the arteriosclerotic process (Ter Avest, Stalenhoef et al. 2007). Aortic PWV was found to be a strong predictor of future cardiovascular events and all-cause mortality (Vlachopoulos, Aznaouridis et al. 2010).

2.5 LONGITUDINAL MOTION OF THE ARTERY WALL

2.5.1 Measurement method

The longitudinal motion of artery wall was detected already in the 1950s when canine abdominal aorta, one example of a large elastic artery, was investigated by Lawton & Greene (Lawton,R, Greene,L 1956). The measured longitudinal motion was considered to be negligible and it took several decades until it was investigated again. At the beginning of the present century, a Swedish research group evaluated longitudinal motion of the common carotid artery wall (CALM) noninvasively in human trials and they found the CALM to have the same magnitude as the radial motion (Persson, Rydén Ahlgren et al. 2003). The delay in understanding that CALM was as relevant phenomenon as radial motion was partly due to technical issues – detecting the longitudinal motion with ultrasound is demanding because of the limited resolution of this technique.

Non-invasive ultrasound imaging has become the golden standard for examining the carotid artery wall motion and several studies have been performed with different methods (Cinthio, Rydén Ahlgren et al. 2005, Gastounioti, Golemati et al. 2011, Gastounioti, Golemati et al. 2013, Golemati, Sassano et al. 2003, Golemati, Stoitsis et al. 2012, Idzenga, Holewijn et al. 2012, Idzenga, Hansen et al. 2012, Larsson, Heyde et al. 2015, Numata, Hasegawa et al. 2007, Zahnd, Orkisz et al. 2011, Zahnd, Orkisz et al. 2013, Zahnd, Salles et al. 2015). Three major motion-tracking methods are used with which to analyse the longitudinal motion from B-mode images: block matching, optical flow and feature matching (Rizi, Au et al. 2020). Block matching is widely used to perform speckle tracking, where the longitudinal motion is evaluated by dynamically following the target. Different variations have been successfully developed, e.g. Cinthio et al. (2005) applied echo tracking using a small block size that enabled accurate tracking of speckles in images (Cinthio, Rydén Ahlgren et al. 2005, Rizi, Au et al. 2020). Yli-Ollila et al. (2013) developed a method based on luminance optimization and a validation of the longitudinal motion tracking method was published; the reproducibility of this approach was shown to be good (Yli-Ollila, Laitinen et al. 2013). In optical flow, velocity fields are determined across different temporal frames; longitudinal motion is evaluated through a static window (Rizi, Au et al. 2020). Feature matching was recently introduced, and it was tested in one study where the procedure was validated with 18 healthy volunteers and 16 patients with carotid plaque and at least one cardiovascular risk factor (Scaramuzzino, Carallo et al. 2017).

In addition to the three aforementioned methods, also other techniques are used, e.g. radiofrequency analysis and velocity vector imaging. Wall motion extraction based on radiofrequency signals involves the computation of a phase shift between two subsequent time steps to determine the corresponding spatial motion (Rizi, Au et al. 2020). Velocity Vector Imaging (VVI) is a commercially available tool and suitable for estimating the total amplitude of CALM but it does not give possibilities

for more accurate analysis of waveforms. Some other commercial or freely available methods have been developed, but evaluation studies are still few. Zahnd et al. have developed a new method which combines previously validated methods for motion estimation and wall segmentation (Zahnd, Orkisz et al. 2013, Zahnd, Kapellas et al. 2017, Zahnd, Saito et al. 2018). Gastounioti et al. have developed a web-based platform named CAROTID which integrates motion-based Computer-Aided Diagnosis functionalities for patients with carotid atherosclerosis (Gastounioti, Kolias et al. 2014, Rizi, Au et al. 2020).

2.5.2 **Driving force and direction of the motion**

Most of CALM studies have focused on measuring the amplitude of the longitudinal motion. The multiphasic waveform of CALM is a newer discovery; this refers to specific motion pattern with its four distinct phases during cardiac cycle: a primary antegrade motion in early systole, a retrograde motion later in systole, a secondary antegrade motion in diastole and then gradual return back to the starting point (Rizi, Au et al. 2020). For example, principal component analysis has offered the possibility to study the relationships with principal components of waveform and pulse pressure, revealing associations with blood pressure, and relationships with the principal components of the waveform and stiffness indices of distensibility and compliance (Yli-Ollila, Tarvainen et al. 2016a). In a study of 19 healthy volunteers aged 19-49, the second principal component was strongly associated with arterial stiffness (Yli-Ollila, Tarvainen et al. 2016a).

Yli-Ollila (Yli-Ollila, Tarvainen et al. 2016b) demonstrated that CALM occurs first in the intima-media complex and this is followed by the CALM of the adventitial layer. It is possible that the elastic fibres drag the adventitial layer along with them, as the intima-media complex moves in the longitudinal direction (Yli-Ollila, Tarvainen et al. 2016b). Cinthio et al. (Cinthio, Rydén Ahlgren et al. 2006) found that the longitudinal motion decreased deeper in the artery wall (adventitia) as compared to the surface, intima-media region resulting in a shear strain of the artery wall.

The physical significance and exact driving force of the carotid artery wall longitudinal motion (CALM) have remained unclear (Au, Bochnak et al. 2018). Cinthio et al. have speculated on the major causes for longitudinal wall motion including shear force from blood flow, although this is unlikely to explain the multiphasic patterns on retrograde motion of wall motion itself (Cinthio, Rydén Ahlgren et al. 2006). Elastic recoil may also have some role in the longitudinal motion, the rotational movement of the heart in systole or reflecting pulse wave from the periphery (Cinthio, Rydén Ahlgren et al. 2006). Blood friction creating the tangential force at the wall surface and blood influx inducing radial systolic stretching have also been mentioned (Zahnd, Balocco et al. 2015). The anterograde motion has been speculated to be caused by the pulse wave (Rizi, Au et al. 2020) and a weak-to-moderate association between left ventricular rotation and retrograde CALM has been found (Au, Bochnak et al. 2018). Breathing is known to influence the reproducibility of measurement of CALM (Cinthio, Rydén Ahlgren et al. 2005). It has

also been hypothesized that the mechanical deformation of the artery wall contributes nutrients and other essential substance intake from blood flow to vessel wall and surrounding tissues (Rizi, Au et al. 2020).

Arterial stiffness has been found to correlate with CALM (Yli-Ollila, Laitinen et al. 2014, Yli-Ollila, Laitinen et al. 2016). Hemodynamic shear stress focusing on the endothelium is considered to be a notable regulator of the acute changes in the vascular diameter and it may influence long-term adaptive vascular wall structural remodelling as a response to different pathophysiological states such as hypertension, diabetes or hyperlipidaemia (Davies 2009). For example, it is also known that arterial bifurcations are more susceptible to flow disturbances and atherosclerotic changes are detected earlier than at sites of laminar flow.

There is evidence from a small-size study with 12 patients with moderate-to-severe carotid artery plaque stenosis and 23 healthy controls that the presence of carotid atherosclerotic plaque alters the direction and magnitude of the longitudinal motion (Tat, Psaromiligkos et al. 2016b). Among healthy study participants, a predominantly retrograde motion was detected in comparison with patients with a plaque burden who had mainly an anterograde motion during systole. Additionally, a significantly greater anterograde motion was detected in patients with severe stenosis compared to those with moderate-stage stenosis (Tat, Psaromiligkos et al. 2016b). An increased atherosclerotic plaque burden in both humans and mice has been associated with low total carotid artery longitudinal motion (Svedlund, Gan 2011a). Bucak & Canic used computational modelling where CALM and longitudinal motion of stenosed coronary arteries were compared; they found longitudinal displacement in stenotic lesions to be highly dependent on the geometry of the stenotic lesions (Bukac, Canic 2013). They also observed longitudinal motion to be smaller in atherosclerotic than in healthy arteries.

A small study conducted in pigs revealed that the length of the common carotid artery wall segment changed during the cardiac cycle (Tozzi, Hayoz et al. 2001) and in a non-invasive ultrasound study conducted in 5 pigs carotid arteries, CALM displayed significant changes in the response to catecholamines (Rydén Ahlgren, Cinthio, Steen et al. 2012). In that study the increase of CALM seemed to be strongly related to α-adrenoreceptor activation. Despite the notable changes in CALM when predisposing study subjects to catecholamines which act within minutes, CALM seemed to be stable over a 4-month period in healthy humans (Rydén Ahlgren, Cinthio, Persson et al. 2012, Rydén Ahlgren, Steen et al. 2015). Also, among young children, intra-subject variability over time (1-year follow-up) was reported to be small with the general shape of the longitudinal motion being preserved (Au, Proudfoot et al. 2019).

2.5.3 Association with cardiovascular risk factors

According to evidence emerging from different studies, CALM may also reflect vascular health e.g. (Svedlund, Gan 2011a, Svedlund, Eklund et al. 2011, Zahnd, Boussel et al. 2011, Tat, Au et al. 2015). The collected CALM -studies concerning

cardiovascular risk factors are presented in Table 2. Studies investigating the associations between cardiovascular risk factors and CALM parameters are still relatively few with study populations differing from each other. The effect of normal aging on CALM still remains unclear, but among 4-7 year old children (n=114) CALM changed rapidly during the 1-year follow-up i.e. increase in the systolic retrograde, diastolic, maximum and total radial-axial displacement with no differences between gender, reflecting the rapidly occurring change in CALM occurring during childhood (Au, Proudfoot et al. 2019). When investigating the association between CALM and arterial stiffness among 5-8 year-old children, Profound et al. found only a weak correlation between arterial stiffness measured by PWV and CALM indicating that CALM was not a good candidate for measuring arterial stiffness in pre-pubertal children (Proudfoot, Au et al. 2019).

In several studies with older study participants, often with a cardiovascular disease status, reduced CALM values have been found among older patients in comparison to younger healthy study subjects (Svedlund, Gan 2011a, Zahnd, Boussel et al. 2011, Zahnd, Vray et al. 2012). Interestingly, Cinthio et al. (Cinthio, Albinsson et al. 2018) investigated changes in the multiphasic pattern of CALM in 135 healthy non-smoking study subjects of different ages; they found middle-aged and older subjects exhibited significantly different CALM patterns than younger individuals, e.g. the appearance of an increasingly prominent antegrade phase.

There is some evidence, that cardiovascular risk factors have correlated with CALM in smaller study groups. In a small study of patients (n=16) with established coronary artery disease and 16 healthy volunteers, the coronary artery disease group displayed a significantly lower total longitudinal motion of the common carotid artery than healthy controls (Svedlund, Gan 2011b). In a small study of 7 individuals with spinal cord injury who are considered at risk of cardiovascular disease and 7 able-bodied participants, the retrograde intramural strain was smaller in the individuals with spinal cord injury and also smaller peak displacements were found in the intima-media and adventitia layers (Tat, Au et al. 2015). In a small study conducted by Svedlund and Gan in Sweden, 10 patients with common carotid artery plaques and 10 healthy controls were compared, and significantly lower total CALM values were observed when there was a greater plaque burden (Svedlund, Gan 2011a). They made the same finding with 46 mice fed a high fat diet – a low CALM was associated with greater plaque burden and, also with higher IMT. In addition, the low CALM mice displayed higher cholesterol-levels. Tat et al. (Tat, Psaromiligkos et al. 2016b) studied the influence of carotid plaque on CALM in a small population of 12 patients with moderate-to-severe carotid stenosis and compared the results to 23 healthy study participants. The outcome was that healthy individuals displayed a predominantly retrograde motion during systole whereas patients with carotid plaques exhibited mostly an antegrade motion. In addition, patients with severe plaque stenosis had a greater antegrade motion than those with moderate stenosis.

Zahnd et al. investigated 126 Indigenous Australians with periodontal disease and 27 age- and sex- matched healthy controls to examine the associations between

CALM and cardiovascular risk factors (Zahnd, Vray et al. 2012). Patients with periodontal disease showed significantly lower CALM than controls independent of other cardiovascular risk factors like smoking status, BMI, HDL-C, non-HDL-C, HBA1c, diastolic blood pressure, age and sex. In trial of 161 healthy adults, 51 older healthy adults and 14 older adults with cardiovascular disease, total amplitude CALM was found to be reduced in older adults and adults with cardiovascular disease as compared to the younger study participants independent of arterial stiffness (Au, Valentino et al. 2017).

In a larger study of 441 patients referred for myocardial perfusion scintigraphy examination for suspected coronary artery disease, low CALM was found to associate with greater clinically determined myocardial ischemia (Svedlund, Eklund et al. 2011). Furthermore, in the same study, high CALM was observed to predict independently 1-year event-free survival with the regard to major adverse cardiovascular events. This study population was divided into tertiles with respect to the magnitude of CALM, and patients with low CALM showed significantly greater BMI than patients with the middle tertile of CALM. In addition, the group with the lowest CALM displayed the highest carotid IMT. However, in that study, CALM did not relate to smoking-habits, the presence of diabetes or hypertension. In a large multi-ethnic cohort of 389 participants (Black 22%, Chinese 14%, Hispanic 22%, White 39%), contrary results were found; greater CALM was associated with greater IMT but no associations were found with other cardiovascular risk factors such as systolic blood pressure, diastolic blood pressure, diabetes, smoking status, age, high-sensitive CRP or arterial stiffness (Gepner, Colangelo et al. 2015). It is not known how breathing was taken to account in that study and ethnicity may also have exerted an influence on CALM. Gepner et al. (Gepner, McClelland et al. 2019) studied 2050 participants and during a 12-year follow-up 791 cardiovascular events occurred. Longitudinal displacement did not show any predictive value for future cardiovascular events. In these studies, the focus has been on the associations between CALM and risk factors, but the direction of the longitudinal motion has not been taken into account, rather the whole amplitude has been the focus of interest.

The recently published expert consensus review of longitudinal motion (Rizi, Au et al. 2020) emphasizes that CALM seems to be a promising marker of vascular health. There is still a need for large clinical trials and long follow-up studies to clarify the importance of findings emerging from the above-mentioned cross-sectional studies. Currently, large studies examining the subclinical phases of vascular diseases are also lacking. This study improves the knowledge of the inter-relationships between the CALM parameters and the conventional measures of arteriosclerosis in a large study population of the young-to-middle-aged adults. It also deepens our understanding of the associations between the CALM parameters and the traditional risk factors of arteriosclerosis as well as MetS.

Table 2. Studies of longitudinal motion and vascular health

Study	Population	Method	Results
Au, Bochnak et al. (Au, Bochnak et al. 2018)	15 healthy men (22 ± 2 y)	Speckle tracking Cross sectional Three interventions (acute changes in sympathetic activation and smooth muscle relaxation): the serial subtraction test; the cold pressor test; exposure to sublingual nitroglycerine.	Serial subtraction test increased pulse pressure apical rotation and carotid shear rate, no changes in CALM. Cold pressor test increased pulse pressure, basal left ventricular rotation and carotid shear rate, no changes in CALM. Sublingual nitroglycerine: no changes in CALM. In further analysis, three analyses were pooled for a change scores, when changes in left ventricular basal rotation were related to changes in systolic retrograde CALM and changes in carotid shear rate were related to changes in diastolic CALM displacement.
Au, Proudfoot et al. 2019 (Au, Proudfoot et al. 2019)	n=114 (65 girls) (5.8 ± 0.9 y) from the Health Outcomes and Physical activity in Pre-schoolers study	Speckle tracking One-year follow-up study	Increases in CALM magnitudes over 1-year follow-up: in systolic retrograde, diastolic, maximum, and total radial-axial displacement with no differences between gender.
Au, Valentino et al. 2017 (Au, Valentino et al. 2017)	161 younger heathy adults (24 ± 5 y), 51 heathy adults (70 ± 5 y), 14 adults with coronary artery disease (67 ± 8 y)	Speckle tracking Cross sectional	All CALM parameters reduced in the old healthy adults and adults with coronary artery disease compared with young healthy adults. Diastolic velocity and maximum diastolic acceleration were further reduced in the adults with coronary artery disease when compared to the older healthy adults. Diastolic CALM parameters were more strongly related to age than systolic CALM parameters.
Cinthio, Albinsson et al. 2018 (Cinthio, Albinsson et al. 2018)	135 (65 men 20-76 y, 85 women 22-73 y), healthy non-obese (BMI<20)	Echo tracking Cross sectional	Investigated different phases of CALM. Patterns seen in middle-aged and older subjects were significantly different from those in young subjects including 2 additional phases and new complex patterns.
Gepner et al. 2015 (Gepner, Colangelo et al. 2015)	389, 59 ± 8.7 y Randomly chosen participants from Multi-Ethnic Study of Arteriosclerosis, free from cardiovascular disease	Velocity vector imaging, Follow-up study, mean follow up 9.5 y CALM measured at baseline	During the follow-up 19 (4.9%) cardiovascular disease events, 3.6% of these being coronary heart events. Greater longitudinal displacement associated with higher IMT. Longitudinal displacement was not associated with other cardiovascular risk factors or markers of arterial stiffness. No significant association between the longitudinal displacement and future coronary heart disease and cardiovascular events.

Gepner Robyn et al. 2019 (Gepner, McClelland et al. 2019)	2050 participants in Cardiovascular disease risk in the Multi-Ethnic Study of Atherosclerosis. 64 ± 10 y.	Velocity vector imaging Follow-up study Median follow-up 12 y	791 cardiovascular events during the 12-year median follow-up. Longitudinal displacement was positively associated with smoking, negative with heart rate and diastolic blood pressure. In the different ethnic groups, there were some differences in CALM. No predictive value was found.
Proudfoot, Au et al. 2019 (Proudfoot, Au et al. 2019)	n=191 5-8 y children, participants in the health Outcomes and Physical activity in Pre-schoolers study	Speckle tracking Cross sectional	Less retrograde systolic and maximum CALM were weakly associated with higher PWV but not with β-stiffness.
Svedlund, Eklund et al. 2011 (Svedlund, Eklund et al. 2011)	n=441, age showed in tertiles by total longitudinal displacement: 62.05-62.37±8.9-9.29 y Suspected coronary artery disease, referred for myocardial perfusion scintigraphy examination.	Velocity vector imaging Cross sectional and follow-up study, median 372 days.	61 major cardiovascular events occurred during follow-up. Low CALM associated with greater clinically determined myocardial ischemia. High longitudinal displacement predicted independently 1-year event-free survival. Study population was divided into tertiles with respect to the magnitude of CALM. Patients with low CALM showed significantly greater BMI than the middle tertile group. The lowest CALM displayed the highest carotid IMT. No relation between CALM and smoking habits, diabetes or hypertension.
Svedlund &Gan 2011 (Svedlund, Gan 2011a)	10 patients with common carotid artery plaques and 10 healthy volunteers. Age not mentioned.	Velocity vector imaging Cross sectional	Significantly lower total CALM was found when there was a greater plaque burden.
Svedlund &Gan 2011 (Svedlund, Gan 2011a)	46 mice with fat-diet	Velocity vector imaging Cross sectional	Low CALM was associated with a greater plaque burden. Low-CALM mice expressed higher IMT and higher cholesterol -levels compared to mice with higher CALM.
Svedlund & Gan 2011 (Svedlund, Gan 2011b)	16 healthy volunteers and 16 patients with established coronary artery disease	Velocity vector imaging Cross sectional	Significantly lower total longitudinal displacement in coronary artery disease group as compared to healthy volunteers.

Tat, Au et al. 2015 (Tat, Au et al. 2015)	7 individuals with spinal cord injury (36.0 ±12.9) who are considered at risk of cardiovascular disease and 7 able-bodied participants (22.3 ± 2.4)	Speckle tracking Cross sectional Common carotid artery longitudinal wall displacement and intramural shear strain were compared to traditional markers of arterial health, common carotid artery stiffness and IMT.	Retrograde intramural shear strain was smaller in individuals with spinal cord injury compared to able-bodied participants and smaller peak displacements in the intima-media and adventitia. No difference in antegrade oriented motion. Group differences observed in retrograde wall motion phase were greater than those observed for stiffness and IMT. Changes in retrograde oriented motion were independent of IMT and stiffness indices.
Tat, Psaromiligkos et al. 2016 (Tat, Psaromiligkos et al. 2016b)	12 patients with carotid plaque causing moderate (50-79%) (67.1 ± 10.4 y) or severe (80-99%) (69.7 ± 9.4 y) stenosis and 23 healthy participants (57.0 ± 7.6 y).	Speckle tracking Cross sectional	Healthy individuals showed predominantly retrograde motion during systole, whereas those with plaque had mostly anterograde motion. Patients with severe plaque stenosis had greater anterograde motion than those with moderate stenosis.
Zahnd, Boussel et al. 2011 (Zahnd, Boussel et al. 2011)	26 Older diabetic patients (57.7 ± 9.2 year) and younger healthy volunteers (25.7 ± 9.0)	(Contour guided) speckle tracking Cross sectional	Longitudinal displacements of distal and proximal wall of common carotid artery were significantly lower in the diabetic subjects.
Zahnd, Vray et al. 2012 (Zahnd, Vray et al. 2012)	126 Indigenous Australians with periodontal disease and 27 healthy controls.	Contour guided speckle tracking Cross sectional	Patients with periodontal disease displayed significantly lower CALM than controls independent of other cardiovascular risk factors, cross-sectional distensibility and carotid IMT. The strongest correlates of CALM due to multivariable model were age, waist, and pulse pressure, independent of other cardiovascular risk factors, cross-sectional distensibility and PWV.

CALM = carotid artery longitudinal wall motion; IMT = intima-media thickness; PWV = pulse wave velocity.

3 AIMS OF THE STUDY

This thesis focuses on the clinical significance and usability of parameters characterizing the longitudinal motion of common carotid artery wall in the assessment of the state of vascular health of the vascular wall. The Cardiovascular Risk in Young Finns Study is an on-going epidemiological study of atherosclerosis risk factors in children and young adults and forms a large and well-characterized study population suitable for this investigation. There is no previously conducted study, where the association between the cardiovascular risk factors and CALM parameters has been assessed in a large study population of this age group i.e. young-to-middle-aged adults. The aim was to investigate the associations between CALM and the subclinical phase of arteriosclerosis. More specifically, the evaluation of the longitudinal motion was divided into three sub-studies:

1. To study the interrelationships between longitudinal motion of the common carotid artery wall and the conventional measures of arteriosclerosis.
2. To clarify the inter-relationships between the carotid artery longitudinal motion parameters and the traditional risk factors of arteriosclerosis.
3. To investigate the associations between carotid artery longitudinal wall motion parameters and metabolic syndrome.

4 SUBJECTS AND METHODS

4.1 SUBJECTS AND STUDY DESIGN

4.1.1 Description of the Cardiovascular Risk in Young Finns Study

The Cardiovascular Risk in Young Finns Study is an ongoing, five-centre follow-up study of atherosclerosis risk factors in Finnish children and adolescents. The first cross-sectional survey was conducted in 1980, when 3596 children and adolescents participated. The original sample age range was from 3 to 18, and participants were randomly chosen from each area in Finland from a national register. During the years 1980-2007, follow-ups have been conducted regularly in this cohort at intervals from 3 to 6 years (Raitakari, Juonala et al. 2008). The study was approved by local ethics committees and subjects provided written informed consent.

Kuopio University Hospital is one of the five centres involved and investigates the population of Eastern Finland. This cross-sectional study consists of the Kuopio centre data from the year 2007, when the subjects were aged 30 to 45. Vascular ultrasound studies were available for 465 subjects. Ultrasound studies from the left common carotid artery were performed to derive values of both IMT and Cdist. In addition, the left brachial artery diameter measurements were performed to assess the FMD.

In 2007, the visits followed a pre-determined protocol, and all the participants received a letter of invitation with advice on how to prepare themselves for venous blood sampling as well as ultrasound imaging and hemodynamical measures. In general, there was one visit to see the personnel in the research centre, first the participant gave the blood sample and after that he/she underwent ultrasound imaging and the hemodynamical assessment. If a study participant was not able to give a blood sample on the same morning, another visit was arranged. Before venous blood sampling, there was an overnight fast from 22.00 on the previous night when eating and drinking were not allowed, except for water. In the previous evening, the participant was advised to avoid heavy alcohol consumption and intensive exercise. The study participant was requested to avoid high fat foods, drinking coffee or cola and vitamins or dietary supplements in the same morning when the ultrasound imaging and hemodynamic measures were made. There was no special resting time described in the study protocol before the participants underwent ultrasound studies and hemodynamical measures, but they were sitting in the waiting room and when in the study room, they were resting in the supine position before the actual measurement, while the electrodes were being positioned and their personal details entered into the imaging equipment. If blood samples and measurements were performed on the same day, the study participants were recommended to bring a light snack with them.

To provide for any possible absences from work (e.g. sick leave, holidays), there were three trained sonographers instead of one in the Kuopio study centre in 2007. These specialists performed the ultrasound studies and the hemodynamic studies. Two adjacent rooms were in use after taking blood sampling. In one room, one of the research nurses performed certain measurements (e.g. weight, height, blood pressure) including PWV, and in the other room, the carotid ultrasound studies and FMD were conducted. Part of study participants visited first the research nurse and came then to the other room for carotid ultrasound studies and measuring FMD, the others came in the reverse order but the order in which the measurements was conducted was of no importance. The aim was to minimize the time that the participants needed to spend to complete all phases of the study, in the other words, a study participant was taken to whichever room was available.

4.1.2 **Design of studies I-III**

In study I, the association between conventional parameters of subclinical arteriosclerosis, IMT, FMD, Cdist and PWV, and CALM parameters were evaluated. Vascular studies were available for 465 study participants. Successful longitudinal motion analysis could be determined in 292 subjects (63 %).

In study II, the association between traditional cardiovascular risk factors and CALM parameters were examined. In this analysis five women were excluded due to pregnancy and thus, the final study population involved 287 participants.

In study III, association between MetS and CALM parameters were studied. Exclusion criteria were pregnancy and type 1 diabetes. Furthermore, there were a lack of anthropometric studies for two study participants i.e. the final study population was 281 participants. Adiponectin data was lacking in seven study participants, hence in the univariate analysis with CALM and adiponectin, a total of 274 study participants were analysed.

4.1.3 **Clinical characteristics**

Height and weight were measured, height to an accuracy of 1 cm and weight to an accuracy of 1 kg. BMI was calculated as weight in kilograms divided by height in meters squared. Waist circumference was measured using an anthropometric tape at the end of expiration at the midpoint between the iliac crest and the lowest rib at the midaxillary line to an accuracy of 0.1 cm and the average of two measurements was used. Systolic and diastolic blood pressures were measured in the sitting position from the brachial artery using a random zero sphygmomanometer (Hawksley & Sons Ltd, Lancin, UK). The average of three measurements was used in the analysis.

Smoking habits, medications, diagnosed diseases and pregnancy were examined with questionnaires. Smoking was processed as dichotomous variable (smoking/non-smoking) and subjects smoking regularly on a daily basis were regarded as smokers.

4.1.4 Biochemical analyses

For the determination of serum lipid, adiponectin, insulin and glucose levels, venous blood samples were drawn after an overnight fast. All measurements of lipid levels as well as glucose, insulin, adiponectin levels, were performed in duplicate in the same laboratory. Standard enzymatic methods were used for measuring levels of serum total cholesterol, triglycerides, and HDL-C. The Friedewald formula was used to calculate the LDL-C concentration for participants with triglycerides < 4mmol/l. The serum insulin concentration was measured by microparticle enzyme immunoassay (IMx insulin reagent, Abbott Diagnostics, USA) on an IMx instrument, and glucose concentrations were analysed enzymatically. Serum adiponectin concentrations were analysed by radioimmunoassay (Human Adiponectin and Leptin RIA kits, Linco Research, Inc, MO, USA). Homeostasis model assessment (HOMA) index was calculated from the formula: fasting glucose (mmol/l) x fasting insulin (μU/ml)/ 22.5.

4.1.5 Assessment of cardiovascular risk factors and measures of metabolic syndrome

Cardiovascular risk factors were defined as BMI ≥ 25.0 kg/m2, LDL-C ≥ 3.0 mmol/l or lipid-lowering medication, systolic blood pressure ≥ 140 mmHg, or antihypertensive medication. The metabolic syndrome was defined according to the Harmonized criteria and the definition included the following criteria: waist circumference ≥ 88cm in women and ≥ 102cm in men, fasting plasma glucose ≥ 5.6 mmol/l or drug treatment, hypertriglyceridemia ≥ 1.7 mmol/l or treatment, HDL-cholesterol ≤ 1.3 mmol/l in women and 1.0 in men or drug treatment, and systolic blood pressure ≥ 130 mmHg or diastolic blood pressure ≥ 85 mmHg or antihypertensive drug treatment. A diagnosis required that any three of the five criteria should be present (Alberti, Eckel et al. 2009). Hyperinsulinemia defined as non-diabetic subjects having a fasting insulin level in the highest quartile, 11.06 mU/l, was used as a cut-off point in this study (cut-off point of the whole study population of the Young Finns Study year 2007). Low adiponectin as a risk factor was defined as the lowest quartile in this study population and it was 6.22 μg/ml. High HOMA-IR as a risk factor was defined as the highest quartile in this study population and it was ≥ 2.44.

4.1.6 Pulse wave velocity

To determine conventional measurement of arteriosclerosis, PWV, a commercially available whole-body impedance cardiograph device (CircMon®, JR Medical Ltd., Tallinn, Estonia) was used. Circmon has a whole-body impedance cardiograph channel, a distal impedance plethysmogram channel and an ECG channel. When the pulse pressure wave enters the aortic arch and the diameter of the aorta changes, the whole-body impedance decreases. This can be measured by the voltage electrodes on the distal parts of the extremities. The software measures the time difference

between the onset of the decrease in impedance in the whole-body impedance signal and the popliteal artery signal (at knee joint level). The PWV in CircMon software is determined from the time difference and distance between the two recording sites (Kööbi, Kaukinen et al. 1997, Kööbi, Kähönen et al. 2003, Aatola, Hutri-Kähönen et al. 2010). Measures of PWV have been found to possess a good repeatability index and a reproducibility index (99% and 87%, respectively) (Tahvanainen, Koskela et al. 2009). The validation study has been reported previously (Kööbi, Kähönen et al. 2003).

4.2 ULTRASOUND IMAGING

Ultrasound studies were performed using Sequoia512 ultrasound scanner (Acuson, Mountain View, Calif) equipped with a 14MHz linear array transducer. Sonographers were trained and they adhered to standardized protocol. Ultrasound imaging of common carotid artery was performed to analyse the conventional parameters of vascular health and longitudinal wall motion. Brachial artery imaging was performed to analyse FMD.

4.2.1 Carotid Ultrasound Imaging

Ultrasound studies were performed using a Sequoia512 ultrasound scanner (Acuson, Mountain View, Calif) equipped with a 14MHz linear array transducer. Sonographers were trained and they followed the standardized protocol. The ECG signal (modified chest lead 5) was recorded and presented alongside with B-mode image sets. The left common carotid artery was scanned and resolution box function was used to record a 25 mm wide and 15 mm high image including the beginning of the carotid bifurcation and the distal common carotid artery. A five second cine loop (25 frames per second) was digitally stored for subsequent off-line analysis. The same image set was used to derive IMT and Cdist as well as radial and longitudinal motions of artery wall. Blood pressure was measured in the supine position with an automated sphygmomanometer (Omron M4, Omron Matsusaka Co., Ltd, Japan) immediately before and after ultrasound imaging for the calculation of the Cdist (Raitakari, Juonala et al. 2003).

4.2.2 Carotid intima-media thickness

The mean IMT was measured by focusing the image on the posterior (far) wall of the left common carotid artery, and gain settings were used to optimize image quality. In an attempt to valuate the greatest distance between the lumen-intima interface and the media-adventitia interface, a magnified image from the angle was recorded. The best-quality end-diastolic frame was selected from the video. At least, four measurements were taken from this image approximately 10 mm proximal to the

bifurcation to derive the IMT. Digitally stored scans were manually analysed by a single reader blinded to the details of the participants.

The reproducibility of the IMT measurements is very good, e.g. the 3-month between-visit coefficient of variation (CV) was 6.4% and the between-observer CV was 5.2% (Raitakari, Juonala et al. 2003).

4.2.3 Carotid artery distensibility

The best-quality cardiac cycle was selected from the 5-second cine loop to assess carotid elasticity indices. The common carotid artery diameter was measured at least twice both at end-diastole and at end-systole by using the callipers of the ultrasound scanner. The means of the measurements were used as the end-diastolic and end-systolic diameters. In the calculation of the indices of arterial elasticity, the following ultrasound and concomitant brachial blood pressure measurements were used:

$$Cdist = ([D_s - D_d]) \div D_d \div (P_s - P_d)$$

where D_s is the systolic diameter, D_d is the diastolic diameter, P_s is systolic blood pressure, P_d is diastolic blood pressure. The reproducibility of the Cdist values used in this study has been shown to be at a good level, e.g. carotid artery between-visit coefficient of variation was 16.3% for Cdist and 2.7% for carotid artery diastolic diameter. Intra-observer CV was 13.6% for Cdist and for carotid artery diastolic diameter 1.4% (Juonala, Järvisalo et al. 2005).

4.2.4 Longitudinal motion

Arterial wall motion analysis was performed using a motion tracking program developed in-house in our research-group (Yli-Ollila, Laitinen et al. 2013). The software was written in Matlab (2007b, The MathWorks Inc., Natic, MA, USA) and it is capable of tracking the longitudinal and radial motion of different arterial layers in the common carotid artery ultrasound video and to synchronize the movements with the simultaneously recorded ECG. The basic method used in the motion tracking was a two-dimensional cross-correlation (block matching) enhanced with a contrast optimization technique to reduce noise from the videos.

In the longitudinal motion analysis, three regions of interest (ROIs) are drawn on the ultrasound image: one on the intima-media complex, one on the adventitial layer and one on surrounding tissue outside adventitia (see Figure 5B). When tracking the radial motion of the arterial wall, the ROIs are drawn on the distal and proximal arterial wall. The motion tracking of the longitudinal motion was considered suitable for analysis if the tracking successfully recorded at least two heart cycles, otherwise the motion data was discarded.

Three different longitudinal motion curves were measured: between the intima-media complex and the adventitial layer (IA, see Figure 5E), between the intima-media complex and the surrounding tissue (IO, see Figure 5C) and between the adventitial layer and the surrounding tissue (AO, see Figure 5D). The curves of the longitudinal motion have been previously shown to vary extensively between

individuals (Yli-Ollila, Laitinen et al. 2013). The longitudinal motion can be divided into three categories: forward-oriented, bidirectional, and backward-oriented curves.

The amplitude of the motion (ampl), the forward (ante) oriented and backward (retro) oriented component of the motion between the different layers of the common carotid artery wall were investigated. In addition, the main deviation of the longitudinal motion (dev) between the different arterial layers was evaluated by computing the average of the motion curve over a cardiac cycle. A parameter called Polydeg was used to estimate the complexity of the longitudinal movement. Polydeg is the degree of the polynomial function needed to fit the function to the data points of the longitudinal movement of intima-media layer (IO curve) in order to obtain a Pearson`s correlation coefficient greater than 0.95. In addition to the longitudinal amplitude and complexity parameters, the diameter change of the common carotid artery was measured to create a distension curve, in which the longitudinal movement of the intima-media complex was plotted against the diameter change during a heart cycle. Our novel stiffness parameter RAlength is the length of this two-dimensional curve. The reproducibility of the longitudinal parameters has been described previously (Yli-Ollila, Laitinen et al. 2013) and the overall reproducibility was estimated as good. For IAante reproducibility CV% was 72.3 and repeatability CV% 46.0. For IAretro reproducibility CV% was 46.1 and repeatability CV% 32.3. For IOante the same values were 36.1 and 28.5, and for IOretro 28.6 and 21.0. For IAampl, the reproducibility CV% was 29.8 and repeatability CV% 19.0. For IOampl, the reproducibility CV% was 16.3 and repeatability CV% was 13.8. For IAdev, the reproducibility CV% was 97.1 and repeatability CV% was 97.2% and for IOdev, the reproducibility CV% was 92.3 and repeatability CV% 117.5.

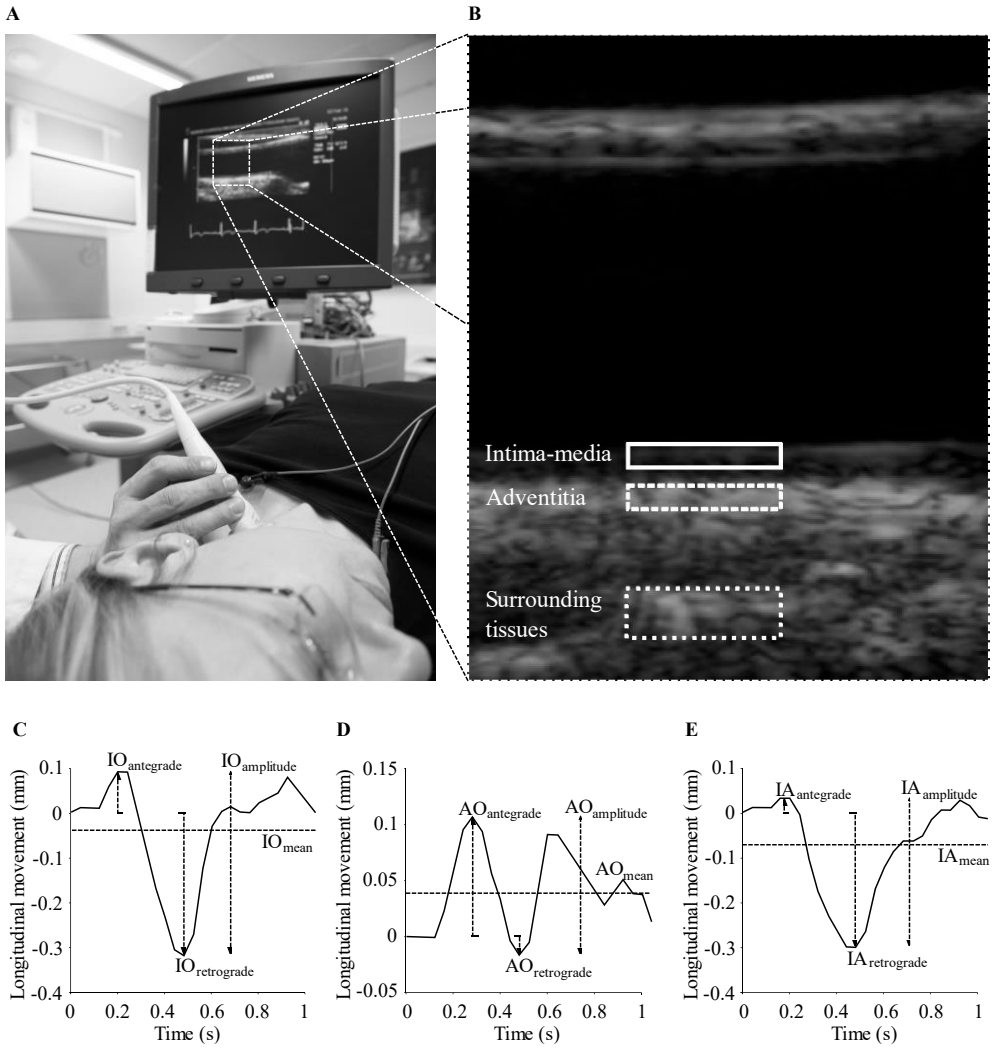

Figure 5. Measuring the longitudinal motion of the left common carotid artery (A) and the carotid artery wall longitudinal motion curves in the different arterial layers. B-mode ultrasound image of the common carotid artery and regions of interests (ROIs) are drawn on the carotid wall (B), where a solid line is drawn on the intima-media -complex, a dashed line on the adventitia layer and a dotted line on the surrounding tissue. In curves (C, D, E), the solid line represents the movement of the curve, and dashed lines refer to the maximum antegrade (ante), retrograde (retro) and peak-to-peak amplitudes (ampl).

4.2.5 **Brachial flow-mediated dilatation**

The brachial artery was imaged by ultrasound according to the guidelines in the measurement of the brachial flow-mediated dilatation (FMD) (Corretti, Anderson et al. 2002). The same ultrasound scanner and transducer as the carotid imaging was used and a segment of the left brachial artery above the antecubital crease was imaged in the longitudinal plane. To assess brachial FMD, the brachial artery

diameter was measured both at rest and during reactive hyperaemia. Increased flow was induced by inflation of a pneumatic tourniquet placed around the forearm to a pressure of 250 mmHg for 4.5 minutes, followed by release (Figure 6). The vessel diameter was derived from the average of 3 measurements at rest and at 40, 60 and 80 seconds after cuff release (Juonala, Viikari et al. 2004). The maximum FMD in scans after reactive hyperaemia was expressed as the percentage relative to resting scan. Brachial artery diameter measurements have a high degree of reproducibility, the 3-month between-visit CV was 3.2%. For FMD measurements the between-visit was 26.0%. Intra-observer CV was 15.3.% for FMD and 1.2% for brachial diameter (Juonala, Viikari et al. 2004).

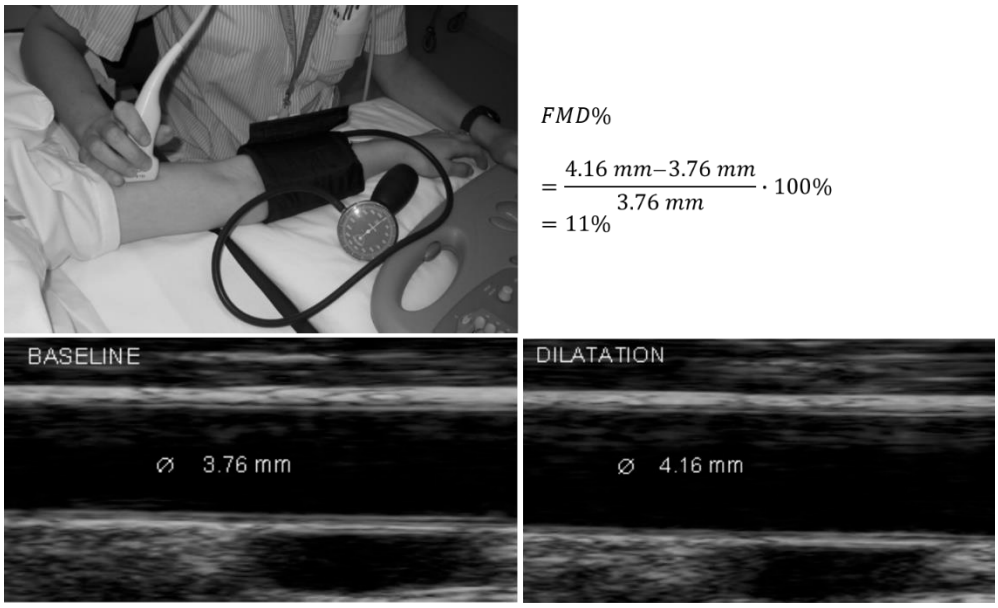

Figure 6. Measuring brachial flow-mediated dilatation. Brachial artery diameter (FMD) is measured at rest and at 40, 60 and 80 seconds after reactive hyperemia. Increased flow was induced by inflation of a tourniquet placed around the forearm to a pressure of 250 mmHg for 4.5 minutes and followed by release.

4.3 STATISTICAL METHODS

In the first study, non-parametric tests were adopted because CALM parameters were not normally distributed; the distribution was slightly skew. Spearman`s rank correlation coefficients were used to define conformities between the indices of longitudinal movement and the conventional parameters of vascular health. In the advanced analyses, the study population was analysed in tertile groups for Cdist and PWV. Statistical significance between these groups was assessed with Kruskal-Wallis

test. Mann-Whitney U-test with Bonferroni correction was used to analyse more accurately the significance of differences between specific tertiles. Statistical analyses were performed with SPSS version (IBM Corp., IBM SPSS Statistics for Windows, Version 22.0, Armonk, NY) and statistical significance was inferred at a 2-tailed value of $p < 0.05$.

In the second and third studies, parametric tests were used, and this enabled adjusting the results with age and gender. Residuals in the linear regression model were normally distributed and the size of the population was large, thus the application of parametric tests was deemed acceptable. A partial correlation analysis adjusted with age and gender was used to assess associations between the indices of longitudinal motion, the conventional parameters of vascular health and traditional cardiovascular risk factors systolic blood pressure, diastolic blood pressure, total cholesterol, LDL-C, HDL-C, triglycerides and BMI. To examine the effects of the accumulation of risk factors on vascular health parameters, we calculated a simple score according to the number of current risk factors (0, 1, 2, and 3 or more risk factors). Risk factors were defined as BMI ≥ 25.0 kg/m2; LDL-C ≥ 3.0 mmol/l or lipid-lowering medications; systolic blood pressure ≥ 140 mmHg or antihypertensive medication, and daily smoking. A comparison between groups was performed using analysis of covariance (ANCOVA) while adjusting for variables with pairwise comparisons being executed. Bonferroni correction was used to analyse more accurately the significance of difference between different score groups. Statistical analyses were performed with SPSS version (IBM Corp., IBM SPSS Statistics for Windows, Version 22.0, Armonk, NY) and statistical significance was inferred at a 2-tailed value of $p<0.05$.

In the third study, independent samples T-test was used to detect differences between study groups with and without MetS. Linear regression model adjusted with age and gender was used to assess associations between the indices of longitudinal motion and the components of the MetS. Multivariate regression model with a stepwise method adjusted for age and sex being used to examine the independent effects of the individual components of MetS and longitudinal motion parameters. Statistical analyses were performed with SPSS version (IBM Corp., IBM SPSS Statistics for Windows, Version 22.0, Armonk, NY) and statistical significance was inferred at a 2-tailed value of $p<0.05$.

4.4 ETHICAL CONSIDERATIONS

The study was approved by Ethics Committee, Hospital District of Southwest Finland and local ethics committees, Ethics Committee of Kuopio University Hospital. A written informed consent was obtained from all the subjects. This study was conducted in compliance with the Declaration of Helsinki.

5 RESULTS

5.1 ASSOCIATIONS BETWEEN CALM PARAMETERS AND CONVENTIONAL MEASURES OF SUBCLINICAL ARTERIOSCLEROSIS

Successful longitudinal motion analyses were performed in 292 subjects out of 465 subjects (63 %). The clinical characteristics are presented in Table 3. Table 4 summarizes the means of the indices of longitudinal motion and conventional measures of subclinical arteriosclerosis. The correlations between longitudinal motion parameters and prevailing parameters reflecting subclinical vascular changes (IMT, Cdist, FMD, PWV) are shown in Table 5.

PWV and Cdist showed a significant correlation with most of the longitudinal motion parameters (Table 5). Only IAante, IOampl and AOampl did not display any statistically significant correlations with PWV. IAante did not also show any statistically significant association with Cdist.

The retrograde motion parameters and amplitude were directly correlated with Cdist and inversely with PWV. Moreover, the parameters describing antegrade motion and deviation were directly correlated with PWV and inversely with Cdist. No significant correlation was seen between the parameters of longitudinal motion and FMD nor with IMT. When associations between IA motion and tertiles of Cdist and PWV were studied, a statistically significant difference was found between the highest and lowest Cdist tertiles in IAampl, IAretro and IAdev and between the highest and lowest PWV tertiles in IAretro and IAdev. No significant difference was found between the middle and the highest tertiles.

Polydeg showed no significant correlations with any of studied measures illustrating the subclinical vascular changes examined in this study (Table 5). The overall distance of intima movements during one heart cycle (RAlength) and IMT did not show any significant correlations, but with Cdist, RAlength correlated directly and with FMD and PWV, inverse correlations were detected (Table 5).

Table 3. Clinical characteristics of the study population.

	Study 1	**Study 2**	**Study 3**	
	N=292	N=287	No MetS N=228	MetS N=53
Age (years)	38 ± 5	38 ± 5	38 ± 5	39 ± 5 NS
Sex (%women)	–	60.3%	63.2%	49.1% NS
Body height (cm)	170 ± 9	170 ± 9	170 ± 9	172 ± 10 NS
Body weight (kg)	75 ± 16	75 ± 16	71 ± 14	90 ± 16***
Waist (cm)	–	–	83.1 ± 10.2	101.1 ± 11.8 ***
Body mass index (kg/m2)	25.7 ± 4.7	25.7 ± 4.7	24.6 ± 3.7	30.5 ± 5.2 ***
Systolic blood pressure (mmHg)	128 ± 14	128 ± 14	126 ± 13	137 ± 14 ***
Diastolic blood pressure (mmHg)	81 ±10	81 ± 10	79 ± 9	89 ± 9 ***
Total cholesterol (mmol/l)	–	5.04 ± 0 .87	4.95 ± 0.83	5.49 ± 0.93 ***
LDL-cholesterol (mmol/l)	–	3.10 ± 0.75	3.04 ± 0.73	3.40 ± 0.81 **
HDL-cholesterol (mmol/l)	–	1.38 ± 0.34	1.42 ± 0.30	1.20 ± 0.43 ***
Triglycerides (mmol/l)	–	1.29 ± 0.74	1.08 ± 0.44	2.05 ± 0.90 ***
Glucose (mmol/l)	–	–	5.20 ± 0.44	5.75 ± 0.60 ***
Insulin (mU/l)	–	–	6.78 ± 4.70	14.26 ± 7.96 ***
HOMA-IR	–	–	1.60 ± 1.26	3.68 ± 2.17 ***
Adiponectin (µg/ml)	–	–	10.90 ± 5.75	7.11 ± 3.18 ***
Smoking	–	17.1 %	16.3 %	22.6 % NS

Values are mean ± SD or prevalence (%). Statistical significances in study 3 between the metabolic syndrome (MetS) and no metabolic syndrome -subgroups: NS= nonsignificant, *p<0.05; **p<0.01, ***p<0.001. HDL = High-density lipoprotein; LDL = Low-density lipoprotein. HOMA-IR = Homeostasis model assessment of insulin resistance.

Table 4. Characteristics of the study population; the conventional parameters of subclinical arteriosclerosis and the longitudinal motion parameters.

	Study1 N= 292	**Study2** (N= 287)	**Study3** No MetS (N=228)	MetS (N=53)
Intima-media -thickness (mm)	0.67 ± 0.10	0.67 ± 0.10	–	–
Carotid artery distensibility (%/10mmHg)	1.98 ± 0.68	1.97 ± 0.68	–	–
Flow-mediated dilatation (%)	9.17 ± 4.27	–	–	–
Pulse wave velocity (m/s)	8.30 ± 1.35	–	–	–
IAante (mm)	0.07 ± 0.06	0.07 ± 0.06	0.06 ± 0.06	0.09 ± 0.07 **
IAretro (mm)	0.10 ± 0.10	0.10 ± 0.09	0.11 ± 0.10	0.07 ± 0.06 ***
IAampl (mm)	0.17 ± 0.09	0.17 ± 0.09	0.17 ± 0.09	0.15 ± 0.07 NS
IAdev (mm)	−0.02 ± 0.05	−0.01 ± 0.05	−0.02 ± 0.05	0.00 ± 0.05 **
IOante (mm)	0.14 ± 0.13	0.15 ± 0.13	0.13 ± 0.12	0.22 ±0.13**
IOretro (mm)	0.26 ± 0.22	0.25 ± 0.22	0.29 ± 0.22	0.12 ± 0.15***
IOampl(mm)	0.40 ± 0.18	0.40 ± 0.18	0.42 ± 0.18	0.34 ± 0.15**
IOdev (mm)	−0.02 ± 0.05	−0.01 ± 0.05	−0.02 ± 0.05	0.00 ± 0.05**
AOante (mm)	0.12 ± 0.11	–	–	–
AOretro (mm)	0.19 ± 0.17	–	–	–
AOampl (mm)	0.31 ± 0.14	–	–	–
AOdev (mm)	−0.02 ± 0.05	–	–	–
Polydeg	7 ± 2	–	–	–
Ralength (mm)	1.58 ± 0.47	–	–	–

Values are mean ± SD or prevalence (%). Statistical significances in study 3 between the metabolic syndrome (MetS) and no metabolic syndrome subgroups: NS= nonsignificant, *p<0.05; **p<0.01, ***p<0.001. The longitudinal motion parameters: IA = longitudinal motion between intima-media -complex and adventitia; IO = longitudinal motion between intima-media -complex and surrounding tissue; AO = longitudinal motion between the adventitial layer and the surrounding tissue. Subindexes ante = forward oriented component of the longitudinal motion; retro = backward oriented component of the longitudinal motion; ampl = amplitude of the

longitudinal motion; dev = the main deviation between the different arterial layers. Polydeg = the degree of the polynomial function needed to fit the function to the data points of the longitudinal motion of intima-media layer, Polydeg estimates the complexity of the longitudinal motion. RAlength = the length of the two-dimensional curve, measures the diameter change.

Table 5. Correlations between the common carotid artery longitudinal motion parameters and conventional parameters of subclinical arteriosclerosis.

	CIMT	Cdist	FMD	PWV
IAante	−0.015	−0.090	0.000	0.099
IAretro	0.048	0.225***	−0.088	−0.171**
IAampl	−0.001	0.213***	−0.046	−0.137*
IAdev	−0.020	−0.182**	0.073	0.168**
IOante	0.072	−0.173**	−0.026	0.162**
IOretro	0.028	0.312***	−0.040	−0.211***
IOampl	0.077	0.267***	−0.043	−0.077
IOdev	−0.014	−0.183**	0.078	0.166**
AOante	0.077	−0.132*	−0.030	0.129*
AOretro	−0.011	0.267***	0.014	−0.151*
AOampl	0.037	0.217***	−0.008	−0.018
AOdev	−0.011	−0.181**	0.066	0.168**
Polydeg	−0.077	−0.014	−0.065	−0.025
RAlength	−0.023	0.471***	−0.153*	−0.224***

Statistical significances: * $p < 0.05$; ** $p < 0.01$, *** $p < 0.001$. The longitudinal motion parameters: IA = longitudinal motion between intima-media -complex and adventitia; IO = longitudinal motion between intima-media -complex and surrounding tissue; AO = longitudinal motion between the adventitial layer and the surrounding tissue. Subindexes ante = forward oriented component of the longitudinal motion; retro = backward oriented component of the longitudinal motion; ampl = amplitude of the longitudinal motion; dev = the main deviation between the different arterial layers. Polydeg = the degree of the polynomial function needed to fit the function to the data points of the longitudinal motion of intima-media layer, Polydeg estimates the complexity of the longitudinal motion. RAlength = the length of the two-dimensional curve, measures the diameter change. CIMT= carotid intima-media thickness; Cdist = carotid artery distensibility; FMD = brachial flow-mediated dilatation (%); PWV = pulse wave velocity.

5.2 INFLUENCE OF CARDIOVASCULAR RISK FACTORS ON THE COMMON CAROTID ARTERY

The clinical characteristics of the study subjects and characteristics of vascular health are shown in Table 3 and Table 4. The risk factor prevalence was: BMI ≥ 25.0 kg/m^2 50.3% (N=144); LDL-C ≥ 3.0 mmol/l 52.3% (N=150) or lipid-lowering medication 2.4% (N=7); systolic blood pressure ≥ 140 mmHg 24.1% (N=69) or antihypertensive medication 7.3% (N=21). Smoking prevalence was 17.1% (N=49).

The correlations between the CALM parameters, CIMT, Cdist and the traditional risk factors are displayed in Table 6. The associations between the traditional risk factors and the motion of different common carotid artery layers displayed parallel results; the significant associations were most evident in IO motion with significant associations being found between CALM and systolic blood pressure, diastolic blood pressure and BMI and also with both antegrade and retrograde motion of IO and total cholesterol as well as triglycerides.

Antegrade motion correlated directly and retrograde motion inversely with the studied cardiovascular risk factors; the only exception was HDL (Table 6). IOante displayed a significant direct correlation with BMI, diastolic blood pressure, total cholesterol, LDL-C and triglycerides but no significant association was seen with systolic blood pressure and HDL-C. A significant inverse correlation was seen between IOretro and systolic blood pressure as well as with IOretro and diastolic blood pressure, total cholesterol, triglycerides and BMI but not with LDL-C nor HDL-C. Both IOdev and IAdev were directly correlated with diastolic blood pressure, BMI, but no statistically significant correlations were detected with lipids. IOampl showed an inverse correlation with systolic blood pressure, diastolic blood pressure and BMI but no statistically significant correlations were detected with lipids. IAampl had a significant, indirect correlation solely with diastolic blood pressure. IAante revealed a significant direct correlation with BMI and an inverse correlation with HDL but no other significant associations were seen between IAante and risk factors. Instead, IAretro displayed an inverse and statistically significant correlation with BMI, systolic and diastolic blood pressure but not with lipids. CIMT correlated directly and significantly with BMI, systolic blood pressure and LDL-C. Cdist demonstrated a significant inverse correlation with systolic and diastolic blood pressure, total cholesterol, triglycerides, and BMI but not with LDL-C and HDL-C.

Antegrade longitudinal motion increased and retrograde longitudinal motion decreased as the number of risk factors increased (Table 7). Reductions in peak-to-peak amplitude as well as in retrograde amplitude of longitudinal motion were found when the number of risk factors increased. The CALM parameters showed also significant differences between score groups with 0 and 1 risk factor (IAampl, IAretro, IAdev, IOdev) and between 0 and 2 risk factors (IAante, IAretro, IAdev, IOante, IOretro, IOdev). When the risk factor load increased up to three or more, only IOante and IOretro still displayed a significant difference. IOante exhibited a significant difference between number of risk factors 1 and 2, and 1 and 3 or more.

CIMT displayed a significant difference in score-groups with 0 and 3 or more risk factors. In this study population, Cdist did not show any significant differences between different score-groups.

Table 6. Age- and sex-adjusted correlations between vascular health parameters and cardiovascular risk factors.

	Body mass index	Systolic blood pressure	Diastolic blood pressure	Total cholesterol	Low-density lipoprotein	High-density lipoprotein	Triglycerides
IAante	0.173**	0.075	0.097	0.043	0.073	−0.142*	0.114
IAretro	−0.140*	−0.149*	−0.200***	−0.109	−0.083	−0.009	−0.109
IAampl	−0.027	−0.106	−0.144*	−0.085	−0.037	−0.109	−0.036
IAdev	0.141*	0.115	0.155**	0.063	0.086	−0.086	0.076
IOante	0.274***	0.109	0.198***	0.224***	0.214***	−0.103	0.255***
IOretro	−0.291***	−0.189**	−0.256***	−0.163**	−0.106	0.008	−0.228***
IOampl	−0.158**	−0.152*	−0.170**	−0.037	0.025	−0.064	−0.094
IOdev	0.146*	0.115	0.155**	0.068	0.091	−0.087	0.078
CIMT	0.252***	0.181**	0.079	0.092	0.126*	−0.132*	0.106
Cdist	−0.142*	−0.338***	−0.365***	−0.130*	−0.074	−0.072	−0.122*

Statistical significances: * $p < 0.05$; ** $p < 0.01$, *** $p < 0.001$. The longitudinal motion parameters: IA = longitudinal motion between intima-media -complex and adventitia; IO = longitudinal motion between intima-media -complex and surrounding tissue. Subindexes ante = forward oriented component of the longitudinal motion; retro = backward oriented component of the longitudinal motion; ampl = amplitude of the longitudinal motion; dev = the main deviation between the different arterial layers. CIMT= carotid intima-media thickness; Cdist = carotid artery distensibility.

Table 7. Relationships between numbers of traditional risk factors (hypertension, obesity, dyslipidaemia, smoking), conventional measurements, and the longitudinal motion parameters.

Number of Risk Factors	**0**	**1**	**2**	**≥3**	
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	p-value
IAante (mm)	0.05 ± 0.05	0.06 ± 0.06	0.08 ± 0.07[b]	0.06 ± 0.06	0.008
IAretro (mm)	0.15 ± 0.13	0.09 ± 0.07[a]	0.09 ± 0.10b	0.09 ± 0.07	0.003
IAampl (mm)	0.20 ± 0.11	0.15 ± 0.06[a]	0.173 ± 0.10	0.15 ± 0.08	0.02
IAdev (mm)	−0.04 ± 0.06	−0.01 ± 0.05[a]	−0.00 ± 0.06[b]	−0.01 ± 0.05	0.002
IOante (mm)	0.11 ± 0.12	0.11 ± 0.10	0.19 ± 0.14[b, d]	0.19 ± 0.12[c, e]	<0.0001
IOretro (mm)	0.36 ± 0.27	0.28 ± 0.19	0.19 ± 0.20[b]	0.18 ± 0.18[c]	<0.0001
IOampl (mm)	0.47 ± 0.23	0.39 ± 0.16	0.38 ± 0.17	0.37 ± 0.13	0.049
IOdev (mm)	−0.04 ± 0.06	−0.01 ± 0.05[a]	−0.00 ± 0.06[b]	−0.01 ± 0.05	0.002
Cdist (%/10mmHg)	2.15 ± 0.84	2.02 ± 0.59	1.95 ± 0.69	1.72 ±0.56	NS
CIMT (mm)	0.66 ± 0.07	0.66 ± 0.09	0.68 ± 0.11	0.72 ± 0.11[c, e]	0.009

Significant differences ($p<0.05$) between score-groups are shown with letters: a: 0-1, b 0-2, c 0-≥3, d 1-2, e 1-3, f 2-≥3. NS = nonsignificant. Univariate analysis of variance was used with Bonferroni correction. The longitudinal motion parameters: IA = longitudinal motion between intima-media -complex and adventitia; IO = longitudinal motion between intima-media -complex and surrounding tissue. Subindexes ante = forward oriented component of the longitudinal motion; retro = backward oriented component of the longitudinal motion; ampl = amplitude of the longitudinal motion; dev = the main deviation between the different arterial layers. Cdist = carotid artery distensibility; CIMT= carotid intima-media thickness.

5.3 METABOLIC SYNDROME´S EFFECT ON CALM PARAMETERS

The clinical characteristics of the study subjects and the differences between study groups with and without MetS are shown in Table 3 and Table 4. Differences in the CALM parameters between these two groups are displayed in Table 3. There were significant differences between MetS and no-MetS groups in all conventional measures of MetS, also including insulin-levels, HOMA-IR and adiponectin. There were no significant differences between the study groups with respect to age, gender and smoking habits. In the CALM parameters, a significant difference between MetS and no-MetS-groups was observed in all examined parameters except for IAampl. In the MetS group, amplitude of antegrade CALM was significant larger and amplitude of retrograde CALM significant smaller than among non-MetS individuals. The study group without MetS showed negative IAdev, whereas subjects having MetS had slightly positive values in deviation. MetS according to the harmonized criteria was detected in 53 subjects (19%).

Linear regression model adjusted with age and gender was used to define univariate conformities between the CALM parameters and the components of the metabolic syndrome (Table 8). The retrograde peak-to-peak amplitude of CALM showed significant negative correlations with hypertension whereas deviations and IOante displayed significant positive correlations. No significant correlations were found between peak-to-peak amplitudes and antegrade amplitudes of IA motion and hypertension. With respect to obesity, significant positive correlations were found with antegrade amplitudes of CALM as well as with deviations. Retrograde amplitudes of CALM and IOampl displayed a significant negative correlation with obesity. IAampl showed no significant correlation with obesity. IOante exhibited a significant positive and IOretro a significant negative correlation with hypertriglyceridemia but no other significant associations were seen with high triglyceride levels and CALM parameters. Dyslipidaemia, low HDL as a risk factor, revealed a significant positive correlation with IAante but with other CALM parameters there were no significant associations. Retrograde motions of IA and IO as well as peak-to-peak amplitudes showed a significant negative correlation with hyperinsulinemia whereas there was a significant positive association between IOante and the presence of hyperinsulinemia. No significant correlations were seen between longitudinal motion parameters and hyperglycaemia nor with adiponectin levels except for that between IOante and adiponectin. Retrograde motions of IA and IO as well as amplitudes showed significant negative associations with HOMA-IR and a significant positive association was found with IOante.

In the multivariate analysis with a stepwise method, hypertension showed a significant inverse correlation with retrograde amplitudes of CALM (Table 9). Hypertension correlated significantly and directly with deviations, but no other significant correlations between CALM parameters and hypertension were observed.

IOante showed significant positive and IOretro significant negative correlation with obesity but no significant associations were shown in obesity and IA parameters. There was a significant positive correlation between low-HDL levels and antegrade as well as peak-to-peak amplitudes of IA but not with the other CALM parameters. Hyperinsulinemia had an inverse correlation with retrograde and peak-to-peak amplitudes of CALM but not with other CALM parameters. Hyperglycaemia did not show any significant correlations in this model.

Table 8. Age- and sex – adjusted relationships between components of MetS and the longitudinal motion parameters in men and women aged 30-45 years.

	IA		IO	
	B ± SE	Beta	B ± SE	Beta
Hypertension (RR ≥ 130/≥85 mmHg or medication)				
Antegrade	0.015 ± 0.008	0.119 NS	0.049 ± 0.016	0.192**
Retrograde	−0.029 ± 0.012	−0.156 *	−0.090 ± 0.028	−0.205**
Amplitude	−0.015 ± 0.012	−0.083 NS	−0.041 ±0.023	−0.116 NS
Deviation	0.014 ± 0.007	0.131 *	0.014 ± 0.007	0.133*
Obesity (waist ≥102cm in men and ≥ 88cm)				
Antegrade	0.022 ± 0.009	0.149 *	0.094 ± 0.018	0.305***
Retrograde	−0.031 ± 0.013	−0.138 *	−0.145 ± 0.030	−0.270***
Amplitude	−0.009 ± 0.013	−0.042 NS	−0.051 ± 0.025	−0.116*
Deviation	0.018 ± 0.008	0.136 *	0.019 ± 0.008	0.145*
Dyslipidaemia (HDL-C < 1.00mmol/L in men,<1,3 in women or medication)				
Antegrade	0.024 ± 0.008	0.177 **	0.030 ± 0.017	0.108 NS
Retrograde	−0.006 ± 0.012	−0.029 NS	−0.021 ± 0.029	−0.043 NS
Amplitude	0.018 ± 0.012	0.092 NS	0.009 ± 0.023	0.023 NS
Deviation	0.014 ± 0.007	0.114 NS	0.014 ± 0.007	0.114 NS
Dyslipidaemia (TG ≥ 1.7mmol/L or medication)				
Antegrade	0.016 ± 0.009	0.102 NS	0.058 ± 0.019	0.184**
Retrograde	−0.022 ± 0.014	−0.093 NS	−0.086 ± 0.032	−0.157**
Amplitude	−0.006 ± 0.013	−0.027 NS	−0.028 ± 0.027	−0.062 NS
Deviation	0.006 ± 0.008	0.045 NS	0.007 ± 0.008	0.050 NS

Hyperinsulinemia (≥11.06 mU/l)				
Antegrade	0.006 ± 0.009	0.037 NS	0.071 ± 0.018	0.230***
Retrograde	−0.036 ± 0.013	−0.158 **	−0.129 ± 0.030	−0.240***
Amplitude	−0.030 ± 0.013	−0.141*	−0.057 ±0.025	−0.132*
Deviation	0.014 ± 0.008	0.109 NS	0.015 ± 0.008	0.113 NS
Hyperglycaemia (≥5,6 mmol/L or treatment)				
Antegrade	0.010 ± 0.009	0.070 NS	0.026 ± 0.018	0.086 NS
Retrograde	−0.018 ± 0.013	−0.081 NS	−0.046 ± 0.031	−0.087 NS
Amplitude	−0.008 ± 0.013	−0.037 NS	−0.020 ± 0.026	−0.046 NS
Deviation	0.008 ± 0.008	0.060 NS	0.008 ± 0.008	0.061 NS
Low adiponectin level (≤6.22 µg/ml)				
Antegrade	0.009 ± 0.009	0.065 NS	0.003 ± 0.018	0.010*
Retrograde	0.002 ± 0.014	0.007 NS	0.001 ± 0.032	0.002 NS
Amplitude	0.011 ± 0.013	−0.052 NS	0.004 ± 0.026	0.010 NS
Deviation	−0.003 ± 0.008	−0.021 NS	0.003 ± 0.008	0.023 NS
HOMA-IR (≥2.44)				
Antegrade	0.003 ± 0.008	0.024 NS	0.064 ± 0.017	0.217***
Retrograde	−0.031 ± 0.013	−0.145*	−0.129 ± 0.030	−0.240***
Amplitude	−0.028 ± 0.012	−0.136*	−0.056 ± 0.024	−0.137*
Deviation	0.009 ± 0.007	0.073 NS	0.009 ± 0.007	0.075 NS

Statistical significances: NS= nonsignificant, * $p < 0.05$; ** $p < 0.01$, *** $p < 0.001$. Unstandardized coefficients B ± Std.Error, Beta, Sig. Abbreviations: The longitudinal motion parameters: IA = longitudinal motion between intima-media -complex and adventitia; IO = longitudinal motion between intima-media -complex and surrounding tissue. HOMA-IR = Homeostasis model assessment of insulin resistance.

Table 9. Multivariate relationships between each component of the metabolic syndrome and longitudinal motion parameters adjusted for age and sex.

	Hypertension	**Obesity**	**Low HDL-C**	**High triglycerides**	**Hyperglycaemia**	**Hyperinsulinemia**
	B ±SE, Beta	**B ± SE, Beta**	**B ± SE, Beta**	**B ± SE, Beta**	**B ± SE, Beta**	**B ± SE, Beta**
lAante (mm)			0.02 ± 0.01, 0.164**			
lAretro (mm)	−0.03 ± 0.01, −0.175**					−0.03 ± 0.01, −0.124*
lAampl (mm)			0.03 ± 0.01, 0.126*			−0.03 ± 0.01, −0.156**
lAdev (mm)	0.02 ± 0.01, 0.145 *					
lOante (mm)		0.09 ± 0.02, 0.278***		0.04 ± 0.02, 0.130*		
lOretro (mm)	−0.06 ± 0.03, 0.127*	−0.10 ± 0.03, −0.184**				−0.08 ± 0.03, −0.151*
lOampl (mm)						−0.06 ± 0.03, −0.131*
lOdev (mm)	0.02 ± 0.01, 0.144*					

Statistical significances: *p<0.05, **p<0.01, ***p<0.001. Unstandardized coefficients B ± Std.Error, Beta, Sig. The longitudinal motion parameters: lA = longitudinal motion between intima-media -complex and adventitia; lO = longitudinal motion between intima-media -complex and surrounding tissue. Subindexes ante = forward oriented component of the longitudinal motion; retro = backward oriented component of the longitudinal motion; ampl = amplitude of the longitudinal motion; dev = the main deviation between the different arterial layers. HDL-C = High-density lipoprotein cholesterol.

6 DISCUSSION

In this thesis, a newly developed non-invasive ultrasound imaging analysis was applied in a clinical research project for the first time. Associations between the CALM parameters and the conventional measures of subclinical arteriosclerosis, atherosclerosis, traditional cardiovascular risk factors and MetS were studied. This thesis also represents a notable step in the validation of the new, CALM measuring analysis, which has been previously developed in our laboratory. It was found that the CALM parameters exhibit a significant relationship with conventional measures of arterial stiffness meaning that they can reflect aspects of arteriosclerosis. In the second substudy, an association was found between CALM parameters and traditional cardiovascular risk factors. The peak-to-peak and retrograde amplitudes of CALM had an inverse correlation with systolic and diastolic blood pressure and BMI. Retrograde CALM correlated indirectly with total cholesterol and triglyceride concentrations. Significant direct associations were also found with antegrade CALM and diastolic blood pressure, LDL-C, total cholesterol, triglycerides and BMI. The clustering of cardiovascular risk factors was associated with the disturbances in the longitudinal motion; antegrade motion increased and retrograde decreased as the number of risk factors increased. In the third substudy MetS was found to associated with alterations in CALM. When studying separately associations with different MetS components, significant independent associations were found with hypertension, dyslipidemia and hyperinsulinemia. Hyperglycaemia or low adiponectin levels did not show any significant correlations with CALM. Insulin resistance was observed to influence the longitudinal motion and caused motion disturbances. MetS was associated with increased antegrade CALM and decreased retrograde CALM, generally both are considered markers of unfavourable vascular health.

6.1 STUDY DESIGN AND GATHERING THE DATA

This cross-sectional study is based on a large, five-centre follow-up study (The Cardiovascular Risk in Young Finns Study). Study participants were randomly chosen from the national register and the Kuopio centre data from 2007 was taken for analysis. The data represents the Eastern Finland subpopulation. Selection bias has not affected the representativeness of the original study cohort in 1980.

Vascular ultrasound studies were available for 465 subjects and successful longitudinal analyses were achieved in 292 subjects. The reason for the relatively large number of unsuccessful scans was that in 2007 the ultrasound imaging protocol had been optimized to measure IMT and Cdist, not to analyse longitudinal motion

parameters. Longitudinal motion analysis of common carotid artery is challenging and requires a good frame-to-frame image quality from the ultrasound video and even the smallest artefacts can disturb the longitudinal motion analysis. When the special demands of motion analysis are taken to account, all the analyses were successful (Yli-Ollila, Laitinen et al. 2013). During the 27 year follow up, there have been approximately 40 % dropouts. In addition, it may be speculated that ultrasound image quality in arteries with advanced arteriosclerotic alterations may have been lower in this data and even represent one reason for unsuccessful scans. These two factors may have affected the representativeness of the study population. Nevertheless, the study population was homogenous with an age-range of 15 years and in this age group, the incidence of arteriosclerosis or atherosclerosis is low. The data was gathered in the year 2007, and according to the national Finterveys survey, some changes have occured in the health status of Finnish adults during recent years (Koponen, Borodulin et al. 2018). For example, the popularity of smoking continued to decline in 30-49-year old adults during the time period 2011-2017. Although LDL-cholesterol has also shown a decreasing trend, unfortunately the prevalence of obesity has increased among the 30-49-year old population.

The first substudy consisted of these 292 subjects. In the second substudy, five women were excluded due to pregnancy, hence the final study population included 287 subjects. In the third substudy, four study participants were excluded because of type one diabetes, five women were excluded due to pregnancy and there was a lack of anthropometric studies for two study participants whereupon the final study population involved 281 participants. Adiponectin data was lacking in seven study subjects, and thus the univariate analysis with CALM and adiponectin evaluated 274 study participants. Although data quality did not allow successful longitudinal motion analysis in all 465 subjects, studied population size (N=281-292) was sufficient to allow reliable statistical analyses. All substudies in this thesis were cross-sectional in their nature, and thus causality cannot be concluded from the results. Our aim was to focus on young adults in the age range 30-45 years and to evaluate the healthy physiologic conditions or subclinical phase of arteriosclerosis. The results may not be generalized to individuals outside the age range or subjects with advanced disease process. This thesis examined white European subjects and for this reason, the results may not be generalized to other ethnic groups. The study population of this thesis is large and well-characterized.

6.2 MAIN FINDINGS IN RELATION TO EARLIER RESEARCH

6.2.1 Interrelationships between indices of longitudinal motion of common carotid artery wall and the conventional measures of subclinical arteriosclerosis (Study I)

The peak-to-peak and retrograde amplitudes of the longitudinal motion were directly correlated with Cdist and inversely correlated with PWV. No significant

correlation was found between CALM and carotid IMT and brachial artery FMD in this study. These findings indicate that arterial stiffening can modulate CALM while other features of arteriosclerosis such as morphological alterations in the intima-media complex or endothelial dysfunction measured by FMD do not have a notable influence on CALM, at least in this population of young adults. A high PWV value represents stiffness of arteries whereas a high Cdist value is evidence of high elasticity in the arteries. PWV is also thought to illustrate the status of the larger vascular tree and Cdist represents the local status of the artery wall. Associations between CALM parameters and conventional measurements of subclinical arteriosclerosis have been investigated in only a few studies where the characteristics of population have differed significantly from those examined here (Table 2). In the study of Zahnd et al., CALM was determined in patients with periodontal disease and a significant association was found with Cdist but not with PWV (Zahnd, Vray et al. 2012). In the present thesis, both Cdist and PWV instead showed a significant correlation with most of the studied CALM parameters. It is noteworthy that the longitudinal amplitude was divided by pulse pressure in the study of Zahnd (2012) and therefore the results may not be directly comparable with the CALM parameters determined in the present study.

While PWV illustrates mainly stiffness along the aortic and aorto-iliac pathway rather than local stiffness (Laurent, Cockcroft et al. 2006), carotid IMT reflects predominantly morphological and local changes in common carotid artery (Ter Avest, Stalenhoef et al. 2007). In contrast to the present findings, decreased longitudinal motion has been found to be associated with increased IMT in only two previous studies (Svedlund, Eklund et al. 2011, Zahnd, Vray et al. 2012). Contrary findings emerged from the large multi-ethnic follow-up study of Gepner et al., where a greater longitudinal displacement was associated with higher carotid IMT (Gepner, Colangelo et al. 2015). It should be noted that Zahnd´s study population consisted of patients with periodontal disease and Svedlund´s with suspected coronary artery disease. Gepner´s study participants were aged 45-84 years, representing an older population. Hence, the results of this thesis are not fully comparable for these published reports as we evaluated young and middle-aged adults without inclusion criteria for cardiovascular diseases.

In 5-8-year old children, there was only a weak correlation between CALM and PWV (Proudfoot, Au et al. 2019). In children, the arteriosclerotic process is either absent or in its very early phase and this may be one reason for the weak correlation although cardiovascular risk factors identified in childhood have been shown to predict increased IMT in adulthood (Raitakari, Juonala et al. 2003). The association between CALM and IMT may be different in later phases of disease process when some degree of structural remodelling has occurred in the vascular wall (see Figures 1 and 2) and a possible atheromatous lesion has developed. In childhood, longitudinal motion changes rapidly and a retrograde shift has been detected during one year of follow-up study (Au, Proudfoot et al. 2019) reflecting the nature of CALM development during growth.

Although FMD is thought to illustrate the early and predominantly functional changes in artery wall (Ter Avest, Stalenhoef et al. 2007) and it has provided a valuable insights into subclinical atherosclerosis (Raitakari, Celermajer 2000); endothelial dysfunction is involved in the entire arteriosclerotic disease process from the beginning to its clinical manifestations (Trepels, Zeiher et al. 2006). Therefore, FMD could reflect both atherosclerotic and non-atheromatous features of arteriosclerosis (see Table 1). One can speculate that in the early subclinical phase of arteriosclerosis, associations between FMD and CALM could have existed in this study population. It may be also possible that FMD is a rather robust marker and subtle changes in endothelial function are not detectable with this method or alternatively the size of the population examined here did not have enough statistical power to reveal these subtle associations. In the literature, the association between CALM and FMD has not studied before.

When the CALM was investigated in detail, motion was found to occur within the vascular wall. A remarkable amount of motion occurred between intima-media complex and adventitia. In the literature, it has demonstrated that the inner part of artery wall, i.e. the intima-media complex moves more in an axial direction than does the adventitial region (Persson, Rydén Ahlgren et al. 2003, Cinthio, Rydén Ahlgren et al. 2006). In this thesis, IOampl was notably larger than AOampl, indicating that the majority of the longitudinal motion occurred within the vascular wall. Previously, Cinthio et al. (Cinthio, Rydén Ahlgren et al. 2006) demonstrated that CALM displayed a biphasic bidirectional pattern where a distinct antegrade motion in the early systole was followed by motion in the opposite direction in later systole. Similar findings were found in the present thesis. The association between antegrade and retrograde components of CALM and stiffness indexes was a new finding. The retrograde component of CALM was approximately double that of the antegrade component of CALM. In the first substudy, associations between conventional measurements and new parameters Polydeg and RAlegth were examined. Ralength showed a significant positive correlation with Cdist and negative with PWV and this reflected the elasticity of the artery wall. An inverse correlation was found between RAlength and FMD, which was surprising, because it would have been expected that good endothelial function is directly correlated with RAlength. The significance of the association was low and requires further research in the future. No significant correlation was found between Polydeg and conventional measures of subclinical arteriosclerosis.

6.2.2 Influence of cardiovascular risk factors on longitudinal motion of the common carotid artery wall (Study II)

The peak-to-peak and retrograde amplitudes of CALM were inversely correlated with systolic and diastolic blood pressure and BMI. The retrograde amplitude of CALM correlated indirectly with total cholesterol and triglyceride levels. The amplitude of the antegrade CALM was directly correlated with BMI, diastolic blood pressure, LDL-C, total cholesterol, and triglyceride concentrations. The antegrade

CALM increased and the retrograde CALM decreased with the increasing number of cardiovascular risk factors. These findings suggest that traditional cardiovascular risk factors influence CALM by increasing the antegrade oriented motion as well as decreasing the retrograde oriented motion. Interestingly, when the cardiovascular risk factor accumulation was higher, the total CALM amplitude was decreased, and this also resulted in a diminishing of the antegrade component.

In the second substudy IA- and IO parameters were investigated. AO parameters were omitted from the further evaluation because AO -parameters reflect the motion occurring outside the vascular wall and our focus of interest was on motion occurring within the artery wall.

In this thesis, the association profiles with cardiovascular risk factors were different in CALM compared to those of Cdist and IMT. Cdist represents local carotid artery stiffening and exhibited the strongest associations with blood pressure. As the first substudy demonstrated, CALM parameters associated significantly with stiffness parameters and the same associations were seen also in the second substudy. Significant correlations were found especially with the longitudinal parameters and diastolic blood pressure but also with retrograde CALM and systolic blood pressure. Interestingly, IOante and IOretro correlated significantly with total cholesterol and triglyceride levels. It is possible that CALM parameters can reflect also different aspects of vascular disease other than diffuse vascular stiffening because in the atherosclerotic process, the influx of LDL-C from blood to the intima layer depends on the presence of sustained plasma LDL-C levels (Tabas, Williams et al. 2007). Triglyceride particles are known to be atherogenic (Sarwar, Danesh et al. 2007). Among young adults, both increased serum triglycerides and low HDL-C have been associated with increased arterial stiffness (Li, Chen et al. 2004). In the present study, there were no significant associations with HDL-C and CALM, only IAante showed a negative correlation with HDL-C. The population size examined by Li, Chen et al. (Li, Chen et al. 2004) was larger (n=835) than in the present study, and this may be one reason why they were able to detect weak correlations with HDL-C as discussed in the next chapter.

In this substudy, significant differences between none and one and none and two risk factors were found more often with the IA motion than with the conventional measures, where a significant difference was found only when three or more risk factors were present. One plausible reason for this is that there is not enough statistical power in our Eastern Finland subpopulation size to reveal true associations, e.g. with Cdist, than can be attained in a larger multicentre study population (Juonala, Järvisalo et al. 2005, Mattsson, Rönnemaa et al. 2008, Juonala, Viikari et al. 2004). BMI and hypertension were the most powerful contributors to the alteration in CALM as they correlated significantly with the 7/8 longitudinal parameters. The finding made in the first substudy that larger retrograde oriented motion was associated with elastic arteries and increased antegrade motion with stiffer arteries was confirmed in the second substudy. Larger retrograde CALM was associated with fewer cardiovascular risk factors and greater antegrade oriented

CALM with more cardiovascular risk factors. Interestingly we found amplitude to decrease when the risk factor load was more than two. This may explain the slight differences in CALM parameters found in the score groups including two or more risk factors.

Associations between cardiovascular risk factors and CALM have been investigated in some previous studies (Table 2), but the study populations and sizes have differed from the present thesis, being 1) mostly smaller, 2) study subjects have been older and 3) the stage of arteriosclerotic process has been more advanced than in the present study population e.g. (Svedlund, Eklund et al. 2011, Svedlund, Gan 2011a, Zahnd, Boussel et al. 2011, Zahnd, Vray et al. 2012). However, these results are mostly in parallel with the results of the current study: cardiovascular risk factors have reduced CALM in the periodontal disease population (Zahnd, Vray et al. 2012), among spinal cord injury subjects (Tat, Au et al. 2015) and among patients with diabetes (type 1 or type 2, diagnosed at least one year before) (Zahnd, Boussel et al. 2011). In addition, a lower CALM in comparison to healthy controls has been detected among patients with established cardiovascular disease (Svedlund, Eklund et al. 2011, Svedlund, Gan 2011b, Au, Valentino et al. 2017). Further, the carotid plaque burden has been shown to associate with a reduced CALM as compared to healthy controls (Svedlund, Gan 2011a) and can influence the direction of the motion, causing a significant anterograde shift in CALM (Tat, Psaromiligkos et al. 2016a). Opposite results were described in one large multi-ethnic cohort (n= 389) where study subjects were free of cardiovascular disease at baseline (Gepner, Colangelo et al. 2015, Gepner, McClelland et al. 2019). In that study, greater CALM was associated with increased IMT, but no significant associations were seen between total CALM and other baseline cardiovascular risk factors including age, systolic blood pressure, diastolic blood pressure, pulse pressure, diabetes mellitus diagnosis or former or current smoking, nor with conventional markers of carotid stiffness (Young`s Elastic Modulus and Distensibility Coefficient) (Gepner, Colangelo et al. 2015). In a 12-year follow-up in the same cohort, no predictive value was found for cardiovascular disease events, although CALM was directly correlated with smoking and indirectly with diastolic blood pressure (Gepner, McClelland et al. 2019). There may be several reasons to explain these opposite outcomes, e.g. the study population in the present thesis was younger and more homogenous. It is known that breathing can modify the CALM in the common carotid artery and it was taken to account in the present thesis but it is not known how breathing was taken to account in Gepner´s et al. cohort study. It is also known that ethnicity may influence CALM. The VVI-imaging technique used in Svedlund´s studies do not detect the multiphasic components of CALM as clearly (Svedlund, Gan 2011a) as in other motion tracking methods (e.g. (Yli-Ollila, Laitinen et al. 2013, Cinthio, Rydén Ahlgren et al. 2006)). However, Gepner et al. used a more recent version of the VVI-algorithm in longitudinal studies which does reveal the multiphasic component. This technique was not developed for motion tracking of heart as was the case with the older VVI-technique. However, this may not explain the majority of the differences in the results. The techniques used by

Gepner et al. measured the motion of intima in relation to the ultrasound scanner and the motion of surrounding tissues was not taken into account. In this thesis the motion of surrounding tissues was taken to account because this may cause motion disturbances.

6.2.3 Carotid artery longitudinal wall motion alterations associated with metabolic syndrome and insulin resistance (Study III)

In the third substudy, there were no significant differences between MetS and no-MetS group in terms of age, sex, body height and smoking habits (Table 3). These two groups were otherwise significantly different in terms of other clinical characteristics. These two groups differed significantly in the studied CALM parameters except for IAampl. The MetS influenced CALM by increasing the antegrade oriented motion and decreasing the retrograde oriented motion. The MetS components affected the CALM parameters in parallel in the IA and IO layers and almost all MetS components were found to influence the different CALM parameters except for hyperglycaemia. Hyperinsulinemia showed significant negative correlations with retrograde longitudinal motion as well as with peak-to-peak amplitudes. Additionally, hyperinsulinemia showed a significant positive correlation with IOante. HOMA-IR, as a marker of insulin resistance, exhibited a significant negative correlation with the retrograde longitudinal motion, as well as with total amplitudes. Finally, HOMA-IR showed a significant positive correlation with IOante. These findings indicate that early disruptions in glucose metabolism seem to affect the longitudinal motion of common carotid artery.

Novel findings were that hyperinsulinemia and insulin resistance displayed significant associations with CALM. No association was found between CALM and hyperglycaemia but disruptions in glucose metabolism influence initially on insulin levels, and in the later phase of the disease when the insulin-mediated control of glucose balance becomes insufficient, hyperglycaemia and the eruption of type 2 diabetes occurs (Ormazabal, Nair et al. 2018). In this study population of 281 participants, only 6 study participants were hyperglycaemic. This low amount of hyperglycaemic cases may also explain non-significant associations with CALM parameters, i.e. there were not enough statistical power to reveal possible true associations between hyperglycaemia and CALM in this study group.

In this study, the participants with type 1 diabetes (4 participants) were excluded but those with type 2 diabetes were included (2 participants). There is no strict boundary between impaired glucose tolerance and the onset of type 2 diabetes diabetes but it is known that the increased risk for cardiovascular diseases begins already when there is a state of impaired glucose tolerance and the risk increases with elevated glucose levels (Emerging Risk Factors Collaboration, Sarwar et al. 2010, Cosentino, Grant et al. 2020) and is evident already in the state of insulin resistance (Nesto 2004, Grundy 2016).

In the pathophysiology of MetS, hyperinsulinemia is accepted to be essential and the overabundance of free fatty acids the considerable contributor to the

development of MetS (Eckel, Grundy et al. 2005). The underlying mechanisms behind significant associations between CALM and insulin as well as HOMA-IR are presumable related to the role of insulin in cellular metabolism, as the regulator of vascular tonus, and its other possible functional roles in vasculature. Under physiological conditions, insulin stimulates NO production and thus it has an important role in the maintenance of an appropriate tonus in the vascular wall (Yki-Järvinen 2003, Ormazabal, Nair et al. 2018). Insulin influences also blood flow to target tissues: it has been demonstrated to increase skeletal muscle blood flow and there was a significant difference between lean and obese study participants; in obese participants, there is a defect in insulin´s action to increase the blood flow to tissues (Laakso, Edelman et al. 1990). In situations of insulin resistance, NO synthesis which is normally stimulated by insulin, is selectively impaired and the compensatory hyperinsulinemia may activate the MAPK pathway (Zhou, M. S., Schulman et al. 2010, Ormazabal, Nair et al. 2018). The outcome is vasoconstriction, inflammation, increased sodium and water retention, further developing an elevation of blood pressure. In addition, NO normally inhibits the proliferation of the smooth muscle cells in the vascular wall and inhibits platelet and leukocyte adhesion (Nesto 2004). The effect of insulin in the vascular wall and its associations with CALM as well as with the cardiovascular disease process may be explained through the aforementioned mechanisms. In clinical settings, measuring insulin is far more complicated than e.g. measuring fasting lipids. The presence of MetS was associated with increased antegrade CALM and decreased the retrograde CALM. MetS has shown to associate with arterial stiffness in several reports (Eckel, Grundy et al. 2005, Li, Chen et al. 2005, Koskinen, Kähönen et al. 2009, Koskinen, Magnussen et al. 2010, Scuteri, Cunha et al. 2014, Prenner, Chirinos 2015, Vágovičová, Mlíková Seidlerová et al. 2015, Gomez-Sanchez, Garcia-Ortiz et al. 2016, Vilmi-Kerälä, Koivistoinen et al. 2017, Topouchian, Labat et al. 2018).

Low adiponectin levels did not show any significant associations with CALM apart from with IOante. There are publications demonstrating that BMI is inversely correlated with adiponectin and there are also publications showing that insulin decreases adiponectin levels (Trujillo, Scherer 2005). Lower levels of adiponectin have been associated with hypertension, the development of MetS and insulin resistance already in a pediatric population (Orlando, Nava et al. 2019). Furthermore, low adiponectin levels have been shown to correlate with most of the components of the MetS in adults (Santaniemi, Kesäniemi et al. 2006). Despite these findings in the literature, and adiponectin´s generally accepted role in both lipid and glucose metabolism in the peripheral tissues, no significant correlation was found between the low adiponectin levels and CALM parameters in this thesis. It is not possible to provide a clear explanation for the weak or absent correlation found here. The interdependence of glucose and lipid metabolism disorders, endothelial dysfunction, obesity, and hypertension has been reported in the literature. However, the goal of this thesis was not to undertake a deeper investigation of the signalling pathways underpinning these pathologies.

The retrograde amplitudes of CALM were inversely correlated with hypertension, obesity, and hyperinsulinemia. Hypertension and hyperinsulinemia as well as obesity, seemed to be the major factors behind the alteration of the CALM parameters. Although there is a clear relationship between obesity and hypertension, the actual mechanism may be multifactorial, involving 1) adipose tissue derived adipokines and cytokines, 2) increases in blood free fatty acid levels, 3) increase of plasma insulin and lipid levels as well as 4) inflammation and 5) a reduction of adiponectin release causing endothelial dysfunction (Kotsis, Jordan et al. 2018). These alterations cause a decrease in baroreflex sensitivity and vasoconstriction leading to an increase of arterial stiffness and changes in the renal tubules where sodium reabsorption increases, leading to hypertension (Kotsis, Jordan et al. 2018).

6.3 FUTURE PROSPECTS

The need for large, well-characterized follow-up studies has been pointed out (Rizi, Au et al. 2020). Interesting associations have been found in cross-sectional study settings including the present thesis as in other studies investigating CALM and the cardiovascular risk factors. CALM seems to be a potential indicator of vascular health, but the number of studies is still small and the predictive value of CALM as a vascular risk measure is still unclear. In future study designs, it would be important to take into account the technical demands concerning the CALM imaging. The novel findings concerning glucose metabolism disorders and CALM may well stimulate interest to study in more detail the interdependence between insulin and subclinical vascular disease.

There is the intriguing possibility that CALM can offer both additive and predictive values in conjunction with traditional measures when evaluating the individual´s vascular health as well as helping to detect signs when the patient is still in the subclinical disease phases but this speculation will need to be confirmed with further investigation, especially with follow-up studies.

The possible interdependence between arteriosclerosis and atherosclerosis is an interesting phenomenon, because they have been previously considered as separate pathological processes. Therefore, a better understanding of these mechanisms is needed. Studying motion disturbance in the longitudinal wall motion in other arteries than common carotid artery would offer interesting information of the longitudinal motion phenomenon. It would be worthwhile investigating longitudinal motion disturbances in some specific arterial disease, for example in patients with dilatation of the ascending aorta or in patients with an increased risk for aortic dissection.

Furthermore, intervention studies are needed to evaluate the usability of CALM as a tool e.g. for measuring the effect of lifestyle interventions on vascular health. The driving force for CALM remains still unclear, and in the future, the role of cardiac systolic function as its driving force should be clarified.

The significant associations found between CALM and subclinical phases of vascular diseases as well as CALM and early glucose metabolism disorders in our young, healthy study population highlight the importance of the early detection of the pathological processes, combined with appropriate interventions as cost effective ways of preventing cardiovascular diseases.

7 CONCLUSIONS

1. Arterial stiffness modulates carotid artery longitudinal motion and measurement of longitudinal motion can be of value in the assessment of vascular health. Longitudinal motion is associated with arterial stiffness and arteriosclerotic process.
2. Cardiovascular risk factors especially systolic and diastolic blood pressure, body mass index, total cholesterol and triglycerides levels associate with carotid artery longitudinal motion parameters. Antegrade longitudinal motion increased whereas retrograde longitudinal motion decreased as the number of risk factors increased. Risk factors of arteriosclerosis associated with the disturbance of the longitudinal motion of the common carotid artery.
3. The presence of the metabolic syndrome and insulin resistance influenced carotid artery longitudinal motion. In particular, hypertension, obesity and hyperinsulinemia were associated with reduced total peak-to-peak amplitude as well as increased antegrade and reduced retrograde amplitudes.

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ORIGINAL PUBLICATIONS (I – III)

I

Interrelationships between indices of longitudinal movement of the common carotid artery wall and the conventional measures of subclinical arteriosclerosis

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Interrelationships between indices of longitudinal movement of the common carotid artery wall and the conventional measures of subclinical arteriosclerosis

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Summary

Our objective was to study the interrelationships between longitudinal movement of the wall of the common carotid artery and the conventional measures of arteriosclerosis in a large and well-characterized study population. Successful longitudinal movement analyses were performed on 292 subjects. The peak-to-peak and retrograde amplitudes of the longitudinal movement were directly correlated with carotid artery distensibility ($r = 0{\cdot}21$, $P<0{\cdot}001$ and $r = 0{\cdot}23$, $P<0{\cdot}001$, respectively) and inversely correlated with pulse wave velocity ($r = -0{\cdot}14$, $P<0{\cdot}05$ and $r = -0{\cdot}17$, $P<0{\cdot}01$, respectively). All longitudinal motion parameters were independent of brachial flow-mediated dilatation and intima-media thickness. Our findings indicate that arterial stiffening modulates longitudinal movement and, therefore, measurement of longitudinal movement can be of value in the assessment of vascular health.

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arterial stiffness; carotid artery distensibility flow-mediated dilatation; intima-media thickness; motion tracking; pulse wave velocity; ultrasound imaging

Introduction

Cardiovascular diseases and their complications are still the most common causes of death all around the world (Murray & Lopez, 1997; Lopez *et al.*, 2006; Joshi *et al.*, 2008). The disease process often begins in childhood or in young adulthood (Raitakari *et al.*, 2003; Juonala *et al.*, 2005; Koskinen *et al.*, 2009; Veijalainen *et al.*, 2013) although the clinical signs of atherosclerosis mainly appear later in life (Perk *et al.*, 2012). Optimal cardiovascular health can still be promoted in adolescence as demonstrated in STRIP-study (Niinikoski *et al.*, 2007, 2009; Pahkala *et al.*, 2013).

It is challenging to detect subclinical changes in vascular beds, but it would provide a major advance, as it could help to identify high-risk individuals as well as to focus preventive strategies on vulnerable members of the population. Arteriosclerosis is a concept, which covers a variety of vascular changes including patchy atherosclerotic lesions but also diffuse non-atheromatous alterations appearing as thickening and hardening of the arterial wall and leading to loss of elasticity of the walls of arteries (Bierman, 1987). To be able to characterize such multiform phenomenon, several non-invasive techniques are used to estimate arterial properties by means of investigating alterations in vascular structure, biomechanics, and function of arteries.

Carotid artery distensibility (Cdist), which is one of the traditional measurements, has been found to decline in young adults with the metabolic syndrome (Mattsson *et al.*, 2008). A number of risk factors identified in childhood and adolescence have also been associated with decreased Cdist values in adulthood (Juonala *et al.*, 2005). The increases in common carotid intima-media thickness (IMT) as measured by ultrasound have been found to correlate with cardiovascular risk factors (Raitakari *et al.*, 2003; Ter Avest *et al.*, 2007), and several risk factors identified in childhood could predict increased IMT in adulthood (Raitakari *et al.*, 2003). Carotid IMT is also a strong predictor of future vascular events (Lorenz *et al.*, 2007). Brachial flow-mediated dilatation (FMD) is another traditional measurement. FMD is considered to be a marker of endothelial function and to reveal early, predominantly functional, changes in the vascular wall (Ter Avest *et al.*, 2007). According to Gokce *et al.* (2002), high-risk patients with impaired FMD values were likely to suffer cardiovascular events. This finding was confirmed in patients with overt coronary disease

(Chan *et al.*, 2003). Moreover, the status of systemic endothelial function is believed to modify the relationships between metabolic risk and atherosclerosis (Juonala *et al.*, 2004; Mattsson *et al.*, 2008). Arterial pulse wave velocity (PWV) is commonly used to estimate arterial stiffness (Laurent *et al.*, 2006), and it is thought to be an index of the arteriosclerotic process (Ter Avest *et al.*, 2007). Many cardiovascular risk factors, identified in childhood and adulthood, are known to correlate with increased PWV in adults (Aatola *et al.*, 2010). Moreover, aortic PWV is also a strong predictor of future cardiovascular events and all-cause mortality (Vlachopoulos *et al.*, 2010).

The longitudinal movement of carotid artery seems to be a potential new indicator of vascular health (Svedlund & Gan, 2011; Svedlund *et al.*, 2011; Zahnd *et al.*, 2011). However, it is not known which pathophysiological or structural changes contribute to alterations in longitudinal movement. To clarify this, it is valuable to examine associations between longitudinal movement of the carotid artery wall and a variety of conventional measures of subclinical arteriosclerosis such as Cdist, IMT, FMD and PWV. In our research group, we have previously developed a new non-invasive method to investigate the properties of the arterial wall by measuring the longitudinal and radial movements of the common carotid artery (CCA) wall simultaneously from an ultrasound video (Yli-Ollila *et al.*, 2013, 2014). In this study, we have applied this method to clinical research, which is a notable step in validating the method by expanding the number of subjects assessed with the technique. The main aim of this study was to investigate whether the prevailing indicators of vascular health would show any associations with parameters of the longitudinal movement of the CCA.

Methods

Subjects and study design

The Cardiovascular Risk in Young Finns Study is an ongoing, five-centre follow-up study of atherosclerosis risk factors in Finnish children and adolescents. The first cross-sectional survey was conducted in 1980, when 3596 children and adolescence participated. The age range of the original sample was from 3 to 18 years, and participants were randomly chosen in each area from a national register. During the years 1980–2007, follow-up studies have been conducted regularly in this cohort at intervals from 3 to 6 years. The study was approved by local ethics committees, and the subjects provided written informed consent.

Kuopio University Hospital is investigating the population of Eastern Finland and is one of the five centres involved. This cross-sectional study consists of the Kuopio centre data from 2007, when the subjects were 30–45 years old. Vascular ultrasound studies were available for 465 subjects. Ultrasound studies from the left CCA were performed to derive values of both IMT and Cdist. In addition, the left brachial artery diameter measurements were performed to assess the FMD.

Clinical characteristics

Height and weight were measured, height to an accuracy of 1 cm and weight to an accuracy of 1 kg. Body mass index was calculated as weight in kilograms divided by height in metres squared. Blood pressure was measured in the sitting position from the brachial artery using a random zero sphygmomanometer (Hawksley & Sons Ltd, Lancin, UK), and the average of three measurements was used in the analysis.

Carotid ultrasound imaging

Ultrasound studies were performed by trained sonographers following the standardized protocol described previously (Raitakari *et al.*, 2003). Carotid and brachial artery imaging was performed using Sequoia512 ultrasound scanner (Acuson, Mountain View, Calif) equipped with a 14 MHz linear array transducer. The ECG signal (modified chest lead 5) was recorded and presented along with B-mode image sets.

The left CCA was scanned using a resolution box function to record a 25-mm-wide and 15-mm-high image including the beginning of the carotid bifurcation and the distal CCA. A 5 s cine loop (25 frames per second) was digitally stored for subsequent offline analysis. The same image set was used to derive carotid IMT and Cdist as well as radial and longitudinal movements of artery wall. For the calculation of the distensibility parameters, blood pressure was measured in the supine position with an automated sphygmomanometer (Omron M4; Omron Matsusaka Co., Ltd, Kyoto, Japan) immediately before and after ultrasound imaging.

Carotid intima-media thickness

The mean IMT was measured by focusing the image on the posterior wall of the left CCA. The best-quality end-diastolic frame was selected from the video. At least, four measurements were taken from this image approximately 10 mm proximal to the bifurcation to derive the maximal carotid IMT. The method has been described previously in detail (Raitakari *et al.*, 2003). The reproducibility of the IMT measurements is very good (Raitakari *et al.*, 2003), for example the 3-month between-visit coefficient of variation was 6·4% and the between-observer coefficient of variation was 5·2% (Raitakari *et al.*, 2003).

Carotid artery distensibility

Cdist measures the ability of the arteries to expand in response to the pulse pressure caused by cardiac contraction and relaxation (Juonala *et al.*, 2005). A cine loop was acquired, and the best-quality cardiac cycle was selected from the 5-s cine loop to assess carotid elasticity indices. The CCA diameter was measured at least twice at both end-diastole and at end-systole using the callipers of the ultrasound scanner. The means of the measurements were used as the end-diastolic and end-systolic diameters. In the calculation of the indices of

arterial elasticity, the following ultrasound and concomitant brachial blood pressure measurements were used, Cdist = $([D_s-D_d]/D_d)/(P_s-P_d)$, where D_s is the systolic diameter, D_d is the diastolic diameter, P_s is systolic blood pressure, P_d is diastolic blood pressure. The reproducibility of the Cdist values used in this study has shown to be at a good level (Juonala *et al.*, 2005), for example between-visit coefficient of variation was 16·3% for Cdist and 2·7% for carotid artery diastolic diameter (Juonala *et al.*, 2005).

Longitudinal movement

Arterial wall movement analysis was performed using our in-house motion tracking programme (Yli-Ollila *et al.*, 2013). The software was written in MATLAB (2007b; The MathWorks Inc., Natic, MA, USA), and it is capable of reading the graphical ECG-information from the ultrasound recording and simultaneously tracking the longitudinal and radial movements of the arterial wall. The basic method used in the motion tracking was a two-dimensional cross-correlation (block matching) enhanced with a contrast optimization technique to reduce noise from the videos.

In the longitudinal movement analysis, regions of interest (ROIs) are drawn on the intima-media complex, on the adventitial layer and on surrounding tissue (see Fig. 1a). When tracking the radial movement of the arterial wall, the ROIs are drawn on the distal and proximal arterial wall (see Fig. 1b). The motion tracking of the longitudinal movement was considered suitable for analysis if the tracking successfully recorded at least two heart cycles, otherwise the movement data were discarded.

We measured three different longitudinal movement curves: the first between the intima-media complex and the adventitial layer (IA, see Fig. 2a), the second between the intima-media complex and the surrounding tissue (IO, see Fig. 2b), and the third between the adventitial layer and the surrounding tissue (AO, see Fig. 2c). The curves of the longitudinal movement have been previously shown to vary extensively between individuals (Yli-Ollila *et al.*, 2013), and thus, the longitudinal movement can be divided into three categories: forward-oriented, bidirectional and backward-oriented curves. We investigated the amplitude of the movement (ampl) as well as the forward (ante) and backward (retro) oriented component of the movement between the different layers of the CCA wall. In addition, we evaluated the main deviation of the longitudinal movement (dev) between the different arterial layers by computing the average of the movement curve over a cardiac cycle. We used a parameter called Polydeg to estimate the complexity of the longitudinal movement. Polydeg is the degree of the polynomial function needed to fit the function to the data points of the longitudinal movement of intima-media layer (IO curve) in order to obtain a Pearson's correlation coefficient >0·95.

In addition to the longitudinal amplitude and complexity parameters, the diameter change of the CCA was measured to create a distension curve, in which the longitudinal movement of the intima-media complex was plotted against the diameter change during a heart cycle (see Fig. 2d). Our novel stiffness parameter RAlength is the length of this two-dimensional curve.

The reproducibility of the longitudinal parameters has been described previously (Yli-Ollila *et al.*, 2013), and the overall reproducibility was estimated as good.

Brachial flow-mediated dilatation

In the measurement of the FMD, the brachial artery was imaged by ultrasound according to the guidelines (Corretti *et al.*, 2002). A segment of the left brachial artery above the antecubital crease was imaged in the longitudinal plane with the same ultrasound scanner and transducer as the carotid imaging. The brachial artery diameter was measured both at

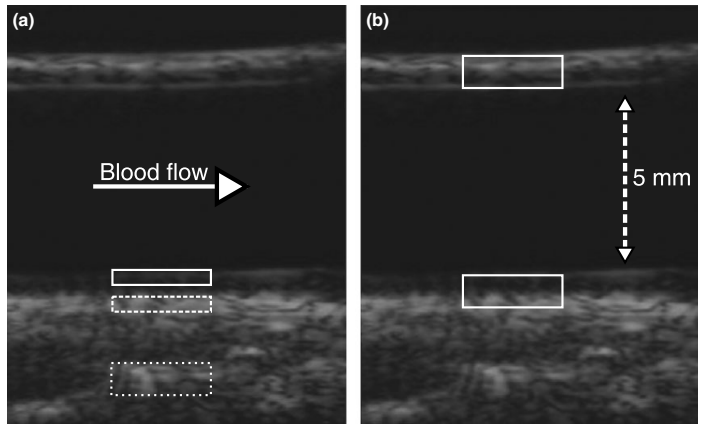

Figure 1 B-mode ultrasound image of a common carotid artery. Regions of interest (ROIs) are drawn on intima-media complex (solid line), on the adventitia layer (dash line) and on the surrounding tissue (dotted line) for longitudinal movement analysis (a). To track the radial movement of the common carotid artery, the ROIs are drawn in the distal and proximal arterial wall (b).

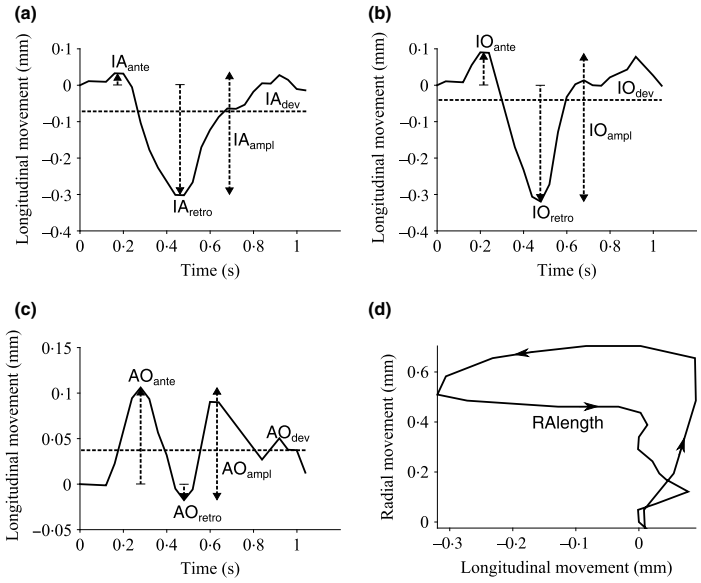

Figure 2 Motion parameters defined from longitudinal movement curves in the different artery wall layers. The solid line represents the movement curve, and dashed lines refer to the maximum antegrade (ante), retrograde (retro) and peak-to-peak amplitudes (ampl) as well as the average deflection from the baseline during a heartbeat. (a) Longitudinal movement of the intima-media complex and the adventitia layer (IA). (b) Longitudinal movement of the intima-media complex and the surrounding tissue (IO). (c) Longitudinal movement between the adventitia layer and the surrounding tissue (AO). (d) Two-dimensional motion curve, where longitudinal movement of intima-media is plotted against the radial diameter change during a heartbeat. Arrows mark the direction of the motion, and RAlength describes the length of the curve in millimetres.

rest and during reactive hyperaemia to assess brachial FMD. Briefly, increased flow was induced by inflation of a pneumatic tourniquet placed around the forearm to a pressure of 250 mmHg for 4·5 min, followed by release. The vessel diameter was derived from the average of three measurements at rest and at 40, 60, and 80 s after cuff release (Juonala *et al.*, 2004). The maximum FMD in scans after reactive hyperaemia is expressed as the percentage relative to resting scan. As described previously, brachial artery diameter measurements have a high degree of reproducibility (the 3-month between-visit CV was 3·2%) and for FMD measurements, the reproducibility was 26·0% (Juonala *et al.*, 2004).

Pulse wave velocity

The whole-body impedance cardiography device (CircMon®; JR Medical Ltd., Tallinn, Estonia) was used to determine PWV. CircMon has a whole-body impedance cardiography channel, a distal impedance plethysmogram channel and an ECG channel. The whole-body impedance decreases when the pulse pressure wave enters the aortic arch and the diameter of the aorta changes. The software measures the time difference between the onset of the decrease in impedance in the whole-body impedance signal and the popliteal artery signal. The PWV is determined from the distance and the time difference between the two recording sites. A more detailed description of the method and the validation study has been reported previously (Kööbi *et al.*, 2003; Aatola *et al.*, 2010). Measures of PWV have been found to possess both good repeatability and reproducibility (99% and 87%, respectively) (Tahvanainen *et al.*, 2009).

Statistical methods

As the longitudinal motion parameters were not normally distributed, nonparametric tests were adopted. Spearman's rank correlation coefficients were used to define conformities between the indices of longitudinal movement and the conventional parameters of vascular health. In the advanced analyses, the study population was analysed in tertile groups for Cdist and PWV. Statistical significance between these groups was assessed with Kruskal–Wallis test. Mann–Whitney U-test with Bonferroni correction was used to analyse more accurately the significance of differences between specific tertiles. Statistical analyses were performed with SPSS version (IBM Corp., IBM SPSS Statistics for Windows, Version 22·0, Armonk, NY), and statistical significance was inferred at a 2-tailed value of $P<0·05$.

Results

Successful longitudinal movement analyses were performed in 292 subjects of 465 subjects (63%). The clinical characteristics of these subjects are presented in Table 1. Table 2 summarizes medians, interquartile ranges (IQR) and ranges of the indices of longitudinal movement. The correlations between longitudinal movement parameters and prevailing parameters reflecting subclinical vascular changes (IMT, Cdist, FMD, PWV) are shown in Table 3.

Cdist and PWV revealed a significant correlation with most of the longitudinal movement parameters (Table 3). Only IA_{ante}, IO_{ampl} and AO_{ampl} did not exhibit any statistically significant correlations with PWV nor did IA_{ante} with Cdist.

Table 1 Clinical characteristics of the study population.

	Median	Interquartile range	Range	*N*
Age (years)	39·0	33–42	15	292
Body height (cm)	169·0	164–177	44	292
Body weight (kg)	72·0	63–85	103	292
Body mass index (kg/m^2)	24·9	22·6–27·8	28·2	292
Systolic blood pressure (mmHg)	128	118–137	101	291
Diastolic blood pressure (mmHg)	80	73–88	69	291
Intima-media thickness (mm)	0·67	0·59–0·72	0·53	292
Carotid artery distensibility (%/10 mmHg)	1·96	1·52–2·34	4·27	292
Flow-mediated dilatation (%)	8·76	6·23–11·55	28·11	291
Pulse wave velocity (m/s)	8·03	7·32–9·15	8·14	254

Table 2 Characteristics of longitudinal movement.

	Median	Interquartile range	Range	*N*
IA_{ante} (mm)	0·047	0·022 to 0·095	0·358	292
IA_{retro} (mm)	0·072	0·035 to 0·147	0·601	292
IA_{ampl} (mm)	0·151	0·104 to 0·209	0·584	292
IA_{dev} (mm)	−0·008	−0·047 to 0·016	0·391	292
IO_{ante} (mm)	0·106	0·035 to 0·229	0·519	292
IO_{retro} (mm)	0·206	0·035 to 0·229	1·169	292
IO_{ampl} (mm)	0·367	0·274 to 0·495	1·192	292
IO_{dev} (mm)	−0·008	−0·045 to 0·017	0·368	292
AO_{ante} (mm)	0·081	0·028 to 0·185	0·533	292
AO_{retro} (mm)	0·156	0·056 to 0·278	0·982	292
AO_{ampl} (mm)	0·281	0·210 to 0·367	1·025	292
AO_{dev} (mm)	−0·009	−0·047 to 0·016	0·391	292
Polydeg	6	6 to 8	11	292
RAlength (mm)	1·512	1·251 to 1·881	2·748	263

Abbreviations and explanations of indices of longitudinal movement of common carotid artery are given in the text.

The parameters describing retrograde movement and amplitude were directly correlated with Cdist and inversely with PWV. Furthermore, the parameters reflecting antegrade movement and deviation were directly correlated with PWV and inversely with Cdist. FMD and IMT did not show any significant correlations with the parameters of longitudinal movement.

The associations between IA movement and tertiles of Cdist and PWV are presented in Figs 3 and 4, respectively. A statistically significant difference was found between the lowest and highest Cdist tertiles in IA_{retro}, IA_{ampl} and IA_{dev} (Fig. 3b–d) and between the lowest and highest PWV tertiles in IA_{retro} and as well as IA_{dev} (Fig. 4b and d). In addition, significant differences were detected between the middle and highest tertiles (Fig. 3c).

Table 3 Correlations between the common carotid artery longitudinal movement parameters and conventional parameters of subclinical arteriosclerosis.

	FMD%	IMT	Cdist	PWV
IA_{ante}	0·000	−0·015	−0·090	0·099
IA_{retro}	−0·088	0·048	0·225§	−0·171‡
IA_{ampl}	−0·046	−0·001	0·213§	−0·137†
IA_{dev}	0·073	−0·020	−0·182‡	0·168‡
IO_{ante}	−0·026	0·072	−0·173‡	0·162‡
IO_{retro}	−0·040	0·028	0·312§	−0·211§
IO_{ampl}	−0·043	0·077	0·267§	−0·077
IO_{dev}	0·078	−0·014	−0·183‡	0·166‡
AO_{ante}	−0·030	0·077	−0·132†	0·129†
AO_{retro}	0·014	−0·011	0·267§	−0·151†
AO_{ampl}	−0·008	0·037	0·217§	−0·018
AO_{dev}	0·066	−0·011	−0·181‡	0·168‡
Polydeg	−0·065	−0·077	−0·014	−0·025
RAlength	−0·153†	−0·023	0·471§	−0·224§

For abbreviations see the text.
Statistical significances: $^{\dagger}P<0·05$; $^{\ddagger}P<0·01$, $^{\S}P<0·001$.

Polydeg displayed no significant correlations with any of recognized measures reflecting subclinical vascular changes examined in this study (Table 3). No significant correlation was also seen between the overall distance of intima movements during one heart cycle (RAlength) and IMT, but RAlength did correlate directly with Cdist and inversely with PWV and FMD (Table 3).

Discussion

Our research group has previously developed a new non-invasive method for investigating the mechanical properties of the arterial wall (Yli-Ollila *et al.*, 2013). In the present study, we detected an association between the longitudinal movement of the CCA wall and Cdist and PWV. In contrast, there was a lack of correlation between measures reflecting longitudinal movement of CCA wall and carotid IMT or brachial artery FMD, in a large and well-characterized study population. Our findings suggest that arterial stiffening modulates longitudinal movement of the CCA wall, while other features of arteriosclerosis such as morphological change in intima-media complex or endothelial dysfunction do not have a notable impact in this respect at least in population of young adults in general population. Because longitudinal movement is clearly linked with biomechanical properties of the CCA wall, its measurement may be useful in the assessment of vascular health.

A high Cdist value represents high elasticity in arteries whereas a high PWV value is associated with stiff arteries. Cdist is also thought to reflect the local status of arterial wall whereas PWV reflects the status of the larger vascular system. Interestingly, Cdist and PWV displayed a significant correlation with most of the longitudinal movement parameters used in this study. In a previous study, where the longitudinal movement in subjects with periodontal disease was studied, a

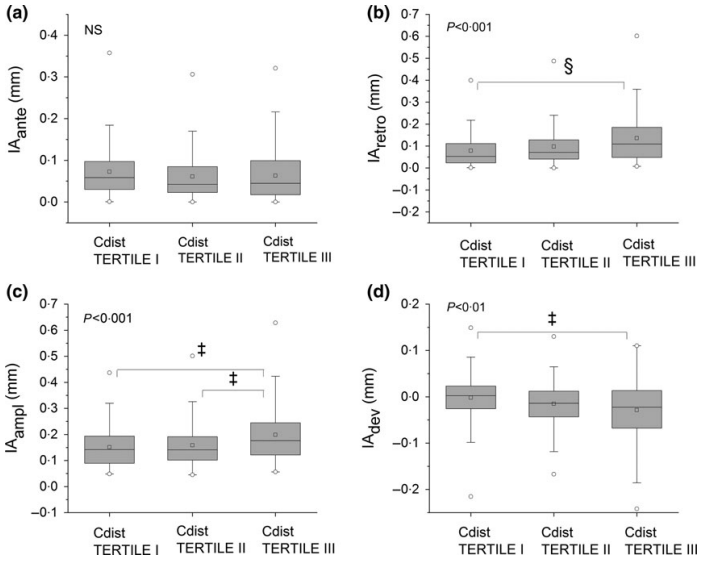

Figure 3 The longitudinal movement values of the intima-adventitia in tertiles of carotid artery distensibility (Cdist). (a) the antegrade movement (IA_{ante}) values in tertiles of Cdist; (b) the retrograde movement (IA_{retro}) values in tertiles of Cdist; (c) the peak-to-peak amplitude (IA_{ampl}) values in tertiles of Cdist; (d) the deviation (IA_{dev}) values in tertiles of Cdist. The 25 and 75th percentiles are displayed as boxes, medians (–), means (□), minimum and maximum values (○). The P-values of the Kruskal–Wallis test are displayed in upper left corner of the subimages, and the following abbreviations have been used to present the P-values of the Mann–Whitney U test with Bonferroni correction: ‡P<0·01, §P<0·001.

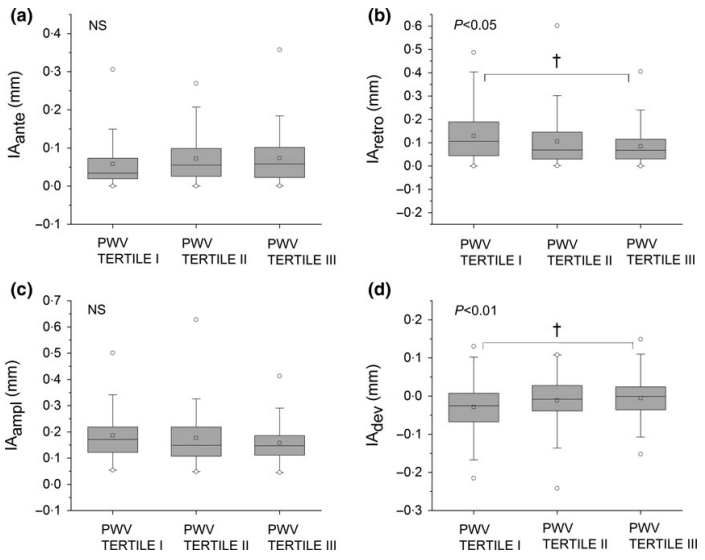

Figure 4 The longitudinal movement values of the intima-adventitia in tertiles of pulse wave velocity (PWV). (a) the antegrade movement (IA_{ante}) values in tertiles of PWV; (b) the retrograde movement (IA_{retro}) values in tertiles of PWV; (c) the peak-to-peak amplitude (IA_{ampl}) values in tertiles of PWV; (d) the deviation (IA_{dev}) values in tertiles of PWV. The 25 and 75th percentiles are displayed as boxes, medians (–), means (□), minimum and maximum values (○). The P-values of the Kruskal–Wallis test are displayed in upper left corner of the subimages, and the following abbreviations have been used to present the P-values of the Mann–Whitney U test with Bonferroni correction: †P<0·05.

similar correlation was obtained with Cdist but not with PWV (Zahnd *et al.*, 2012). Nevertheless in that study, the longitudinal amplitude was divided by pulse pressure and thus the results are not directly comparable with longitudinal movement parameters assessed in our study. Whereas PWV illustrates mainly arterial stiffness along the aortic and aorto-iliac pathway rather than local or segmental arterial properties (Laurent *et al.*, 2006), carotid IMT reflects predominantly local morphological changes in the CCA (Ter Avest *et al.*, 2007). Endothelium-dependent FMD has been considered as a surrogate marker of atherosclerosis (Sørensen *et al.*, 1997) but in general, endothelial dysfunction contributes the whole pathophysiological cascade of arteriosclerotic disorders (Trepels *et al.*, 2006). Therefore, FMD could reflect specifically arterial endothelial function and indirectly both atherosclerotic and non-atheromatous features of arteriosclerosis. In this study, FMD and IMT displayed no significant correlations with the longitudinal movement parameters. In contrast to our findings, attenuated longitudinal movement has been found to be associated with higher IMT values in two previous studies

(Svedlund *et al.*, 2011; Zahnd *et al.*, 2012). However, Svedlund's study population consisted of patients with suspected coronary artery disease and Zahnd's population suffered from periodontal disease, whereas our study was based on a population based sample of young and middle-aged adults without any inclusion criteria for vascular diseases. Therefore, we speculate that the relationship between longitudinal movement and IMT may be different in healthy subjects from that in patients with more advanced vascular diseases.

When we investigated the longitudinal movement in detail, we found that movement occurs intrinsically in the vascular wall. A considerable amount of longitudinal movement occurs between intima-media complex and adventitia. It has been shown previously that the inner part of the vessel wall, the intima-media complex, displays a larger longitudinal movement than the outer part of the vascular wall, the adventitial region (Persson *et al.*, 2003; Cinthio *et al.*, 2006). In our study, we also found that IO_{ampl} was considerably larger than AO_{ampl}. This seems to be an intrinsic feature of the vascular wall and reflects the biomechanics inside the artery. Cinthio *et al.* (2006) reported that the amplitude of the longitudinal movement of the arterial wall is of the same magnitude as the diameter change occurring in the CCA of healthy humans. They also reported previously that the longitudinal movement shows a multiphasic bidirectional pattern with a distinct antegrade movement in early systole and retrograde movement (i.e. opposite direction) in later systole (Cinthio *et al.*, 2006) with similar findings being found in this study.

We found that the retrograde movement of arterial wall was associated with flexible arteries. In other words, there was a significant direct correlation between the Cdist value and retrograde movement and an inverse correlation between retrograde movement and PWV. It is noteworthy that the antegrade component of the movement showed the opposite result and this is evidence of the presence of stiff arteries. IO_{ante} and AO_{ante} exhibited a direct correlation with PWV and inverse with Cdist. The association between the antegrade and retrograde components of the longitudinal movement and stiffness indices is a novel finding. Retrograde longitudinal movement seems to be approximately twofold greater than the antegrade movement assessed in our population. In contrast to the IO_{ante} and AO_{ante}, the retrograde and peak-to-peak amplitudes of the longitudinal movement both have a direct correlation with Cdist and an inverse correlation with PWV. However, these findings may be explained by the fact that the amplitude of the retrograde movement is larger in comparison with the antegrade movement.

RAlength and polydeg are new parameters which have not been used previously in clinical studies. Here, RAlength displayed a significant negative correlation with PWV and a positive correlation with Cdist, reflecting the elasticity of the vascular wall. While the inverse correlation between RAlength and FMD was discordant with Cdist and PWV, one would have predicted that good endothelial function would be directly associated with RAlength. The significance of the correlation was low, and it should be confirmed in future studies before any conclusions can be made about its significance. Polydeg did not display any significant correlations with conventional parameters of vascular health.

The longitudinal movement phenomenon in the arterial wall is a new field of research, and the physiological significance of the longitudinal movement is still unclear. It still remains to be clarified whether longitudinal movement of CCA is describing completely unknown properties of the vascular wall. It has been debated whether the longitudinal shear strain in CCA is caused by the pulsating blood flow, the physical movement of the heart or the cyclic diameter change of the artery (Cinthio *et al.*, 2006; Yli-Ollila *et al.*, 2013). Stratification of the vascular wall seems to influence the movement of the wall layers, but its physiological significance, especially that of the retrograde movement, is still unknown. Our results indicated that it was associated with the elasticity of arteries, but it is also possible that large repeated distensions could be a risk factor for plaque rupture in atherosclerotic arteries. Some evidence has been found of risk factors correlating with the amplitude of the longitudinal movement, for example (Zahnd *et al.*, 2011), but there is also one report that impairment of carotid longitudinal movement would be independent of some cardiovascular risk factors (Zahnd *et al.*, 2012). Thus, the true clinical significance of longitudinal movement of arterial wall as well as its physiological background, for example physiological correlates of longitudinal movement, will require further investigation.

Our study was executed in a large, well-characterized study population consisting young adults of age of 30–45 years. Our aim was to focus on young adults so that we could evaluate the subclinical phase of arterial disease; hence, the association between longitudinal movement and conventional measures of arteriosclerosis or arterial stiffness markers in population with advanced vascular disease remains unclear. Our study population consists of white European subjects, and for that reason, these results may not be generalized to other ethnic groups. Ultrasound imaging was performed and analysed in a large population (N = 465), with successful longitudinal analysis being achieved in 292 subjects. There is a good reason for the relatively large number of unsuccessful scans, since in 2007 the ultrasound CCA imaging protocol was optimized to measure IMT and Cdist, not to analyse the longitudinal carotid wall movement. Longitudinal motion analysis is challenging and requires a good frame to frame image quality from the ultrasound video as even the smallest image artefacts can disturb the motion analysis. Thus, it is likely that the motion tracking success rate would have been higher if the study protocol had been designed for the longitudinal motion analysis. In our recent studies in which the special demands of this analysis have been taken into account, all the analyses were successful (Yli-Ollila *et al.*, 2013). Nevertheless, the final number of subjects in the present study is large and for the majority, the signal quality of the longitudinal movement was good.

Conclusion

We studied the interrelationships between longitudinal parameters of the arterial wall and the conventional measures of arteriosclerosis in a large, well-characterized study population. Our findings emphasize the influence of arterial stiffening on longitudinal movement of the arterial wall. This observation supports the idea that measurements of longitudinal movement can be of value in the assessment of vascular health.

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Conflict of interest

The authors have no conflict of interests.

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II

Influence of cardiovascular risk factors on longitudinal motion of the common carotid artery wall

Taivainen H, Yli-Ollila H, Juonala M, Kähönen M, Raitakari OT, Laitinen TM, Laitinen TP

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Influence of cardiovascular risk factors on longitudinal motion of the common carotid artery wall

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ABSTRACT

Background and aims: Carotid artery longitudinal wall motion (CALM) is a new biomarker, which can be measured together with carotid intima-media thickness and distensibility measurements in the same session. Our objective was to study the relationship between these indicators of vascular health and cardiovascular risk factors in a large and well-characterized study population.
Methods: The study population consisted of 465 subjects aged 30–45 years. Successful measurements were performed in 287 participants.
Results: The peak-to-peak and retrograde amplitudes of the longitudinal motion were inversely correlated with systolic blood pressure (SBP; $r = -0.152$, $p<0.05$ and $r = -0.189$, $p<0.01$), diastolic blood pressure (DBP; $r = -0.170$, $p<0.01$ and $r = -0.256$, $p<0.001$) and body mass index (BMI; $r = -0.158$, $p<0.01$ and $r = -0.291$, $p<0.001$). In addition, retrograde amplitude of longitudinal motion indirectly correlated with total cholesterol and triglycerides ($r = -0.163$, $p<0.01$ and $r = -0.228$, $p<0.001$, respectively). Amplitude of antegrade longitudinal motion was directly correlated with DBP, total cholesterol, LDL-cholesterol, triglycerides and BMI ($r = 0.198–0.274$, $p<0.001$ for all). Antegrade longitudinal motion increased and retrograde longitudinal motion decreased with the increasing number of cardiovascular risk factors.
Conclusions: The magnitude of correlation coefficients between CALM parameters and risk factors was comparable with those for carotid intima-media thickness and distensibility. However, the correlation profile for various risk factors was different and CALM gives additional information regarding arteriosclerosis and risk factors.

1. Introduction

Several non-invasive imaging techniques have been developed to investigate alterations representing different features of the arteriosclerotic and atherosclerotic process [1]. Carotid intima-media thickness (CIMT) measured by ultrasound is a widely used method to reveal atherosclerotic plaque disease and structural changes in the vascular wall [1], and it is known to correlate with cardiovascular risk factors, LDL-cholesterol, triglycerides, systolic and diastolic blood pressure, body mass index and smoking [2]. Carotid artery distensibility (Cdist) is used to evaluate the local stiffness of the vascular wall. A number of cardiovascular risk factors identified in childhood have been associated with decreased

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Cdist values in adulthood [3]. Cdist has been found to decline in young adults with metabolic syndrome in both sexes [4]. Decreased Cdist has been implicated also as an independent predictor of cardiovascular events in the elderly [5,6].

In our previous studies, we found that arterial stiffening is associated with alterations in carotid artery longitudinal motion (CALM) [7–9]. Some evidence suggests that CALM may also reflect vascular health [10–13]. Some evidence has been found on cardiovascular risk factors correlating with CALM in smaller study groups; significantly lower common carotid artery (CCA) wall longitudinal motion mean amplitudes have been shown in older patients with type 2 diabetes [12], and patients with CCA plaques have shown significantly lower CALM compared to controls [10]. Patients with periodontal disease also show significantly lower CALM than healthy controls [14]. In addition, there is evidence that low CALM may be a significant independent 1-year predictor of major adverse cardiovascular events in patients with suspected coronary disease [11].

However, associations of CALM with traditional risk factors of cardiovascular diseases have not been studied before in a large study population of young adults representing healthy subjects or subclinical disease phase. To investigate the associations between CALM and cardiovascular risk factors, we measured CALM in a large population where individuals were participants in the Cardiovascular Risk in Young Finns Study.

2. Materials and methods

2.1. Subjects and study design

The Cardiovascular Risk in Young Finns Study is a five-center ongoing follow-up study of atherosclerosis risk factors in Finnish children and adolescents. The first cross-sectional survey was conducted in 1980 with 3596 children and adolescents participants. The original sample age range was from 3 to 18 years, and participants were randomly chosen from each area in Finland from a national register. In this cohort, follow-up studies were conducted at regular intervals from 3 to 6 years during the period 1980–2007. The study was approved by local ethics committees. The subjects provided written informed consent.

Kuopio University Hospital is one of the five centers involved and investigates the population of Eastern Finland. The present cross-sectional study consists of the Kuopio center data from 2007, when the subjects were aged 30–45 years and vascular ultrasound studies were available for 465 subjects. Successful longitudinal motion analyses were performed for 292 subjects. Five women were excluded due to pregnancy, hence the final study population included 287 participants.

2.2. Assessment of risk factors

Height was measured to an accuracy of 1 cm and weight to an accuracy of 1 kg. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. Systolic and diastolic blood pressures (SBP, DBP) were measured from the brachial artery using a random zero sphygmomanometer (Hawksley & Sons Ltd, Lancin, UK) in the sitting position. The average of three measurements was used in the analysis. Smoking habits were ascertained with a questionnaire. Smoking was processed as dichotomous variable (smoking/non-smoking), according to a regular daily use of tobacco products.

Venous blood samples were drawn after an overnight fast for the determination of serum lipid levels and all measurements of lipid levels were performed in duplicate in the same laboratory. To measurelevels of serum total cholesterol, triglycerides, and high-density lipoprotein cholesterol (HDL-C), standard enzymatic methods were used. Friedewald formula was used to calculate the low-density lipoprotein cholesterol (LDL-C) concentration. Details of these methods have been described previously [15].

2.3. Carotid ultrasound imaging

Ultrasound studies were performed by trained sonographers following the standardized protocol described previously [2]. Carotid artery imaging was performed using Sequoia512 ultrasound scanner (Acuson, Mountain View, Calif) equipped with a 14 MHz linear array transducer and the ECG signal (modified chest lead 5) was recorded and presented alongside with B-mode image sets.

A resolution box function was used to scan the left common carotid artery (CCA) to record a 25 mm wide and 15 mm high image, including the beginning of the carotid bifurcation and the distal CCA. For subsequent off-line analysis, a 5 s cine loop (25 frames per second) was digitally stored. To derive CIMT and Cdist as well as radial and longitudinal motions of artery wall, the same image set was used. Blood pressure was measured in the supine position with an automated sphygmomanometer (Omron M4, Omron Matsusaka Co., Ltd, Japan) immediately before and after ultrasound imaging, for the calculation of the distensibility parameters.

2.4. Longitudinal motion

Carotid artery wall motion analysis was performed using an in-house motion tracking program developed by our research-group [16]. The software was written in Matlab (2007b, The MathWorks Inc., Natic, MA, USA). It is capable of reading the graphical ECG-information from the ultrasound recording and simultaneously tracking the longitudinal and radial motions of the arterial wall. The basic method used in the motion tracking was a two-dimensional cross-correlation (block matching) enhanced with a contrast optimization technique to reduce noise from the videos.

In the longitudinal motion analysis, regions of interest (ROIs) are drawn on the ultrasound image on the intima-media complex, on the adventitial layer and on the surrounding tissue outside the adventitia. When tracking the radial motion of the arterial wall, ROIs are drawn on the distal and proximal arterial wall. The motion tracking of the longitudinal motion was considered suitable for analysis if the tracking successfully recorded at least two heart cycles, otherwise the motion data was discarded. Details of the methods have been described earlier [7,16].

We measured two different longitudinal motion curves: between the intima-media complex and the adventitial layer (IA) and between the intima-media complex and the surrounding tissue (IO). The curves of the longitudinal motion have been previously shown to vary extensively between individuals [16]. We investigated the amplitude of the motion (ampl), the forward (ante) oriented, and backward (retro) oriented component of the motion between the different layers of the CCA wall. We evaluated also the main deviation of the longitudinal motion (dev) between the different arterial layers by computing the average of the motion curve over a cardiac cycle.

2.5. Conventional measurements of vascular health

CIMT was measured by focusing the image on the posterior wall of the left CCA and the best-quality end-diastolic frame was selected from the video. To derive the maximal CIMT, at least four measurements were taken from this image, approximately 10 mm proximal to the bifurcation. The method has been described previously in detail [2]. Cdist measures the ability of the arteries to expand in response to the pulse pressure caused by cardiac

contraction and relaxation [3]. The best-quality cardiac cycle was selected from the 5-s cine loop to assess carotid elasticity indices. The CCA diameter was measured at both end-diastole and end-systole, at least twice, using the calipers of the ultrasound scanner. The means of the measurements were used as the end-diastolic and end-systolic diameters. The following ultrasound and concomitant brachial blood pressure measurements were used in the calculation of the indices of arterial elasticity: Cdist = $([D_s\text{-}D_d]/D_d)/(P_s\text{-}P_d)$, where D_s is the systolic diameter, D_d is the diastolic diameter, P_s is systolic blood pressure, P_d is diastolic blood pressure.

2.6. Statistical methods

Partial correlation analysis adjusted with age and gender was used to define conformities between the indices of longitudinal motion, the conventional parameters of vascular health and traditional cardiovascular risk factors SBP, DBP, total cholesterol, LDL-C, HDL-C, triglycerides and BMI. Distributions of longitudinal motion parameters were only slightly skewed and residuals of the model were normally distributed, thus parametric tests were considered acceptable to use. To examine the effects of risk factors cumulation on vascular health parameters, we calculated a simple score according to the number of current risk factors (0, 1, 2, and 3 or more risk factors). Risk factors were defined as BMI $\geq$25.0 kg/m^2; LDL-C $\geq$ 3.0 mmol/l or lipid-lowering medications; SBP $\geq$140 mmHg or antihypertensive medication, and daily smoking. Comparison between groups was performed using analysis of covariance (ANCOVA) with adjusting variables age and gender, and pairwise comparisons were executed. Bonferroni-correction was used to analyze more accurately the significance of the difference between different score-groups. Statistical analyses were performed with SPSS version (IBM Corp., IBM SPSS Statistics for Windows, Version 22.0, Armonk, NY) and statistical significance was inferred at a 2-tailed value of $p<0.05$.

3. Results

The clinical characteristics of the study subjects and characteristics of vascular health are shown in Table 1. Smoking prevalence was 17.1% (N = 49) and other risk factors prevalence was: BMI $\geq$ 25.0 kg/m^2, 50.3% (N = 144); LDL-C $\geq$ 3.0 mmol/l, 52.3% (N = 150) or lipid-lowering medication 2.4% (N = 7); SBP $\geq$ 140/- mmHg, 24.1% (N = 69) or antihypertensive medication 7.3% (N = 21). The correlations between CALM parameters, CIMT, Cdist and traditional risk factors are shown in Table 2. The associations between motion of different CCA layers and traditional risk factors showed parallel results and significant associations were most clearly seen in IO motion. The results display retrograde motion correlating inversely and antegrade motion correlating directly with risk factors, with the exception of HDL (Table 2). IO_{ante} showed a significant direct correlation with DBP, total cholesterol, LDL-C, triglycerides and BMI, but not with SBP and HDL-C. IO_{retro} revealed a significant inverse correlation with systolic and diastolic blood pressures, total cholesterol, triglycerides and BMI but not with LDL-C and HDL-C. IO_{dev} showed a significant direct correlation with DBP and BMI as well as IA_{dev}, but no statistically significant correlations were seen with lipids. IO_{ampl} was inversely correlated with SBP, DBP and BMI but no statistically significant correlations were seen with lipids; IA_{ampl} showed a significant inverse correlation only with DBP. IA_{ante} showed a significant direct correlation with BMI and an inverse correlation with HDL but not with other risk factors. IA_{retro} instead correlated inversely and significantly with SBP, DBP and BMI, but not with lipids. CIMT revealed a significant direct correlation with SBP, LDL-C and BMI. Cdist showed a significant inverse correlation with SBP and DBP, total cholesterol, triglycerides and BMI, but not with LDL-C and HDL-C.

Antegrade longitudinal motion increased and retrograde longitudinal motion decreased with the increasing number of risk factors (Table 3). A decrease in peak-to-peak amplitude as well as in retrograde amplitude of longitudinal motion was detected when the number of risk factors increased. CALM parameters displayed significant differences between score groups with 0 and 1 risk factor (IA_{retro}, IA_{ampl}, IA_{dev}, IO_{dev}) and between 0 and 2 risk factors (IA_{ante}, IA_{retro}, IA_{dev}, IO_{ante}, IO_{retro}, IO_{dev}). Between 0 and 3 risk factors, only IO_{ante} and IO_{retro} showed a significant difference. IO_{ante} revealed a significant difference between the number of risk factors 1 and 2, and 1 and 3. CIMT showed a significant difference in score-groups with zero and 3 or more risk factors. Cdist showed no significant differences between different score-groups in this study population. The influence of risk factors on CALM parameters was similar in men and women (i.e. no significant gender interaction was observed in relationships).

4. Discussion

In the present study, we found an association between CALM and traditional cardiovascular risk factors, especially SBP, DBP and BMI. In addition, we found a significant correlation between both antegrade and retrograde motion of IO and total cholesterol as well as triglycerides. IO_{ante} correlated also with LDL-C. In general, antegrade motion correlated directly with the measured risk factors and retrograde motion had an inverse correlation. When cumulative risk loads was taken into account, we observed the total amplitude of longitudinal motion to decrease as the number of risk factors increased. Our findings suggest that traditional cardiovascular risk factors modulate CALM by diminishing the retrograde longitudinal motion and by increasing the antegrade oriented motion. However, when the risk factor load grows higher, the total longitudinal motion amplitude decreases, resulting in attenuation of its antegrade component as well.

Yli-Ollila et al. [17] demonstrated that in a healthy population, CALM occurs first in the intima-media complex and is followed by the longitudinal motion of the adventitial layer. An external elastic

Table 1
Clinical characteristics of the study population and vascular health characteristics.

	Mean (SD)	Median	Minimum	Maximum
Age (years)	38.1 (4.8)	39	30	45
Body height (cm)	170 (8.8)	169	150	194
Body weight (kg)	74.9 (15.9)	72.0	44	147
BMI (kg/m^2)	25.7 (4.7)	25.0	17.7	45.9
SBP (mmHg)	128 (14)	128	99	199
DBP (mmHg)	81 (10)	80	55	125
Total cholesterol (mmol/l)	5.04 (0.87)	4.90	3.10	8.20
LDL-C (mmol/l)	3.10 (0.76)	3.00	1.48	5.38
HDL-C (mmol/l)	1.38 (0.34)	1.36	0.48	3.50
Triglycerides (mmol/l)	1.29 (0.74)	1.05	0.34	6.66
IA_{ante} (mm)	0.066 (0.061)	0.048	0.000	0.358
IA_{retro} (mm)	0.101 (0.093)	0.072	0.000	0.602
IA_{ampl} (mm)	0.167 (0.088)	0.150	0.045	0.628
IA_{dev} (mm)	−0.014 (0.054)	−0.008	−0.242	0.149
IO_{ante} (mm)	0.146 (0.127)	0.108	−0.002	0.517
IO_{retro} (mm)	0.254 (0.219)	0.204	0.000	1.169
IO_{ampl} (mm)	0.400 (0.178)	0.366	0.099	1.291
IO_{dev} (mm)	−0.014 (0.053)	−0.008	−0.219	0.149
CIMT (mm)	0.67 (0.10)	0.67	0.48	1.01
Cdist (%/10 mmHg)	1.97 (0.68)	1.94	0.02	4.29

SBD, systolic blood pressure; DBP, diastolic blood pressure; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; BMI, body mass index calculated as weight in kilograms divided by square of height in meters. Smoking prevalence: smoking daily or more often. Abbreviations and explanations of indices of longitudinal motion of common carotid artery are given in the text.

Table 2
Age- and sex-adjusted correlations (correlation coefficient r) between vascular health parameters and cardiovascular risk factors.

	SBP	DBP	Total cholesterol	LDL-C	HDL-C	Triglycerides	BMI
IA_{ante}	0.075	0.097	0.043	0.073	−0.142*	0.114	0.173**
IA_{retro}	−0.149*	−0.200***	−0.109	−0.083	−0.009	−0.109	−0.140*
IA_{ampl}	−0.106	−0.144*	−0.085	−0.037	−0.109	−0.036	−0.027
IA_{dev}	0.115	0.155**	0.063	0.086	−0.086	0.076	0.141*
IO_{ante}	0.109	0.198***	0.224***	0.214***	−0.103	0.255***	0.274***
IO_{retro}	−0.189**	−0.256***	−0.163**	−0.106	0.008	−0.228***	−0.291***
IO_{ampl}	−0.152*	−0.170**	−0.037	0.025	−0.064	−0.094	−0.158**
IO_{dev}	0.115	0.155**	0.068	0.091	−0.087	0.078	0.146*
CIMT	0.181**	0.079	0.092	0.126*	−0.132*	0.106	0.252***
Cdist	−0.338***	−0.365***	−0.130*	−0.074	−0.072	−0.122*	−0.142*

Statistical significances: *$p < 0.05$; **$p < 0.01$, ***$p < 0.001$. For abbreviations see the text.

Table 3
Age and sex–adjusted relationships between numbers of traditional risk factors, conventional measurements and longitudinal movement parameters.

Number of risk factors	0	1	2	≥3	*p*-value
	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	
IA_{ante} (mm)	0.049 (0.045)	0.061 (0.058)	0.082 (0.069)[b]	0.063 (0.061)	0.008
IA_{retro} (mm)	0.147 (0.125)	0.091 (0.067)[a]	0.091 (0.098)[b]	0.089 (0.067)	0.003
IA_{ampl} (mm)	0.196 (0.113)	0.152 (0.063)[a]	0.173 (0.096)	0.152 (0.076)	0.02
IA_{dev} (mm)	−0.039 (0.060)	−0.011 (0.046)[a]	−0.003 (0.056)[b]	−0.014 (0.048)	0.002
IO_{ante} (mm)	0.111 (0.122)	0.110 (0.098)	0.187 (0.143)[b,d]	0.185 (0.121)[c,e]	<0.0001
IO_{retro} (mm)	0.358 (0.272)	0.284 (0.189)	0.193 (0.201)[b]	0.182 (0.175)[c]	<0.0001
IO_{ampl} (mm)	0.469 (0.230)	0.394 (0.159)	0.380 (0.172)	0.366 (0.130)	0.049
IO_{dev} (mm)	−0.039 (0.060)	−0.011 (0.046)[a]	−0.003 (0.055)[b]	−0.014 (0.048)	0.002
CIMT (mm)	0.658 (0.074)	0.661 (0.093)	0.679 (0.107)	0.722 (0.108)[c,e]	0.009
Cdist (%/10 mmHg)	2.145 (0.836)	2.019 (0.586)	1.951 (0.687)	1.715 (0.561)	NS

Significant differences ($p < 0.05$) between score-groups are shown with letters: a: 0–1, b 0–2, c 0-≥3, d 1–2, e 1–3, f 2-≥3. For abbreviations see the text. Univariate analysis of variance was used with Bonferroni correction.

lamina connects the media layer to the adventitia and is composed of condensed sheets of elastic fibers. Thus it is possible that the elastic fibers drag the adventitia layer along, as the intima-media complex moves in the longitudinal direction [17]. We decided to inspect both IA and IO parametersto get a better insight of this new phenomenon. Longitudinal motion between intima and adventitia (IA) represents the kinetics within the vascular wall and IO represents the total arterial movement occurring between intima and surrounding tissues. In the present study, we found both IA and IO amplitude of the longitudinal motion of CCA to diminish when the number of risk factors increased. Interestingly, the antegrade component of IA and O increased as risk factors cumulated, but when the number of risk factors reached three or more, the antegrade component of CALM seemed to stop increasing or diminished. One interpretation for this may be that changes in the longitudinal motion represent an early phase alteration of the carotid artery wall and the delicate motion may diminish while the risk factor load is big enough. Because the driving force for CALM is still unclear, and because a clear perception of cardiovascular disease risk factors' impact on CALM is newly taking shape, it is advantageous to observe this phenomenon extensively.

Elastic properties of arteries and the role of arterial stiffness in the development of cardiovascular diseases have been of great interest during the last years [18]. One of the traditional measurements, Cdist, has shown to predict upcoming cardiovascular events, cardiovascular mortality and all-cause mortality [19]. Cdist has also been found to decline in young adults with metabolic syndrome [4] and several cardiovascular risk factors identified in childhood have been associated with decreased Cdist values in adulthood, such as childhood blood pressure, skinfold thickness, high LDL-cholesterol (at or above 80th persentile) and smoking ($p<0.001$) [3]. In adulthood, relationships between current risk factors (SBP, LDL-C, triglycerides and BMI) and Cdist were significant in males and females [3]. Decreasing trend in Cdist ($p < 0.001$) across groups with an increasing number of childhood risk factors in young adults aged 24–39 years was found in the same cross-sectional study [3]. A follow-up study displayed baseline and 6-year changes in waist circumference, baseline insulin change in systolic blood pressure, independently associating with decreasing Cdist levels in young adults [20]. In the study of Koskinen et al. at baseline, an inverse correlation between BMI, SBP, DBP, triglycerides, total cholesterol, LDL-c and a straight correlation with HDL and Cdist were significant [20]. Cdist was found to be significantly lower and carotid stiffness higher among non-diabetic and type 2 diabetic hypertensive adults and older patients *versus* healthy normotensives [21].

CIMT, as measured by ultrasound, has been found to correlate with cardiovascular risk factors [1,2]. Raitakari et al. [2] have shown that risk factor profile (LDL-C, SBP, BMI and smoking) assessed in 12- to 18-year-old adolescents is directly related to CIMT measured in young adulthood. In multivariable analysis for current risk variables, SBP, BMI and smoking were all significantly associated with CIMT in the model adjusted for age and sex. CIMT has been shown to be thicker among young middle-aged and middle-aged smokers than non-smokers [22]. Men (age 37 ± 4 years) with borderline hypertension have been shown to have significantly higher CIMT and increased levels of LDL-diene conjugation as a marker of LDL oxidation, but similar FMD-measured endothelial function [23]. Berni et al. [24] investigated whether each conventional cardiovascular risk factor *per se* has an effect on IMT on adults (aged 34–90); obesity emerged as the greatest risk factor with regard to intima-media thickening in CCA and femoral artery, followed by hypertension, hypercholesterolemia, smoking and overweight.

We found association-profiles with risk factors to differ between CALM and conventional measurements. Cdist, representing local carotid artery stiffening, had strongest associations with blood pressure. As shown in our previous study, CALM correlates

significantly with conventional stiffness parameters [7], and the significant association was also seen in this study, especially with longitudinal parameters and DBP, but also with retrograde longitudinal motion and SBP. The significant association between total cholesterol and triglycerides with antegrade and retrograde longitudinal motion of the intima-media complex (IO) was an interesting finding and suggests that CALM parameters may represent other aspects of vascular disease than just diffuse stiffening, as hypercholesterolemia is associated with lipid and free and esterified cholesterol accumulation to vascular wall, especially in the focal atherosclerotic process [25]. In the present study, a significant difference between none and one or none and two risk factors were more often seen with the IA longitudinal motion than with the conventional measures, where a significant difference was identified only when three or more risk factors were included. The interpretative factor for this is also that there is not enough power in our Eastern Finland subpopulation size to reveal true associations, e.g. with Cdist, that was found before in a larger multicenter study population (e.g. Refs. [3,4,26]). Hypertension and BMI seemed to be the most powerful contributors for the alteration of longitudinal movement as associating significantly with 7/8 longitudinal parameters (Table 2).

According to our previous study, biomechanical properties of vascular wall contribute to the direction of CALM: larger retrograde oriented motion was associated with elastic arteries, while augmented antegrade oriented motion was associated with stiffer arteries [7]. This finding is analogical with results of the present study, showing larger retrograde oriented motion in association with less risk factors and larger antegrade oriented longitudinal motion in association with more risk factors. However, we found the total amplitude of longitudinal motion to diminish when the risk load increases to more than two. This may explain why only slight differences in CALM were observed in the risk score groups including two or more risk factors.

In other studies, focusing on relations between cardiovascular disease or cardiovascular risk factors and CALM, the direction of motion has not been evaluated in detail, rather the whole amplitude is observed. Associations between cardiovascular risk factors and CALM have been investigated earlier in some studies, but the characteristics of the study population differs from each other: Svedlund et al. [11] showed, in a large study population with suspected coronary artery disease, that low total longitudinal motion associated with more advanced clinically determined myocardial ischemia in one year follow-up and also with a higher CIMT. Population was divided into tertiles as for magnitude of CALM, and patients with low CALM displayed significantly greater BMI compared with patients with middle tertile CALM, but no significant differences were found between different CALM tertiles and hypertension, diabetes mellitus diagnosis or smoking habits [11]. However, diabetic patients displayed reduced longitudinal motion amplitudes in another smaller study population of young healthy volunteers and older diabetic patients (26 + 26), illustrating the effect of the arteriosclerotic process on the artery wall [12]. In other study design with 46 mice, attenuated CALM was associated with greater plaque burden in the brachiocephalic artery and larger IMT [10]. The same study also showed that higher cholesterol levels were in association with a low longitudinal motion in mice. In a small study (10 patients and 10 controls), patients with atherosclerotic plaques had lower CALM compared to controls and in the other report, a small group of patients with coronary artery disease ($n = 16$) had significantly lower total longitudinal motion than healthy volunteers ($n = 16$) [27]. Zahnd et al. [14] investigated 126 study participants with periodontal disease and 27 healthy controls to study the association of CALM with cardiovascular risk factors. The amplitude of CALM was markedly lower in the periodontal disease group, but the direction of the motion was not separated as in our study. The significant difference between groups was independent of cardiovascular risk factors (age, sex, smoking status, BMI, HDL-C, nonHDL-C, HbA1c and DBP), cross-sectional distensibility and CIMT, suggesting it may represent an independent marker of vascular health [14]. Our study population stands out, as participants were young and middle-aged adults without any inclusion criteria for vascular diseases and the size of the study population was larger than in most of the previous mentioned studies.

Opposite results have been published in a multi-ethnic large cohort where greater CALM was associated with greater CIMT, but no significant association was seen between total longitudinal motion and cardiovascular risk factors age, SBP, DBP, pulse pressure, diabetes mellitus or former or current tobacco use, nor with traditional markers of carotid stiffness [28]. Interpretative factors for opposite results may be several, i.e. our study population was younger, breathing can modify the longitudinal motion of CCA, and it is not known how breathing was taken into account in the study by Gepner et al. also ethnicity may have its influence on CALM. In addition, the aforementioned study was performed with velocity vectory imaging (VVI)-technique, which previously could not show multiphase components of CALM as clearly [10] as in other motion tracking methods (e.g. Refs. [16,29]). These contradictory findings emphasize the need for further investigation. The physiological background of CALM remains still unclear, although more detailed knowledge of the determinants of CALM [30] and a more accurate analysis of longitudinal motion curves has been reported [9].

Our aim was to focus on young adults to evaluate the healthy physiologic or subclinical phase of arterial disease. Our study was executed in a large and well-characterized study-population consisting of young adults of 30–45 years. The results may not be generalizable to people outside this age range. Our study consists of white European subjects and for this reason the results may not be generalizable to other ethnic groups. The number of the study population was large but notably smaller than in traditional risk factor reports of The Cardiovascular Risk in Young Finns Study (e.g. Refs. [2,26]). Longitudinal motion analysis of CCA is challenging and requires a good frame to frame image quality from ultrasound video as even the smallest image artefacts can disturb the motion analysis. Ultrasound imaging was performed and analysed in 465 subjects, with a successful longitudinal analysis achieved in 292 subjects. The reason for the relative large number of unsuccessful scans was that in 2007 the ultrasound CCA imaging protocol was optimized to measure Cdist and CIMT, not to analyze CALM, as discussed in the previous study [7]. When the special demands of the analysis have been taken into account, all the analyses were successful [16]. The final number of study subjects in the present study is large nevertheless, and for the majority, the signal quality of data was good.

4.1. Conclusion

We studied the inter-relationships between the CALM parameters, conventional measures of arteriosclerosis and traditional risk factors of cardiovascular diseases in a large, well-characterized study-population. The traditional risk factors modulate CALM by diminishing the retrograde longitudinal motion and increasing the antegrade oriented motion. Our findings support the hypothesis that subclinical arteriosclerosis alters CALM and thus measurements of CALM can be used in the assessment of the vascular health. Several vascular health evaluating methods allow wide perception of the vascular state and provide some diverse information needed to better understand the complicated phenomenon occurring within vessel walls.

Conflicts of interest

The authors declared they do not have anything to disclose regarding conflict of interest with respect to this manuscript.

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III

Carotid artery longitudinal wall motion alterations associated with metabolic syndrome and insulin resistance

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Carotid artery longitudinal wall motion alterations associated with metabolic syndrome and insulin resistance

Short title: Metabolic Syndrome and Longitudinal Motion of the Common Carotid Artery

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Summary

Background and aims: Our objective was to study relationships between the new biomarker of vascular health, carotid artery longitudinal wall motion (CALM) and metabolic syndrome (MetS).

Methods: Carotid ultrasound and assessment of MetS and its components were performed with 281 subjects aged 30–45 years. In the longitudinal motion analysis, the amplitude of motion and the antegrade-oriented and retrograde-oriented components of motion between the intima-media complex and adventitial layer of the common carotid artery wall were assessed.

Results: MetS, according to the harmonized criteria, was detected in 53 subjects (19%). MetS was significantly associated with increased antegrade and decreased retrograde longitudinal motion in the carotid artery wall. Augmented antegrade amplitude of longitudinal motion was associated with obesity ($\beta = 0.149$, $P < 0.05$) and low HDL-cholesterol ($\beta = 0.177$, $P < 0.01$). Attenuated retrograde amplitude of longitudinal motion was associated with hypertension ($\beta = -0.156$, $P < 0.05$), obesity ($\beta = -0.138$, $P < 0.05$) and hyperinsulinemia ($\beta = -0.158$, $P < 0.01$). Moreover, insulin resistance (homeostasis model assessment index above 2.44) was associated with adverse changes in CALM.

Conclusion: MetS and insulin resistance were associated with alterations in CALM. In particular, hypertension, obesity and hyperinsulinemia were associated with reduced total peak-to-peak amplitude as well as increased antegrade and reduced retrograde amplitudes, all of which might be markers of unfavourable vascular health.

Keywords: arterial stiffness, cardiovascular risk factors, hyperinsulinemia, hypertension, insulin resistance, motion tracking, ultrasound imaging.

Introduction

Metabolic syndrome (MetS) is a cluster of multiple cardiovascular risk factors such as central obesity, hypertension, dyslipidemia, glucose intolerance and insulin resistance (Eckel *et al.*, 2005). MetS is associated with increased risk of cardiovascular diseases and all-cause as well as cardiovascular disease mortality (Gami *et al*., 2007; Mottillo *et al*., 2010). Mechanisms through which MetS increases cardiovascular risk involve several pathophysiological changes in the arterial wall (Qiao *et al*., 2007). Although MetS components are interrelated, each component may act independently through different mechanisms, with adverse effects on the structure and function of the vascular system. Therefore, when investigating consequences of MetS, parallel use of methods that characterize the structure and function of blood vessels provides opportunity for a comprehensive evaluation of pathophysiological changes. Carotid artery longitudinal wall motion (CALM) is a relatively new biomarker reflecting vascular health that can be measured by using carotid ultrasound imaging together with assessment of carotid intima-media thickness and distensibility measurement in the same session (Yli-Ollila *et al*., 2013). CALM has not been studied systematically in subjects with MetS.

One important consequence of MetS is arterial stiffening, which is known to contribute to prognosis in diabetic patients (Prenner & Chirinos, 2015). Components of MetS have different associations with arterial stiffness parameters (Vagovicova *et al*., 2015). Furthermore, distinct clusters of components of MetS show differing patterns of associations with arterial stiffness (Scuteri *et al*., 2014). The results of our previous studies suggest that arterial stiffening is associated with alterations in CALM (Taivainen *et al*., 2015; Yli-Ollila *et al*., 2016; Yli-Ollila *et al*., 2016). Therefore, arterial stiffening is a potential link between CALM and MetS.

In addition to visceral adiposity, a key feature of MetS is insulin resistance (Salmenniemi *et al*., 2004). Insulin itself has obvious vascular effects (Yki-Järvinen, 2003). Insulin resistance is accompanied closely by endothelial dysfunction, which is thought to be an important mechanism through which insulin resistance results in harmful effects on the vasculature (Yki-Järvinen, 2003; Nesto, 2004). Adiponectin is an insulin-sensitizing and anti-inflammatory adipokine, the concentration of which decreases with weight gain; its levels are indirectly associated with insulin resistance (Trujillo & Scherer, 2005). Because adiponectin is protective against the development of arteriosclerosis, it is an interesting possible link between MetS and CALM.

To investigate associations between CALM parameters and components of MetS, we performed carotid ultrasound imaging and measured CALM in a large population of individual participants in the Cardiovascular Risk in Young Finns Study. Furthermore, CALM in association with insulin resistance, hyperinsulinemia and low adiponectin concentration was studied.

Methods

Subjects and study design

The Cardiovascular Risk in Young Finns Study is an ongoing, five-centre follow-up study of atherosclerosis risk factors in Finnish children and adolescents. The first cross-sectional survey was conducted in 1980, when 3596 3- to 18-year-old children and adolescents participated. Participants were randomly chosen from each area in Finland through a national register. With this cohort, follow-up studies were conducted regularly at intervals of from 3 to 6 years during the years 1980–2007 (Raitakari *et al*., 2008). The study was approved by the Ethics Committee, Hospital District of Southwest Finland. The participants provided written informed consent.

Kuopio University Hospital investigates the population of Eastern Finland and is one of the five centres involved. The present cross-sectional study consists of Kuopio centre data from 2007, when the subjects were 30 to 45 years of age. Vascular ultrasound studies were available for 465 subjects, and successful CALM analysis was performed for 292 subjects. Five female individuals were excluded due to pregnancy, and four individuals were excluded because of type 1 diabetes. Furthermore, there was a lack of anthropometric data for two individuals; thus, the final study population included 281 participants. Adiponectin data were lacking for seven participants. Hence, in the univariate analysis of CALM and adiponectin, 274 participants were analysed.

Assessment of risk factors

Height was measured to an accuracy of 1 cm and weight to an accuracy of 1 kg. Body mass index (BMI) was calculated as weight in kilograms divided by height in metres squared. Waist circumference was measured using an anthropometric tape at the end of expiration at the midpoint between the iliac crest and the lowest rib, with an accuracy of 0.1 cm, and the average of two measurements was used. Systolic and diastolic blood pressure were measured in the sitting position from the brachial artery using a random zero sphygmomanometer (Hawksley & Sons Ltd, Lancin, UK). The average of three measurements was used in the analysis. Cigarette smoking, medications, diagnosed diseases and pregnancy were measured with questionnaires, and smoking was processed as a dichotomous variable (smoking/non-smoking). Subjects smoking regularly daily were regarded as smokers.

Venous blood samples were drawn after an overnight 12 h fast for the determination of serum lipid, adiponectin, insulin and glucose levels. All measurements of lipid levels as well as glucose, insulin and adiponectin levels were performed in duplicate in the same laboratory. To measure levels of serum total cholesterol, triglycerides and high-density lipoprotein cholesterol (HDL-C), standard enzymatic methods were used. The Friedewald formula was used to calculate low-density lipoprotein cholesterol (LDL-C) concentration for participants with triglycerides <4 mmol/L. Details for these methods have been described previously (Juonala *et al*., 2004; Raiko *et al*., 2010). Serum insulin concentration was measured through microparticle enzyme immunoassay (IMx insulin reagent, Abbott Diagnostics, USA) on an IMx instrument, and glucose concentrations were analysed enzymatically (Raiko *et al*., 2010). The homeostasis model assessment (HOMA-IR) index was calculated using the following formula: fasting glucose (mmol/L) × fasting insulin (μU/mL)/22.5. Serum adiponectin concentrations were analysed through radioimmunoassay (Human Adiponectin and Leptin RIA kits, Linco Research, Inc, MO, USA; Saarikoski *et al*., 2010).

Definition of metabolic syndrome, hypertension, hyperglycaemia, hyperinsulinemia and insulin resistance

Metabolic syndrome was defined according to the harmonized criteria, and the definition included the following: waist circumference ≥88 cm in women and ≥102 cm in men; fasting plasma glucose ≥5.6 mmol/L or drug treatment; hypertriglyceridemia ≥1.7 mmol/L or treatment; HDL-C ≤1.3 mmol/L in women and 1.0 in men or drug treatment; and systolic blood

pressure ≥130 mmHg or diastolic blood pressure ≥85 mmHg or antihypertensive drug treatment. A diagnosis required that any three of the five criteria be present (Alberti *et al*., 2009). Hyperinsulinemia was defined as non-diabetic subjects having fasting insulin level in the highest quartile, where 11.06 mU/L was used as the cut-off point (cut-off point of the entire study population of the Young Finns Study, year 2007). Low adiponectin as a risk factor was defined according to the lowest quartile in this study population; the cut-off value was 6.22 μg/mL. High HOMA-IR as a risk factor was defined as the highest quartile in this study population and it was ≥2.44.

Carotid ultrasound imaging

Ultrasound studies were performed by trained sonographers following the standardized protocol described previously (Raitakari *et al*., 2003). Carotid artery imaging was performed using a Sequoia512 ultrasound scanner (Acuson, Mountain View, CA, USA) equipped with a 14 MHz linear array transducer. The ECG signal (modified chest lead 5) was recorded and presented alongside B-mode image sets. The left common carotid artery (CCA) was scanned using a resolution box function to record a 25 mm-wide and 15 mm-high image, including the beginning of the carotid bifurcation and the distal CCA. A 5-s cine loop (25 frames per second) was digitally stored for subsequent offline analysis.

Longitudinal motion

Assessment of CALM was performed in line with recently published practical guidelines (Rizi *et al*., 2020). Carotid artery wall motion analysis was performed using an in-house motion tracking program developed by our research group (Yli-Ollila *et al*., 2013). The software is written in MATLAB (2007b, The MathWorks Inc., Natic, MA, USA) and is capable of reading the graphical ECG-information of the ultrasound recording and simultaneously tracking the longitudinal and radial motions of the arterial wall. The basic method used in the motion tracking was a two-dimensional cross-correlation (block matching) enhanced with a contrast optimization technique to reduce noise from video.

In the longitudinal motion analysis, regions of interest were drawn on the ultrasound image on the intima-media complex, on the adventitial layer and on the surrounding tissue outside the adventitia. The motion tracking of the longitudinal motion was considered suitable for analysis

if the tracking successfully recorded at least two heart cycles, otherwise the motion data were discarded. Details of the method have been described (Yli-Ollila *et al*., 2013; Taivainen *et al*., 2015).

We measured longitudinal motion curves between the intima-media complex and the adventitial layer (IA). The curves of longitudinal motion have been previously shown to vary extensively between individuals (Yli-Ollila *et al*., 2013). We investigated the amplitude of the motion (IAampl), the forward-oriented (IAante) and the backward-oriented (IAretro) component of the motion between the different layers of the CCA wall. Furthermore, we evaluated the main deviation of the longitudinal motion (IAdev) between the arterial layers by computing the average of the motion curve over a cardiac cycle. A schematic figure showing the different measured parameters of carotid artery longitudinal wall motion in two original registrations is presented in Figure 1.

Statistical methods

Distributions of longitudinal motion parameters were only slightly skewed and residuals in models were normally distributed, thus parametric tests were considered acceptable to use. Independent samples *t*-test was used to determine the significance of differences between study groups with and without metabolic syndrome. Linear regression model adjusted for age and sex was used to define conformities between the indices of longitudinal motion and the components of metabolic syndrome, HOMA-IR as well as adiponectin. A multivariate regression model with stepwise method adjusted for age and sex was used to find independent effects of the individual components of metabolic syndrome and longitudinal motion parameters.

Results

Clinical characteristics of subjects with MetS (MetS+) and without (MetS−) are shown in Table 1. Significant differences between these two groups were found in all characteristic measures of MetS, including levels of insulin, glucose and adiponectin as well as in HOMA-IR. For smoking, age and sex, no statistically significant difference was found between the study groups. Differences in the CALM parameters between the MetS+ and MetS− groups are shown in Figure 2. Among the CALM parameters, significant differences between the MetS+ and MetS− groups were found in all examined parameters except IAampl. In subjects belonging to the MetS+ group, IAante was larger ($P < 0.01$) and IAretro was smaller ($P < 0.001$) than in the MetS− group. Individuals without MetS showed negative IAdev, whereas subjects in the MetS+ group had slightly positive values in IAdev. Representative examples of carotid artery longitudinal wall motion in a subject without MetS and with MetS are presented in Figure 1.

Table 2 presents associations (linear regression model adjusted for age and gender) between the CALM parameters and the components of MetS. IAdev showed positive and IAretro negative correlations with hypertension ($P < 0.05$ for both). No significant correlations were found between IAante, IAampl and hypertension. IAante and IAdev exhibited positive correlations with obesity ($P < 0.05$ for both). IAretro showed a negative correlation with obesity ($P < 0.05$). IAampl showed no significant correlation with obesity. No significant associations were found between CALM parameters and hypertriglyceridemia. Dyslipidemia, with low HDL-C a risk factor, showed a positive correlation ($P < 0.01$) with IAante, but there were no statistically significant associations with other CALM parameters. No significant correlations were observed between longitudinal motion parameters and either hyperglycaemia or adiponectin. IAretro and IAampl exhibited negative correlations with hyperinsulinemia ($P < 0.01$ and $P < 0.05$, respectively). IAretro and IAampl showed negative correlations with HOMA-IR ($P < 0.05$ for both).

In the multivariate analysis with a stepwise method, hypertension was associated with IAretro ($\beta = -0.175$, $P < 0.01$) and IAdev ($\beta = 0.145$, $P < 0.05$), but no other significant associations between CALM parameters and hypertension were seen. Low HDL-C was associated with IAante ($\beta = 0.164$, $P < 0.01$) and IAampl ($\beta = 0.126$, $P < 0.05$) but not with other CALM parameters. Hyperinsulinemia exhibited an inverse association with IAretro ($\beta = -0.124$, $P < 0.05$) and IAampl ($\beta = -0.156$, $P < 0.01$) but not with other CALM parameters.

Hyperglycaemia and obesity did not show any significant associations with CALM in these multivariate models.

Discussion

The novel finding of this study is that MetS is associated with alterations in CALM among young (30–45-year-old) adults. We found statistically significant differences between MetS+ and MetS− groups in all examined CALM parameters except IAampl. When studying separately associations of different components of MetS with CALM, statistically significant independent associations were found for hypertension, dyslipidemia and hyperinsulinemia. Hyperglycaemia or low adiponectin did not exhibit any significant associations with CALM parameters. In general, MetS and adverse profile in its components were associated with augmented antegrade and attenuated retrograde motion of intima-media complex in relation to the adventitia layer, which might be markers of unfavourable vascular health (Taivainen *et al*., 2015; Taivainen *et al*., 2018).

The relation between MetS and CALM parameters has not previously been systematically evaluated. However, there have been reports demonstrating significant associations between MetS and arterial stiffness (Li *et al*., 2005; Koskinen *et al*., 2009; Koskinen *et al*., 2010; Gomez-Sanchez *et al*., 2016; Vilmi-Kerälä *et al*., 2017; Topouchian *et al*., 2018). Thus, our finding of altered CALM in subjects with MetS is not surprising. One article reported CALM in type 2 diabetic subjects (Zahnd *et al*., 2011). In older type 2 diabetic subjects, mean amplitudes of CALM were lower than in young, healthy subjects. However, the study groups were not well comparable since, in older diabetic subjects, atherosclerotic process is more pronounced due to arterial ageing compared with a younger reference population. Therefore, it was not possible to detect a possible independent role of diabetes behind altered CALM. The influence of normal ageing process to CALM has been of interest to Cinthio and colleagues, who studied 150 healthy non-obese patients aged 20–76 years and determined that the antegrade-oriented phase of longitudinal motion increased with ageing and was earlier in men than in women (Cinthio *et al*., 2018). An important advantage of the present study is that MetS+ and MetS− groups were comparable with regard to age and sex distributions. Furthermore, statistically significant associations between MetS components and CALM were detected even after adjustment for age and sex.

We have reported previously relationships between CALM and cardiovascular risk factors in a study which is based on the same research population than this (Taivainen *et al*., 2018). In the present study, many of the same risk factors were included as components of MetS. In our previous study, systolic and diastolic blood pressure, BMI, total cholesterol and LDL-C showed significant associations with CALM (Taivainen *et al*., 2018). This is in line with the present study demonstrating changes in CALM to be associated with hypertension, obesity and dyslipidemia. When investigating the different MetS components of the harmonized criteria, the results were parallel to those of our previous study – antegrade longitudinal motion increased and retrograde longitudinal motion decreased with the existence of cardiovascular risk factors and, now, with MetS components (Taivainen *et al*., 2018).

The present study also included assessments of hyperinsulinemia, insulin resistance and low adiponectin, which are closely related to impaired glucose metabolism and therefore enable elaborate evaluation of metabolic disorders related to MetS. Novel findings are that hyperinsulinemia and insulin resistance also showed significant associations with CALM. No significant association was identified between hyperglycaemia and CALM in the present study, but reasonable, insulin-metabolism, and on demand hyperinsulinemia, regulates glucose levels and aim is to maintain euglycemia. Hyperinsulinemia is not included in all MetS criteria although it has been reported to be an important feature of MetS in the literature (e.g. Kassi *et al*., 2011).

Hyperinsulinemia is accepted to be essential in the pathophysiology of MetS, and an overabundance of free fatty acids is acknowledged to be a considerable contributor to the development of hyperinsulinemia (Eckel *et al*., 2005). In glucose metabolism, defects of insulin action contribute to glucose uptake and metabolism in insulin-sensitive tissues such as muscle and adipose tissue, and attenuates the insulin capability to suppress glucose production of the liver (Eckel *et al*., 2005). Furthermore, among some insulin-resistant individuals who secrete enough insulin to maintain near normal or normal glucose tolerance and do not acquire type 2 diabetes compensatory hyperinsulinemia can also act on in a way that predisposes the development of essential hypertension (Reaven, 2011). Some mechanisms behind essential hypertension in individuals with insulin resistance may arise from the fact that not all tissues are equally insulin-resistant; that is, the kidney is not resistant to the influence of insulin, and insulin enhances renal sodium retention in hyperinsulinemic/insulin-resistant individuals (Facchini *et al*., 1999). This could predispose to elevated blood pressure and in long term add risk to vascular changes.

In our study, adiponectin levels were significantly lower and HOMA-IR significantly higher in the MetS+ group compared with the MetS− group. Adiponectin levels decrease with visceral fat accumulation, and adiponectin has been found to protect against hypertension, type 2 diabetes, inflammation and atherosclerotic diseases (Matsuzawa, 2010; Ohashi *et al*., 2011). Among young adults in the Young Finns Study (n = 1693), high adiponectin levels were shown to associate with decreased incidence of MetS (Juonala *et al*., 2011). Despite significant univariate correlations with obesity, hypertension, hyperinsulinemia and insulin resistance, we did not find any significant correlations between adiponectin levels and CALM parameters.

Mechanisms underlying the relationship between MetS and CVD are likely to occur via direct or indirect influences of different components on endothelial function, the deposition of LDL cholesterol (LDL-C) or the recruitment, migration and proliferation of monocytes in smooth muscle cells in the arterial wall (Qiao *et al*., 2007). MetS can promote arterial stiffening through a variety of mechanisms including increased sympathetic activity, enhanced activity of the renin-angiotensin-aldosterone system, increased production of inflammatory cytokines and reactive oxygen species and reduction of nitric oxide availability (Saladini & Palatini, 2018). When evaluating the mechanisms behind the association between MetS and CALM, these same mechanisms should also be taken into consideration.

Our study was undertaken with a large, well-characterized study population comprising 30- to 45-year-old adults. The population consisted of white European subjects, and, for that reason, these results may not be generalizable to other ethnic groups. The size of the study population was large but notably smaller than in many other reports of The Cardiovascular Risk in Young Finns Study. Longitudinal motion analysis of CCA is challenging and requires good frame-to-frame image quality in ultrasound videos. In 2007, the ultrasound imaging protocol was optimized to measure carotid intima-media thickness and distensibility but not to assessments of CALM, and this is the reason for the relatively large number of unsuccessful scans, as described previously (Taivainen *et al*., 2018). However, the final number of study subjects in the study herein is large, and, for the majority, the signal quality of the data was good.

Conclusion

Our findings support the hypothesis that MetS alters CALM. In particular, hypertension, obesity and hyperinsulinemia were associated with reduced total peak-to-peak amplitude,

increased antegrade and reduced retrograde amplitudes, all of which might be markers of unfavourable vascular health.

Conflict of Interest

The authors have no conflict of interest.

Author Contributions

HT, HY, TML and TPL designed the experiment. HT, HY, TML and TPL contributed to the analysis and interpretation of data. HT, HY, TML, TPL, MJ, MK and OTR participated in the elaboration of the manuscript and gave final approval for its submission and publication, being accountable for all aspects of the work herein.

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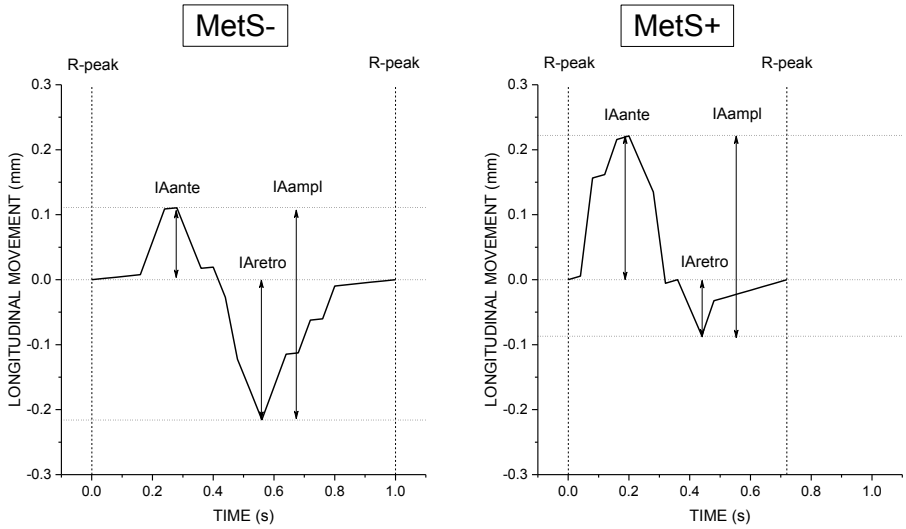

Figure 1. Representative examples of carotid artery longitudinal wall motion in a subject without metabolic syndrome (MetS-) and another with metabolic syndrome (MetS+). Time point 0.0 seconds corresponds to end-diastolic frame (incident with the R-wave on a continuously recorded electrocardiogram). In MetS+ antegrade oriented motion was larger and retrograde oriented was smaller than in MetS-. Abbreviations: IAante = Antegrade amplitude of the longitudinal motion between intima-media and adventitia layers, IAretro = Retrograde amplitude of the longitudinal motion between intima-media and adventitia layers, and IAampl = Peak-to-peak amplitude of the longitudinal motion between intima-media and adventitia layers.

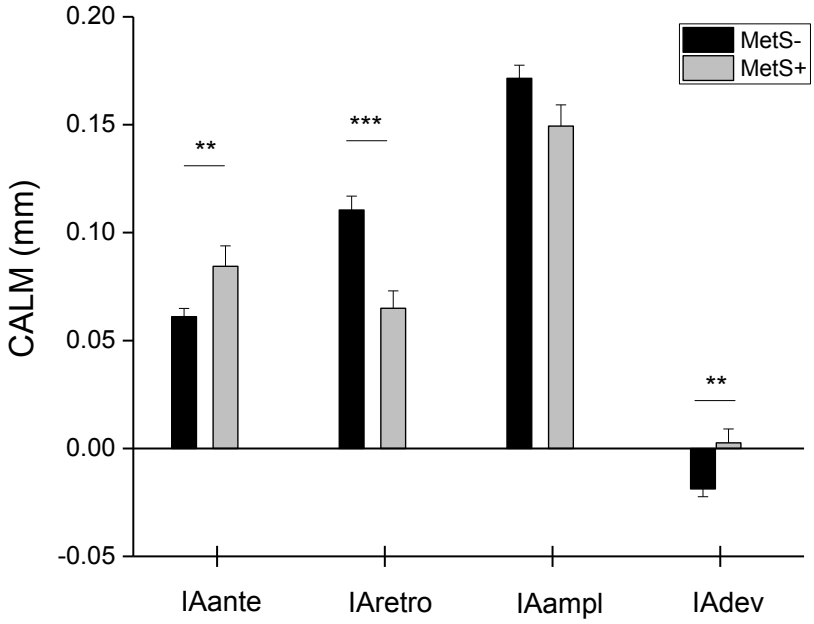

Figure 2. Carotid artery longitudinal motion (CALM) parameters between subjects without (MetS−) and with (MetS+) metabolic syndrome. IAante = Antegrade amplitude of the longitudinal motion between intima-media and adventitia layers, IAretro = Retrograde amplitude of the longitudinal motion between intima-media and adventitia layers, IAampl = Peak-to-peak amplitude of the longitudinal motion between intima-media and adventitia layers, IAdev = Average deviation of the longitudinal motion between intima-media and adventitia layers.

Table 1. Clinical characteristics of subjects without (MetS−) and with (MetS+) metabolic syndrome.

	MetS− (n = 228)	MetS+ (n = 53)
Age (years)	37.9 (4.9)	39.1 (4.5)
Sex (% women)	63.2%	49.1%
Smoking (%)	16.3%	22.6%
Body mass index (kg/m^2)	24.6 (3.7)	30.5 (5.2) ***
Waist circumference (cm)	83.1 (10.2)	101.1 (11.8) ***
Systolic blood pressure (mmHg)	126 (13)	137 (14) ***
Diastolic blood pressure (mmHg)	79 (9)	89 (9) ***
Total cholesterol (mmol/L)	4.95 (0.83)	5.49 (0.93) ***
Low-density lipoprotein cholesterol (mmol/L)	3.04 (0.73)	3.40 (0.81) **
High-density lipoprotein cholesterol (mmol/L)	1.42 (0.30)	1.20 (0.43) ***
Triglycerides (mmol/L)	1.08 (0.44)	2.05 (0.90) ***
Glucose (mmol/L)	5.20 (0.44)	5.75 (0.60) ***
Insulin (mU/L)	6.78 (4.70)	14.26 (7.96) ***
HOMA-IR	1.60 (1.26)	3.68 (2.17) ***
Adiponectin	10.90 (5.75)	7.11 (3.18) ***

Values are mean (SD) / %. Significances: ** = $P < 0.01$, *** = $P < 0.001$. Abbreviations: HOMA-IR = Homeostasis model assessment of insulin resistance.

Table 2. Age- and sex-adjusted relationships between components of metabolic syndrome and longitudinal motion parameters.

	B (SE)	Beta
Hypertension (Blood pressure ≥ 130/85 or medication)		
IAante	0.015 (0.008)	0.119
IAretro	−0.029 (0.012)	−0.156 *
IAampl	−0.015 (0.012)	−0.083
IAdev	0.014 (0.007)	0.131 *
Obesity (Waist circumference ≥ 102 cm in men and ≥ 88 cm in women)		
IAante	0.022 (0.009)	0.149 *
IAretro	−0.031 (0.013)	−0.138 *
IAampl	−0.009 (0.013)	−0.042
IAdev	0.018 (0.008)	0.136 *
Dyslipidemia (Triglycerides ≥ 1.7 mmol/L or medication):		
IAante	0.016 (0.009)	0.102
IAretro	−0.022 (0.014)	−0.093
IAampl	−0.006 (0.013)	−0.027
IAdev	0.006 (0.008)	0.045
Dyslipidemia (HDL-C < 1.00mmol/L in men and < 1.3 in women or medication)		
IAante	0.024 (0.008)	0.177 **
IAretro	−0.006 (0.012)	−0.029
IAampl	0.018 (0.012)	0.092
IAdev	0.014 (0.007)	0.114
Hyperglycemia (Glucose ≥ 5.6 mmol/L or treatment)		
IAante	0.010 (0.009)	0.070
IAretro	−0.018 (0.013)	−0.081
IAampl	−0.008 (0.013)	−0.037
IAdev	0.008 (0.008)	0.060

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Hyperinsulinemia (Insulin ≥ 11.06 mU/L)		
IAante	0.006 (0.009)	0.037
IAretro	−0.036 (0.013)	−0.158 **
IAampl	−0.030 (0.013)	−0.141 *
IAdev	0.014 (0.008)	0.109
Insulin resistance (HOMA-IR ≥ 2.44)		
IAante	0.003 (0.008)	0.024
IAretro	−0.031 (0.013)	−0.145 *
IAampl	−0.028 (0.012)	−0.136 *
IAdev	0.009 (0.007)	0.073
Low adiponectin (≤ 6.22 µg/mL)		
IAante	0.009 (0.009)	0.065
IAretro	0.002 (0.014)	0.007
IAampl	0.011 (0.013)	−0.052
IAdev	−0.003 (0.008)	−0.021

Significances: *P < 0.05, **P < 0.01. Abbreviations, IAante = Antegrade amplitude of the longitudinal motion between intima-media and adventitia layers, IAretro = Retrograde amplitude of the longitudinal motion between intima-media and adventitia layers, IAampl = Peak-to-peak amplitude of the longitudinal motion between intima-media and adventitia layers, and IAdev = Average deviation of the longitudinal motion between intima-media and adventitia layers, HDL-C = high-density lipoprotein cholesterol, HOMA-IR = Homeostasis model assessment of insulin resistance.

Table 3. Multivariate relationships between each component of metabolic syndrome and longitudinal motion parameters adjusted for age and sex.

	Hypertension B ± SE, Beta	Obesity B ± SE, Beta	Low HDL-C B ± SE, Beta	High triglycerides B ± SE, Beta	Hyperglycaemia B ± SE, Beta	Hyperinsulinemia B ± SE, Beta
IAante (mm)			0.02 ± 0.01, 0.164 **			
IAretro (mm)	−0.03 ± 0.01, −0.175 **					−0.03 ± 0.01, −0.124 *
IAampl (mm)			0.03 ± 0.01, 0.126 *			−0.03 ± 0.01, −0.156 **
IAdev (mm)	0.02 ± 0.01, 0.145 *					

Statistical significances: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. Unstandardized coefficients B ± Std.Error, Beta, Sig. Abbreviations, IAante = Antegrade amplitude of the longitudinal motion between intima-media and adventitia layers, IAretro = Retrograde amplitude of the longitudinal motion between intima-media and adventitia layers, IAampl = Peak-to-peak amplitude of the longitudinal motion between intima-media and adventitia layers, and IAdev = Average deviation of the longitudinal motion between intima-media and adventitia layers, HDL-C = high-density lipoprotein cholesterol.

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The longitudinal motion of the common carotid artery is a new promising index of vascular health. Here, a motion tracking analysis software, previously developed in our laboratory, was applied for the first time in clinical research. It was demonstrated that carotid stiffness parameters were associated with the longitudinal motion and the presence of the metabolic syndrome and insulin resistance as well as compensatory hyperinsulinemia caused longitudinal motion disturbances in the carotid artery.

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