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University of Turku

CARDIAC LEFT VENTRICULAR MASS AND ARTERIAL STIFFNESS FROM CHILDHOOD TO EARLY ADULTHOOD – ASSOCIATION WITH CARDIOMETABOLIC RISK FACTORS

The Special Turku Coronary Risk Factor Intervention Project
(STRIP)

Hanna Mikola



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To My Family

ABSTRACT

Hanna Mikola (née Hietalampi). Cardiac left ventricular mass and arterial stiffness from childhood to early adulthood – association with cardiometabolic risk factors. The Special Turku Coronary Risk Factor Intervention Project (STRIP).

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Background: Cardiac left ventricular mass and arterial stiffness are subclinical markers of cardiovascular disease which are associated with cardiovascular disease and clinical events in adulthood.

Aims: The aim of this thesis was to study left ventricular mass and arterial stiffness in childhood and their determinants in terms of cardiometabolic risk factors and ideal cardiovascular health. This thesis also aimed to investigate the effect of a dietary and lifestyle intervention on ideal cardiovascular health and subclinical markers of cardiovascular disease.

Subjects and methods: This thesis is a part of the prospective, randomized Special Turku Coronary Risk Factor Intervention Project (STRIP) that has followed initially over 1,000 children for 27 years. Their cardiometabolic risk factors have been repeatedly measured from childhood to adulthood. Children in the intervention group (n=540) have received dietary and lifestyle counseling aimed at improving the quality of dietary fat. Subclinical markers of atherosclerotic cardiovascular disease were examined using noninvasive ultrasonic measurements of carotid and aortic distensibility, pulse wave velocity, carotid intima-media thickness and cardiac left ventricular mass. This study analyses the data of the children who participated in the arterial ultrasound evaluations from the age of 11 to 19 years (n=420–503), and in the cardiac ultrasound assessments from the age of 15 to 19 years (n= 394–420).

Results: Cardiac left ventricular mass and arterial stiffness increased with increasing age from childhood to early adulthood. In boys, the arterial stiffness increased more than in girls. It was found that current pulse pressure, weight and physical activity along with birth weight were associated with left ventricular mass in adolescence; overall these factors explained nearly 50% of the variation in left ventricular mass. Childhood blood pressure, body mass index, LDL cholesterol concentration and insulin resistance were associated with arterial stiffness. The dietary and lifestyle intervention was associated with ideal cardiovascular health in adolescence, however, it had no effect on left ventricular mass or arterial stiffness.

Conclusions: Marked age-related arterial stiffening is evident already in childhood, particularly in boys. Several cardiometabolic risk factors determine arterial stiffness and cardiac structure already at an early age. Although the dietary and lifestyle intervention promoted ideal cardiovascular health in adolescence, the intervention did not change the subclinical markers of cardiovascular disease at this early age.

Keywords: Childhood, cardiovascular disease, prevention, left ventricular mass, arterial stiffness, longitudinal

TIIVISTELMÄ

Hanna Mikola (o.s. Hietalampi). Sydämen vasemman kammion massa ja valtimoiden jäykistyminen lapsuudesta varhaiseen aikuisuuteen – yhteys kardiometabolisiin riskitekijöihin. Sepelvaltimotaudin Riskitekijöiden Interventioprojekti (STRIP).

Lääketieteellinen tiedekunta, kardiologia ja kardiiovaskulaarilääketiede, Turun kliininen tohtoriorjelmä, Sydäntutkimuskeskus, Turun yliopisto, Turku, Suomi.

Tausta: Sydämen vasemman kammion massa ja valtimoiden jäykistyminen ovat valtimoterveyden varhaismerkkejä, jotka ovat yhteydessä sydän- ja verisuonisairauksien päätapahtumiin aikuisiällä.

Tavoite: Väitöskirjatutkimuksen tavoitteena oli tutkia vasemman kammion massaa ja valtimoiden jäykistymistä lapsuudessa, sekä selvittää niitä määrittäviä tekijöitä kardiometabolisten riskitekijöiden ja ihanteellisen sydänterveyden mittareiden avulla. Lisäksi tutkimuksen tavoitteena oli selvittää ravitsemus- ja elämäntapaneuvonnan yhteyttä ihanteelliseen sydänterveyteen ja valtimoterveyden varhaisiin muutoksiin.

Menetelmät: Väitöskirjatutkimus on osa prospektiivista, satunnaistettua Sepelvaltimotaudin Riskitekijöiden Interventioprojekti (STRIP) -tutkimusta, jossa on seurattu 27 vuoden ajan alun perin yli 1000 lasta. Tutkittavien lasten sydän- ja verisuonisairauksien riskitekijät on mitattu toistuvasti lapsuudesta aikuisuuteen. Ateroskleroottisen sydän- ja verisuonitaudin varhaisia muutoksia tutkittiin mittaamalla ultraäänellä kaulavaltimon ja aortan distensibiliteetti, pulssiaallon etenemisnopeus, kaulavaltimon intima-median paksuus sekä sydämen vasemman kammion massa. Interventoryhmän lapset (n=540) ovat saaneet ravitsemus- ja elämäntapaneuvontaa, jonka päätavoitteena on ollut ruokavalion rasvan laatuun vaikuttaminen. Tässä työssä käytettiin niiden lasten tietoja, jotka osallistuivat valtimoiden ultraäänitutkimukseen 11–19 vuoden iässä (n=420–503) ja sydämen ultraäänitutkimukseen 15–19 vuoden iässä (n=394–420).

Tulokset: Sydämen vasemman kammion massa kasvoi ja valtimot jäykistyivät lapsuudesta varhaiseen aikuisuuteen. Pojilla valtimoiden jäykistyminen oli voimakkaampaa kuin tytöillä. Tutkimuksessa havaittiin, että pulssipaine, paino ja liikunta sekä syntymäpaino olivat yhteydessä vasemman kammion massaan nuoruudessa selittäen lähes 50 % variaatiosta. Lapsuudessa mitattu verenpaine, painoindeksi sekä seerumin LDL-kolesterolipitoisuus ja insuliiniresistenssi olivat yhteydessä valtimoiden jäykistymiseen lapsuudesta varhaiseen aikuisuuteen. Ravitsemus- ja elämäntapaneuvonta oli yhteydessä ihanteelliseen sydänterveyteen nuoruudessa, mutta sillä ei havaittu olevan yhteyttä valtimoiden jäykistymiseen tai vasemman kammion massaan.

Johtopäätökset: Valtimot jäykistyvät iän myötä jo lapsuudessa - erityisesti pojilla. Useat kardiometaboliset riskitekijät ovat yhteydessä valtimoiden jäykistymiseen ja sydämen rakenteeseen jo varhaisella iällä. Ravitsemus- ja elämäntapaneuvonnalla voidaan edistää ihanteellista sydänterveyttä nuoruudessa, vaikkakaan se ei ollut yhteydessä valtimotaudin varhaismerkkeihin näin varhaisella iällä.

Avainsanat: Lapsuus, sydän- ja verisuonitaudit, ennaltaehkäisy, vasemman kammion massa, valtimoiden jäykistyminen, pitkittäistutkimus

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ABBREVIATIONS

AHA	The American Heart Association
Adist	aortic distensibility
Apo A1	apolipoprotein A1
Apo B	apolipoprotein B
BMI	body mass index
BSA	body surface area
CATCH	Child and Adolescent Trial for Cardiovascular Health
DISC	Dietary Intervention Study in Children
Cdist	carotid distensibility
E%	percentage of energy intake
HDL	high-density lipoprotein
HOMA-IR	homeostasis model assessment of insulin resistance
IMT	intima-media thickness
LDL	low-density lipoprotein
LVM	left ventricular mass
MET	metabolic equivalent
PANIC	Physical Activity and Nutrition in Children
PDAY	Pathological Determinants of Atherosclerosis in Youth Study
PCSK9	protein convertase subtilisin kexin 9
PUFA	polyunsaturated fatty acids
PWV	pulse wave velocity
(PUFA+MUFA)/SAFA	polyunsaturated fatty acid + monounsaturated -to-saturated fatty acid ratio
SAFA	saturated fatty acids
SES	socioeconomic status
STRIP	Special Turku Coronary Risk Factor Intervention Project

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications, which are referred to in the text by Roman numerals I-IV.

- I. **Mikola H**, Pahkala K, Rönnemaa T, Viikari JS, Niinikoski H, Jokinen E, Salo P, Simell O, Juonala M, Raitakari OT. Distensibility of the aorta and carotid artery and left ventricular mass from childhood to early adulthood. *Hypertension*. 2015; 65(1):146–152.
- II. **Mikola H**, Pahkala K, Niinikoski H, Rönnemaa T, Viikari JS, Jula A, Juonala M, Raitakari OT. Cardiometabolic determinants of carotid and aortic distensibility from childhood to early adulthood. *Hypertension*. 2017; 70(2): 452–460.
- III. **Hietalampi H**, Pahkala K, Jokinen E, Rönnemaa T, Viikari JS, Niinikoski H, Heinonen OJ, Salo P, Simell O, Raitakari OT. Left ventricular mass and geometry in adolescence: early childhood determinants. *Hypertension*. 2012; 60(5): 1266–1272.
- IV. Pahkala K, **Hietalampi H**, Laitinen TT, Viikari JS, Rönnemaa T, Niinikoski H, Lagström H, Talvia S, Jula A, Heinonen OJ, Juonala M, Simell O, Raitakari OT. Ideal cardiovascular health in adolescence: effect of lifestyle intervention and association with vascular intima-media thickness and elasticity (the Special Turku Coronary Risk Factor Intervention Project for Children [STRIP] study). *Circulation*. 2013; 127(21): 2088–2096.

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1 INTRODUCTION

Atherosclerosis is a chronic inflammatory disease characterized by the slow development of lipid accumulates and plaques, and stiffening, of artery walls. (Ross 1999) The atherosclerotic process begins early in life although atherosclerotic cardiovascular disease usually manifests as coronary heart disease, cerebrovascular complications and peripheral arterial disease several decades later in adulthood.(Berenson et al. 1998; Barker 1995; McGill et al. 2000a) Subclinical markers of cardiovascular disease provide a window to study the early pathophysiological changes occurring in the arteries which are relevant to the development of atherosclerosis.(Oliver and Webb 2003; Salonen and Salonen 1991; Davis et al. 2001; Urbina et al. 2009)

The first findings of early atherosclerotic process in arterial wall were recognized in autopsy studies of young German men killed in World War I.(Mönckeberg 1915) Later observations from the soldiers dying in World War II, as well as the Korean and Vietnam Wars strengthened these findings.(Enos et al. 1986; McNamara et al. 1971) Long term cardiovascular studies have been conducted in children and young adults, e.g. the Bogalusa Heart Study and the Pathological Determinants of Atherosclerosis in Youth Study, have demonstrated subclinical atherosclerosis to be evident in early childhood.(Berenson et al. 1998; Wissler 1991) Moreover, an association between childhood risk factors and the development of atherosclerosis has been detected in several studies.(Van Horn et al. 2005; Åkerblom et al. 1985; Simell et al. 2009; Golding et al. 2001) For example, risk factors identified in childhood have been associated with subclinical markers of cardiovascular disease later in adulthood.(Raitakari et al. 2003; Li et al. 2004a; Li et al. 2004b; Juonala et al. 2005)

In Finland, in the 1960s, cardiovascular disease morbidity was highest in the world, particularly in men. At the same time, the main risk factors had been identified in large epidemiological studies, such as the Seven Countries Study and the Framingham Heart Study. (Keys et al., 1986; Kannel et al., 1964) Concomitantly, preventive efforts and studies started to develop gradually. In 1972, the North Karelia Project began in Finland.(Puska, 1973) This comprehensive community-based intervention aimed to achieve population-wide changes in dietary and smoking habits so as to eventually shift the population distribution of cardiovascular risk factors. The North Karelia Project was followed by a remarkable reduction in coronary heart disease risk and mortality: from the 1960s to 2012, the mortality from coronary heart disease in Finland decreased among working-age men and women by over 80%.(Jousilahti et al. 2016) The decrease in mortality has been estimated to be explained mainly by reductions in smoking prevalence, blood pressure and blood lipid levels due to the favorable dietary and lifestyle changes.(Jousilahti et al. 2016; Laatikainen et al. 2005) In addition, improved treatment

methods during that time period have been estimated to account for approximately a quarter of the decrease.

Despite improved prevention and treatment, cardiovascular disease still remains the leading cause of disability and death all around the world.(The Global Burden of Disease Study 2016 Causes of Death Collaborators, 2017; Murray et al. 2012) Nowadays, there are trials which have supported the benefits of adopting an early prevention strategy with interventions starting early in childhood, even as early as infancy or prenatally. (Hanson and Gluckman 2014; Lloyd-Jones et al. 2006) It is claimed that these kinds of strategies can be effective in preventing cardiovascular disease over the life course, set individuals on the best possible trajectories of lifelong cardiovascular health and reduce health care costs.(Allen et al., 2017; Hanson and Gluckman, 2014)

2 REVIEW OF THE LITERATURE

The left ventricle of the heart and the arterial tree are the main components of the human cardiovascular system that maintain the systemic circulation. With every heartbeat, the left ventricle ejects blood into the aorta, which is the main artery of the human body. Conduit arteries, such as the aorta and carotid artery, function as a physiological buffer during cardiac contraction due to their distensible walls. (Nichols and O'Rourke 2005) This cushioning function is described as arterial distensibility; its reciprocal is designated as arterial stiffness.(O'Rourke 2007) During the pulsations generated by the pumping heart, the kinetic energy is stored as motion in the conduit artery walls maintaining tissue perfusion during diastole. (Safar and O'Rourke 2006) During systolic contraction, 40% of stroke volume is transferred directly to peripheral tissues, whereas the rest of the stroke volume distends the conduit arteries. (Nichols and O'Rourke 2005) The compliant conduit arteries in young individuals reduce the afterload and ventricular stroke work and this optimizes arterial-ventricular coupling, ensuring a steady and continuous peripheral blood flow throughout the whole cardiac cycle.(London 1998) Left ventricular mass (LVM) reflects cardiac adaptation to the workload of the heart.

2.1 Subclinical markers of cardiovascular disease in childhood

The cardiovascular system undergoes morphological and functional changes throughout the lifespan.(Lakatta et al. 1987) Although it is recognized that atherosclerosis starts early in life, even in utero, rather little is known about the development and adaptation of cardiovascular system during childhood.(Barker, 1995; Berenson et al. 1998) It has been demonstrated that various cardiometabolic risk markers alter the development of arterial and cardiac structure in children who are obese or have familial hypercholesterolemia.(Aggoun et al. 2000; Tounian et al. 2001; Chinali et al. 2006) Arterial stiffness, intima-media thickness and left ventricular hypertrophy have been implicated as potential markers of cardiovascular risk in young adults and children.(Koskinen et al. 2012; Urbina et al. 1995; Chinali et al. 2006; Li et al. 2003)

To study early development of cardiovascular disease a better understanding of interrelations between cardiometabolic risk factors and subclinical markers of cardiovascular disease in healthy children is required. Therefore, this thesis specifically focuses on arterial stiffness, intima-media thickness and LVM in children and adolescents.

2.1.1 Arterial stiffness

Arterial stiffness is a dynamic property of the artery that is dependent on arterial structure, function and arterial pressure.(Urbina et al. 2009) Local arterial stiffness, arterial distensibility, is a measure of the ability of the arteries to expand (i.e. compliance) as a response to the pressure alterations generated by the heart. The distensibility of conduit arteries mainly results from the elastin to collagen ratio in their walls, and is dependent on the distending pressure.(Avolio et al. 1998) As the distending pressure increases, there is a greater recruitment of relatively inelastic collagen fibers and consequently, a reduction in distensibility (Oliver and Webb 2003). Thus, the relationship between distensibility and blood pressure is partly physiological. Arterial stiffening develops from the complex interaction between stable and dynamic changes involving structural and cellular elements of the arterial wall (Zieman et al. 2005). Local arterial stiffness reflects the manner in which pressure, blood flow, and arterial diameter change during the cardiac cycle.

The artery walls comprise three major layers: the tunica intima, tunica media, and tunica adventitia.(Kielty et al. 2007) The media consists of contractile smooth muscle cells, elastic fibers, collagen and proteoglycans. The elastic fiber and smooth muscle cell lamellae confer elastic recoil and contractility on artery walls. The number of elastic lamellae varies along the arterial tree. (Chirinos 2012) The elastin to collagen ratio decreases in artery walls from the aorta towards peripheral arteries, leading to increasing arterial stiffness from the central towards the distal arteries.(Latham et al. 1985) The aorta and distensible conducting arteries have between 40 and 70 elastic lamellae; they expand in response to systolic blood pressure and have a passive elastic recoil that allows for the maintenance of pressure during diastole. Coronary, peripheral and other muscular arteries have fewer (3–40) elastic lamellar layers. Collagen is much stiffer than elastin; at low strain levels, elastin is the factor dominating the composite behavior, while at high strain levels, collagen becomes increasingly important. In addition to the passive mechanical properties of artery wall structures, arterial stiffness can be modulated by functional components related to cellular processes in the arterial wall.(Townsend et al. 2015) Over-activation of different matrix metalloproteases decreases the elastin to collagen ratio.(Zieman et al. 2005) Calcification and accumulation of advanced glycation end-products are important determinants of arterial stiffness. The endothelial cells initiate the remodeling of arteries by monitoring the blood flow and pressure inside the artery lumen and transmitting this signal further to the smooth muscle cells and fibroblast cells. In addition, the sympathetic nervous system and endothelium-derived nitric oxide control changes in smooth muscle tone, and are able to actively modulate the stiffness of the arterial wall, even in large arteries such as the aorta.(Failla et al. 1999)

The elastic fibers of the artery wall allow stretch and expand upon the movement of a pulse through the lumen. Branching points of the arterial tree, such as aortic bifurcation

and branches of renal arteries, are areas where arterial stiffness changes (from an elastic artery to a muscular artery), and higher resistance arteries can give rise to a wave reflection.(Nichols and O'Rourke 2005) This heterogeneity in the arterial stiffness has important physiological and pathophysiological consequences. A pressure wave, which propagates rapidly along a viscoelastic tube with numerous branches is progressively amplified, from central to distal conduit arteries due to the wave reflections. Particularly, in peripheral arteries, wave reflections can amplify the pressure wave because the reflection locations are closer to peripheral sites than to central arteries, and pulse wave velocity (PWV) is higher in a peripheral stiffer artery. The net result is that the amplitude of the pressure wave is higher in peripheral arteries than in central arteries, the so-called 'amplification phenomenon'. In healthy young adults, the reflected wave normally returns to the central aorta in diastole and is able to boost coronary flow without adding to the systolic load.(Laurent et al. 2006)

Conduit arteries stiffen with age.(Safar and O'Rourke 2006) Arterial stiffening with age, arteriosclerosis, is partly considered as a physiological change, related to the loss of elastic fibers in the arterial wall caused by continuous cycles of expansion and contractions.(O'Rourke and Hashimoto 2007; Greenwald 2007) The changes are most marked in the aorta, and its major proximal branches, and are less extensive in the peripheral muscular arteries (Avolio et al. 1983) However, it has been estimated that it takes 30 years until elastin breakdown becomes relevant.(O'Rourke 2007) Regardless of the physiological age-related changes at older ages, studies have reported arterial stiffening with age already in children and adolescents.(Hauser et al., 2013; Doyon et al. 2013) In cross-sectional studies examining 2- to 18-year-old children, a systematic decrease in aortic distensibility was found, beginning from the age of 2 years.(Hauser et al. 2013; Sarkola et al. 2012) In contrast, a cross-sectional study demonstrated that the effect of age on aortic distensibility was not linear; distensibility increased from birth and was greatest at about the age of ten years, thereafter decreasing with advancing age, with distensibility equal to that present at birth being usually present at the age of 18 years.(Laogun and Gosling 1982) With regard to the carotid artery, a decrease in its distensibility has been reported to begin from the age of 6 years.(Doyon et al. 2013; Jourdan et al. 2005; Sarkola et al. 2012)

Boys may have greater arterial stiffness than girls already in childhood. A cross-sectional study in young adults, aged 18 years and older, demonstrated lower brachial artery distensibility in men than women.(Urbina et al. 2002) In addition, cross-sectional trials have revealed that aortic distensibility was similar in both sexes from birth to 14-15 years of age but thereafter boys had lower distensibility and this phenomenon extended until they were in their late forties.(Laogun and Gosling 1982) Another cross-sectional study conducted in 6 to 18 year old subjects detected a sex-difference in carotid distensibility starting from the age of 15 years.(Doyon et al. 2013)

Somatic growth during childhood increases the aortic lumen diameter. (Sarkola et al. 2012) A larger diameter reduces impedance to pulsatile flow and helps maintain pulse pressure in a physiological range but also amplifies the mean and pulsatile tensions impinging on the aortic wall. Increased wall tension leads to increased wall stress that may increase arterial stiffness through elastin fragmentation followed by deposition of stiffer matrix components. (Lakatta et al. 2009; Zachariah et al. 2014)

Arteriosclerosis is considered as a diffuse hardening or stiffening of the artery wall involving adventitia and media; it is dilatatory in nature, and it can lead to increased left ventricular afterload and stroke work resulting in inefficient ventriculo-arterial coupling. Atherosclerosis is viewed as a multifocal and occlusive arterial wall thickening resulting from luminal atheroma and plaque formation, starting in the intima, with its ischaemic consequences clinically seen in coronary, carotid, renal, and lower limb arteries. Because arteriosclerosis may activate a number of mechanisms involved also in atherogenesis, it has been postulated that arterial stiffening in childhood may increase the risk for future cardiovascular disease. (Palombo and Kozakova 2015)

Arterial stiffness is related to traditional cardiovascular disease risk factors; it predicts cardiovascular events in adults and has been implicated as a potential marker of subclinical atherosclerosis in apparently healthy persons. (Lehmann 1993; Koskinen et al. 2012; Haluska et al. 2010) The pioneering Bogalusa Heart Study revealed that carotid artery stiffness was greater in adolescents with a parental history of myocardial infarction or diabetes. (Riley et al. 1986) Children with elevated total and low-density lipoprotein (LDL) cholesterol concentrations, obesity, elevated leptin levels, increased blood pressure, obesity-related hyperinsulinemia, type 1 diabetes mellitus, and exposure to tobacco smoke have been reported to have increased arterial stiffness. (Kallio et al. 2009; Urbina et al. 2009) A favorable association of fitness with arterial distensibility has been found in adolescents. (Pahkala et al. 2013) Childhood cardiovascular disease risk factors, such as elevated blood pressure, are suggested to predict arterial stiffness in adulthood. (Juonala et al. 2005)

In stiffer arteries, the pulse wave reflections return earlier to the heart; as a consequence, aortic systolic pressure rises, diastolic pressure decreases, and pulsations of flow extend further into smaller arteries of organs. The elevated pulse pressure increases left ventricular afterload, myocardial oxygen demand and reduces coronary perfusion in diastole, imposing an increased stress on small arteries, particularly those in brain and kidney. These changes may lead to left ventricular hypertrophy and evoke damage in fragile microvessels (principally in brain and kidney) leading to micro-infarcts and micro-hemorrhages, with damage to these specialized cells e.g. causing a cognitive decline or renal failure. (O'Rourke and Hashimoto 2007; Lakatta and Levy 2003)

Assessment of arterial stiffness

Arterial stiffness can be evaluated at the systemic, regional and local levels. (Townsend et al. 2015) Measurements of arterial stiffness can be estimated with tonometry, oscillometry, ultrasonography or with MRI-based approaches.(Pereira et al. 2015) The PWV between the carotid and femoral artery is described as the 'gold standard' measurement of arterial stiffness.(Laurent et al. 2006) It is typically measured with a pressure transducer or with applanation tonometry, but also ultrasound and impedance can be used. The central pressure waveform can be estimated from the radial pressure pulse.(Millasseau et al. 2003) Cardiac magnetic resonance imaging represents a novel way of assessing arterial stiffness. A magnetic resonance imaging device is used to obtain images from the ascending aorta, the proximal aorta, and the distal descending aorta. Arterial distensibility is then calculated by dividing the difference of the aortic area at the end of the systole and diastole by the product of aortic area at the end of diastole and brachial pulse pressure.(Nelson et al. 2009) Local arterial stiffness can also be measured in the aorta and common carotid artery by ultrasound. The change in artery diameter during the cardiac cycle is measured with ultrasound and related to pulse pressure achieved with brachial blood pressure measurements.(Koivistoinen et al. 2012)

The aorta is of particular interest since postmortem analyses have indicated that atherosclerotic alterations are first seen in the abdominal aorta, and furthermore the aorta makes the largest contribution to the arterial buffering function.(McGill et al. 2000b) An assessment of local carotid stiffness may also provide important prognostic information as the carotid artery is a frequent site of atheroma formation.(Safar and O'Rourke 2006)

This thesis specifically focuses on arterial distensibility, which is defined as the change in arterial diameter during the cardiac cycle divided by the pulse pressure (Figure 1). Lower distensibility signifies increased arterial stiffness and thus is interpreted as an elevated cardiovascular risk.(Oliver and Webb 2003) Distensibility measures the ability of arteries to expand (i.e. compliance) in response to the pulse pressure caused by cardiac contraction and relaxation.

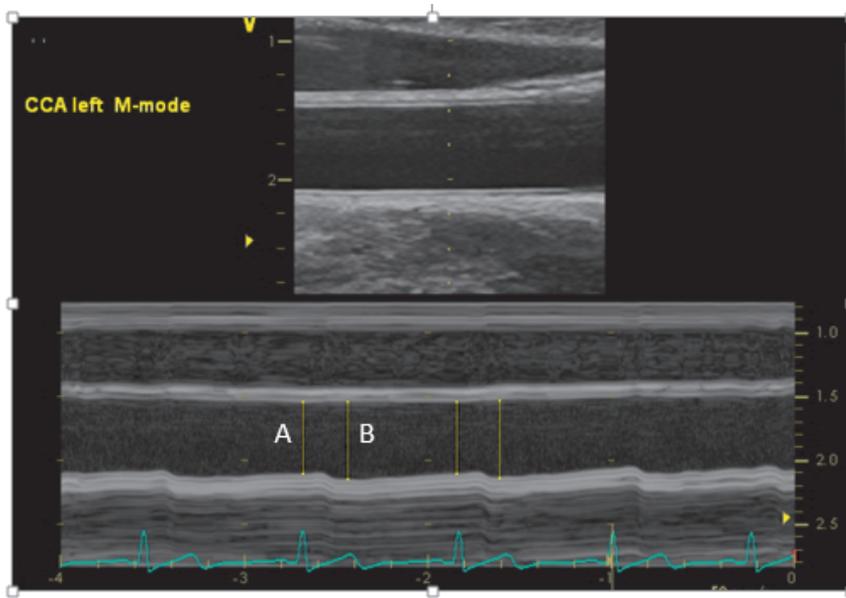


Figure 1. Ultrasound image obtained during measurement of carotid distensibility. The change in arterial diameter during diastole (A) and systole (B) related to pulse pressure is used to describe arterial distensibility.

2.1.2 Arterial intima-media thickness

Arterial intima-media thickness (IMT) reflects arterial structure and morphological alterations of the arterial tree. The first signs of atherosclerosis include lipid deposits, resulting in the appearance of fatty streaks in the intima of systemic arteries. Advanced atherosclerotic lesions arise from these fatty streaks, and their progression during childhood and adolescence is accelerated in the presence of cardiovascular risk factors.(McGill and McMahan, 1998; Berenson et al., 1998)

Arterial IMT can be measured at various sites in the arterial tree. In many studies, the carotid artery has been the target because it is located superficially on the neck and can be readily visualized by ultrasound. For example, an assessment of IMT has also been used extensively in children and young adults with known risk factors for cardiovascular disease in studies investigating early, subclinical disease.(Urbina et al. 2009) It has been proposed that measurement of carotid IMT should be added to cardiovascular risk factors since this could improve the individual risk assessment.(Lorenz et al. 2007)

Physiological aging is the main determinant of carotid IMT and it increases throughout an individual's lifetime.(Engelen et al. 2013; Doyon et al. 2013; Sarkola et al. 2012) In children, systolic blood pressure, as well as pulse pressure, is related to carotid

IMT.(Doyon et al. 2013) Carotid IMT has been shown to be elevated in children with familial hypercholesterolemia, hypertension, obesity, exposure to tobacco smoke, type 1 diabetes, and metabolic syndrome. (Aggoun et al. 2000; Urbina et al. 2009; Kallio et al. 2010) Longitudinal studies have revealed that the presence of cardiovascular risk factors in childhood is associated with increased carotid IMT in young adults.(Li et al., 2003; Raitakari et al., 2003; Davis et al., 2001; Laitinen et al., 2012) A linear increasing relationship has been demonstrated between obesity in childhood and carotid IMT in young adults.(Oren et al. 2003; Freedman et al. 2004) One study investigating the impact of diet and exercise in obese children detected a significant decrease in carotid IMT within one year of the intervention.(Woo et al. 2004)

Although carotid artery IMT is typically investigated, autopsy studies have suggested that the first atherosclerotic lesions actually begin to develop in the abdominal aorta.(McGill et al. 2000b) A few pediatric studies have measured IMT in the aorta. Furthermore, this technique has proved useful even in neonates and young children.(Urbina et al. 2009) With abdominal ultrasound, elevated aortic IMT has been associated with low birth weight, intrauterine growth restriction, exposure to tobacco smoke, familial hypercholesterolemia, and seropositivity to Chlamydia pneumoniae. (Koklu et al. 2007; Skilton et al. 2005; Gunes et al. 2007; Järvisalo et al. 2001; Volanen et al. 2006; Kallio et al. 2010) Physical activity has been favorably associated with aortic IMT in adolescents.(Pahkala et al. 2011)

The Muscatine offspring study examined the relationships between cardiovascular risk factors and both aortic IMT and carotid IMT. Although cardiovascular risk factors were associated with both aortic and carotid IMT, it appeared that the strength of the associations was greater for aortic IMT than for carotid IMT in those <18 years of age, suggesting that measurement of aortic IMT may allow detection of the atherosclerotic process at an earlier age than is possible with carotid IMT.(Dawson et al. 2009)

Assessment of intima-media thickness

Arterial IMT can be measured relatively simply and noninvasively with ultrasonography.(de Groot et al., 2004) The measurement of the arterial IMT by ultrasonography was first described by Pignoli.(Pignoli et al. 1986) Since then, the technique has been widely used for the assessment of arterial wall thickness *in vivo*.(Pignoli et al 1986)

The assessment of the arterial IMT with conventional B-mode high resolution ultrasound is based on the recognition of the double line pattern in the image and involves a simple distance measurement between the leading edges of the far wall lumen-intima and media-adventitia ultrasound interfaces (Figure 2). The ultrasound system should be operated

with a linear broadband transducer of above 7 MHz.(Dalla Pozza et al. 2015) In smaller children, especially for neonatal arteries, it is recommended that special transducers with higher frequencies should be used.(Sundholm et al. 2015)

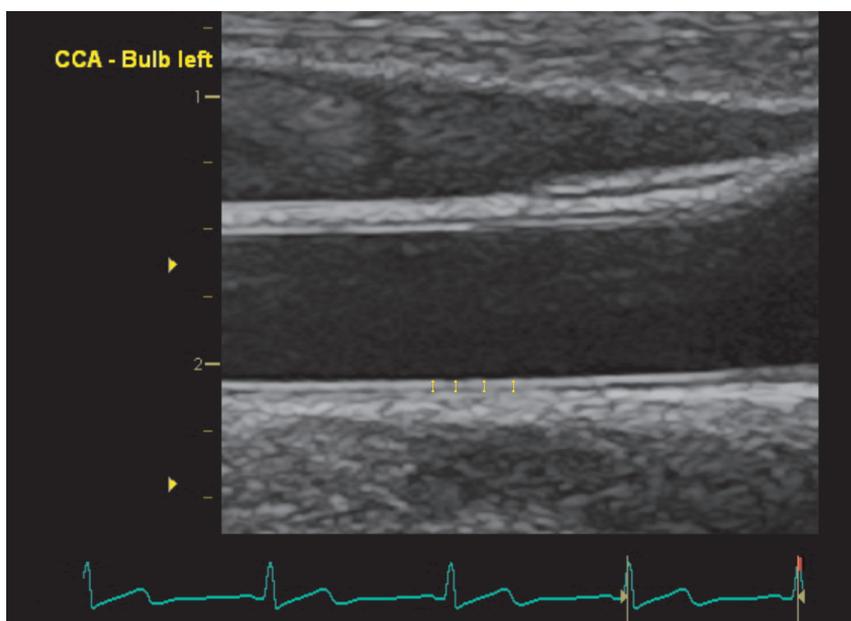


Figure 2. Ultrasound image of assessment of carotid intima-media thickness. The image is taken proximal to the carotid bifurcation.

2.1.3 *Left ventricular mass*

Left ventricular hypertrophy is associated with increased morbidity and mortality in both adults and children.(Daniels et al. 1998; Levy et al. 1990; Mitsnefes et al. 2003; Chinali et al. 2006) During childhood, cardiac growth is closely associated with somatic growth.(Lindstedt and Schaeffer 2002; Urbina et al. 1995) The other factors that influence cardiac structures in childhood are i.e. age, genetics, sex, pubertal stage, ethnicity, physical activity, body composition, basal metabolic rate, blood pressure, diabetes and exposure to tobacco smoke.(Gidding et al. 2013; Malcolm et al. 1993; Bella and Göring 2012; McClean et al. 2017)

An increase in LVM, as related to cardiac remodeling, can be a consequence of either an adaptive or a maladaptive process.(Cohn et al., 2000) Since LVM is related to body growth, the indexing of LVM allows a better comparison of subjects with different body sizes (de Simone et al. 1992; Chinali et al. 2016). While LVM is strongly determined by lean body mass (Foster et al. 2016.), lean body mass is not easily measured in the clinical

setting. Therefore, other measures, including height, weight, and BSA, have been used as surrogates for lean body mass. (Khoury et al 2009.)

In early infancy, LVM is mainly determined by the number of cardiomyocytes (hyperplasia).(Zak 1974) After the age of 1 year, cardiomyocytes have reached their final number and changes in LVM reflect the growth of myocytes (hypertrophy) as a response to increased pressure or volume load.(Kehat and Molkenin 2010) The physiological growth of the left ventricle (eutrophy) related to a child's somatic growth is mediated through growth factor signaling (e.g. growth hormone, insulin-like growth factor-1) while pathological cardiac remodeling is stimulated by neurohormones (e.g. atrial natriuretic peptide and renin-angiotensin-aldosterone) and involves fibrosis of cardiac tissue.(Dorn and Force 2005) Furthermore, activation of inflammatory pathways, and hemostatic and fibrinolytic mechanisms have been described in left ventricular remodeling.(Conen et al. 2006; Velagaleti et al. 2008) In obese adolescents, left ventricular growth is thought to be a compensatory response to an increased cardiac workload but also responses to non-hemodynamic metabolic factors (e.g. insulin levels) seem to play a significant role.(Chinali et al. 2006) In healthy children, birth weight and early growth are associated with LVM between the ages of 2 and 9 years.(de Jonge et al. 2011; Jiang et al. 2006; Geelhoed et al. 2009) Early cardiac programming due to placental insufficiency, sustained hypoxia, nutrition, and cardiac pressure/volume overload during fetal growth may partially explain the mechanisms behind cardiac adaptation related to birth weight.(Sarvari et al. 2017) Nonetheless, it is not inevitable that these changes would necessarily increase an individual's long-term cardiovascular risk.(Arnott et al. 2015)

In adults, it has been hypothesized that there are causative links between pulse pressure, arterial stiffness and LVM. With advancing age, pulse pressure increases due to arterial stiffening. The elevated pulse pressure increases the left ventricular afterload and thus also the myocardial oxygen demand as well as reducing coronary perfusion.(Vlachopoulos et al. 2006) These changes may trigger left ventricular hypertrophy.(Boutouyrie et al. 1995) Left ventricular hypertrophy at the levels associated with excess mortality in adults has been detected in children with severe obesity and impaired glucose tolerance.(Gidding et al. 2004)

Measurements of left ventricular size and function are essential in the assessment of patients with congenital and acquired heart diseases.(Lopez et al. 2010) Changes in left ventricular geometry are common in various heart diseases. This thesis focused on LVM and left ventricular geometry in healthy children.

Assessment of left ventricular mass

Echocardiography is a well-documented imaging modality used to assess LVM. In hypertensive children, echocardiographic measurement of the left ventricle has been recognized as a technique capable of detecting a left ventricular target organ injury. (Flynn et al. 2017) Ultrasonically assessed transthoracic echocardiography is the preferred method for cardiac assessment in children, because it is a noninvasive way of obtaining detailed anatomic, hemodynamic, and physiologic information about the pediatric heart. (Armstrong et al. 2012; Lopez et al., 2010) Other methods include cardiac magnetic resonance and transesophageal echocardiography. In the echocardiographic measurement of cardiac structures, the relationship between heart and body size needs to be taken into account. (de Simone et al. 1992; Chinali et al. 2016) Commonly, LVM is indexed to lean body mass, height or BSA, although, the best method for indexing LVM in children is still an area of active investigation. (Chinali et al. 2016; Foster 2016)

It has been recommended that the measurement of left ventricular dimensions should be performed at the end of diastole by using M-mode or 2D imaging (Figure 3). (Lai et al. 2006; Lang et al. 2015) The parasternal short axis at the midpapillary level is the preferred site for measurement of the left ventricular chamber size and wall thickness, but the subcostal short axis can also be exploited. The ventricular septum, left ventricular dimension, and left ventricular posterior wall can be used to determine left ventricular size and to exclude hypertrophy.

LVM, the estimated weight of the ventricular muscle, can be assessed by several techniques. LVM calculated from the M-mode has been applied extensively in adult clinical trials and epidemiologic studies. (Lopez et al. 2010) It has also been used in children, though accuracy and reproducibility data, especially in infants, are scant. LVM is calculated by subtracting the endocardial volume from the epicardial volume and multiplying this difference (the myocardial volume) by the myocardial-specific density (approximately 1.05 g/mL). (Lopez et al. 2010; Lang et al. 2015)

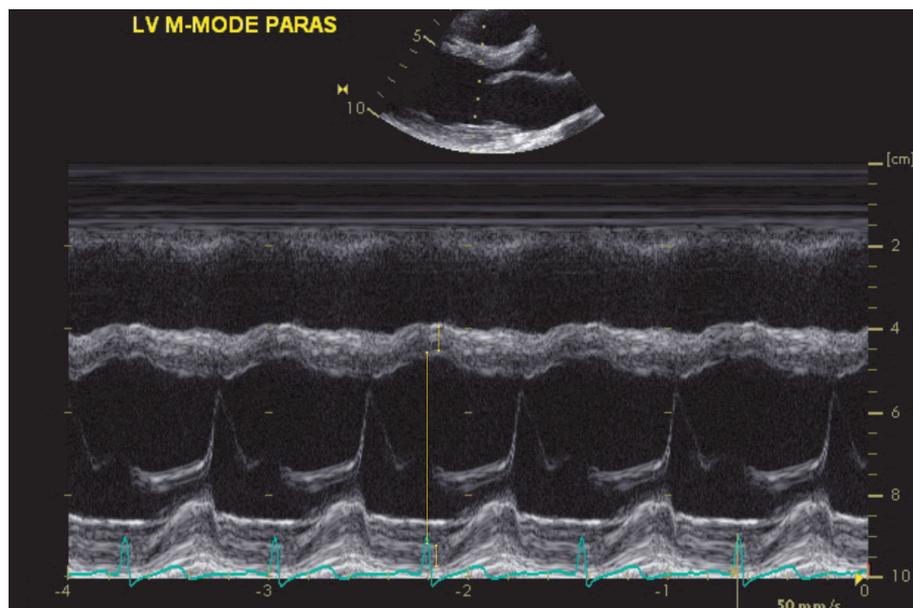


Figure 3. M-mode ultrasound image obtained during measurement of cardiac left ventricular mass.

2.2 Determinants of cardiovascular disease in childhood

Cardiovascular disease begins in childhood, even as early as during fetal life, and it is related to the presence and intensity of risk factors that are both genetic and environmental in origin. Typically the risk factors occur together, i.e. they cluster. Clinical events such as myocardial infarction, stroke and peripheral arterial disease are the culmination of the lifelong process of atherosclerosis.

2.2.1 Birth size and early growth

Some of the first human studies pointing to an association between the intrauterine environment, early life, and later cardiovascular health outcomes were published forty years ago.(Forsdahl 1977) The epidemiologist David Barker was one of the earliest proponents of the theory of fetal origins of adult disease, prompting the theory to be denoted as ‘Barker's hypothesis’. In 1986, Barker published findings proposing a direct link between prenatal nutrition and late-onset coronary heart disease.(Barker and Osmond 1986) Since the publication of Barker's initial findings, other researchers have detected several associations between early growth and cardiovascular risk factors.(Gillman 2005; Barker et al. 2005; Singhal 2010) The associations are thought to be consequences of

developmental plasticity, the phenomenon by which one genotype can give rise to a range of different physiological or morphological states in response to different environmental conditions during development.

Birth size reflects intrauterine growth and development – a small birth size often is a reflection of a suboptimal intrauterine environment. However, in contrast, intrauterine challenges are not always associated with reduced fetal growth and low birth weight. There is epidemiological data indicating that the relationship between birth weight and later disease risk is U-shaped; a high birth weight is also associated with a greater disease risk.(Hanson and Gluckman 2015)

In addition to nutritional factors, fetal development is affected by a number of other circumstances, including maternal hormonal and metabolic factors, placental function, and external environmental exposures (e.g. maternal smoking).(Fowden et al. 2006) Animal studies have also demonstrated that the timing, duration, and the exact nature of the insult during pregnancy are important in determining the specific physiological phenotype. Epigenetic modification of non-imprinted genes, affecting gene expression and evoking phenotypic changes, is thought to be a key mechanism underlying the intrauterine environment's influence on the developing fetus.(Gluckman and Hanson 2004) Fetal adaptive responses to the intrauterine environment take place during plastic periods of development. Fetal adaptations, as a response to environmental cues, develop in order to correspond to the predicted postnatal environment. These adaptations can have immediate beneficial effects and promote survival in the short term but can also lead to increased disease susceptibility in the long term.(Gluckman and Hanson 2004) A low birth weight has been associated with postnatal hypertension, glucose intolerance, and alterations in the functioning of a number of endocrine axes, including the pancreatic islets, the renin-angiotensin system, and the hypothalamic pituitary- adrenal axis.(Fowden et al. 2005)

Recent studies have described normal or increased IMT in adolescents and young adults with fetal growth restriction.(Skilton et al. 2015; Skilton et al. 2011) Intrauterine growth restriction has been associated with a narrowing of the diameter of central arteries in adolescence, but results related to arterial stiffness are controversial.(Brodzki et al. 2005; Gardiner et al. 2001; Veille and Sivakoff, 1989) An abnormal postnatal growth, characterized by excessive weight gain, has been associated with increased arterial IMT and increased arterial stiffness.(Evelein et al. 2013) Conversely, a low birth weight has been associated with changes in cardiac shape in childhood and pre-adolescence.(Crispi et al. 2010; Sarvari et al. 2017) An increased birth weight has been associated with ventricular hypertrophy in infants of mothers with diabetic mellitus.(Ullmo et al. 2007) Furthermore, early growth has been linked with LVM in childhood.(de Jonge et al. 2011; Toemen et al. 2016) The mechanisms that combine birth weight and early growth with subclinical markers of cardiovascular disease in later life are largely unknown. Recent

data emerging from the Cardiovascular Risk in Young Finns Study indicated that the impaired fetal growth was associated with subtle increases the cardiac structure in adults, which were less marked than those that have been described in childhood.(Arnott et al. 2015)

2.2.2 Body weight

Overweight and obesity is fundamentally the result of a long-term positive energy balance. Today, obesity among children, adolescents and adults has emerged as one of the most serious public health issues. The global prevalence of childhood obesity has increased dramatically over the past four decades. In 2013, 23.8% of boys and 22.6% of girls in the developed countries were overweight or obese; in the developing countries, the corresponding rates were 12.9% and 13.4%, respectively.(Ng et al., 2014) In Finland, in children aged 2–18 years, 12% of girls and 22% of boys have been reported to be overweight, and 2 % of girls and 4 % of boys are estimated to be obese. (Saari et al. 2011) Recently, the rising trends children's BMI values have plateaued in many high-income countries, albeit at high levels. (NCD Risk Factor Collaboration 2017)

Several methods and criteria are used to define overweight and obesity in adolescents.(Saari et al. 2011; Cole et al. 2000) In children, the current guidelines recommend assessment of BMI, as a marker of general adiposity, commencing at the age of 2 years.(Expert Panel on Integrated Guidelines for Cardiovascular Health and Risk Reduction in Children and Adolescents 2011) In Finland, weight-for-height and BMI-for-age are used to monitor the growth of children in well-baby clinics and in school health care on an annual basis. (Saari et al. 2011)

Childhood obesity tracks into adulthood and is associated with a clustering of childhood cardiometabolic risk factors and the consequent deterioration in cardiovascular health.(Lakshman et al. 2012; Baker et al. 2007; Singh et al. 2008) For example, childhood overweight is a powerful risk factor for hypertension, dyslipidemia and insulin resistance in childhood.(Skinner et al. 2015) Furthermore, being overweight during childhood has been associated with cardiometabolic risk factors, diabetes and subclinical atherosclerosis in adulthood.(Srinivasan et al. 1996; Schubert et al. 2009; Juonala et al. 2011) A study of approximately 300,000 Danish school children aged 7 to 13 years revealed an association between childhood BMI with coronary heart disease events during adulthood.(Baker et al. 2007)

Overweight and obese children have been reported to have increased carotid IMT and arterial stiffness as compared with their normal weight control counterparts. (Iannuzzi et al. 2004; Tounian et al. 2001; Juonala et al. 2011) This may be due to a rearrangement of the materials present in the arterial wall in obese children. Several studies have reported

an association between obesity and greater LVM in children and adolescents, and this has been detected as early as 2 years of age (Chinali et al. 2006; de Jonge et al. 2011; Gidding et al. 2014).

Many of the specific mechanisms between obesity and subclinical markers of cardiovascular disease are incompletely understood in children and likely involve complex interactions between several factors. Obesity is associated with increased metabolic demand due to the greater amount of adipose tissue, increasing blood volume, and increased preload to the heart. In addition, arterial remodeling related to arterial stiffness increases the afterload to the heart, and may influence myocardial function through ventricular-vascular interactions.(Cote et al. 2013; Cote et al. 2015) Autonomic function is sensitive to changes in adiposity, and may continue to be altered as atherosclerosis-related structural changes in large arteries impede vagal inputs. (Grassi et al. 1998; Jennings et al. 2002) Furthermore, metabolic dysfunction, such as insulin resistance, may alter the sympathetic-vagal balance via effects on arterial smooth muscle.(Singhal 2005) Finally, a direct effect of myocardial lipid accumulation on myocardial function has been postulated as an alternative mechanism.(Kass 2005; Koopman et al. 2012)

2.2.3 Blood pressure

Hypertension is regarded as one of the strongest risk factors for cardiovascular disease. Pediatric hypertension may be secondary to some other disease process or it may be essential hypertension, which is rare. Epidemiological studies over the past 20 years have shown that blood pressure levels in children and adolescents have increased, and this has been attributed, at least in part, to the rise in obesity.(Expert Panel on Integrated Guidelines for Cardiovascular Health and Risk Reduction in Children and Adolescents 2011; Zachariah et al. 2014; de Moraes et al. 2014) Elevated blood pressure is often a life course problem that can develop during childhood and progress through adolescence and then into adulthood.(Chen and Wang, 2008) In addition to genetic factors, there are several lifestyle factors, including increased energy intake, physical inactivity, and high salt intake which are thought to be primarily responsible for the rise in blood pressure, as well as other factors such as intrauterine growth retardation which might also play a role.

Hypertension and elevated blood pressure are undiagnosed in the pediatric population.(Hansen et al., 2007) Consensus guidelines define hypertension during childhood as blood pressure at three or more visits measured ≥ 95 th percentile for age, sex, and height. (National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents 2004) There has been a debate about what should be the criteria of hypertension and the utility of screening for increased blood pressure in asymptomatic children and adolescents.(Moyer and Force 2013; Xi et al.

2017; Flynn et al. 2017) The current guidelines recommend routine measurement of blood pressure during health care visits for children over three years.(Expert Panel on Integrated Guidelines for Cardiovascular Health and Risk Reduction in Children and Adolescents 2011) For children from birth to age 2 years, blood pressure should be measured when there is a suspicion of renal disease, coarctation of the aorta, or some other condition that may be contributing to the blood pressure elevation. In Finland, blood pressure is measured routinely during visits to the well-baby clinic at the age of 4 years and in school health care at the age of 7, 11 and 14 years.

Elevated blood pressure in childhood has been associated with subclinical markers of cardiovascular disease.(Rao 2016; Kollias et al. 2014) In previous studies in children and adolescents, elevated blood pressure has been linked with increased arterial stiffness and increased IMT. (Whincup et al. 2005; Li et al. 2003; Juonala et al. 2010) Analyses from four longitudinal childhood cohort studies revealed that the association of elevated blood pressure in childhood with carotid intima-media thickness was markedly reduced if these individuals became normotensive adults.(Juhola et al. 2013) Hypertension and elevated blood pressure in children and adolescents have also been associated with increased LVM. (Stabouli et al. 2009; Urbina et al. 2011; Gidding et al. 2014)

The mechanisms and physiological homeostatic relationship between blood pressure and arterial stiffness are complex (AlGhatrif et al. 2017). Elevated blood pressure accelerates atherosclerosis, collagen synthesis, arterial smooth muscle hyperplasia and hypertrophy, which contribute to arterial stiffening. (Franklin 2005) In addition, stiffening of the artery wall and improper matching between diameter and flow have been associated with unfavorable alterations in pulsatile hemodynamics, including an increase in arterial pressure wave amplitude, which increases the pulse pressure. Since there are stiffer arteries, systolic blood pressure increases in particular. There has been a debate about whether there is a causality between elevated blood pressure and arterial stiffness. There are reports that childhood elevated blood pressure can be linked with arterial stiffness in adulthood (Juonala et al. 2005a; Aatola et al. 2010a; AlGhatrif et al. 2013), suggesting that increased blood pressure may induce adverse arterial effects, e.g. signs of the atherosclerotic process, in the arterial wall and lead to stiffening of the artery. Thus, increased blood pressure would seem to precede arterial stiffness especially at younger ages. On the other hand, the longitudinal community-based Framingham Offspring Study in an older age cohort (mean age 60 years) demonstrated that higher aortic stiffness associated with a higher risk of incident hypertension, indicating that arterial stiffness may precede and contribute to the development of higher blood pressure (Kaess et al. 2012). In the light of these previous studies, the association between blood pressure and arterial stiffness may be bidirectional and the putative causal direction between blood pressure and arterial stiffness could change with increasing age.

2.2.4 *Lipoproteins and lipids*

Lipids, such as cholesterol and triglycerides, are transported in lipoprotein particles in blood plasma. The lipoprotein transport system serves multiple functions including the transportation of dietary fats from intestine to liver and the movement of processed cholesterol particles to peripheral tissues for membrane synthesis and steroid hormone production, as well as the processing of free fatty acids which all have an essential role in human physiology.(Genest 2003) Lipid and lipoprotein levels change at various periods during the growth of the child and are predictive of future adult lipoprotein profiles.

Physiologically, it has been stated that the LDL receptor functions optimally at an LDL cholesterol concentration of 1.5 mmol/l in plasma. When LDL cholesterol is present above this level, the cells respond by reducing their number of LDL receptors to ensure that the rate of uptake of LDL cholesterol does not increase. This postulated 'appropriate' level of LDL cholesterol (1.5 mmol/l) is similar to the mean level of LDL cholesterol measured in eight other mammalian species that are not naturally susceptible to atherosclerosis. Moreover, this level is approximately the mean level of LDL cholesterol observed in normal human newborn infants at a time before dietary and other environmental stresses have elevated its level.(Goldstein and Brown 1977; O'Keefe et al. 2004)

The most common dyslipidemias in childhood are combined dyslipidemia and familial hypercholesterolemia.(Expert Panel on Integrated Guidelines for Cardiovascular Health and Risk Reduction in Children and Adolescents 2011) There are some secondary reasons that can evoke childhood dyslipidemia e.g. hypothyroidism, anorexia nervosa, diabetes, chronic liver or kidney disease, and other endocrine diseases causing high cortisol or steroid hormone levels.

The pronounced increase in the prevalence of obesity has led to a much larger population of children with dyslipidemia. Combined dyslipidemia characterized by an elevation in the triglyceride level, normal-to-mild elevation in the LDL cholesterol level, and a reduced high-density lipoprotein (HDL) cholesterol level, is estimated to be prevalent in more than 40% of obese adolescents.(Kavey 2015)

Familial hypercholesterolemia is a common genetic disorder of cholesterol metabolism that leads to lifetime high LDL cholesterol levels and a markedly increased risk of coronary heart disease. Mutations in the LDL receptor gene lead to defective LDL receptor function, diminished catabolism of LDL particles and thus to severely elevated serum LDL cholesterol levels. Other genes that may be affected are those coding for protein convertase subtilisin kexin 9 (PCSK9) and apolipoprotein B. Current estimates of the prevalence of heterozygous familial hypercholesterolemia range from 1:200 to 1:600

but this disorder appears to be largely underdiagnosed and undertreated all around the world. (Nordestgaard et al. 2013; de Ferranti et al. 2016; Lahtinen et al. 2015) Conversely, also ≥ 3 other PCSK9 mutations have been identified that result in lower LDL cholesterol levels and a decreased risk of coronary heart disease. (Cohen et al. 2006; Hallman et al. 2007; Huang et al. 2009) It has been proposed that these mutations protect against coronary heart disease by producing significant lifelong decreases in LDL cholesterol levels and that the duration of decrease in LDL cholesterol may be as important as its magnitude in decreasing the coronary heart disease risk already from childhood. (Hallman et al. 2007; Qiu et al. 2017)

High plasma concentrations of total cholesterol and LDL cholesterol are fundamental risk factors of cardiovascular disease. In several studies, a clear correlation has been observed between lipoprotein disorders and the onset and severity of atherosclerosis in children, adolescents, and young adults. The Bogalusa Heart Study and PDAY Study showed that dyslipidemias were associated with atherosclerotic lesions and accelerated atherosclerosis already during the first two decades of life. (Berenson et al. 1998; Wissler 1991)

Hypercholesterolemia in childhood is associated with increased arterial stiffness and arterial IMT. (Leeson et al. 2000; Aggoun et al. 2000; Järvisalo et al. 2001) In addition, childhood lipid levels have been shown to predict subclinical atherosclerosis in adulthood. (Davis et al. 2001; Li et al. 2003; Raitakari et al. 2003; Juonala et al. 2005) In animal models, the rate of aortic cholesterol accumulation correlated with aortic collagen synthesis activity, an observation suggesting that elevated collagen synthesis may be associated with increased accumulation of cholesterol in the arterial wall. (Palombo and Kozakova 2015) Moreover, extracellular matrix stiffening has been shown to promote a disruption of the integrity of the endothelial barrier, thus increasing endothelial permeability and cholesterol uptake in the arterial wall, which is a critical step in the formation of atherosclerotic plaques. (Huynh et al. 2011) These mechanisms may in part explain the associations between hypercholesterolemia and increased arterial stiffness.

Universal lipid screening in the pediatric population is recommended at age of 9 – 11 years and 17 – 21 years. (Expert Panel on Integrated Guidelines for Cardiovascular Health and Risk Reduction in Children and Adolescents 2011) For high risk children, the screening is recommended at the age of 2 – 8 years. In Finland, lipid screening in pediatric population is recommended only for high risk children who have a family member who has been diagnosed with coronary heart disease at an early age (men < 55 years and women < 65 years) or who has a genetic disorder of cholesterol metabolism.

2.2.5 *Glucose-homeostasis*

Diabetes is an established risk factor for early cardiovascular disease.(Expert Panel on Integrated Guidelines for Cardiovascular Health and Risk Reduction in Children and Adolescents 2011; Selvin et al. 2004) Metabolically, diabetes is characterized by an elevated blood glucose concentration, i.e. hyperglycemia, due to impaired glucose-insulin homeostasis. Insulin is the key stimulator of glucose uptake in cells. In type 1 diabetes, autoimmune destruction of pancreatic beta cells leads to insufficient insulin production, which causes hyperglycemia. Impaired glucose uptake in cells due resistance to the action of insulin, combined with beta cell dysfunction, can lead to impaired glucose tolerance, hyperglycemia and eventually to type 2 diabetes. (Schofield and Sutherland 2012; Rizza et al. 1985; Festa et al. 2006) Both genetic and environmental factors contribute to the development of type 2 diabetes.(DeFronzo 2009)

Type 2 diabetes in adolescence has become a disorder with a rapidly increasing incidence. Prior to 1992, type 2 diabetes was a disease of ageing and considered to affect only adults. The disease has now been convincingly described in adolescents across the globe; its appearance has been driven by changes in nutrition and health related to the rapid economic development and the social changes taking place over the past 30 years.(Viner et al. 2017; Hannon and Arslanian 2015) Nowadays, type 2 diabetes accounts for nearly 50% of new diabetes cases in adolescents and its incidence and prevalence continue to rise.(Hannon and Arslanian 2015)

Children with hyperglycemia and insulin resistance have been shown to have subclinical evidence of atherosclerosis characterized by increased carotid IMT and increased arterial stiffness.(Järvisalo et al. 2002; Urbina et al. 2012) Left ventricular hypertrophy has been also demonstrated in children with impaired glucose tolerance.(Gidding et al. 2004; Gidding et al. 2013; Toprak et al. 2008) Insulin resistance and chronic hyperinsulinemia increase the local activity of the renin–angiotensin–aldosterone system and elevate the expression of angiotensin II receptors in arterial tissue, which may lead to arterial wall hypertrophy and fibrosis.(Brillante et al. 2009; Jesmin et al. 2003) Impaired glucose tolerance also enhances non-enzymatic glycation and cross-linking of collagen, leading to stiffening of the arteries.(Brownlee et al. 1988)

2.2.6 Lifestyle

Diet

Dietary habits influence cardiometabolic health, including various risk factors such as obesity, lipoprotein concentrations, blood pressure, glucose-insulin homeostasis, oxidative stress, inflammation, hepatic function, adipocyte metabolism, cardiac function, and the gut microbiome.(Mozaffarian 2016; Micha et al. 2017) The primary features of so-called “heart-healthy eating” involve both dietary quality and energy balance.(Steinberger et al. 2016) The development of food preferences in childhood is important because early preference patterns have a long-term influence on dietary intakes later in life.(Mikkilä et al. 2005)

Vegetables, fruits, fish, whole grains, nuts, low-fat dairy products, and vegetable oils have been associated with a lower risk of cardiovascular disease. Conversely, red and processed meats, and foods rich in refined grains, starch, added sugars, salt, and trans fat have been associated with an increased risk of cardiovascular disease.(Mozaffarian et al. 2011). The effects of foods likely reflect complex, synergistic contributions and interactions between food structure, preparation methods, fatty acid profile, carbohydrate quality (e.g. fiber content, absorption rate), protein type, micronutrients, and phytochemicals.(Mozaffarian 2016)

In Finnish children, a high intake of added sugar, sodium and saturated fat, as well as the growing amount of animal protein in the diet, are regarded as nutritional challenges (National Nutrition Council 2018). As an example of the dietary health effects in children, lower consumption of vegetable oils and a higher consumption of sugar-sweetened beverages have been shown to associate with a higher metabolic risk determined as a risk score based on the components of the metabolic syndrome.(Eloranta et al. 2014; Jääskeläinen et al. 2012) Fruit and vegetable consumption in childhood has been shown to associate with arterial IMT and arterial stiffening in young adulthood.(Aatola et al. 2010b; Kaikkonen et al. 2012; Kaikkonen et al. 2013) Excess sodium intake, independent of blood pressure, was demonstrated to associate with left ventricular hypertrophy as well as fibrosis in the heart, kidneys, and arteries.(Appel et al. 2011)

Physical activity

Over the last decades, there has been a steady decrease in the amount of time that children spend being physically active and an accompanying increase in the time spent undertaking sedentary activities.(Expert Panel on Integrated Guidelines for Cardiovascular Health and Risk Reduction in Children and Adolescents 2011) The health

benefits of physical activity have been known at least since the time of Hippocrates. Nowadays, physical inactivity is an established modifiable risk factor for cardiovascular disease.(Shiroma and Lee 2010; Ahmed et al. 2012) In children, physical activity has been favorably associated with cardiometabolic risk factors.(Ekelund et al. 2012; Pahkala et al. 2011) Physical activity is related to increased insulin sensitivity, a favorable lipid profile, a decreased prevalence of the metabolic syndrome, and lower blood pressure (Rizzo et al. 2008; Mansikkaniemi et al. 2012). Higher levels of moderate-to-vigorous physical activity and the avoidance of sedentary behavior have been associated with a slower age-related progression of aortic stiffness in adults.(Ahmadi-Abhari et al. 2017) In children, physical activity and cardiorespiratory fitness have been favorably associated with arterial stiffness and IMT.(Haapala et al. 2017; Pahkala et al. 2011; Pahkala et al. 2013) Importantly, a moderate increase in physical activity decelerates the progression of IMT.(Pahkala et al. 2011) Furthermore, in boys aged 9- to 15-years and in young adults, physical activity has been favorably associated with carotid stiffness later in adulthood.(Pälve et al. 2014)

These effects may be mediated through reduction in α -adrenergic receptor-mediated arterial tone and the release of nitric oxide via increased shear stress during or immediately after physical activity.(Kingwell et al. 1997) Physical activity may increase the elastin content of the aortic wall and since it has been speculated to enhance arterial dilatation, it might delay the age-associated reduction or fraction of elastic lamellae in the arterial wall.(Matsuda et al. 1993; Schlatmann and Becker 1977) After three months of aerobic exercise training, carotid distensibility was enhanced and this change corresponded with a reduction in plasma endothelin-1 concentration, which is a vasoconstrictor peptide.(Maeda et al. 2009) Physical activity has also been associated with structural and functional cardiac adaptations known as the 'athlete's heart' already during childhood.(Pluim et al. 2000; McClean et al. 2017)

Exposure to tobacco smoke

Although public health programs have led to a substantial decrease in the prevalence of tobacco smoking, it is still undoubtedly a modifiable risk factor to health, contributing to premature cardiovascular morbidity and mortality worldwide.(Pencina et al. 2009; Centers for Disease Control and Prevention 2008) Tobacco smoke contains over 4,000 chemicals including nicotine, carbon monoxide, acrolein, and oxidative compounds.(Csordas and Bernhard 2013) Exposure to tobacco smoke induces multiple pathological effects on the arterial wall and vasculature, including arterial dysfunction, atherogenesis, oxidation, inflammation and thrombosis.(Ambrose and Barua 2004) Moreover, exposure to tobacco smoke has been linked to accelerated atherosclerosis.(Celermajer et al. 1996; Howard et al. 1994)

Exposure to tobacco smoke has adverse cardiovascular consequences as early as the first decade of life, and this extends to fetal exposure to tobacco smoke in utero.(Simonetti et al. 2011; Geelhoed et al. 2011) Childhood exposure to tobacco smoke has been linked to impaired cardiac autonomic function and changes in heart rate variability. In addition, childhood exposure to tobacco smoke exposure was associated with a clustering of cardiometabolic risk factors.(Raghuv eer et al. 2016) The cardiovascular effects of exposure to tobacco smoke in childhood are occult but long-lasting and substantial. Exposure to tobacco smoke in childhood has been linked to accelerated arterial stiffness in children at the age of 11 years.(Kallio et al. 2009). Children who have been exposed to tobacco smoke in utero have been reported to have increased carotid IMT.(Geerts et al. 2012) A recent study in children and youths exposed to tobacco smoke indicated that its consequences persist into adult life.(West et al. 2015) In addition, actual smoking in adolescence has been associated with increased carotid IMT. (Dratva et al. 2013)

2.2.7 Clustering of cardiometabolic risk factors in childhood

Cardiometabolic risk factors have a tendency to cluster in the same individuals, even in childhood.(Viitasalo et al. 2014; Magnussen et al. 2010) Children with clustering of cardiometabolic risk factors have an increased risk of adulthood metabolic syndrome, type 2 diabetes and cardiovascular disease.(Morrison et al. 2007; Magnussen et al. 2010) These consequences of obesity, such as metabolic syndrome and type 2 diabetes, once thought to appear only in middle age, are now expected to be present in childhood.(Dalla Valle et al. 2015) When obese Finnish children were examined, prediabetes was present in 34.7% of those investigated (in 28.4% of girls and in 40.2% of boys).(Dalla Valle et al. 2015) A risk factor cluster, characterized by central obesity, insulin resistance, hyperglycemia, dyslipidemia and hypertension, known as the metabolic syndrome, has been associated with subclinical atherosclerosis in adults and in children.

The focus in identifying cardiovascular risk factors has gradually shifted towards favorable cardiovascular profiles and the clustering of favorable cardiovascular factors and behaviors beginning in early childhood.(Lloyd-Jones et al. 2010) It has been demonstrated that low-risk cardiovascular profiles are associated with significant reductions in cardiovascular morbidity and mortality.(Stamler et al. 1999)

2.3 Promotion of cardiovascular health in childhood

At a young age, the two main goals of prevention are 1) to prevent the development of risk factors (primordial prevention) and 2) to recognize and manage those children and adolescents at high risk due to the presence of one severe risk factor or multiple risk

factors (primary prevention). The prevention of cardiovascular disease requires a life-course health policy with strategies aimed at various levels e.g. individuals, families, communities, and they involve also legislative policies influencing public health care, schools, the food industry, and the environmental planning.

2.3.1 *Ideal cardiovascular health in childhood*

The American Heart Association (AHA) defined the concept of ideal cardiovascular health in 2010 as part of their 2020 impact goals for cardiovascular health promotion and disease reduction.(Lloyd-Jones et al. 2010) This definition includes seven ideal cardiovascular health metrics: four favorable health behaviors related to smoking, BMI, physical activity, and diet, and three favorable health factors: total cholesterol concentration, blood pressure, and fasting blood glucose levels. Ideal cardiovascular health was defined as the simultaneous presence of all of these seven metrics at ideal levels. For the pediatric population, the AHA health behavior criteria are: abstinence from smoking, a BMI <85th percentile, ≥ 60 minutes of moderate or vigorous physical activity daily, and adherence to a diet emphasizing fruits, vegetables, fish, whole grains, low sodium, and few sugar-laden foods and drinks. The AHA-recommended health factor metrics for ideal cardiovascular health in children are as follows: total cholesterol <4.4mmol/l (<170 mg/dL), blood pressure <90th percentile, and a fasting plasma glucose level <5.6mmol/l (<100 mg/dL). The definition of ideal cardiovascular health was intended to be simple and easy-to-measure and monitor over time. Moreover, it was meant to be accessible and actionable and allow all patients, clinicians, and communities to focus and develop the capability of improving cardiovascular health.

2.3.2 *Dietary and lifestyle intervention in preventing the development of cardiovascular disease beginning in childhood*

A great number of dietary interventions have been conducted aiming to lower the prevalence of cardiovascular disease risk factors and consequently to reduce cardiovascular disease morbidity, incidence and mortality. Intervention strategies include community mobilization, social marketing, school-based health education, worksite health promotion, screening and referral of those individuals at high risk, education of health professionals, and the direct education of adults and modification of physical environments. In children, a few studies have investigated the effect of dietary intervention on cardiovascular risk markers, such as lipid levels and blood pressure, and subclinical markers of cardiovascular disease; some of them have been implemented as randomized controlled trials.

A major cardiovascular disease prevention program in children and adolescents was conducted in North Karelia, Finland, during 1978 – 1980. (Puska et al. 1982) The school and community based intervention aimed to influence health behavior and cardiovascular disease risk factors in 13 to 15 year-old children. The study was carried out for 2 years at two levels: an intensive direct intervention in two schools, and a general country-wide intervention. The intervention encouraged children to adopt healthy lifestyles that would lead to prevention or reduction of risk factors and also otherwise promote cardiovascular health. The main focus was on prevention of smoking and the introduction of dietary changes influencing serum cholesterol and blood pressure levels (reduction in saturated fat, partial substitution of saturated fat by polyunsaturated fats, increase in vegetable consumption, and reduction in sodium intake). Physical activity, reduction or prevention of overweight, and some other principles of a healthy diet (i.e., a reduction in sugar intake) were also promoted. In the two schools, butter used on bread was replaced by soft margarine (with P/S ratio >1). Whole milk (2.9-3.9% fat) was replaced by skimmed milk (fat <0.05%), buttermilk (fat <0.08%), or water. Vegetable oil was used for salad dressings and cooking. Low-fat meat products were preferred. The consumption of fish, poultry, various vegetables, and fresh salads was promoted, and egg yolks were avoided. Food industries serving the schools were requested to reduce the salt content of their products and the salt used in cooking was substituted with a special mineral salt in which approximately 30% of the sodium had been replaced by magnesium and potassium. These changes reduced the percentage of energy from fat from 37% to 32% and increased the P/S ratio from 0.13 to 0.6 in the average school diet. Added sodium was reduced by approximately 1 g NaCl. At the beginning of the project, the mean serum total cholesterol was 5.1 mmol/l for all boys and girls; it declined by 0.5 mmol/l in all boys, by 0.35 mmol/l in intensive intervention girls, by 0.35 mmol/l in country-wide intervention girls, and by 0.21 mmol/l in reference girls. The educational program was relatively effective in preventing any increase in smoking among children at this critical age.

The Dietary Intervention Study in Children (DISC), a multicenter prospective randomized trial in 663 preadolescent children aged 8 to 10 years with elevated LDL cholesterol values, was started in 1987 in the United States. The study was designed to test the efficacy and safety of a dietary intervention to lower saturated fat and cholesterol intake among children, who were randomly assigned to an intervention or usual care group, and entered into the study which had a duration of 2.5 years, followed by a mean of 7.4 years follow-up.(Van Horn et al. 2005). LDL cholesterol decreased from 3.38 mmol/l in both the intervention and usual care groups to 2.84 mmol/l and 2.90 mmol/l at 5 years, respectively, but increased at the last visit in both groups. Differences between the two groups were statistically significant at 1 and 3 years, but not at 5 years or at the last visit. Total serum cholesterol followed a similar pattern as the LDL cholesterol and was significantly lower in the intervention than the usual care group at 1 and 3 years, but not at 5 years or the last visit. Sexual maturation had the most significant association with LDL cholesterol. The results showed that a modification of dietary fat can be achieved

and safely sustained in pubertal children, with no adverse effects observed up to 7.4 years later. Furthermore, LDL cholesterol levels could be improved significantly if one undertakes a 3 year long intensive dietary intervention, although the results were no longer significant over 5 years with a lower-intensity maintenance intervention.(Van Horn et al. 2005)

The Child and Adolescent Trial for Cardiovascular Health (CATCH) was an intervention study of 5106 initially 9 year-old children in 56 intervention and 40 control public schools located in California, Louisiana, Minnesota, and Texas carried out during 1991 – 1994.(Luepker et al., 1996) The study was aimed to assess the outcomes of health behavior interventions, focusing on the elementary school environment, classroom curricula, and home programs, for the primary prevention of cardiovascular disease. At the school level, the two primary end points were changes in the fat content of food service lunch offerings and the amount of moderate-to-vigorous physical activity in the physical educational programs. At the level of the individual student, the serum cholesterol change was the primary end point and was used in the power calculations of the study. Individual level secondary end points included psychosocial factors, recall measures of eating and physical activity patterns, and other physiologic measures. The percentage of energy intake from fat declined significantly more in the intervention school lunches (from 38.7% to 31.9%) than in control lunches (from 38.9% to 36.2%). The intensity of physical activity in physical educational classes during the CATCH intervention increased significantly in the intervention schools compared with the control schools. Self-reported daily energy intake from fat among students in the intervention schools was significantly reduced (from 32.7% to 30.3%) compared with that consumed by students in the control schools (from 32.6% to 32.2%). Intervention students reported significantly more daily vigorous activity than controls (58.6 minutes vs 46.5 minutes). Blood pressure, body size, and cholesterol measures did not differ significantly between treatment groups. No evidence was observed for any deleterious effects of this intervention on growth or development. The CATCH intervention was thus able to modify the fat content of school lunches, increase moderate-to-vigorous physical activity in physical education, and improve dietary and physical activity behaviors in children during three school years.(Luepker et al. 1996)

The Special Turku Coronary Risk Factor Intervention Project (STRIP) is a prospective, randomized intervention trial investigating the effect of a family-based dietary intervention starting during infancy and extending to early adulthood. The study targets primarily dietary fat quality and secondarily fruit, vegetable, whole grain, sodium and sucrose intake to influence biological and behavioral risk factors of atherosclerosis.(Simell et al. 2009) Families of 5-month-old infants were recruited into the study at well-baby clinics in Turku, Finland, in 1990–1992. At age 7 months, 1,062 infants were randomized to an intervention group or to a control group. The intervention group has repeatedly received individualized dietary and lifestyle counseling with the

main aim being to replace saturated fat with unsaturated fat in the diet. Dietary counseling has been shown to associate with lower saturated fat intake and leading to lower serum LDL cholesterol concentrations from infancy until 19 years of age. (Niinikoski et al. 2012) Between the ages of 13 months and 9 years, both intervention girls and boys had a greater dietary fiber intake than controls.(Ruottinen et al. 2010) The intervention has also been associated with improved insulin sensitivity, lower blood pressure, and a reduced risk for the metabolic syndrome.(Oranta et al. 2013; Niinikoski et al. 2009; Nupponen et al. 2015)

The Physical Activity and Nutrition in Children (PANIC) Study is a long-term controlled physical activity and dietary intervention trial in 512 Finnish children. The study assessed the lifestyle, health and wellbeing of children 6-8 years of age and allocated the children to either the intervention or control group in the baseline study in 2007-2009. The children in the intervention group and their parents received individualized and family-based physical activity and dietary counseling during the first two years. The intervention also included after-school sports clubs for the children. This study is continuing with less intensive physical activity and dietary intervention until adulthood. The first follow-up was carried out at the 2-year study in 2009-2011; the 8-year follow-up study is being carried out in 2016-2018 with the 13-year follow-up study planned to be conducted in 2021-2023.(Eloranta et al. 2014)

3 AIMS OF THE STUDY

This thesis is based on findings from The Special Turku Coronary Risk Factor Intervention Project. The main purpose was to study cardiovascular health in children and adolescents by examining the association of age, sex, cardiometabolic risk factors and lifestyle with subclinical markers of cardiovascular disease.

The specific aims were:

1. to study the longitudinal progression of arterial stiffness and LVM from childhood to early adulthood (Study I, Study III)
2. to study cardiometabolic determinants of arterial stiffness and LVM in childhood (Study II, Study III)
3. to examine ideal cardiovascular health in childhood, and its association with aortic intima-media thickness and aortic stiffness (Study IV)
4. to investigate the effect of dietary and lifestyle intervention on ideal cardiovascular health and subclinical markers of cardiovascular disease. (Study I, III, and IV)

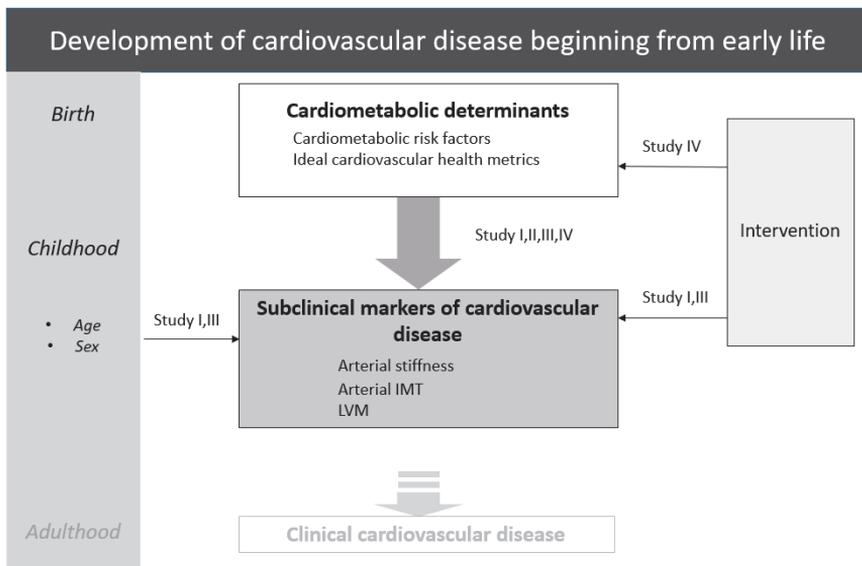


Figure 4. Schematic presentation of the aims of the study.

4 SUBJECTS AND METHODS

The study primarily included subjects who participated in the longitudinal Special Turku Coronary Risk Factor Intervention Project (STRIP) at the age of 11 to 19 years of age and provided data on ultrasonic arterial and cardiac measures.

4.1 The Special Turku Coronary Risk Factor Intervention Project (STRIP)

The STRIP study is a prospective, randomized intervention trial, which began in childhood with the aim to prevent the development of cardiometabolic risk factors primarily through a heart-healthy diet.(Simell et al. 2009; Niinikoski et al. 2012) The study was launched nearly 30 years ago in 1989. Families of 5-month-old infants were recruited into the study at well-baby clinics in Turku, Finland, between February 1990 and June 1992. At the age of 7 months, 1062 infants (56.5% of the eligible age-cohort) were randomly allocated to a dietary intervention (n=540) or control (n=522) group (Figure 5). The study is still ongoing.

Families randomized to the intervention group visited the study center at 1- to 3-month intervals until the child was 2 years old, and biannually thereafter until 20 years of age. Until the child was 7 years old, the intervention was targeted primarily at the parents, but afterwards, separate sessions were organized for the child. The control group families were seen biannually until the child was 7 years of age, after which they were seen annually until the child was aged 20 years. The intervention group has repeatedly received individualized heart-healthy dietary and lifestyle counseling with the main aim being to replace saturated fat with unsaturated fat in the diet. The control families did not receive any dietary intervention. During study visits, anthropometric and laboratory data were collected together with other data e.g. on food consumption. Arterial ultrasound studies were initiated when the children were 11 years old and cardiac ultrasound studies at the age of 15 years.

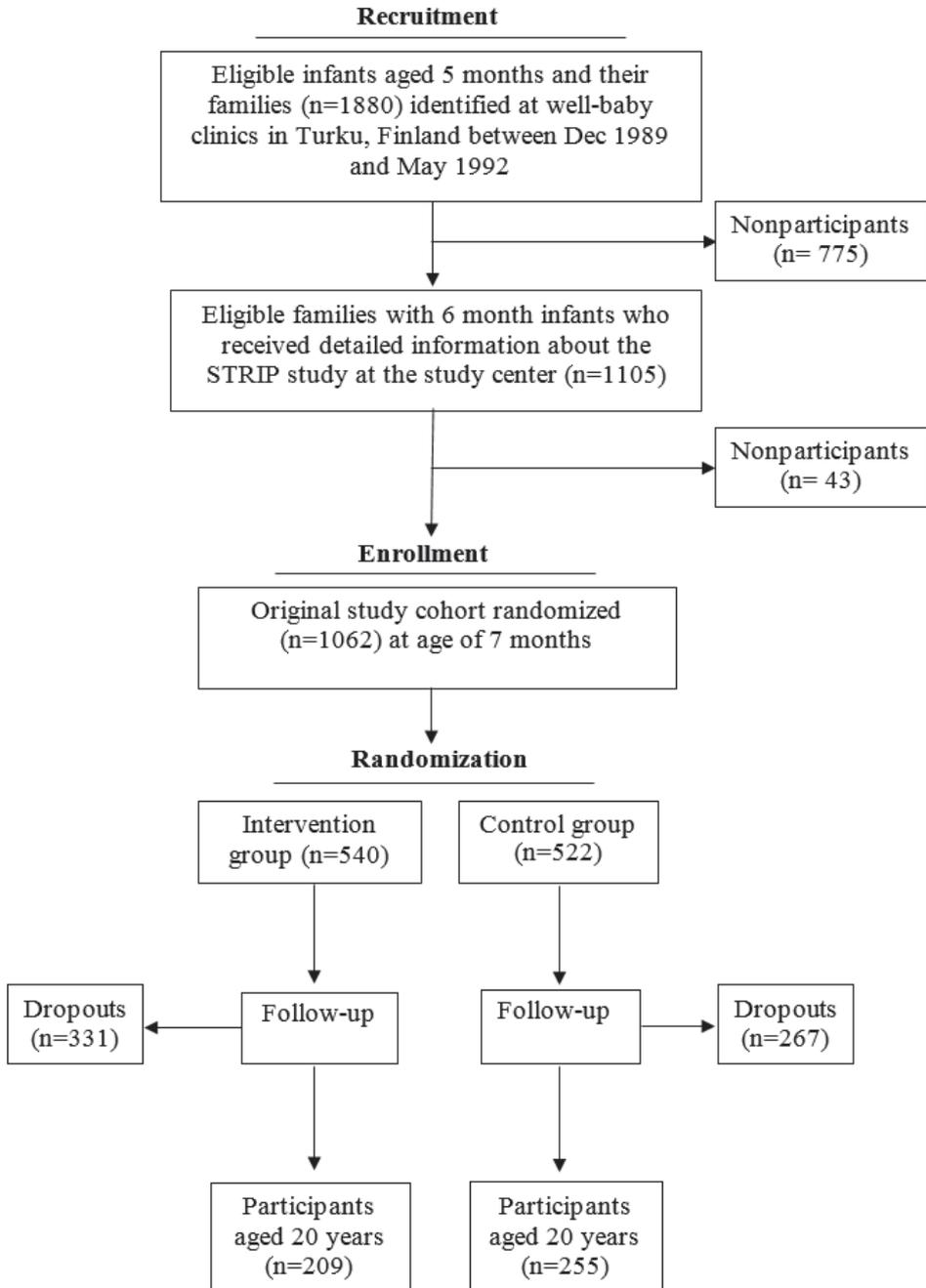


Figure 5. Flow chart of the entire STRIP cohort. The intervention period of the study continued until the age of 20 years.

4.2 Study design and subjects

The subjects included in the studies of this thesis consisted of children and adolescents who participated in the STRIP study at the age of 11 to 19 years (2001 to 2012), and provided ultrasonic data on subclinical markers of cardiovascular disease.

Study I examined the association of age and sex with carotid and aortic stiffness and LVM from childhood to early adulthood. This study cohort comprised 503 adolescents for whom arterial and/or cardiac ultrasound data was available between the ages 11 of 19 years. The stiffness of the abdominal aorta and common carotid artery was measured repeatedly at the age of 11 ($n_{\text{cdist}}=420$, $n_{\text{adist}}=407$), 13 ($n_{\text{cdist}}=483$, $n_{\text{adist}}=476$), 15 ($n_{\text{cdist}}=503$, $n_{\text{adist}}=475$), 17 ($n_{\text{cdist}}=468$, $n_{\text{adist}}=456$) and 19 ($n_{\text{cdist}}=427$, $n_{\text{adist}}=422$) years, and LVM at the age of 15 ($n=418$), 17 ($n=394$) and 19 ($n=420$) years. Among the subjects, the effect of the dietary and lifestyle intervention on arterial stiffness and LVM was also evaluated.

Study II examined among 503 adolescents the association of cardiometabolic risk markers with ultrasonically measured carotid and aortic stiffness at the age of 11, 13, 15, 17 and 19 years.

In study III, childhood determinants of LVM were examined. The study comprised 418 adolescents for whom cardiac ultrasound data was available at the age of 15 years.

Study IV examined ideal cardiovascular health in 394 adolescents at the age of 15, 17 and 19 years of age. The effect of the dietary and lifestyle intervention on ideal cardiovascular health was studied, and furthermore, the association of ideal cardiovascular health with aortic intima-media thickness and aortic stiffness was examined. Of the adolescents with complete ideal cardiovascular health score, 354 (90%), 349 (93%) and 286 (96%) had undergone an aortic ultrasound measurement at the ages of 15, 17 and 19 years.

4.3 Anthropometric measures

4.3.1 Physical examination

Physical examination begun at age 7 months comprised measurement of height, weight, and systolic and diastolic blood pressure. Height was measured to the nearest 0.1 cm using a Harpenden stadiometer (Holtain, Crymych, UK). Until age 21 months, recumbent length was measured with a baby board (Bekvil, Paljerakenne, Helsinki, Finland). Weight was measured to the nearest 0.1 kg using an electronic scale (Soehnle S10; Soehnle, Murrhardt, Germany). An infant scale (Seca 725; Hamburg, Germany) was used until age 15 months. Supine lengths were recorded on a baby-board until the age of 2 years

(Bekvil, Paljerakenne, Helsinki, Finland). BMI was calculated as kilograms per meter squared (kg/m^2). The adolescents were classified as being overweight if their BMI exceeded the international age- and sex-specific criteria. (Cole et al., 2000) Waist circumference was measured midway between iliac crest and lowest rib at the midaxillary line with a flexible measuring tape since age 7 years. BSA (body surface area) was calculated according to Du Bois formula: $\text{BSA} = 0.007184 * (\text{weight} * 0.425) * (\text{height} * 0.725)$.

Data on birth size of the child were collected from the records of the well-baby clinics. Fetal growth restriction was defined as a birth weight below the 10th percentile for gestational age and sex of the total STRIP study cohort.

Seated blood pressure was measured after an appropriate rest of ≥ 15 minutes on the right arm using an age appropriate cuff-size. The blood pressure was measured once until 7 years of age and thereafter at least twice using an oscillometric noninvasive blood pressure monitor (Criticon Dinamap 1846 SX until 2001, thereafter Criticon Dinamap Compact T). Pulse pressure was calculated as systolic blood pressure minus diastolic blood pressure and mean arterial pressure as diastolic blood pressure plus pulse pressure/3.

Pubertal status was classified using Tanner staging (M1-M5/girls, G1-G5/boys) since age 9 years; M1/G1 were considered prepubertal and other stages pubertal.

4.3.2 Biochemical analyses

A venous blood sample was drawn at 7 months, 13 months, and 2 years of age and annually thereafter (except at ages 6 and 8 years). From age 5 years onward, fasting blood samples were obtained. After the blood was clotted at room temperature and subjected to low-speed centrifugation (3,400 g; 12 minutes, serum was separated and stored at -25°C for up to a few weeks, and finally at -70°C . This study utilized the dietary data on saturated fatty acids (SAFA), polyunsaturated fatty acids (PUFA), monounsaturated fatty acids (MUFA), the (PUFA+MUFA)-to-SAFA ratio, and intake of sodium and fiber. These nutrients were closely linked to the dietary intervention targets.

Leisure-time physical activity was assessed with a self-administered questionnaire from the age of 13 to 19 years to calculate a total metabolic equivalent (MET) index in MET h/week. One MET corresponds to an energy consumption of 1 kcal/weight kg/h during rest. For leisure-time physical exercise, data on frequency, duration and intensity were collected. Leisure-time physical activity was calculated by multiplying the mean frequency, duration and intensity of weekly physical activity (multiple of the resting metabolic rate; MET). (Pahkala et al. 2011) The questionnaire has been widely used in

studies involving children, adolescents and adults. (Raitakari et al. 1996; Leino et al. 1999; Lehtonen-Veromaa et al. 2000) It correlates moderately well with objective physical activity measures (accelerometers: $r=0.26-0.40$; pedometers: $r=0.30-0.39$) in young adults, and also with directly assessed maximal exercise capacity ($r=0.49-0.53$). (Yang et al., 2006; Mansikkaniemi et al., 2012) From the leisure-time physical activity data, tertile cut-off points were calculated and the adolescents were divided into three groups of physical activity level: 1) <11.8 MET h/week (level 1); 2) ≥ 11.8 and <32 MET h/week (level 2); 3) ≥ 32 MET h/week (level 3).

Smoking habits were assessed with a questionnaire where the adolescents reported how many cigarettes they had smoked during their life. Family socioeconomic status (SES) was defined as the highest educational level obtained by subjects' parents when the subjects were 9 years old, ranging from one to four: one indicating the lowest educational level and four the highest educational level (1=elementary school, 2=high school, 3=vocational education, 4=higher academic level or university degree).

4.4 Ultrasonographic assessment of arterial stiffness, intima-media thickness and left ventricular mass

The ultrasonography studies were performed in silence in a temperature-controlled clinical research laboratory. On the measurement day, the adolescents were advised to refrain from smoking, from consuming caffeinated drinks, juice, high-fat meals and vitamin supplementation.

4.4.1 *Distensibility of the common carotid artery and abdominal aorta*

Carotid artery distensibility and aortic distensibility were studied with ultrasonography (Acuson Sequoia 512 mainframe; Acuson, Mountain View, CA). Arterial distensibilities were assessed from M-mode ultrasound images with a concomitant measurement of blood pressure in brachial artery. The diameters of the carotid artery and aorta were measured with ultrasonic calipers twice at end-diastole and twice at end-systole. The mean of the end-diastolic and end-systolic diameters, along with blood pressure, were used to calculate the arterial distensibility. Distensibility was defined as $[(D_s - D_d)/D_d]/(SystBP - DiastBP) \times 1000$, where D_s indicates the end-systolic and D_d the end-diastolic diameter of the artery. SystBP stands for systolic and DiastBP for diastolic brachial blood pressure. Lower distensibility signifies an increased arterial stiffness. The between-visit coefficient of variation was 2.7% for carotid artery diastolic diameter, 16.3% for carotid artery distensibility. (Juonala et al. 2005)

Because of pulse pressure amplification between central and peripheral arteries, the use of brachial pressures as a surrogate for aortic or carotid pressures may over-estimate pulse pressure in central arteries.(McEniery et al. 2008) Thus, guidelines recommend using local blood pressures for the determination of arterial distensibility.(Laurent et al. 2006) Due to the lack of direct measurement of central aortic blood pressure in this study, we estimated the central pulse pressure values for 17 and 19 year-olds by using the ratio of peripheral to central pulse pressure reported in the Anglo-Cardiff Collaborative Trial(McEniery et al. 2005) that measured both brachial and central pressures in adolescents and young adults. The formula to calculate estimated central pulse pressure for girls was $[(\text{brachial SystBP}-\text{DiastBP}) * 25] / 41$, and for boys $[(\text{brachial SystBP}-\text{DiastBP}) * 29] / 50$.(McEniery et al. 2005) By utilizing the estimated central pulse pressures, modified aortic and carotid distensibility values were calculated (Study I).

4.4.2 Arterial pulse wave velocity and stroke volume

A whole-body impedance cardiography device (CircMon, JR Medical Ltd) was used to determine PWV and stroke volume at the age of 19 years. CircMon has a whole-body impedance cardiography channel, a distal impedance plethysmogram channel, and an electrocardiography channel.(Kööbi et al., 2003) When the pulse pressure wave enters the aortic arch and the diameter of the aorta changes, the whole-body impedance decreases. The CircMon software measures the time difference between the onset of the decrease in the whole-body impedance signal and subsequently in the distal plethysmogram signal from a popliteal artery at knee joint level. The measurement is triggered by the R wave of the electrocardiography. A repeatability index of 99%, and a reproducibility index of 87% have been reported for the method. (Kööbi et al., 2003)

4.4.3 Intima-media thickness

IMT was studied with ultrasonography (Acuson Sequoia 512 mainframe; Acuson, Mountain View, CA) at the age of 11, 13, 15, 17, and 19 years. For the abdominal aortic intima-media thickness measurements, the most distal 15 mm of the abdominal aorta was scanned with a linear array transducer using a scanning frequency of at least 10 MHz. The image was focused on the far wall (dorsal arterial wall) and image quality was optimized with gain settings. Images 15 mm in width were magnified with a resolution box. All images were taken at end diastole, incident with the R-wave. An image of the most distal 15 mm aortic far wall was captured and the image was stored for subsequent offline analysis. Ultrasonic calipers were used to take 4 measurements of the IMT covering the entire far wall segment and the average of these measurements was used.

The inter-observer coefficient of variation for the aortic IMT measurements was 3.9% and the coefficient of variation for between-visits was 4.9%.(Järvisalo et al. 2001)

4.4.4 *Left ventricular mass*

Echocardiography was performed at the age of 15, 17 and 19 years. Transthoracic 2-dimensional echocardiography was performed with ultrasonography (Acuson Sequoia 512 mainframe, Acuson, Mountain View, CA), using a 3.5 MHz scanning frequency phased-array transducer. Caliper measurements of end-diastolic interventricular septal wall thickness (SWTd), end-diastolic posterior wall thickness (PWTd), and end-diastolic left ventricular internal diameter (LVIDd) were obtained from M-mode tracings. The uncorrected formula to calculate LVM was $1.04[(LVIDd+PWTd+SWTd)^3-LVIDd^3]$. The LVM correction was made according to the formula recommended by the American Society of Echocardiography: $0.8(1.04[(LVIDd+PWTd+SWTd)^3-LVIDd^3])+0.6$ grams.(Lang et al., 2015) LVM was indexed to BSA (LVM/BSA, g/m^2) and to height (LVM/height^{2.7}, $g/m^{2.7}$). In order to assess the reproducibility of the LVM measurements, the analyses were re-read in a subcohort of 57 subjects. The between-observer coefficient of variation for LVM was 8.5%.

4.4.5 *Patterns of left ventricular geometry*

Left ventricle geometric patterns were defined using LVM index (LVM/height^{2.7}, $g/m^{2.7}$) and relative wall thickness at end-diastole calculated as (SWTd+PWTd)/LVIDd, which allows further classification of left ventricular remodeling as either concentric or eccentric remodeling. There are no recommended cut-off points for LVM index and relative wall thickness in adolescents, thus in this study, cut-off points derived from the cohort were used (sex-specific 90th percentile). The LVM index of 34.02 $g/m^{2.7}$ in girls and 37.08 $g/m^{2.7}$ in boys represented the sex-specific 90th percentiles in the cohort. Relative wall thickness >0.36 for boys and girls, respectively, in the cohort represented the 90th percentile. Based on the 90th percentiles of LVM index and relative wall thickness in the subjects, we constructed four groups for left ventricular geometric patterns: a) LVM index <34.02 $g/m^{2.7}$ for girls and <37.08 $g/m^{2.7}$ for boys and relative wall thickness ≤ 0.36 was classified as normal left ventricular geometry; b) normal LVM index with increased relative wall thickness (>0.36) was classified as concentric remodeling; c) increased LVM index (girls ≥ 34.02 $g/m^{2.7}$, boys ≥ 37.08 $g/m^{2.7}$) and normal relative wall thickness (<0.36) was defined as eccentric remodeling; and d) increases in both variables were designated as concentric, increased LVM (Figure 6).

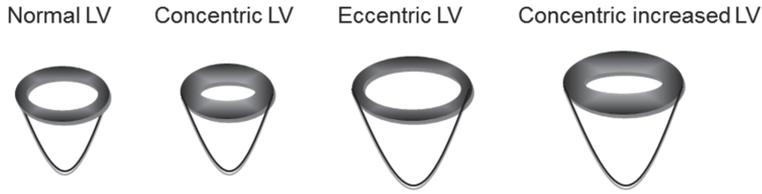


Figure 6. Patterns of left ventricular geometry. LVM index and relative wall thickness are used to define left ventricular (LV) geometric patterns (modified from (Ganau et al., 1992). Reproduced from Hypertension (Study III) with permission of Wolters Kluwer Health.

4.5 Assessment of the ideal cardiovascular health metrics

The childhood ideal cardiovascular health metrics, defined by the AHA included seven ideal cardiovascular health metrics: four favorable health behaviors related to smoking, BMI, physical activity, and diet, and three favorable health factors: total cholesterol concentration, blood pressure, and fasting blood glucose levels. (Lloyd-Jones et al., 2010) Ideal cardiovascular health was defined as the simultaneous presence of all of these seven metrics at ideal levels. For the pediatric population, the AHA health behavior criteria are: abstinence from smoking, a BMI <85th percentile, ≥ 60 minutes of moderate or vigorous physical activity daily, and adherence to a diet emphasizing fruits, vegetables, fish, whole grains, low sodium, and few sugar-laden foods and drinks. The AHA-recommended health factor metrics for ideal cardiovascular health in children are as follows: total cholesterol <4.4 mmol/l (<170 mg/dL), blood pressure <90th percentile, and a fasting plasma glucose level <5.6 mmol/l (<100 mg/dL). In children, BMI and blood pressure normally change with age, growth and development. Therefore, instead of a single threshold, the use of percentiles is recommended when defining higher risk levels for BMI and blood pressure in childhood. (Forouzanfar et al., 2015) In STRIP, complete data on ideal cardiovascular health metrics were available at the age of 15 (n=394), 17 (n=376) and 19 (n=298) years. The metrics for ideal cardiovascular health in children defined by the AHA were adhered to as precisely as possible (Table 1). All percentile limits used in the present study are age- and sex-specific.

4.5.1 Health behaviors

The health behaviors included in the definition of ideal cardiovascular health were BMI, smoking habits (non-smoking), physical activity and diet. Smoking habits were assessed with a questionnaire where the adolescents reported how many cigarettes they had smoked during their life. Those who reported never having smoked a cigarette were

categorized as having an ideal smoking status. Ideal BMI was classified as <85th and ideal physical activity level was defined as leisure time physical activity >30 MET h/wk, which corresponds to >1 h/day of moderate intensity physical activity.

The ideal cardiovascular health criteria for diet define the intake of fruits and vegetables, fish, fiber-rich whole grains, sodium and sugar-sweetened beverages. The intake goals are expressed for a 2000-kcal diet and should be scaled accordingly for other levels of energy intake. Having at least 4 of the 5 dietary components is required for having an ideal diet. The criteria for ideal diet was followed as closely as possible and determined the dietary goals as: 1) fruits and vegetables ≥ 450 g/day, 2) fish ≥ 200 g/wk, 3) at least two 28.35 g (equal to two 1 oz) servings of whole grain bread/day, 4) sodium ≤ 1500 mg/day, and 5) ≤ 450 kcal/week from sugar-sweetened beverages. All intakes were scaled for energy intake to determine if the criterion for ideal diet was met. Potatoes, juices and jams were excluded from the intake of fruits and vegetables. Two servings were used as the minimum for whole grain intake because only the consumption of whole grain bread (≥ 5 g of fiber/100 g bread) was applied.

4.5.2 Health factors

Health factors included total cholesterol and fasting glucose concentration, and blood pressure. (Lloyd-Jones et al. 2010) In childhood, ideal total cholesterol status was defined as <4.40 mmol/l (<170 mg/dl), ideal blood pressure status as systolic blood pressure <90th percentile and diastolic blood pressure <90th percentile, and ideal fasting plasma glucose concentration was classified <5.6 mmol/l (<100mg/dl).

Table 1. Definition of the ideal cardiovascular health metrics (age <20 years) as defined by the American Heart Association (Lloyd-Jones et al. 2010) and the criteria used in this study.

	Ideal metric	Ideal metric
	The American Heart Association's definition	Definition in this study
Health behaviors		
Smoking	Never tried; never smoked whole cigarette	Never smoked a cigarette
Body mass index	<85 th percentile	<85 th percentile
Physical activity	≥60 min of moderate- or vigorous-intensity activity every day	≥60 min of moderate-intensity activity/day (>30 MET h/wk)
Diet	4-5 components*: Fruit and vegetables: ≥4.5 cups/day Fish: ≥two 3.5-oz† servings/week Fiber-rich whole grains: ≥three 1-oz-equivalent servings/day Sodium: <1500 mg/day Sugar-sweetened beverages: ≤450 kcal (36 oz)/week	4-5 components*: Fruit and vegetables: ≥450 g/day Fish: ≥200 g/wk Fiber-rich whole grains: ≥two 28.35 g servings of whole grain bread/day Sodium: <1500 mg/day Sugar-sweetened beverages: ≤450 kcal/week
Health factors		
Total cholesterol	<170 mg/dL (<4.40 mmol/l)	<4.40 mmol/l
Blood pressure	<90 th percentile	<90 th percentile
Plasma glucose	<100 mg/dL (<5.6 mmol/l)	<5.6 mmol/l

*Intake goals expressed for a 2000-kcal diet. Scaled accordingly for other levels of energy intake.

†1 oz: 28.35 g. MET, metabolic equivalent.

4.5.3 *Ideal cardiovascular health score*

Using the metrics and criteria for individual ideal health factors and behaviors, the ideal cardiovascular health score corresponding to the AHA's concept was calculated. (Lloyd-Jones et al., 2010) To create the score, a value of 1 was assigned for each metric if the criterion for ideal cardiovascular health was met. In case the criterion was not met, a value of 0 was assigned. The range of the score was thus 0 to 7, a higher score being indicative of a better cardiovascular health profile.

4.6 Dietary and lifestyle intervention

In the STRIP study, the main aim of the dietary counseling has been the replacement of SAFA with unsaturated fat in the child's diet (targeted ratio of SAFA to MUFA and PUFA 1:2). In addition, the counseling promoted the intake of vegetables, fruits, and whole-grain products, and a low intake of salt. During the first years, the counseling was given primarily to the parents. From 7 years of age onwards, progressively more information was given directly to the child. The parents were carefully informed about the contents of the child's counseling session, and they were encouraged to further discuss the same food-related topics with the child at home. The counseling was given by a nutritionist at every study visit (3- to 6-month intervals). The intervention was individualized and the child's recent food record was used as a basis for suggestions. A fixed diet was never ordered. Most of the counseling material was developed for the STRIP study because ready-made materials for children were sparse. The dietary recommendations were based on the most recent Nordic Nutrition Recommendations.

Child-oriented counseling aiming at the primary prevention of smoking began at 8 years of age. The counseling was based on supporting the self-image of the child and on understanding the health risks associated with smoking. At 16.5 years of age, the detrimental effects of exposure to tobacco smoke were also discussed. A physically active lifestyle was also encouraged but it was not a structured, continuous part of the counseling.

The children in the control group were seen biannually until 7 years of age and annually thereafter. The control families did not routinely receive any detailed intervention focused on the prevention of atherosclerosis risk factors such as dietary fat intake or smoking.

4.7 Statistical methods

In all statistical analyses, the results were considered statistically significant at values of $P < 0.05$.

Aim I: To study the longitudinal progression of arterial stiffness and LVM from childhood to early adulthood

Studies I, II

Repeated measures analysis of variance (RMANOVA) with time as a repeating factor was used to study the association of STRIP study intervention-control group with arterial distensibility. Since the study group was not associated with distensibility of the aorta or carotid artery, the STRIP intervention and control groups were studied as a combined cohort.

RMANOVA was used to evaluate the association of sex and age with arterial distensibility, its components and LVM. In addition, RMANOVA with pubertal stage as repeating factor was used to study the association of pubertal stage with aortic and carotid distensibility. If a child stayed in a certain puberty stage for several years, the measures within that pubertal stage were averaged for the analyses. The shapes of significant interactions were studied with t-tests for differences between sexes within each age point. Stability of distensibility over time and associations between distensibility of aortic and carotid artery were measured using Pearson correlations. Values for aortic and carotid distensibility and PWV were log-transformed before analyses owing to a skewed distribution. The analyses were performed with the SAS 9.3 (SAS Institute, Cary, NC).

Aim II: To study cardiometabolic determinants of arterial stiffness and LVM in childhood

Study II

Aortic distensibility and carotid distensibility as well as the levels of triglycerides, serum insulin and HOMA-IR values were log-transformed for the analyses. The association of cardiometabolic risk markers with aortic distensibility and carotid distensibility between 11 and 19 years of age was studied with a linear mixed-effects model for repeated measures using compound symmetry covariance structure. All models included age and sex as covariates. Since insulin and glucose were first measured at the age of 15 years, the first main model included ages 15, 17 and 19 years.

The arterial distensibilities and risk markers were standardized (z scored) by age and sex for the analyses. When significant associations between the risk markers and arterial distensibility were found, sex×risk marker interactions were studied to investigate whether the associations were similar in boys and girls. In the case that there were significant sex interactions, sex-stratified analyses were conducted. Risk markers that showed an association with either aortic distensibility or carotid distensibility were included in the multivariate model. BMI was standardized (z scored) for multivariable analyses. In additional multivariable models, BMI was replaced with weight and height, respectively, and systolic blood pressure with mean arterial pressure and diastolic blood pressure, respectively, and birth weight or STRIP study intervention/control group were added to the models.

To categorize subjects into those individuals with 1) low distensibility and 2) normal distensibility (respectively for the aortic and carotid artery), we first selected those subjects with at least three of the maximum five measurements for the respective age points of arterial distensibility ($n_{\text{aorta}}=433$, $n_{\text{carotid}}=442$). Second, their aortic distensibility and carotid distensibility values were standardized (z scored) by age and sex, and the mean of the standardized values was calculated. The lowest 20th percentile of the mean values was then used to define subjects with low distensibility (group 1). The rest of the

subjects with distensibility >20th percentile were determined as having normal distensibility (group 2). From the subjects with normal distensibility, we further categorized those with high distensibility using the 80th percentile as the cut-off point (group 3).

The analyses were not corrected for multiple statistical testing since based on the data from previous studies related to arterial stiffening, the used covariates can be hypothesized to associate with arterial distensibility, making the analyses pre-specified. In addition, the number of analyses is limited than rather than explorative. Analyses were performed with the SAS 9.4 (SAS Institute, Cary, NC).

Study III

T-test was used for continuous and Cochran-Mantel-Haenszel 2-test for categorical response variables to study sex differences. Univariate linear regression analyses were performed to assess determinants of LVM. Furthermore, a multivariable model adjusted for sex and weight was used. From the sex and weight adjusted models, significant determinants of LVM were further entered into a multivariable linear regression analysis with backward selection (exclusion criteria $P < 0.15$) when assessing independent determinants of LVM. In the final model, birth length and current waist circumference were also used instead of birth weight and current weight, respectively. Linear regression analysis was used to study associations of the septal and posterior wall thickness with the independent determinants of LVM as well as continuous determinants of left ventricular geometric patterns (normal left ventricular geometry; concentric remodeling; eccentric remodeling; and concentric, increased LVM).

Cochran-Mantel-Haenszel 2-test was used to study associations between categorical determinants of left ventricular geometric patterns. The Tukey-Kramer method was used in post-hoc analyses following statistically significant ANOVAs. Analyses were performed with the SAS,9.2 (SAS Institute, Cary, NC).

Aim III: To study ideal cardiovascular health in childhood, and its association with aortic intima-media thickness and aortic stiffness

Study IV

Due to the low prevalence of adolescents with values of 0 or 1 of the ideal cardiovascular health metrics, adolescents with 0, 1 and 2 scores were combined for the analyses. A low ideal cardiovascular health score was defined as having ≤ 3 ideal metrics. For aortic IMT and distensibility, high risk variables were formed using age and sex specific 85th/15th percentile cut-off points (IMT $\geq 85^{\text{th}}$ percentile, distensibility $\leq 15^{\text{th}}$ percentile). There was no sex difference in the STRIP study groups ($P=0.45$; adjusted for age).

The association between categorical variables was studied with Cochran-Mantel-Haenszel statistics (row mean scores differ), age and sex included in the analyses when applicable. Repeated measures linear regression analysis adjusted for age and sex was used to study the association of the ideal cardiovascular health score with aortic IMT and distensibility. There was no sex-by-ideal cardiovascular health score interaction when aortic IMT ($P=0.71$) or distensibility ($P=0.36$) was the outcome variable, indicating that the effect of the score on the vascular variables was similar in girls and boys. Therefore the sexes were combined in the analyses.

Aim IV: To study the effect of dietary and lifestyle intervention on ideal cardiovascular health and subclinical markers of cardiovascular disease.

Studies I, III, and IV

The risk of STRIP study control group of having low ideal cardiovascular health score was studied with a Poisson regression model with a generalized estimating equation estimation for repeated measures [risk ratios (RR) calculated for STRIP control vs. intervention group; adjusted for age and sex]. Similar analyses were used to study the association of a low ideal cardiovascular health score with the risk of having high risk IMT or distensibility (risk ratios calculated for low vs. high score). SAS 9.3 (SAS Institute, Cary, NC) was used for the analyses.

RMANOVA was used to assess differences between intervention and control groups over time (interaction).

4.8 Ethics

The study was approved by the Joint Commission on Ethics of the Turku University and the Turku University Central Hospital. Written informed consent was obtained from the parents in the beginning of the study and from the children at ages of 15 and 18 years.

5 RESULTS

This study examined the association of age, sex and cardiometabolic risk factors with arterial distensibility and LVM from childhood to early adulthood. The association of ideal cardiovascular health with aortic IMT and distensibility was also evaluated, and the study further reports the effect of dietary and lifestyle intervention on ideal cardiovascular health, arterial distensibility and LVM.

5.1 Characteristics of the subjects

The characteristics of the study subjects at the age of 11 and 19 years are described in Table 2. At the age of 11 years, 20% of the girls and 17% of the boys were overweight, whereas at the age of 19 years, 15% of the girls and 19% of the boys reached the criteria for being considered as overweight.

Table 2. Characteristics of the study cohort by age and sex. Data are given as mean (SD).

Variable	11y		19y	
	Girls	Boys	Girls	Boys
N/n	270/193	298/227	224/213	222/214
Birth weight, g	3485.0 (472.6)	3664.6 (516.7)	3494.5 (475.4)	3668.3 (523.2)
Weight, kg	40.4 (9.0)	38.7 (7.4)	62.8 (11.7)	74.1 (12.1)
Height, cm	148.2(7.1)	147.1 (6.3)	167.4 (6.2)	181.2 (6.3)
BMI, kg/m ²	18.2 (3.1)	17.8 (2.7)	22.4(3.9)	22.6 (3.5)
Waist circumference, cm	63.7 (7.8)	64.5 (7.4)	74.1 (9.4)	80.8 (8.7)
Stage of puberty (M/G), range 1-5	1.7 (0.6)	1.6 (0.5)	5.0 (0.0)	5.0 (0.2)
Systolic blood pressure, mmHg	107.0 (10.6)	105.8 (9.8)	114.6 (11.6)	127.3 (12.5)
Diastolic blood pressure, mmHg	58.2 (6.0)	58.7 (6.4)	64.9 (7.1)	66.0 (8.0)
Pulse pressure, mmHg	48.8 (9.6)	47.1 (8.4)	49.7 (8.7)	61.3 (10.5)
Total cholesterol, mmol/L	4.6 (0.8)	4.4 (0.7)	4.6 (0.8)	4.1 (0.8)
LDL-C, mmol/L	2.9 (0.7)	2.8 (0.6)	2.6 (0.7)	2.4 (0.7)
HDL-C, mmol/L	1.27 (0.2)	1.3 (0.3)	1.5 (0.3)	1.2 (0.2)
Triglycerides, mmol/L	0.86 (0.5)	0.7 (0.4)	1.1 (0.5)	1.1 (0.6)
Glucose, mmol/L			4.7 (0.3)	4.9 (0.4)
Insulin, mU/L			7.2 (3.2)	7.3 (3.4)
HOMA-IR			1.5 (0.7)	1.6 (0.8)
Sodium, g/day	2.4 (0.6)	2.7 (0.6)	2.5 (0.7)	3.6 (1.1)
SAFA, E%	12.4 (2.9)	12.3 (2.7)	11.8 (2.7)	12.3 (3.0)
PUFA, E%	5.5 (1.4)	5.7 (1.6)	6.0 (1.8)	6.3 (2.0)
(PUFA+MUFA)/SAFA	1.4 (0.4)	1.4 (0.3)	1.5 (0.4)	1.5 (0.4)
Fiber, g/day	13.9 (4.2)	14.5 (4.2)	15.6 (6.2)	17.4 (6.9)
Physical activity, MET h/wk			21.7 (19.9)	26.1 (24.4)
Smoking, \geq once a week, %			16.8	22.1
Ideal CVH score, range 0-7			4.0 (1.2)	4.0 (1.1)
Aortic distensibility, %/10mmHg	5.7 (1.8)	5.5 (1.7)	3.2 (1.0)	2.3 (0.8)
Carotid distensibility, %/10mmHg	4.9 (1.0)	4.6 (1.1)	3.7 (0.8)	3.1 (0.6)
Aortic IMT, mm	0.5 (0.1)	0.5 (0.1)	0.5 (0.1)	0.5 (0.1)
PWV, m/s			6.4 (0.7)	6.8 (1.2)
LVM, g			109.6 (22.8)	145.2 (33.8)
LVM /BSA			64.3 (11.1)	74.8 (14.9)
LVM index, g/m ^{2.7}			27.3 (5.6)	29.2 (6.8)

BMI, body mass index; BSA; body surface area, E% percentage of energy intake; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment of insulin resistance; Ideal CVH score, Ideal cardiovascular health score; IMT, intima-media thickness; LDL-C, low-density lipoprotein cholesterol; LVM, left ventricular mass; MET metabolic equivalent; N, cohort size; n, number of subjects participating in ultrasonography, PUFA, polyunsaturated fatty acids; (PUFA+MUFA)/SAFA, the polyunsaturated fatty acid (PUFA)+monounsaturated (MUFA)-to-saturated fatty acid (SAFA) ratio; PWV, pulse wave velocity; SAFA, saturated fatty acids

5.2 Arterial stiffness and left ventricular mass from childhood to early adulthood

5.2.1 Longitudinal progression of arterial distensibility (Study I)

Between the ages of 11 and 19 years, both the aortic and carotid distensibility decreased with advancing age (for both arteries: $P < 0.0001$) (Figure 7a, b and 8a, b). Boys generally had lower distensibility than girls ($P < 0.0001$), and they showed a steeper decrease in distensibility with age (age \times sex interaction: for both arteries $P < 0.01$).

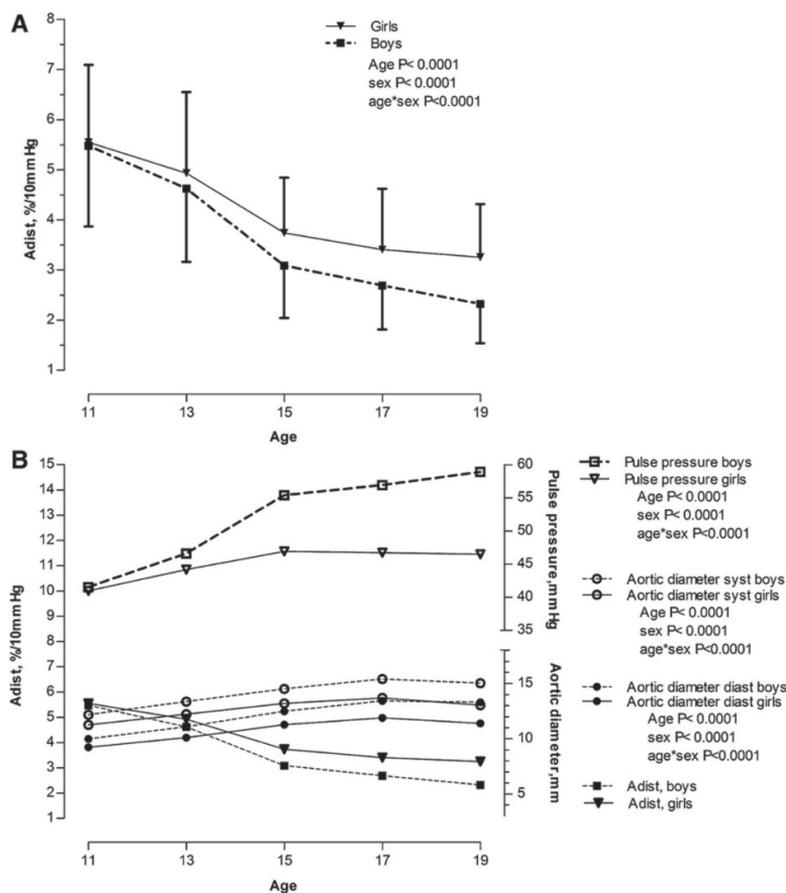


Figure 7a. Mean \pm SD aortic distensibility (Adist) between ages 11 and 19 years in girls and boys. Reproduced from Hypertension (Study I) with permission of Wolters Kluwer Health.

Figure 7b. Components of aortic distensibility (Adist) between the ages 11 and 19 years in girls and boys. Reproduced from Hypertension (Study I) with permission of Wolters Kluwer Health.

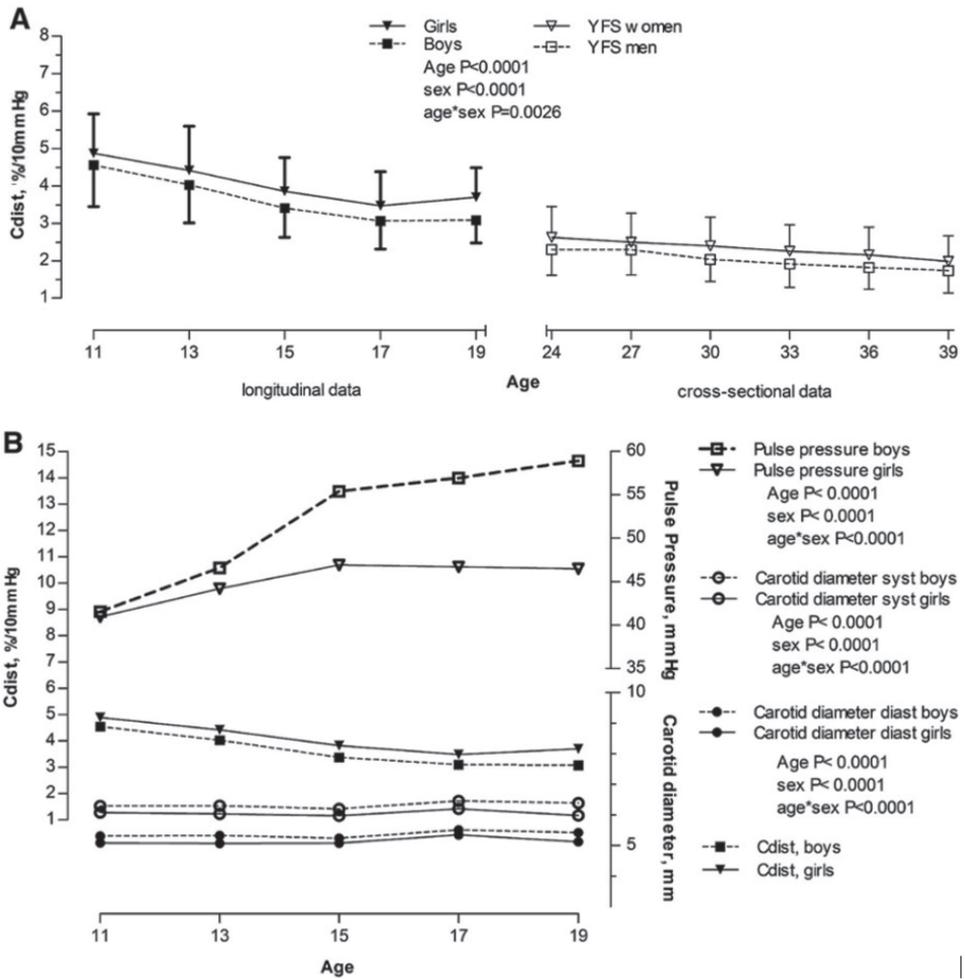


Figure 8a. Mean±SD carotid distensibility (Cdist) between ages 11 and 19 years in the STRIP study participants, and carotid distensibility in women and men of the Cardiovascular Risk in Young Finns Study.(Juonala et al. 2005). Reproduced from Hypertension (Study I) with permission of Wolters Kluwer Health.

Figure 8b. Components of the carotid distensibility (Cdist) between ages 11 and 19 years in boys and girls. Reproduced from Hypertension (Study I) with permission of Wolters Kluwer Health.

Since the significant age×sex interaction indicated that the effect of age on arterial distensibility was different in boys and girls, this sex difference was studied in more detail. At the age of 11 years, aortic distensibility was similar in boys and girls ($P=0.53$), whereas at the age of 13 years, the difference between boys and girls became borderline significant ($P=0.055$), and thereafter boys had lower distensibility than girls ($P<0.0001$). The difference between boys and girls in carotid distensibility was evident already at the age of 11 years ($P=0.002$) and it became even more pronounced with advancing age (13 years: $P=0.0005$; 15 to 19 years: $P<0.0001$). The sex difference remained highly significant when the modified aortic and carotid distensibilities using estimated central pulse pressure were evaluated (data not shown).

To gain more insights into the sex difference in distensibility, the components of arterial distensibility, pulse pressure and arterial diameter, were studied by including them in the analyses as explanatory variables (Figure 7b and 8b). The model including age and sex was first adjusted for pulse pressure, and secondly for arterial diameter. When pulse pressure was added into the model, the sex difference in both aortic and carotid distensibility disappeared ($P_{\text{aorta}}=0.14$, $P_{\text{carotid}}=0.075$) whereas when the adjustment was made for arterial diameter, the sex difference persisted in both arteries (aortic systolic and diastolic diameter: P for sex <0.0001 and P for sex $=0.020$; carotid artery systolic and diastolic diameter: both P for sex <0.0001). Furthermore, the effect of age on pulse pressure differed in boys and girls (age×sex interaction; $P<0.0001$): in girls, pulse pressure increased by 5.8 mmHg between the ages 11 and 19 years, whereas in boys, the increase was more than three times greater, 17.5 mmHg (Figure 7b and 8b).

In an attempt to gain deeper insights into the sex difference in pulse pressure, and distensibility, it was studied if the greater increase in pulse pressure in boys was driven by larger changes in the boys' body size as compared with the girls. After adjustment for weight and height, male sex remained significantly associated with lower aortic and carotid distensibility (both $P<0.0001$). These results indicate that the sex difference in pulse pressure is not only a reflection of the greater increase in boys' body size.

The underlying factors of the sex difference in pulse pressure and distensibility were examined further by assessing the stroke volume as it is one of the determinants of pulse pressure. In this study, these data were available at one time point, at age 19 years. Boys had a greater stroke volume than girls (mean±SE for boys: 95.15±14.86 ml; girls: 74.45±12.97 ml, $P<0.0001$), but the sex difference in distensibility persisted after adjustment for stroke volume (aortic distensibility: $\beta=-0.77$, $P<0.0001$, carotid distensibility: $\beta=-0.40$, $P<0.0001$), indicating that the sex difference in distensibility is unlikely to be explained by a difference in stroke volume.

In addition to studying what lies behind the sex difference in distensibility and pulse pressure, the association of pubertal stage with distensibility was investigated to find out if pubertal stage explained the decrease in distensibility with age. Aortic distensibility

decreased with increasing pubertal stage while no association was found between pubertal stage and carotid distensibility when age and sex were included into the analyses (aortic: $P=0.047$; carotid: $P=0.16$). With respect to aortic distensibility, the pubertal stage \times sex interaction was significant ($P<0.0001$) and therefore sex-stratified analyses were performed. In the sex-stratified analysis, the association of pubertal stage was, however, no longer significant in boys ($P=0.15$) or in girls ($P=0.24$).

The stability of aortic and carotid distensibility was studied by examining the correlations between the repeated distensibility measurements. Aortic and carotid distensibility measured at the age of 11 years correlated moderately with subsequent measurements at age 13, 15, 17, and 19 years (Figure 9, aortic distensibility: all $P<0.05$, carotid distensibility: all $P<0.02$). The correlations between the distensibility of the aorta and carotid artery were also estimated and they ranged from $r=0.25$ to $r=0.38$ during the follow-up (all $P<0.0001$).

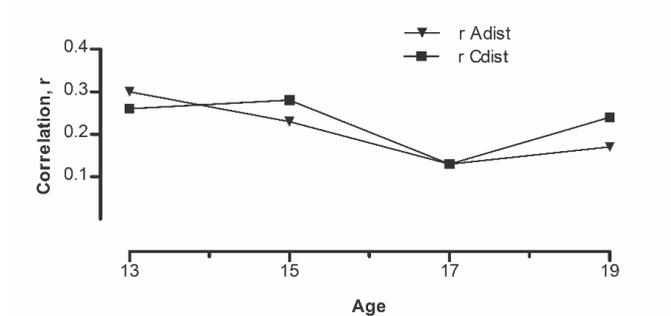


Figure 9. Correlations of aortic (Adist) and carotid distensibility (Cdist) measured at the age of 11 years with subsequent measurements at the age of 13, 15, 17 and 19 years.

PWV

In addition to longitudinal data on aortic and carotid distensibility, PWV was assessed at the age of 19 years. Boys had a higher PWV than girls, further evidence that males have stiffer arteries than females in early adulthood (median [interquartile range]; girls: 6.30 [4.50] m/s, boys: 6.50 [5.80] m/s; $P<0.0001$).

5.2.2 Longitudinal progression of left ventricular mass (Study I)

Between the ages of 15 to 19 years, LVM increased with advancing age and boys had a greater LVM than girls (both $P<0.0001$, Figure 10). In girls, LVM increased by 3.2 g between the age of 15 and 19 years (from 106.4 g to 109.6 g), whereas in boys, the increase was 13.9 g (from 131.3 g to 145.2 g) (P for age \times sex interaction <0.0001).

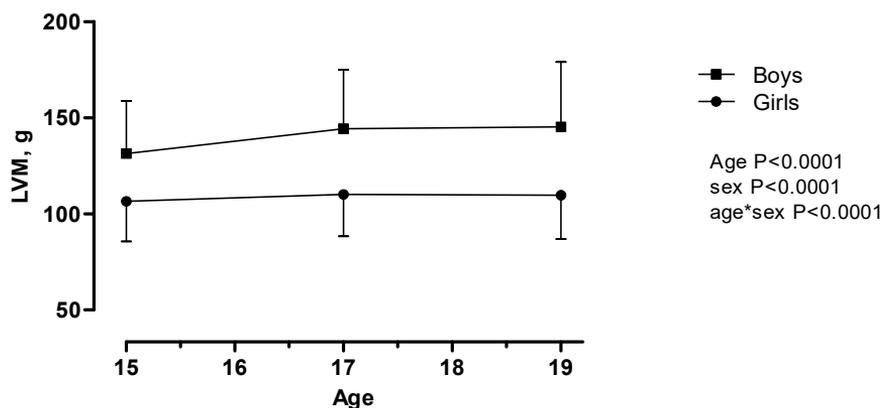


Figure 10. Mean±SD LVM in between ages 15 and 19 years in boys and girls. Reproduced from Hypertension (Study I) with permission of Wolters Kluwer Health.

5.2.3 Association between arterial distensibility and LVM (Study I)

Aortic and carotid distensibility were inversely associated with LVM (aorta: $\beta = -4.28$, SE 1.75, $P = 0.015$; carotid: $\beta = -5.81$, SE 2.59, $P = 0.025$; adjusted for sex and age). When LVM was indexed for BSA, there was no longer any association between the distensibilities and LVM (aorta: $\beta = -1.12$, SE 0.90, $P = 0.21$; carotid: $\beta = -1.60$, SE 1.32, $P = 0.23$; adjusted for sex and age).

5.3 Cardiometabolic determinants of subclinical markers of cardiovascular disease

5.3.1 Cardiometabolic determinants of arterial distensibility (Study II)

In the age and sex adjusted analyses, BMI, waist circumference, and systolic and diastolic blood pressure were inversely associated with carotid and aortic distensibility between ages 11 and 19 years (Figure 11). The components of BMI, weight and height, were also inversely associated with carotid distensibility (weight: $\beta = -0.0031$, SE 0.00079, $P < 0.0001$; height: $\beta = -0.0043$, SE 0.000573, $P < 0.0001$) and aortic distensibility (weight: $\beta = -0.0095$, SE 0.0011, $P < 0.0001$; height: $\beta = -0.0082$, SE 0.00080, $P < 0.0001$). Additionally, serum levels of total cholesterol, LDL cholesterol, apo B and triglycerides were inversely associated with carotid distensibility. LDL cholesterol, apo B and triglycerides also tended to show an inverse association with aortic distensibility. Serum HDL cholesterol displayed a direct association with aortic distensibility.

Serum glucose, insulin and HOMA-IR, studied beginning when the subjects were aged 15, were inversely associated with carotid distensibility. Insulin and HOMA-IR showed also a tendency towards an inverse association with aortic distensibility.

Dietary sodium intake was inversely associated with aortic distensibility. No other associations between dietary variables or physical activity with arterial distensibility were found. Family SES was also not associated with carotid distensibility [age and sex adjusted mean (SE) for SES group 1: 1.26 (0.043), 2: 1.32 (0.014), 3: 1.30 (0.012), 4: 1.30 (0.012), P=0.47] or aortic distensibility [age and sex adjusted mean (SE) for SES group 1: 1.27 (0.061), 2: 1.27 (0.020), 3: 1.29 (0.016); 4: 1.25 (0.016), P=0.36].

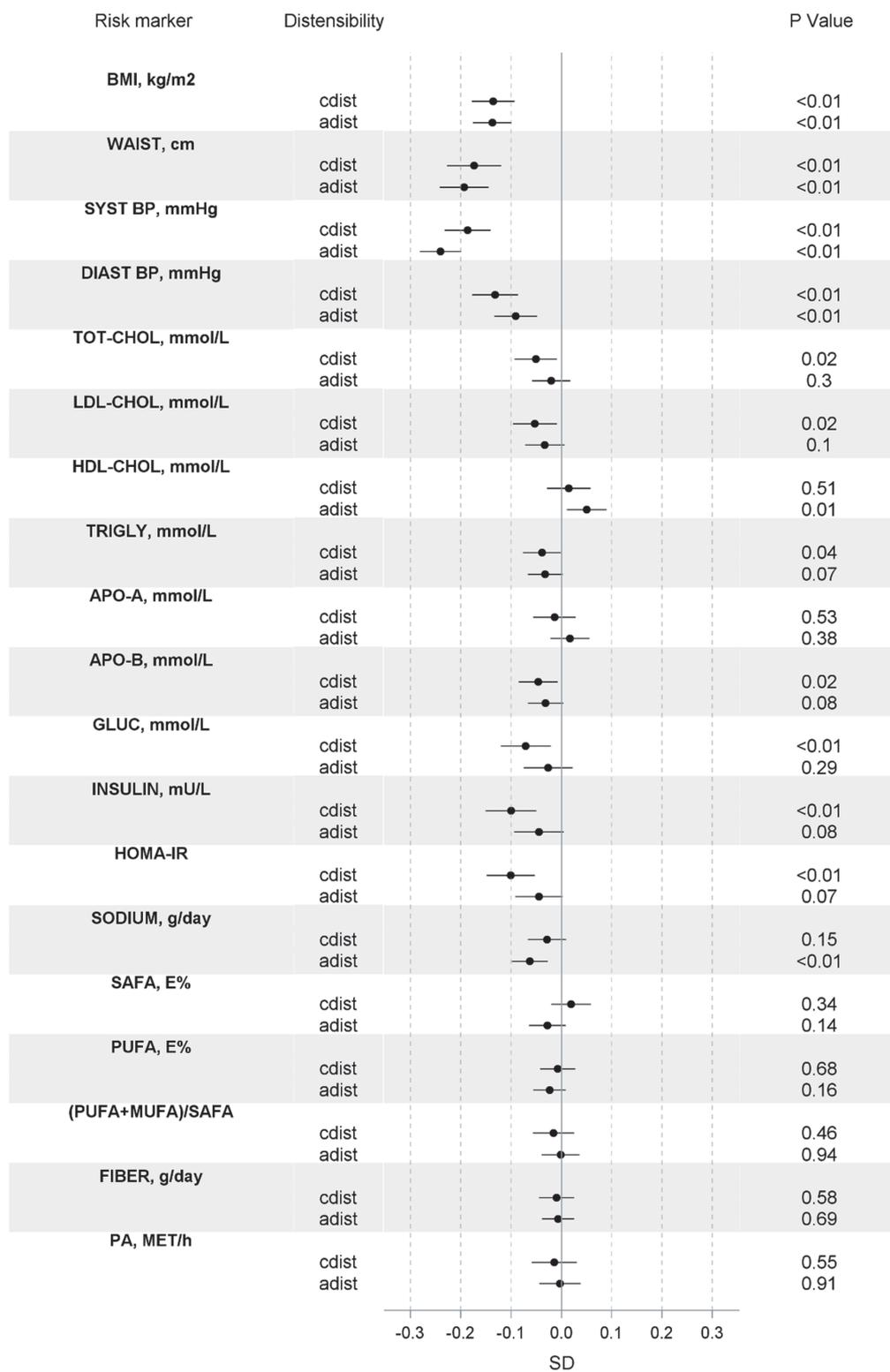


Figure 11. Age and sex adjusted model for standardized cardiometabolic and dietary risk marker associations with standardized carotid and aortic distensibility. The figure shows the association of 1 SD change in the risk marker with SD scaled change in arterial distensibility. Error bars indicate 95% confidence intervals. Bars on the right side of the middle line (SD 0.0) indicate that the association between the risk marker and distensibility is direct and bars on the left side of the middle line indicate an inverse association. Confidence interval bars crossing the middle line indicate a statistically non-significant association. P-values are obtained from a linear mixed-effects model for repeated measures.

Adist, aortic distensibility; Apo-A1, apolipoprotein A1, Apo-B, apolipoprotein B; BMI body mass index; Cdist, carotid distensibility; Diast BP, diastolic blood pressure; E% percentage of energy intake; Gluc, serum glucose; HDL-CHOL, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment of insulin resistance; LDL-CHOL, low-density lipoprotein cholesterol; MET, metabolic equivalent; PA, physical activity; PUFA, polyunsaturated fatty acids; (PUFA+MUFA)/SAFA, the polyunsaturated fatty acid (PUFA)+monounsaturated (MUFA)-to-saturated fatty acid (SAFA) ratio; SAFA, saturated fatty acids; Syst BP, systolic blood pressure; TG, serum triglycerides; TOT-CHOL, serum total cholesterol.

Risk markers that were associated with carotid or aortic distensibility in the age and sex adjusted analyses (Figure 11) were further studied in an attempt to detect independent associations. Systolic blood pressure was chosen to represent blood pressure, BMI body size and HOMA-IR glucose homeostasis. Since serum glucose and insulin data were available from age 15 years, two analyses were performed.

In the first main multivariable model between ages 15 and 19 years, higher systolic blood pressure, LDL cholesterol, age and male sex were independently associated with lower carotid and aortic distensibility (Table 3). Additionally, higher values of HOMA-IR and higher BMI were independently associated with lower carotid distensibility. When BMI was replaced with waist circumference and LDL cholesterol with Apo B or total cholesterol, and birth weight was added to the model, the results were essentially the same (data not shown).

In the second model including ages 11 and 13, higher systolic blood pressure and age were independently associated with lower carotid distensibility and aortic distensibility. Additionally, male sex was associated with lower carotid distensibility and higher BMI with lower aortic distensibility.

Table 3. Multivariable models between ages 15-19 years of the cardiometabolic and dietary risk marker associations with arterial distensibility (log-transformed, %/10mmHg). The models include risk markers showing a significant association with carotid or aortic distensibility in age and sex adjusted models (Figure 11). Systolic blood pressure represents blood pressure and HOMA-IR glucose homeostasis.

Risk marker	Carotid distensibility			Aortic distensibility		
	β^*	SE	<i>P</i>	β^*	SE	<i>P</i>
BMI, kg/m²	-0.0056	0.0027	0.037	-0.0063	0.0039	0.10
Syst BP, mmHg	-0.0025	0.00065	0.0001	-0.0069	0.00097	<0.0001
LDL-C, mmol/L	-0.026	0.012	0.034	-0.039	0.018	0.031
HDL-C, mmol/L	-0.053	0.031	0.092	-0.045	0.046	0.32
Triglycerides, mmol/L	0.0037	0.019	0.85	0.0069	0.029	0.81
HOMA-IR	-0.048	0.018	0.0071	0.011	0.027	0.68
Sodium, g/day	0.0000019	0.0000085	0.82	-0.0000077	0.000013	0.55
Age, y			<0.0001			<0.0001
15y	0.049 [†]	0.018		0.15 [†]	0.028	
17y	-0.058 [†]	0.017		0.051 [†]	0.026	
Sex (girls vs. boys)	0.13	0.021	<0.0001	0.19	0.030	<0.0001
Model R²			0.33			0.41

*) Regression coefficients (SE) for a 1-unit change in the covariates. The regression coefficients can be transformed by using the equation $x=e^{\beta}$ and then interpreted as a percentage change in non-log-transformed distensibility. †) Compared with 19 years of age

BMI, body mass index; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment of insulin resistance; LDL-C, low-density lipoprotein cholesterol; Syst BP, systolic blood pressure. R²: R-Square.

Subjects with low carotid or aortic distensibility as determined using the repeated data had higher BMI values, and higher systolic and diastolic blood pressures since early childhood compared to those determined to have a normal distensibility (Figure 12). An additional comparison of extreme distensibility groups (those in the lowest and highest quintile) showed even more pronounced differences in life-course BMI and blood pressure (Figure 12).

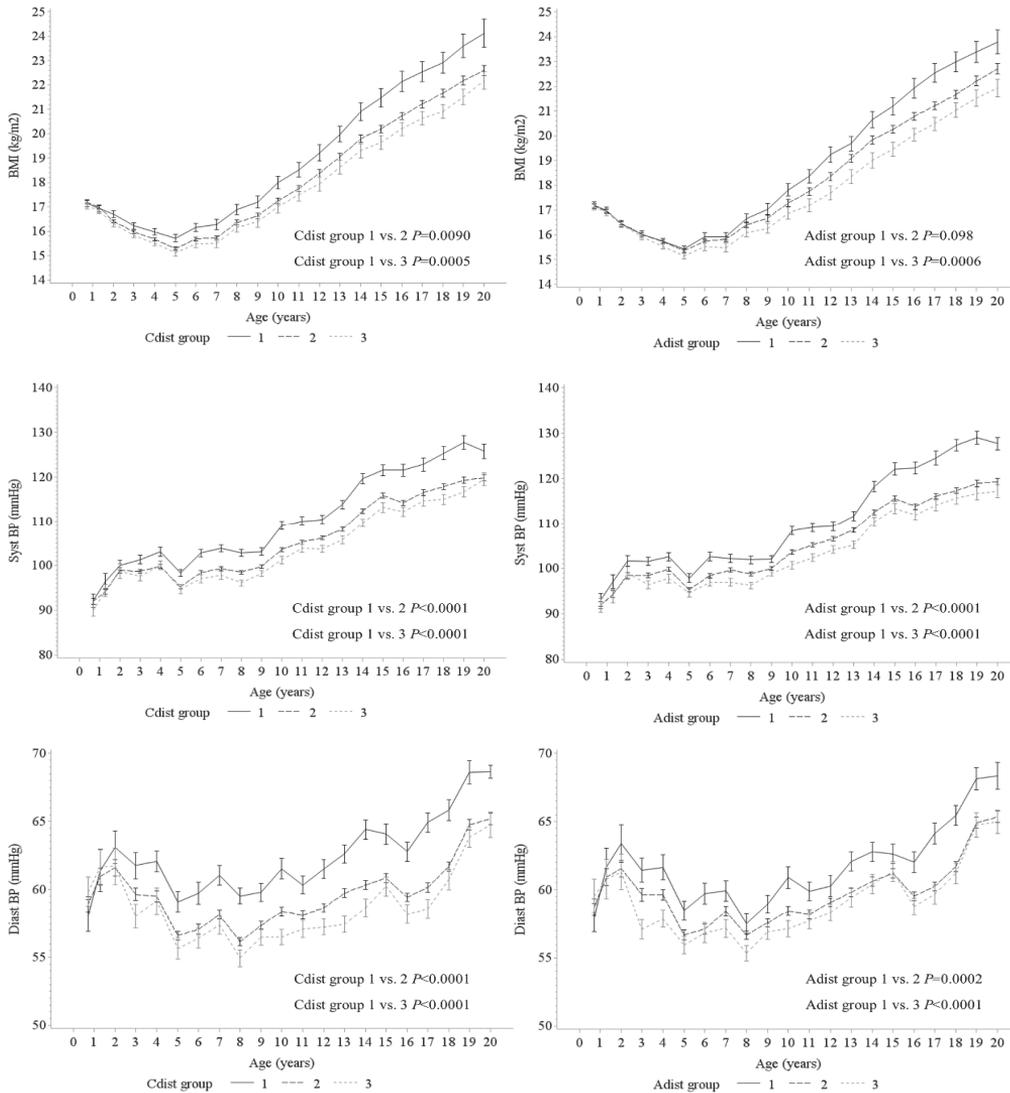


Figure 12. BMI, and systolic and diastolic blood pressure measured from early childhood in the subjects with low (group 1) or normal (group 2) carotid or aortic distensibility (Cdist or Adist, respectively). Low arterial distensibility was defined as the lowest 20th percentile of the age and sex standardized mean values (group 1, solid black line). Subjects with distensibility >20th percentile were considered to have normal distensibility (group 2, dashed black line). Of the subjects with normal distensibility, those with high distensibility were further categorized using 80th percentile as the cut-off point (group 3, dashed gray line). In the analyses, comparisons are made to the low distensibility group 1. Reproduced from Hypertension (Study II) with permission of Wolters Kluwer Health.

5.3.2 Determinants of left ventricular mass (Study III)

Birth weight, birth height and early growth (child's weight gain from birth to the age of 2 years and the increase in height from birth to the age of 7 months) were directly associated with LVM at age 15 years (univariate analyses, Table 4). LVM was also directly associated with male sex, current weight, height, BMI and waist circumference. Adolescents who were overweight had an LVM that was 16.6 grams heavier than those with normal BMI ($P < 0.0001$). LVM increased with increasing blood pressure; with respect to the blood pressure variables, pulse pressure showed the strongest association with LVM. Pulse pressure since the age of 7 months until the age of 15 years (lifetime pulse pressure) was also directly associated with LVM in adolescence. Furthermore, LVM increased with increasing physical activity level.

Table 4. Correlates of LVM at 15 years of age (boys n=217, girls n=201).

Study variables	Univariate model			Adjusted model ^(*)		
	$\beta^{\dagger, \ddagger} \pm SE^{\S}$	<i>P</i>	R ²	$\beta \pm SE^{\S}$	<i>P</i>	R ²
Birth weight, g	0.015±0.002	<0.0001	0.083	0.0077±0.002	0.0001	0.46
	5			0		
Birth height, cm	3.75±0.58	<0.0001	0.092	1.51±0.47	0.0014	0.45
Δweight; birth to 7 mo, kg	6.91±1.65	<0.0001	0.041	-1.89±1.36	0.16	0.44
Δweight; birth to 13 mo, kg	6.22±1.41	<0.0001	0.045	-1.89±1.18	0.11	0.44
Δweight; birth to 2 y, kg	4.84±1.065	<0.0001	0.049	-1.026±0.93	0.27	0.45
Δheight; birth to 7 mo, cm	1.68±0.64	0.0088	0.017	-0.63±0.50	0.21	0.44
Δheight; birth to 13 mo, cm	1.12±0.59	0.056	0.008	-0.69±0.45	0.13	0.44
Δheight; birth to 2 y, cm	0.97±0.52	0.064	0.008	-0.73±0.42	0.083	0.45
			5			
Male sex	24.5±2.4	<0.0001	0.21	20.4±2.0	<0.0001	0.43 ^(*)
Weight, kg	1.40±0.11	<0.0001	0.29	1.25±0.096	<0.0001	0.43 ^(*)
BMI, kg/m ²	2.95±0.41	<0.0001	0.11	3.48±0.35	<0.0001 ^()	0.36 ^()
Waist, cm	1.43±0.16	<0.0001	0.17	1.21±0.14	<0.0001 ^()	0.32 ^()
Height, cm	1.71±0.14	<0.0001	0.26	0.40±0.17	0.017	0.44
Syst BP mmHg	1.25±0.13	<0.0001	0.19	0.31±0.13	0.016	0.44
Diast BP, mmHg	0.60±0.26	0.024	0.012	-0.0028±0.20	0.99	0.43
PP, mmHg	1.64±0.16	<0.0001	0.21	0.50±0.16	0.0021	0.45
Lifetime PP, mmHg	7.69±2.41	0.0015	0.024	-0.081±1.98	0.97	0.43
Stage of puberty (M/G)	na	0.0044	0.033	na	0.078	0.46
Physical activity level (group 1-3)	5.51±1.65	0.0009	0.030	4.41±1.27	0.0006	0.43
STRIP intervention group	-0.98±2.66	0.71	0.000	-0.89±2.00	0.65	0.44
			3			
FGR	15.2±3.83	<0.0001	0.038	5.65±2.99	0.059	0.44

Diast BP, diastolic blood pressure; FGR, fetal growth restriction; birth weight below the 10th percentile for gestational age and sex of the total STRIP-study cohort; PP, pulse pressure; RWT, relative wall thickness at end diastole; R²: partial R-square; Syst BP, systolic blood pressure

^(*) Adjusted for sex and weight, baseline R² for adjusted model including sex and weight 0.43

^(†) Regression coefficients for a change in LVM when the explanatory variable increases one unit

^(‡) Difference of group means for dichotomous variables

^(§) Estimated regression coefficients±SE for a 1-unit change in the covariate.

^(||) Adjusted only for sex

Due to the sex difference in LVM and the strong impact of weight on LVM, an adjustment was made for sex and weight (in the cases of waist circumference and BMI, the adjustment was made only for sex) (Table 4, adjusted model). After the adjustment, birth weight ($P=0.0001$), birth height ($P=0.0014$), current height ($P=0.017$), waist circumference ($P<0.0001$), BMI ($P<0.0001$), systolic blood pressure ($P=0.016$), pulse pressure ($P=0.0021$) and physical activity level ($P=0.0006$) remained associated with LVM. For every 100 grams increase in birth weight, LVM increased by 0.77 grams and for every 1 mmHg increase in pulse pressure, LVM increased by 0.50 grams. After adjusting for sex and weight, early growth, pubertal status and diastolic blood pressure were no longer associated with LVM. The results remained similar when BMI was used instead of weight.

The variables that were associated with LVM after adjusting for sex and weight were entered into a multivariable model. For parallel variables (e.g. systolic blood pressure, diastolic blood pressure and pulse pressure) that had a strong correlation ($r>0.75$) with LVM, only the one with the highest P-value was incorporated into the model. Thus, sex, weight, height, pulse pressure, physical activity level and birth weight were included in the final model (Table 5). In the analysis, all these determinants, with the exception of height, were independently associated with LVM. The results were similar when weight was replaced with waist circumference (Model $R^2=0.44$) or when birth height was used instead of birth weight (Model $R^2=0.46$) in the final model as shown in Table 5. The associations also remained similar when the final model was further adjusted for gestational age (data not shown).

Table 5. Determinants of LVM at the age of 15 years (a multivariable model).

Covariates	$\beta \pm SE^*$	P	R^2
Birth weight, 100 g	0.74 \pm 0.0021	0.0004	0.025
Male sex	12.0 \pm 2.58	<0.0001	0.13
Weight, kg	1.0 \pm 0.13	<0.0001	0.29
Height, cm	0.34 \pm 0.18	0.055	0.00
Pulse pressure, mmHg	0.42 \pm 0.17	0.013	0.010
Physical activity level (groups 1 to 3)	3.8 \pm 1.23	0.0024	0.016
			Total model
			0.47

R^2 : Partial R-square

*Estimated regression coefficients \pm SE for a 1-unit change in the covariate.

In order to gain further insights into this phenomenon, the association of the independent determinants of LVM (Table 5) with left ventricular septal thickness and posterior wall thickness was investigated (Table 6). In the analyses, weight was directly associated with both left ventricular septal thickness and posterior wall thickness, and physical activity level and birth weight were directly associated with posterior wall thickness. Boys had a thicker septum than girls. Sex, current body size, blood pressure, pubertal stage, physical activity level or birth size were not associated with the relative wall thickness of the left ventricle.

Table 6. Association of the independent determinants of LVM with the septal thickness and posterior wall thickness of the left ventricle.

Study variables	Septum			Posterior wall		
	$\beta \pm SE^*$	<i>P</i>	R^2	$\beta \pm SE^*$	<i>P</i>	R^2
Birth weight, 100 g	0.0015 \pm 0.00098	0.13	0.0061	0.000025 \pm 0.000093	0.0066	0.017
Male sex	0.032 \pm 0.012	0.0092	0.034	0.020 \pm 0.012	0.085	0.024
Weight, kg	0.0026 \pm 0.00059	<0.0001	0.099	0.0027 \pm 0.00056	<0.0001	0.12
Height, cm	0.00010 \pm 0.00084	0.90	0.0000	-	0.93	0.0000
PP, mmHg	0.00003 \pm 0.00080	0.97	0.0000	0.000066 \pm 0.00080	0.18	0.0041
Physical activity level (group 1-3)	0.0089 \pm 0.0059	0.13	0.0056	0.0010 \pm 0.00076	0.0017	0.030
			Total model			Total model
			0.14			0.20

PP: Pulse pressure; R^2 : partial R-Square

*Estimated regression coefficients \pm SE for a 1-unit change in the covariate.

Of several potential determinants, weight, BMI and waist circumference were associated with LVM geometric patterns. Adolescents who had eccentric remodeling or concentric, increased LVM had a higher BMI than those with normal left ventricular geometry.

5.4 Ideal cardiovascular health and its association with subclinical markers of cardiovascular disease (Study IV)

5.4.1 Ideal cardiovascular health in adolescence

None of the adolescents had all 7 ideal cardiovascular health metrics and the number of ideal metrics decreased between the studied ages from 15 to 19 years (Figure 13). Boys and girls had similar ideal cardiovascular health scores (mean±SD score: girls 4.29±1.16, boys 4.37±1.11; P=0.44). If one considers the individual metrics, then nearly all of adolescents had an ideal glucose concentration, while the criterion for ideal diet was least often met. When the components included in the criteria for ideal diet were investigated, an ideal intake of whole grains was met by half of the adolescents, but practically none of them met the goal of ideal sodium intake. Adolescents with a low ideal cardiovascular health score (≤ 3 ideal metrics) at the age of 15 had a higher risk of having a low score also at the age of 19 when compared with their 15-year-old peers with a higher score (RR=1.89, 95%CI=1.13 to 3.14, P=0.015).

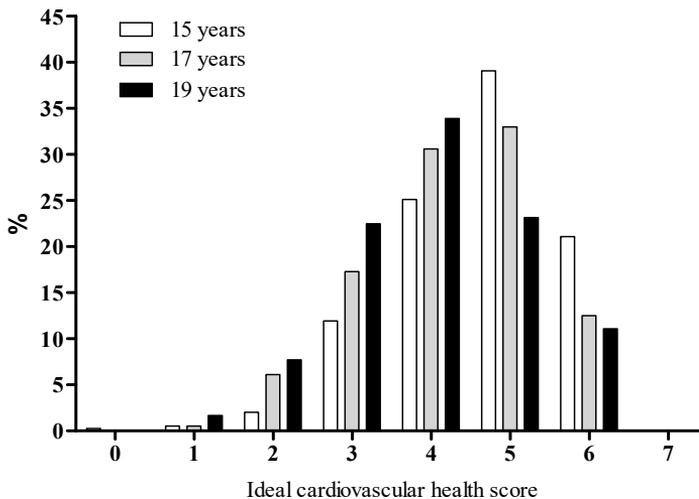


Figure 13. Ideal cardiovascular health score at the age of 15, 17 and 19 years. The number of ideal metrics decreased with age ($P < 0.0001$, sex included in the analysis). Reproduced from *Circulation* (Study IV) with permission of Wolters Kluwer Health.

5.4.2 Association of ideal cardiovascular health with aortic intima-media thickness and distensibility

The ideal cardiovascular health score was favorably associated with aortic IMT and distensibility during adolescence (Figure 14). There was no age×score interaction (IMT $P=0.24$, distensibility $P=0.63$) indicating that the association of the ideal cardiovascular health score was similar at ages of 15, 17 and 19. The association of the score with aortic IMT and distensibility was similar also in the intervention and control group participants (study group×score interaction: IMT $P=0.56$, distensibility $P=0.71$). The risk of having a high aortic IMT was increased in adolescents with a low ideal cardiovascular health score compared with those with a higher score (RR=1.78, 95%CI=1.31 to 2.43, $P=0.0002$; adjusted for age and sex).

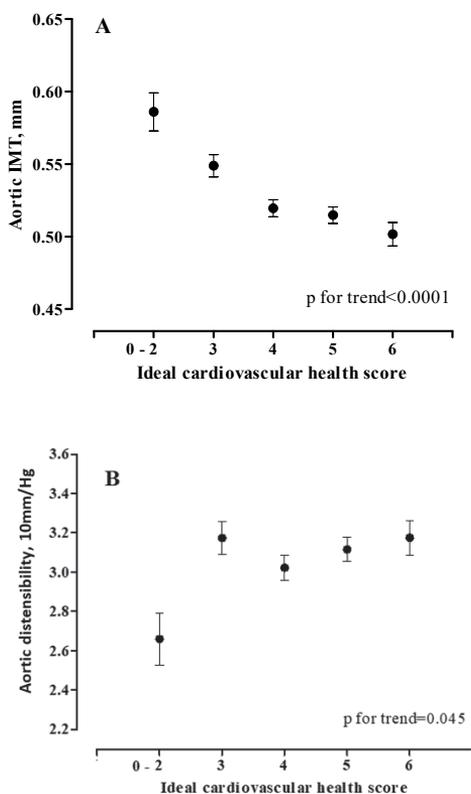


Figure 14. Age and sex adjusted mean (SE) aortic intima-media thickness (A) and distensibility (B) according to the number of ideal cardiovascular health metrics. Adolescents with 0 or 1 ideal metrics are combined with those who have 2 ideal metrics. Reproduced from *Circulation* (Study IV) with permission of Wolters Kluwer Health.

5.5 Effect of dietary and lifestyle intervention (Studies I, II, IV)

Adolescents in the STRIP intervention group had more often high than low ideal cardiovascular health scores compared with the control group participants (Figure 15). The risk of having a low ideal cardiovascular health score was 1.35 times higher in the control children than in the intervention children (RR=1.35, 95%CI=1.04 to 1.77, P=0.026).

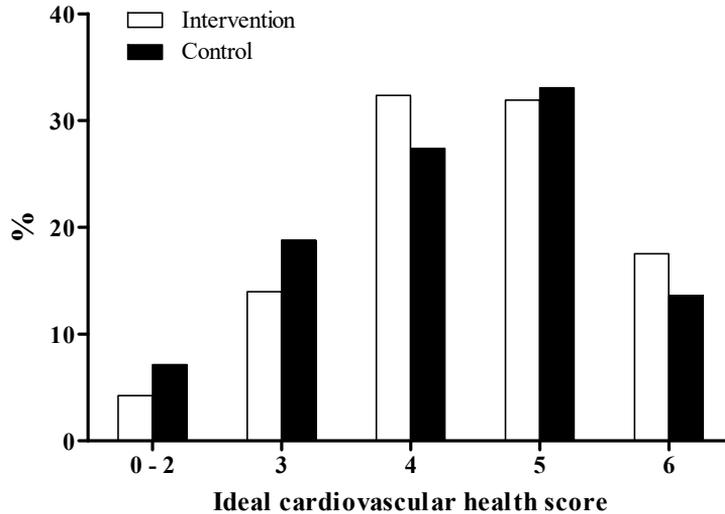


Figure 15. Mean prevalence of ideal cardiovascular health score at age 15, 17 and 19 years in the intervention and control group participants (P=0.034, age included in the analysis). Adolescents with 0 or 1 ideal metrics are combined with those who have 2 ideal metrics. Reproduced from *Circulation* (Study IV) with permission of Wolters Kluwer Health.

If one examines the individual metrics, then adolescents in the intervention group had more often ideal cholesterol (P=0.027, mean prevalence 71.4% vs. 64.2%) and blood pressure (P=0.041, mean prevalence 85.6% vs. 81.0%) than the control group participants. The intervention effect on ideal BMI was of borderline significance (P=0.077). No intervention effect was found for ideal smoking or physical activity. The association of the intervention with diet and glucose was not analyzed due to the low prevalence of those having an ideal diet and the high prevalence of those with ideal glucose.

The dietary counseling given in the STRIP study was not associated with carotid or aortic distensibility [age and sex adjusted mean (SE) for log-transformed values; carotid artery: intervention group 1.31 (0.010), control group 1.31 (0.010); $P=0.84$; aorta: intervention group 1.28 (0.014), control group 1.26 (0.013); $P=0.24$]. Participation in STRIP study intervention also exhibited no association with PWV ($P=0.32$; STRIP study group \times sex interaction: $P=0.56$) or with LVM ($P=0.79$; STRIP study group \times sex interaction $P=0.15$).

6 DISCUSSION

The present study shows that arterial stiffness and LVM increase with age already in childhood. Boys have stiffer arteries and greater LVM compared to girls. In this study, current pulse pressure, weight and physical activity, and birth weight were associated with LVM in adolescence, explaining nearly 50% of the variation. Already at a young age, several cardiometabolic risk factors, such as BMI, blood pressure, LDL cholesterol and insulin sensitivity predict increased arterial stiffness. The study also found that ideal cardiovascular health decreased between the ages of 15 and 19 years and those adolescents who had more ideal cardiovascular health metrics at the recommended level had better arterial distensibility and thinner intima-media. Interestingly, cardiovascular health benefits were gained by having at least 4 of the 7 ideal cardiovascular health metrics at the recommended level. Furthermore, adolescents who received dietary and lifestyle intervention had more often better ideal cardiovascular health. The intervention was not, however, related with arterial distensibility or LVM. Figure 16 highlights the main findings of the study.

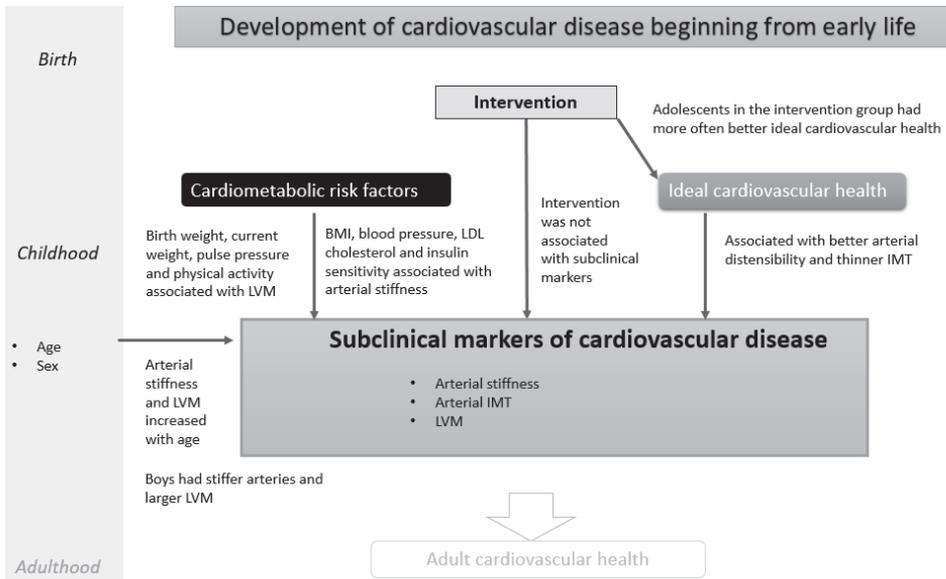


Figure 16. Main findings of the study.

6.1 Study design and subjects

The STRIP study is an extensive longitudinal intervention trial that has continued for over 25 years. It offers a unique possibility to assess subclinical markers of cardiovascular disease, and moreover, to reveal the associations with cardiometabolic risk factors from childhood to early adulthood. The evident strengths of the study are its unique 20-year intervention design with roots in infancy, and the long follow-up of the subjects. The dietary intervention was family-based, which is currently largely viewed as the gold standard for behavioral lifestyle interventions.(Coppock et al. 2014) Furthermore, the relatively large number of subjects have been well phenotyped with well-established methods e.g. physical, laboratory and ultrasound data.

The limitations of the study include loss-to-follow-up, which is inevitable in such long-term studies as the STRIP. In the STRIP study, the main reasons for discontinuance have been moving from the community, a child's recurrent infections, and reluctance to undergo blood sampling. The baseline characteristics of those participating in the study and those lost to follow-up have been compared repeatedly and no major differences have been found e.g. with regard to body weight, BMI, serum total cholesterol or saturated fat intake.(Simell et al. 2009) In addition, loss-to-follow-up analyses regarding components of a cardiometabolic risk factor cluster, the metabolic syndrome, and the STRIP study group showed that discontinuation in the study was not affected by these characteristics.(Nupponen et al. 2015) In addition, those subjects who attended the ultrasound studies did not differ from the subjects in the entire STRIP study cohort with respect to their cardiometabolic risk factors.(Raitakari et al. 2005)

Overall, although a randomized controlled trial is considered the gold standard for a clinical study, a potential limitation is the generalizability of the results at a population level e.g. due to the limited cohort size. In clinical studies, there may also exist unintentional selection of the participants and an unintentional intervention concerning the subjects' interest towards health studies. It is noteworthy that due to the long follow-up period of 20 years, those families continuing in the study may be more health conscious and thus both the intervention and control participants might have better cardiovascular health behaviors and factors than their peers not involved in the study. The participation in a study where all participants are given information on cholesterol levels may have meant that also the control group has been unintentionally intervened, making it more difficult to detect significant differences between the groups.

6.2 Methodological considerations

The key methodological considerations in this study are related to the assessment of arterial stiffness and LVM. Here, the arterial stiffness was assessed locally with

ultrasonography which is a surrogate method rather than the gold standard for these measures in adults.(Laurent et al. 2006) While the measurement of PWV has become the most popular modality for assessment of arterial stiffness in pediatric populations, no validation studies of arterial stiffness measures have been reported in children and adolescents.(Townsend et al. 2015; Urbina et al. 2009) The advantage of locally measured arterial stiffness is that it can be measured directly and non-invasively, at various sites along the arterial tree and it is based on direct measurements of the arterial wall.(Laurent et al. 2006) The aorta is a site of major interest when determining arterial stiffness because the thoracic and abdominal aorta makes the largest contribution to the arterial buffering function, and aortic stiffness has been demonstrated to be an independent predictor of cardiovascular disease in a variety of populations.(Mattace-Raso et al. 2006) Measurement of local carotid stiffness may also provide important prognostic information, since the carotid artery is a frequent site of atheroma formation. Another advantage of ultrasonically assessed arterial stiffness is that arterial IMT and LVM can be measured with the same device, conferring feasibility, which is a desired feature for an assessment method in large studies involving children.

A limitation of this study is that the pulse pressure used to derive distensibility was measured from the brachial artery, not from the artery in question (carotid artery, aorta). The use of brachial pressures may overestimate pulse pressure in central arteries, and furthermore, arterial stiffness may be overestimated. Thus, the guidelines recommend that one should use local pressures in the determination of arterial distensibility.(Laurent et al. 2006) To compensate for the lack of central blood pressure measurement, arterial distensibility was also derived using estimated central pulse pressures.(Mikola et al. 2015) When these distensibility measures were used, the results related to the associations remained similar to those when brachial blood pressure was used, suggesting that the use of brachial data is permissible.(Mikola et al. 2015) Furthermore, previous studies have revealed an excellent correlation between invasively measured blood pressures from the ascending aorta and non-invasively measured blood pressures from the brachial artery, supporting the use of brachial artery pulse pressure when deriving aortic and carotid distensibility.(Borow and Newburger 1982; Reneman et al. 1986) It is also noteworthy that this study and others have reported that the correlations between repeated distensibility measures are relatively weak.(Raiko et al. 2010; Mikola et al. 2015) Instead of being attributable to a measurement error, this discrepancy may be linked to physiological fluctuations in crucial constitutive factors, especially blood pressure and heart rate.

Echocardiography and cardiac magnetic resonance are the best documented imaging modalities which have been used to assess myocardial mass. Although cardiac magnetic resonance imaging has become the gold standard, the echocardiographic measurement of LVM is still a preferred method in clinical practice because it offers the combination of feasibility, accuracy, low cost, and good accessibility.(Armstrong et al. 2012) Especially,

when there is a need to screen or study large populations, the echocardiographic M-mode method has advantages because it is simple, quick, and subject to less measurement variability.(Lang et al. 2015) Overall, there is a large body of evidence to support the accuracy of this method and most studies that relate LVM to prognosis of cardiovascular disease have been based on this method.(Armstrong et al. 2012). However, there are some limitations; the method is based on the assumption that the left ventricle has the shape of an ellipsoid with a symmetric distribution of hypertrophy. Since linear measures are cubed, even small measurement errors have an impact on accuracy. Thus, it is critical that the wall thickness and left ventricular dimensions are measured perpendicular to the long axis of the left ventricle. During the original validation studies of the M-mode technique, it was found that the method overestimated LVM, and therefore the LVM estimation formula includes a correction to allow for an approximated 20% overestimation.(Lang et al. 2015) In this study, the reproducibility of the ultrasonic assessment of LVM was analysed in a subcohort of 57 subjects with the between-observer coefficient of variation for LVM being determined to be 8.5%.

In children the best method for indexing LVM is an area of active investigation.(Chinali et al. 2016) LVM is strongly determined by lean body mass.(Foster et al. 2016) However, most large population studies reporting LVM have indexed to BSA. Because BSA depends clearly on weight, it may obscure the left ventricular hypertrophy associated with obesity. Scaling LVM to height may result in an underestimation of relative LVM in thin individuals and an overestimation among overweight individuals.(Foster et al. 2016) Studies among adults suggest that indexing LVM to height raised to allometric powers such as 1.7, 2.13 and 2.7 offers advantages over indexing to BSA, especially when attempting to predict events in obese patients.

The methods for measuring cardiometabolic risk factors such as blood pressure, weight, height, BMI, and LDL cholesterol and glucose levels are well standardized. Lifestyle factors were assessed with subjective methods that may introduce limitations. For example, physical activity was assessed with self-reported questionnaire and this is prone to difficulties in the estimation of the duration or intensity of physical activity. Dietary intake was collected by using food records, which is a well-established method. However, self-reported food records are prone not only to under-reporting but also to social-desirability bias and social approval, which could result in over-reporting of healthy foods and underreporting of unhealthy foods. In addition, the available food composition data bank may pose limitations, such as natural variability in nutrient content of both biological and processed foods, missing values as well as the limited coverage of certain foods/nutrients, and bioavailability of nutrients. In the STRIP study, the dietary data bank has been continuously updated throughout the study to overcome this shortcoming. Furthermore, the food records were reviewed by a trained nutritionist and entered into the database software by one individual, thereby reducing potential errors and increasing the quality of the data.(Braakhuis et al. 2003)

6.3 Results

6.3.1 *Subclinical markers of cardiovascular disease from childhood to early adulthood*

The process of arterial stiffening with age starts already in childhood and continues relatively steadily. In this study, arterial distensibility decreased with age between the ages of 11 and 19 years. These results are in line with data from the Cardiovascular Risk in Young Finns Study, i.e. indicating that the stiffening process continues until middle age.(Juonala et al. 2005) In a previous cross-sectional study examining 2- to 18-year-old children, age was inversely associated with aortic distensibility, beginning from the age of 2 years.(Hauser et al. 2013) In contrast, it has been demonstrated in a cross-sectional setting that the effect of age on aortic distensibility is not linear; distensibility increased from birth and peaked at about the age of ten years, thereafter decreasing with advancing age, and a distensibility equal to that present at birth was usually evident at the age of 18 years.(Laogun and Gosling, 1982) With regard to the carotid artery, a decrease in its distensibility has been reported to begin from the age of 6 years.(Doyon et al. 2013; Jourdan et al. 2005)

In this study, boys had stiffer arteries than the girls. This is in line with a previous cross-sectional study in young adults aged 18 years and older reporting lower brachial artery distensibility in men than in women.(Urbina et al. 2002) A significant sex-difference beginning at the age of 15 years was observed in aortic distensibility. In line with these results, a previous study has demonstrated that aortic distensibility was similar in both sexes from birth to 14-15 years of age but thereafter boys had lower distensibility, a phenomenon that extended into their late forties.(Laogun and Gosling 1982) In an evaluation of 97 children aged 5-18 years, no sex difference was detected in aortic distensibility.(Sarkola et al. 2012) In this study, carotid distensibility was lower in boys already at the age of 11 years and the sex-difference became more pronounced with increasing age. Previously, in a study of children aged 6 to 18 years, a sex-difference in carotid distensibility was reported from the age of 15 years.(Doyon et al. 2013) Taken together, based on the studies of this thesis and previous findings, the evolution of the arterial stiffness in childhood seems to be related to age and sex.

In this study, LVM increased between the ages of 15 to 19 years and boys had greater LVM than girls throughout the follow-up. Similar results have been reported previously, in children aged 4 months to 18 years.(Sarkola et al. 2012) In adults, arterial stiffness is a risk factor for left ventricular hypertrophy. In this study, arterial distensibility was associated with LVM in adolescence. However, when LVM was indexed to BSA, the association became diluted. Therefore, it is unclear whether the relationship between arterial distensibility and LVM is causative in this age group or confounded by body size.

6.3.2 *Cardiometabolic determinants of arterial stiffness and left ventricular mass in childhood*

This thesis demonstrated that several cardiometabolic risk factors associate with stiffer arteries already in childhood. Previously, cardiometabolic risk factors, such as high LDL cholesterol, elevated blood pressure, and obesity, have been associated with arterial stiffness in adults.(Koskinen et al. 2012) Obese children and those with familial hypercholesterolemia have been found to have stiffer arteries at an early age compared with their lean, healthy peers in cross-sectional settings.(Tounian et al. 2001; Aggoun et al. 2000) The results of this study in healthy children indicate that increased blood pressure, BMI, LDL cholesterol and insulin resistance are related to arterial stiffening. Moreover, subjects with low arterial distensibility assessed between ages 11 and 19 years had higher BMI, and higher systolic and diastolic blood pressure already from early childhood. In previous studies, higher blood pressure levels in childhood have been associated with stiffer arteries later in adulthood.(Li et al. 2004a; Juonala et al. 2005; Ferreira et al. 2012) Together these results suggest that already at an early age, increased blood pressure induces adverse arterial effects. In addition, the results emerging from this study in predominantly normal weight children add further evidence to previous cross-sectional findings on the association between adiposity and decreased arterial distensibility in overweight or obese children. In the light of these findings, maintaining normal body weight in childhood may prevent an acceleration of arterial stiffening.

In this study, higher LDL cholesterol concentrations were also associated with lower arterial distensibility between 15 and 19 years of age but this association was not evident at earlier ages. Similar to these findings, higher LDL cholesterol has been shown to associate with stiffer arteries in adults and in children.(Juonala et al. 2005; Whincup et al. 2005; Leeson et al. 2000) Collectively, the data indicate that attempts to maintain normal LDL cholesterol concentration since childhood can be beneficial in preventing arterial stiffening.

Previous reports in adults have shown that insulin resistant subjects have decreased arterial distensibilities.(Salomaa et al. 1995; Giltay et al. 1999; Juonala et al. 2005) Our study further confirms the previous observations between insulin resistance and arterial distensibility and further reveal that insulin resistance may play a role in arterial stiffening already early in life. In the Hoorn study conducted in adults, both impaired glucose metabolism and type 2 diabetes were associated with decreased arterial distensibility.(Henry et al. 2003) Obesity-associated hyperinsulinemia has also been postulated to relate with decreased arterial distensibility in adults.(Salomaa et al. 1995) Similar to the findings of this study, HOMA-IR was found to be an independent determinant of arterial stiffness measured with brachial-ankle PWV in a cross-sectional sample of Japanese adolescents.(Miyai et al. 2009) In addition, in adolescents aged 13 to 15 years, HOMA-IR showed an inverse association with brachial distensibility, while at

earlier ages, no association was observed.(Whincup et al. 2005) In the Cardiovascular Risk in Young Finns Study, higher childhood insulin concentrations predicted lower carotid distensibility in adulthood, but this association disappeared when adjustment was made for the other risk factors associated with distensibility.(Juonala et al. 2005)

To summarize, these findings show that several cardiometabolic risk factors induce adverse effects on arterial stiffness already at an early age and thus further emphasize that cardiovascular disease prevention efforts should be targeted also towards children.

Left ventricular hypertrophy is known to associate with increased morbidity and mortality in both adults and children.(Daniels et al. 1998; Levy et al. 1990; Mitsnefes et al. 2003; Chinali et al. 2006) During childhood, cardiac growth is closely linked with somatic growth.(Lindstedt and Schaeffer 2002; Urbina et al. 1995) In this study, current weight, pulse pressure, physical activity and birth weight were independently associated with LVM in adolescence explaining nearly half of the variation. Current weight was the strongest determinant of LVM, accounting for nearly 30% of the variation. Similarly, weight was associated with the thicknesses of interventricular septum and posterior wall. Here, adolescents with eccentric or concentric left ventricular remodeling had significantly increased BMI and waist circumference compared to those with normal geometry, in line with previous findings in adults.(Toprak et al. 2008) The results from the studies conducted in this thesis provide evidence that the role of normal body weight is particularly important in preventing unfavorable cardiac growth and remodeling already in adolescence.

Previously childhood blood pressure has been associated with LVM in non-hypertensive adolescents.(Li et al. 2004b) In this study, pulse pressure was associated with LVM, but had a weaker explanatory role for LVM than birth weight. For every 100 grams increase in birth weight, LVM increased by 0.77 grams and for every 1 mmHg increase in pulse pressure, LVM increased by 0.50 grams. There are not many studies in healthy children which have investigated the association between birth weight and LVM. Increased birth weight (macrosomia) has been related to ventricular hypertrophy in infants of diabetic mothers.(Ullmo et al. 2007) In contrast, low birth weight is associated with an increased risk for cardiovascular disease in adulthood and also with changes in the cardiac shape even in childhood.(Barker et al. 2005; Crispi et al. 2010) In this study, the association of birth weight with LVM was evident also in children with normal birth weight for gestational age. In a study among 280 healthy African-American adolescents, aged 13 to 18 years, no associations were detected between birth weight and LVM.(Gidding et al. 2014) In that study, data on birth weight were obtained by self-report of the parent, guardian, or participant and they were not verified from birth records as was the case in this study. Moreover, birth weight could not be obtained from 30% of the subjects. The mechanisms that link birth weight and LVM in later life are largely unknown. Changes in stroke volume, cardiac output or afterload due to hemodynamic factors related to birth

weight might contribute to left ventricular growth.(Wong et al., 2004) In addition, endocrine and metabolic programming during intrauterine growth related to the development of the cardiovascular system may partially represent the underlying mechanisms. (Gluckman et al. 2008; Fowden et al. 2005) The association of birth weight with LVM may also be explained by the neurohormonal effects of metabolic factors related to birth size.(Chinali et al. 2006)

In the Athlete's heart, adaptation to the increased hemodynamic load due to physical activity triggers physiological changes in cardiac morphology.(Naylor et al. 2008) In this study, physical activity was directly associated with LVM and posterior wall thickness, showing that also in healthy adolescents, physical activity increases LVM and is specifically associated with the growth of the left ventricle posterior wall.

In summary, the studies included in this thesis provide further evidence for the role of body weight, birth weight, blood pressure and physical activity on LVM. This knowledge will be useful for encouraging implementation of early life interventions targeting these factors.

6.3.3 *Promoting cardiovascular health in childhood*

Ideal cardiovascular health in childhood and its association with subclinical markers of cardiovascular disease

In this study, none of the adolescents had ideal cardiovascular health as listed in the AHA's definition. This was somewhat surprising considering that half of them had received repeated counseling aiming at a healthy diet and primary prevention of smoking. Accordingly, a failure to reach all 7 ideal metrics was reported in 12- to 18-year-old Finnish adolescents studied in 1986.(Laitinen et al. 2012) Previous studies in adults have also reported either a very low or even a non-existing prevalence of ideal cardiovascular health.(Folsom et al. 2011; Yang et al. 2012; Bambs et al. 2011; Ford et al. 2012) According to the National Health and Nutrition Examination Survey 2005-2010 data, only 1.2% of the adults met all 7 metrics while 8.8% of them had 0 to 1 of the ideal behaviors or factors.(Yang et al., 2012) Similar to this study, adherence to an ideal diet was reported as the least often (<1%) met component of the score.(Yang et al. 2012) Similarly, Dong et al. (Dong et al. 2012) showed that only 0.4% of the participants (mean age 69±10 years) were consuming an ideal diet. In particular, the optimum level of sodium intake seems to be very difficult to achieve. In this longitudinal study, the ideal cardiovascular health score declined with age. This is worrisome and reflects the increasing levels of physical inactivity during adolescence, as has been observed also in other cohorts(Tudor-Locke et al. 2010) while fewer adolescents report never to have

smoked with advancing age. Having a low ideal cardiovascular health score at the age of 15 was found to increase the risk of having a low score also 4 years later. These data further support the early initiation of preventive efforts to promote cardiovascular health.

In adults, the ideal cardiovascular health concept has been shown to predict the incidence of cardiovascular disease (Franklin 2005; Lahtinen et al. 2015) and all-cause mortality.(Yang et al. 2012; Palombo and Kozakova 2015) In our study, an ideal cardiovascular health score was favorably associated with arterial IMT and distensibility in adolescents. In adolescents with 3 or fewer of the ideal metrics, the risk of high aortic IMT was 1.8 times higher than in those with at least 4 of the metrics. In line with these results, a previous report from the Cardiovascular Risk in Young Finns study has shown that a higher number of the ideal cardiovascular health metrics in childhood was associated with a reduced risk of high-risk carotid IMT in adulthood.(Laitinen et al. 2012) In addition, a recent report from STRIP and the Cardiovascular Risk in Young Finns study detected an inverse association of ideal cardiovascular health with LVM.(Laitinen et al. 2017) Furthermore, having ideal cardiovascular health in young adulthood and middle age significantly delayed the incidence of major all-cause and cardiovascular morbidity by an average of 4.5 and 6.9 years, respectively, in comparison with individuals with ≥ 2 high risk factors.(Allen et al., 2017) In addition to the incidence of morbidity, on average, individuals who had ideal cardiovascular health lived 3.9 years longer than those with ≥ 2 risk factors at high levels, and they experienced a lower cumulative morbidity burden accompanied with reduced average annual healthcare costs.

Interestingly, in this study, the effect of the score on IMT seemed to almost plateau if an individual had 4 ideal metrics at the recommended level. With respect to distensibility, a similar phenomenon was seen after meeting 3 of the metrics. The results are further evidence on the importance of these health behaviors and factors on cardiovascular health – already in adolescence. This study thus highlights an important public health message that one does not have to reach all 7 recommended health metrics in order to gain cardiovascular health benefits in adolescence, instead the key is to avoid having only a few ideal cardiovascular health metrics at the recommended level. Consequently, the data suggest that preventive efforts could be focused on those with only few of the ideal health behaviors or factors.

Effect of the STRIP intervention on ideal cardiovascular health and subclinical markers on cardiovascular disease

In this study, the risk of having a low ideal cardiovascular health score was reduced in the adolescents belonging to the intervention group compared with the control adolescents. There are no previous studies on the effect of dietary and lifestyle intervention on ideal cardiovascular health in children, adolescents, or adults. In terms of

the behavioral components of the ideal cardiovascular health score, numerous studies have been conducted with the intention to promote a healthy lifestyle in adolescents but with varying success.(Metcalf et al. 2012; Obarzanek et al. 2001) The unique feature of the STRIP study intervention is that it began in infancy and has been provided repeatedly for 20 years. The findings of this study on reaching the individual ideal cardiovascular health metrics are in line with prior results from the STRIP study on risk factors and behaviors. A beneficial intervention effect on LDL cholesterol and blood pressure, for example, metabolic syndrome has been reported whereas there has been a lack of any association between the study group and physical activity.(Niinikoski et al. 2009; Niinikoski et al. 2012; Nupponen et al. 2015; Pahkala et al. 2011) The STRIP study intervention has focused mainly on diet, however, the intervention effect on having an ideal diet could not be studied because of the very low prevalence of those achieving the ideal diet metric. When modified criteria for having an ideal diet were applied (at least 2 components required instead of 4), the intervention adolescents met the dietary goal more often than their control group peers. In previous studies, the intervention has favorably affected the intake of saturated fat, fiber, and vegetables.(Ruottinen et al. 2010; Niinikoski et al. 2012; Talvia et al. 2006) One could suspect that the intervention effect on the individual ideal health factors is in part due to these dietary differences.

The effect of dietary counseling was not reflected on either arterial distensibility or LVM. The underlying reason for the lack of any intervention effect may be that the dietary counseling was targeted to reduce the development of atherosclerosis mainly by mechanisms related to lipid lowering, and such an intervention in the early stages of atherosclerosis may not correlate with arteriosclerosis or cardiac structure. Nevertheless, childhood risk factors have been shown to predict carotid stiffness in adulthood - therefore dietary and lifestyle intervention targeting these risk factors beginning from childhood should not be dismissed as a means of promoting lifelong cardiovascular health.(Juonala et al. 2005)

Clinical and future research perspectives

The risk of developing cardiovascular disease is influenced not only by genetic and adult lifestyle factors but also by biological, lifestyle and environmental factors beginning from early life. In children and adolescents with no overt cardiovascular diseases, the use of noninvasive intermediate end points provides a better risk stratification.(Townsend et al. 2015) This study supports the previous findings in children that arterial stiffness and LVM increase with increasing age. Sex, blood pressure and body size are the main determinants of arterial stiffness and LVM in adolescence. In addition, cardiometabolic risk markers such as LDL cholesterol and insulin sensitivity are related with arterial stiffness at this early age, and, birth weight may exert a long lasting impact on LVM. In this study, none of the adolescents met all 7 ideal cardiovascular health metrics. These findings add

knowledge of the development of cardiovascular diseases from early age and highlight the need for early evaluation of cardiometabolic risk markers and health behaviors. Further studies extending to later life may reveal the associations between cardiometabolic risk markers in early life and the clinical outcomes of cardiovascular disease. In this study, the dietary and lifestyle intervention was not associated with arterial stiffness or LVM in adolescence. It will be interesting to examine these associations in the near future when the cohort children have reached young adulthood.

Assessing subclinical markers of cardiovascular disease in healthy children provides a platform for identifying novel mechanisms driving arteriosclerosis because the long-lasting influence of cardiovascular risk factor exposure, atherosclerosis and drug therapy should be minimized. In this study, children with better ideal cardiovascular health were found to possess better arterial wall health as assessed by arterial stiffness and intima-media thickness. Further research will be needed to provide more detailed knowledge of the role of individual ideal cardiovascular health metrics separately.

Cardiovascular disease prevention in childhood addresses a disease process in its early stages, and it aims to prevent the development of risk factors (primordial prevention) and to prevent future cardiovascular disease by effective management of identified risk factors (primary prevention). This study has shown that ideal cardiovascular health can be promoted through dietary and lifestyle interventions. The promotion of cardiovascular health should occur at various levels of the society and combine diverse approaches including legislation, taxation and organizational change. Along with the principle of the Ottawa Charter, health promotion should make the healthy choice, the easy one. In children, promotion of cardiovascular health must be family-based and integrated into environments surrounding the child. The prevention strategies should thus be applicable in well-baby clinics, daycare centers and schools. For instance, the national Finnish Schools on the Move program was launched in 2010 to implement nation-wide physical activity recommendations in all comprehensive schools and to make the school day more active and pleasant. Non-profit organizations can also play an important role – e.g. the Smart Family Project developed by the Finnish Heart Association provides support on healthy lifestyle counselling for professionals and families. Furthermore, with improved links between health policy workers, environmental experts and representatives of the food industry, cardiovascular health can be improved. The knowledge that an effective dietary and lifestyle intervention can promote ideal cardiovascular health can convince politicians to provide resources to support early life interventions. More data is however needed concerning the clinical usefulness of noninvasive measurement of preclinical atherosclerosis in apparently healthy individuals.

Furthermore, our rapidly changing environment, particularly in areas of technology, work and urbanization, could introduce novel ways to maintain a heart-healthy lifestyle. One can speculate that these preventive strategies may have important biological, medical, and socioeconomic implications and improve the cardiac health of future generations.

7 SUMMARY AND CONCLUSIONS

The novelty of this thesis lies in its longitudinal intervention design in healthy children, investigating subclinical markers of cardiovascular disease, and the associations with cardiometabolic risk factors from childhood to early adulthood.

More specifically:

1. The study reported the longitudinal progression of repeatedly measured arterial stiffness and LVM from childhood to early adulthood (**Study I**) and found that already at this young age, a marked age-related increase in arterial stiffness was evident and this was more pronounced in boys. The effect of sex on arterial stiffness was driven by the difference in pulse pressure. LVM increased with age, and it was greater in boys.
2. Already at a young age, several cardiometabolic risk factors, such as blood pressure, BMI, LDL cholesterol and insulin resistance predict arterial stiffness. (**Study II**). Current pulse pressure, weight, physical activity and birth weight determine LVM in adolescence explaining nearly half of the variation (**Study III**).
3. Ideal cardiovascular health decreases with increasing age in adolescence. Those subjects with a higher number of ideal cardiovascular health metrics had thinner aortic intima-media and less stiff aorta already at this young age. Although having all 7 metrics is the ideal target, cardiovascular health benefits are gained by the avoidance of a low number of ideal cardiovascular health metrics. (**Study IV**).
4. The adolescents who received dietary and lifestyle counseling had more often a higher number of ideal cardiovascular health metrics compared with the control adolescents. The effect of the dietary and lifestyle counseling was not reflected in either arterial stiffness or LVM. (**Study I, III, IV**).

This study shows that age-related arterial stiffening is evident already at a young age, and stiffening is more pronounced in boys than girls. Maintaining normal blood pressure and body weight are key beneficial factors in terms of arterial stiffness and cardiac structure of adolescents. Furthermore, the study highlights an important public health message that one does not have to achieve ideal cardiovascular health in order to gain cardiovascular health benefits in adolescence; the key is to avoid having as few as possible ideal cardiovascular health metrics not at the recommended level. In order to achieve better cardiovascular health, it is evident that dietary and lifestyle counseling is effective, although the benefits are currently not appreciable in terms of subclinical markers of cardiovascular disease.

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