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THE ASSOCIATION OF CORONARY HEART DISEASE RISK FACTORS FROM ADOLESCENCE TO ADULTHOOD WITH CORONARY ARTERY CALCIFICATION AND EPICARDIAL FAT

The Cardiovascular Risk in Young Finns Study

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ABSTRACT

Background: Atherosclerosis and coronary heart disease (CHD) are leading causes of mortality that have their origins in childhood and may develop for decades without clinical symptoms. Coronary artery calcification (CAC) is a marker of subclinical atherosclerosis that confirms the presence of atherosclerotic plaque in the coronary arteries. Epicardial fat, adipose tissue surrounding the heart and coronary arteries, has been suggested to influence the development of CHD.

Aims: The aim of this study was to investigate the associations of cardiovascular disease risk factor levels measured from adolescence to adulthood with CAC and epicardial fat volume (EFV) and the associations of EFV and CAC.

Participants and Methods: This thesis is part of the Cardiovascular Risk in Young Finns Study (Young Finns Study). Cardiovascular disease risk factor levels were measured intermittently from 1980 to 2007 and a computed tomography study to quantify CAC and EFV was performed on 589 participants in 2008.

Results: Higher low-density lipoprotein cholesterol measured in adolescence associated with CAC in adulthood independent of 27-year change in levels. In addition to this risk factor, mean longitudinal values of systolic blood pressure, apolipoprotein B and total cholesterol were also higher among those with, versus those without, CAC. EFV was most strongly associated with body-mass index, while most other risk factor associations were not statistically significant after adjustment for body-mass index. EFV was not independently associated with CAC.

Conclusions: Risk factor levels measured in adolescence and throughout the life-course are associated with CAC. The association of EFV on development of CHD is at least partly explained by its strong relation with overall adiposity.

KEYWORDS: Coronary heart disease, risk factors, coronary artery calcification, epicardial fat

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TIIVISTELMÄ

Tausta: Ateroskleroosi ja sepelvaltimotauti ovat merkittäviä kansantauteja, joiden kehitys alkaa jo lapsuudessa ja jatkuu oireettomana vuosikymmeniä. Sepelvaltimoiden kalkkeutuminen on osoitus aterosklerootttisten plakkien olemassaolosta. Sydäntä ympäröivä rasva on mahdollinen myötävaikuttaja sepelvaltimoplakkien syntymisessä.

Tavoite: Tutkimuksen tavoitteena oli tutkia nuoruudessa mitattujen sepelvaltimotaudin riskitekijöiden yhteyttä aikuisiällä mitattuun sepelvaltimoiden kalkkeutumiseen ja sydämen ympärysrasvan määrään sekä niiden keskinäistä yhteyttä.

Menetelmät: Väitöskirjatutkimus toteutettiin osana Lasten Sepelvaltimotaudin Riskitekijät (LASERI) –tutkimusta. Sepelvaltimotaudin riskitekijätasoja mitattiin vuodesta 1980 vuoteen 2007 toistuvasti ja vuonna 2008 589 tutkittavalle tehtiin sydämen tietokonetomografiatutkimus, jolla mitattiin sepelvaltimoiden kalkkeutumista sekä sydäntä ympäröivän rasvan määrää.

Tulokset: Nuoruudessa mitattu LDL-kolesteroli oli yhteydessä sepelvaltimoiden kalkkeutumiseen aikuisuudessa. Sen lisäksi myös kokonaiskolesterolin ja apolipoproteiini B:n pitoisuudet sekä systolinen verenpaine olivat keskimäärin korkeammat seuranta-aikana niillä, joille kehittyi kalkkiplakkeja verrattuna niihin, joilla niitä ei todettu. Sydäntä ympäröivän rasvan määrä oli vahvimmin yhteydessä kehon painoindeksiin, jolla vakioimisen jälkeen yhteydet useimpien muiden riskitekijöiden kanssa menettivät tilastollisen merkitsevyytensä.

Johtopäätökset: Nuoruudessa ja pitkin elämänkaarta mitatut riskitekijätasot ovat yhteydessä subkliiniseen valtimonkovettumautiin. Sydäntä ympäröivän rasvan vaikutusta valtimonkovettumataudin kehittymiseen selittää osaltaan sen vahva yhteys lihavuuteen.

AVAINSANAT Sepelvaltimotauti, riskitekijät, sepelvaltimoiden kalkkeutuminen, sydämen ympärysrasva

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Abbreviations

Apo-A1 = Apolipoprotein A1
Apo-B = Apolipoprotein B
BMI = Body mass index
BP = Blood pressure
CAC = Coronary artery calcification
CARDIA = Coronary Artery Risk Development in Young Adults
CDAH = Childhood Determinants of Adult Health
CHD = Coronary heart disease
CRP = C-reactive protein
CT = Computed tomography
CVD = Cardiovascular disease
DBP = Diastolic blood pressure
EFV = Epicardial fat volume
EPFV = Extra-pericardial fat volume
FMD = Flow-mediated dilatation
HDL-C = High-density lipoprotein cholesterol
HOMA = Homeostatic model assessment
i3C = International Childhood Cardiovascular Cohort
IMT = Intima-media thickness
LDL-C = Low-density lipoprotein cholesterol
MetS = Metabolic syndrome
OR = Odds ratio
SBP = Systolic blood pressure
SD = Standard deviation
STRIP = Special Turku coronary Risk factor Intervention Project
TTFV = Total thoracic fat volume

List of Original Publications

This dissertation is based on the following original publications, which are referred to in the text by their Roman numerals:

I Hartiala O, Magnussen CG, Kajander S, Knuuti J, Ukkonen H, Saraste A, Rinta-Kiikka I, Kainulainen S, Kähönen M, Hutri-Kähönen N, Laitinen T, Lehtimäki T, Viikari JS, Hartiala J, Juonala M, Raitakari OT. Adolescence risk factors are predictive of coronary artery calcification at middle age: The Cardiovascular Risk in Young Finns Study. *Journal of the American College of Cardiology*, 2012; vol. 60, no. 15: pp. 1364-1370.

II Hartiala O, Kajander S, Knuuti J, Ukkonen H, Saraste A, Rinta-Kiikka I, Kainulainen S, Kähönen M, Hutri-Kähönen N, Laitinen T, Lehtimäki T, Viikari JS, Hartiala J, Juonala M, Raitakari OT, Magnussen CG. Life-course risk factor levels and coronary artery calcification. *The Cardiovascular Risk in Young Finns Study. International Journal of Cardiology*, 2016; vol. 225: pp. 23-29

III Hartiala O, Magnussen CG, Bucci M, Kajander S, Knuuti J, Ukkonen H, Saraste A, Rinta-Kiikka I, Kainulainen S, Kähönen M, Hutri-Kähönen N, Laitinen T, Lehtimäki T, Viikari JS, Hartiala J, Juonala M, Raitakari OT. Coronary heart disease risk factors, coronary artery calcification and epicardial fat volume in the Young Finns Study. *European Heart Journal: Cardiovascular Imaging*, 2015; vol. 16, no. 11: pp. 1256-1263.

1 Introduction

Atherosclerotic cardiovascular diseases (CVD), such as coronary heart disease (CHD), stroke and peripheral vascular disease have their origins in childhood, decades before the clinical presentation of symptoms. In developed and developing countries, CHD is the leading cause of death (World Health Organization 2011). The association of CVD risk factors, such as high low-density lipoprotein cholesterol (LDL-C) and blood pressure (BP) levels, and smoking, on atherosclerotic lesions found in adolescents and young adults have been demonstrated in autopsy studies (Berenson et al. 1998). However, limited data exists on the association of childhood or adolescent CVD risk factors on CHD or cardiovascular events in adulthood. Large cohort studies, such as the Cardiovascular Risk in Young Finns (Raitakari et al. 2008), the Bogalusa Heart (Srinivasan et al. 1976), and Muscatine (Lauer et al. 1975) studies have followed participants from childhood into adulthood. But as the oldest participants in these studies are only in their forties or fifties, CHD incident rates are still relatively low.

The Cardiovascular Risk in Young Finns Study is a multicenter population-based follow-up study on atherosclerosis risk factors that began in 1980 when 3,596 participants aged 3-18 years took part in the first cross-sectional survey. In this thesis, data from the 27-year follow-up of participants that was performed in 2007 are used. Because of the young age (39-45 years) of participants at follow-up, an alternative end-point to CHD, coronary artery calcification (CAC) measured from computed tomography (CT) scans, is used. CAC is a direct marker of, and is correlated with, the extent and severity of coronary atherosclerosis and CHD events and mortality (Detrano et al. 2008; LaMonte et al. 2005; Kondos 2003). In addition, epicardial fat volume (EFV) measurements also obtained from the CT scans is used. Epicardial fat is thought to influence the development of coronary atherosclerosis through local signaling due to its proximity to the coronary arteries (Sacks et al. 2007). In this thesis, the main aims were to study the association of CVD risk factors measured throughout the life-course from adolescence to adulthood on the development of CAC and EFV and to investigate the associations of EFV and CAC.

2 Review of the Literature

2.1 Development of atherosclerosis

Atherosclerosis has been shown to begin early in the life-course and is the etiological precursor to CHD and other CVDs, such as ischemic stroke and peripheral artery disease. The development of atherosclerotic lesions often begins decades before its clinical manifestations. This has been demonstrated in casualties of both the Korean and Vietnam Wars as well as in autopsy studies of young, primarily trauma, victims (Berenson et al. 1998; Enos et al. 1953; McGill et al. 2000; McNamara et al. 1971). Signs of atherosclerosis in the coronary arteries, mainly fatty streak lesions or fibrous thickening without significant luminal narrowing, were observed in the majority of subjects in these studies and their prevalence increased with age. Furthermore, CVD risk factor levels and the number of CVD risk factors were significantly associated with the extent of atherosclerotic changes in the aorta and coronary arteries in the Bogalusa Heart Study and the Pathobiological Determinants of Atherosclerosis Study (Berenson et al. 1998; McGill et al. 2000).

Atherosclerosis is considered to be a chronic inflammatory disease. The development of atherosclerotic plaque begins with the deposition of lipoproteins containing apoliprotein B (Apo-B) in the arterial wall as a result of endothelial dysfunction, followed by an increased migration of macrophages to the site causing adaptive intimal thickening (Sniderman et al 2019; Wolf & Ley 2019; Lusis 2000). Fatty streaks, the first visible atherosclerotic lesions, are observed in humans in the first decade of life. They consist of lipid-laden macrophages, called foam cells, and lipid-rich smooth muscle cells of the subendothelium. Fatty streaks become more advanced lesions, atheromas and fibroatheromas, by the accumulation of extracellular lipid droplets, lipid-rich necrotic debris, and smooth muscle cells as well as fibrosis, and calcification. Fibroatheromas may be largely calcified or contain mainly fibrous tissue without significant lipid or calcium accumulation. The presence of a fissure, hematoma, and/or thrombus is indicative of a complicated lesion. (Wolf & Ley 2019; Stary et al. 1995)

The formation of vascular calcification is ultimately caused by the deposition of calcium phosphate in the arterial wall, myocardium or cardiac valves in the form of hydroxyapatite. It is an active and complex process that has been attributed to

numerous mechanisms and is comparable to that observed in bone tissue biomineralization (Demer & Tintut 2008; Karwowski et al. 2012). Bone-related proteins, such as osteopontin and osteocalcin which serve as nuclei for hydroxyapatite, cells that have osteogenic potential as well as cartilage and bone formation have all been observed in calcified lesions, which indicates that vascular calcification is driven by osteogenic processes. (Demer & Tintut 2008). Vascular calcification occurs in advanced atherosclerosis but also in diabetes or renal failure in the absence of other advanced atherosclerotic changes in the vascular system (Karwowski et al. 2012; Block et al. 2005; Budoff et al. 2005). Furthermore, a lack of mineralization inhibitors normally expressed in blood vessels, such as nucleotide pyrophosphate and matrix Gla protein, has been shown to cause vascular calcification in human and mouse genetic studies (Luo et al. 1997; Rutsch et al. 2003).

Clinical symptoms and complications are related to more advanced lesions, while early lesions may develop silently for decades (Stary et al. 1995). Acute occlusions due to the formation of a thrombus, causing myocardial infarction and stroke, are the most important clinical complications of atherosclerosis. Early structural and functional changes in subclinical atherosclerosis can be identified by ultrasonic assessment of increased carotid artery intima-media thickness (IMT), decreased arterial elasticity, decreased flow-mediated dilatation (FMD), while detection of CAC using CT imaging represents more advanced changes.

The associations of childhood and early adulthood risk factors, lifestyle, and atherosclerotic changes in the vasculature have been the focus of several longitudinal follow-up studies, such as the Muscatine Study (Lauer et al. 1975), Bogalusa Heart Study (Srinivasan et al. 1976), Cardiovascular Risk in Young Finns Study (Raitakari et al. 2008), Childhood Determinants of Adult Health (CDAH) study (Gall et al. 2009.), Coronary Artery Disease Risk Development in Young Adults (CARDIA) study (Cutter et al. 1991), and the Special Turku coronary Risk factor Intervention Project (STRIP) (Lapinleimu et al. 1995) (Table 1). Data from these studies have indicated that the development of atherosclerosis is influenced by the same risk factors found to be operating in adulthood for CHD and that those at risk of developing CHD later in life can be identified using these risk factors measured early in life.

Table 1. Major longitudinal studies on CVD risk factors in children and young adults and subclinical atherosclerosis

Study	Year started	Description of initial cohort	End-points
Muscatine	1971	N=3650, age 8-18	Ultrasound measurements, CAC
Bogalusa Heart	1973	N=3524, age 5-14	Ultrasound measurements
Cardiovascular Risk in Young Finns	1980	N=3596, age 3-18	Ultrasound measurements, CAC
PDAY[1]	1985	N=2786, age 15-34	Autopsy
CARDIA[2]	1986	N=5115, age 18-30	Ultrasound measurements, CAC, incident CHD
CDAH[3]	1985	N= 8,498, age 7-15	Ultrasound measurements
STRIP[4]	1990	N=1062, age 7 months	Ultrasound measurements

[1]PDAY = Pathobiological Determinants of Atherosclerosis, [2]CARDIA = Coronary Artery Risk Development in Young Adults, [3]CDAH = Childhood Determinants of Adult Health, [4]STRIP = Special Turku coronary Risk factor Intervention Project

2.2 Coronary artery calcification

Coronary artery calcification is an intermediate end-point of coronary atherosclerosis most frequent in advanced atherosclerotic lesions and among older individuals (Greenland et al. 2007; Rumberger et al. 1994). The method for measuring CAC by CT was presented by Agatston et al. in 1990. The method takes into account the number, area, and density of calcified plaques in the coronary arteries (the right, left main, descending and circumflex arteries) to give a continuous numerical score (Table 2). Calcified plaques are defined as areas within the region of interest that have a minimum CT density of 130 Hounsfield units and an area of at least 1 mm^2, thus eliminating noise. A factor of 1–4 is then assigned based on the highest CT density (1 for 130–199, 2 for 200–299, 3 for 300–399 and 4 for ≥400 Hounsfield units) and the total area in mm^2 of the lesion is multiplied by this factor. (Agatston et al. 1990) The total CAC score is the sum of all lesion scores of the individual. Other systems for measuring CAC have also been developed, namely the volume score, mass score, and density score. The volume score is calculated as the area of calcification multiplied by the slice thickness. The CT density of the calcium deposit is not taken into account in the volume score (Callister et al. 1998). The mass score offers a calculation of the actual mass of calcium hydroxyapatite in the artery walls. (Oudkerk et al. 2008) The density score conveys the average density of the calcium deposits, which has shown additional prognostic value among individuals with comparable calcium volumes in the Multi-Ethnic Study of Atherosclerosis (Sandfort & Bluemke. 2017; Criqui et al. 2014). The CAC score measured using the

Agatston method is the one most commonly used as a reference in large population studies and risk stratification tool studies and therefore, most widely used in research and clinical practice (Greenland et al. 2018).

Table 2. Calculation of the CAC score using the Agatston method

Lesion density	Weight	Lesion score[1]	Total score
130-199	1		
200-299	2	= Weight x lesion area in mm^2	= Sum of all lesion scores
300-399	3		
>400	4		

[1]For lesions of at least 1 mm^2 in area

The amount of CAC is correlated with the total extent and severity of coronary atherosclerosis (Sangiorgi et al. 1998). A direct association between CAC measured with CT and CHD events, e.g., myocardial infarction or death from CHD, has been shown in a large number of studies. A CAC score of 101 to 300 or over 300 increased the adjusted risk of a coronary event by a factor of 7.73 and 9.67, respectively, compared to those without CAC in a population-based multiethnic U.S. cohort of over 6,700 individuals (Detrano et al. 2008). Among 1,172 asymptomatic patients undergoing coronary CT scans, odds for coronary events were 14.4, 19.7, and 20.2 for CAC scores of >80, >160, and >600 vs 0, respectively, compared to those without CAC (Arad et al. 2000). In 5,600 asymptomatic self-referred low- to intermediate-risk adults, presence of CAC was associated with cardiac events (risk ratio 10.5 for men and 2.6 for women) (Kondos et al. 2003). In the CARDIA study, those with any measurable CAC at age 32-46 years were at a 5-fold risk of experiencing a CHD event during 12.5-years follow-up compared to those without CAC. The hazard ratios for incident CHD were 2.6, 5.8, and 9.8 for CAC scores of 1-19, 20-99, and >100 vs. 0, respectively (Carr et al. 2017).

CAC measurement may be useful in improving risk stratification in those are at intermediate risk of CHD based on CVD risk factor levels and thus serve as a guide for the selection of appropriate treatment. Among those with low CHD risk, CAC measurement is not recommended due to possible negative factors involved; such as radiation exposure, time, and costs. Similarly, patients with high CHD risk are not considered to benefit from CAC screening in clinical practice (Oudkerk et al. 2008; Greenland 2018).

The presence of CAC in a CT study is a sensitive marker of presence of atherosclerotic plaque, while the absence of atherosclerotic plaque is substantially more likely among asymptomatic individuals with a negative CT scan result

(Oudkerk et al. 2008; Simons et al. 1992; Greenland 2007). In contrast, the correlation of arterial calcification and luminal narrowing is weaker than its correlation with plaque burden, since calcium deposits can be present in both obstructive and non-obstructive lesions due to arterial remodeling. (Sangiorgi et al. 1998; Oudkerk et al. 2008; Beckman et al. 2001). A negative or very low (<10) CAC score implies a low probability of CHD events (Taylor et al. 2005; Raggi et al. 2000) and all-cause mortality, even in those with established CVD risk factors such as presence of hypercholesterolemia, diabetes, hypertension, smoking and family history of premature coronary heart disease (Shaw et al. 2003; Budoff et al. 2007). However, cessation of preventive treatment based on a low CAC score in the presence of substantial conventional risk factors is not recommended (Oudkerk et al. 2008).

The association of CAC and plaque instability is less clear. Based on pathology studies, it has been suggested that different types of calcification may have varying effects on plaque vulnerability with spotty "microcalcification" being more prone to rupture than sheet-like "macrocalcification" more commonly seen in stable angina pectoris (Demer & Tintut 2008; Pugliese et al. 2015). Similar findings have been reported in studies on patients with acute coronary syndromes undergoing pre- or post-interventional intravascular ultrasound imaging (Beckman et al. 2001; Ehara et al. 2004). In addition, higher CAC density was inversely associated with CHD and CVD risk in the Multi-Ethnic Study of Atherosclerosis (Criqui et al. 2014).

2.2.1 Prevalence of coronary artery calcification

Prevalence of CAC is higher among men and increases with age. Two large studies have reported age- and sex-stratified percentile points for CAC scores in over 53,000 individuals who were either referred by their physicians or self-referred for a CT study (Hoff et al. 2001; Mitchell et al. 2001) (Table 3). The prevalence of CAC among those aged 40–45 years was approximately 3-fold among men compared with women in the CARDIA study (Loria et al. 2007) and Muscatine Heart Study (Mahoney et al. 1996). The distribution of CAC in the major coronary arteries is comparable to that of the natural development of atherosclerosis in that calcification is most prevalent in the left anterior descending artery, followed by the right coronary artery, left circumflex, and left main arteries (Schmermund et al. 2001; Halon et al. 1983).

Table 3. Previously reported CAC prevalence or CAC score percentile points

Study reference	Number of participants	Age range, years	Prevalence of CAC	CAC score[1]	CAC score percentile			
					50th	75th	90th	95th
Mahoney et. al. 1998	387	29–37	20.4 %					
Loria et. al. 2007	3,043	33–45	9.6 %					
	1,579	40–45	13.3 %					
Hoff et. al. 2003	4,238 men	40–44		27 (120)	1	9	59	
	1,024 women			8 (97)	0	1	4	
Mitchell et. al. 2001	1,551 men	40–44			0	3		142
	726 women				0	0		7

[1]Mean (SD)

2.3 Epicardial fat

Fat surrounding the heart can be distinguished into separate entities. Epicardial fat is visceral fat tissue surrounding the heart and coronary arteries lying between the outer wall of the myocardium and the visceral layer of the pericardial sac. Extra-pericardial fat is adipose tissue overlying the pericardium. Epicardial fat has been suggested to influence the development of CHD through metabolic and inflammatory pathways due to its anatomical proximity to the coronary arteries and myocardium and the lack of a physical barrier between these tissues (Sacks et al. 2007; Nagy et al 2017). Epicardial fat is metabolically active and shows elevated expression and secretion of inflammatory mediators and infiltration by macrophages, lymphocytes, and basophils compared to subcutaneous fat tissue (Mazurek et al. 2003; Sacks et al. 2007). Furthermore, levels of pro-inflammatory markers are higher in the epicardial fat tissue and those of anti-inflammatory markers, such as adiponectin, are lower in CHD patients compared to those without CHD (Hirata et al. 2011; Iacobellis et al. 2005). EFV can be measured non-invasively using CT or magnetic resonance imaging and epicardial fat tissue thickness can be measured using transthoracic two-dimensional echocardiography.

2.3.1 Epicardial fat, coronary heart disease prevalence and events

Several studies have shown an association between EFV or epicardial fat thickness and CHD events (Ding et al. 2009; Mahabadi et al. 2013; Nagy et al. 2017), presence as well as severity of CHD (Eroglu et al. 2009), CAC (Rosito et al. 2008), carotid IMT (Rego et al. 2011; Sengul et al. 2011), arterial stiffness (Kim & Kang 2013;

Brinkley et al. 2011), and CVD risk factor levels including elevated triglyceride and lower high-density lipoprotein cholesterol (HDL-C) concentrations, hypertension, and impaired fasting glucose (Mahabadi et al. 2013; Rosito et al. 2008). EFV is increased among individuals with obesity, type 1 and 2 diabetes, and metabolic syndrome (MetS) (Baker et al. 2006; Wang et al. 2009; Pierdomenico et al. 2011). However, associations between the presence of subclinical atherosclerosis and EFV have been diluted in several studies after adjustment for CVD risk factors, especially body mass index (BMI), waist circumference or overall visceral adipose tissue volume. In the Heinz Nixdorf Recall Study, Mahabadi et al. reported that although CAC scores increased with increasing EFV, after adjusting for CVD risk factors, a trend towards lower EFV was seen with increasing CAC. They concluded that shared risk factors ultimately explained the association of CAC and EFV (Mahabadi et al. 2013). Similarly, Bucci et al. found that associations between EFV and CAC became statistically non-significant after adjustment for age and sex (Bucci et al. 2011). An association between EFV and CAC was found only among those with low BMI, but not overall, in individuals undergoing coronary angiography (Gorter et al. 2008). Although the majority of studies conducted on EFV have reported a significant association with cardiac outcomes (Spearman et al. 2015), as highlighted by the aforementioned studies, the following gap in the literature was recognized: whether EFV is truly an independent risk factor for CHD or rather a marker of overall body adiposity or other shared risk factors influencing the development of CHD.

2.4 Ultrasound measurements

2.4.1 Carotid intima-media thickness

Ultrasonic measurement of arterial wall IMT, introduced by Pignoli et al., has been shown to correlate closely to histological measurements in excised aorta samples (Pignoli et al. 1986). The carotid artery, being an easier vessel to study, has become the standard for IMT measurements. Increased carotid IMT is considered a surrogate marker of subclinical atherosclerosis as it is associated with concurrent and childhood CVD risk factor levels (Davis et al. 2001; Raitakari et al. 2003) and severity and extent of CHD (Burke et al. 1995). Furthermore, increased IMT has been shown to predict myocardial infarction and stroke (OR 1.43 per 1 standard deviation (SD) (0.163 mm) increase for myocardial infarction in the Rotterdam Study and a relative risk of 1.15 per 0.10 mm increase for myocardial infarction and 1.26 per 1 SD increase for stroke in a meta-analysis of 8 studies). (Bots et al. 1997; Lorenz et al. 2007).

2.4.2 Flow-mediated dilatation

Regulation of vascular tone is one of the many functions of the endothelium. Endothelial dysfunction precedes structural changes in the arterial wall and thus serves as an early indication of the atherosclerotic process (Raitakari & Celermajer 2000). The non-invasive method for measuring brachial FMD, a functional marker of endothelial health, was first introduced in 1992 (Celermajer et al. 1992). Artificially induced increased blood flow in the brachial artery is followed by a dilatation response detectable by ultrasound mainly mediated by release of nitric oxide from the endothelial cells of the artery wall (Mullen et al. 2001). Sonographically measured FMD is strongly correlated with measurements obtained using invasive methods (Anderson et al. 1995) and is associated with prevalence of atherosclerosis and cardiovascular events (risk ratio 0.88 per 1 SD increase in FMD for cardiovascular events (Matsuzawa et al. 2015)).

2.4.3 Arterial distensibility

Decreased elasticity of large arteries is an early marker of atherosclerotic changes in the vasculature (Oliver & Webb 2003). Degeneration of arterial wall elastic fibers and an increase in collagen content caused by aging and CVD risk factors causes decreased arterial elasticity (Avolio et al. 1998; Liang et al. 2001). An estimate of arterial elasticity can be obtained by measuring arterial distensibility with ultrasound. Carotid compliance, a non-invasive measure of arterial elasticity, reflects the ability of the carotid artery to expand as a response to the pulse pressure caused by cardiac contraction and relaxation (Riley et al. 1992). Decreased arterial distensibility has been shown to predict CHD events and mortality (hazard ratio 1.22 per 1 SD for cardiovascular events and 1.51 for all-cause mortality (van Sloten et al. 2014)).

2.5 Risk factors and subclinical atherosclerosis

2.5.1 Cardiovascular disease risk factors and coronary artery calcification

The association between CAC and CVD risk factor levels has been shown in numerous studies, both in follow-up studies as well as cross-sectional studies. Large cross-sectional studies on asymptomatic samples have shown robust associations between traditional CVD risk factor levels, such as elevated blood pressure (BP), LDL-C, BMI, and smoking, and CAC. As noted previously, age and male sex are significantly associated with CAC. The presence of hypertension, diabetes and high cholesterol in 9341 asymptomatic U.S. participants aged 35-88 years were

independently associated with CAC score (Pletcher et al. 2004), while in over 31,000 Korean participants aged over 20 years, CAC was associated with the presence of hypertension, diabetes, obesity, chronic kidney disease, and smoking among males and hypertension, diabetes, and obesity among females (Jang et al. 2016). In another large study of over 30,000 U.S. participants (aged 30–90 years), all measured risk factors, including prevalence of hypercholesterolemia, diabetes, hypertension, smoking, and family history of CHD were independently associated with CAC, with the magnitude of the odds ratio (OR) similar to those reported for risk factor levels on clinical CHD (Hoff et al. 2003). CAC also improved the predictive utility of future coronary events beyond CVD risk factor levels (Detrano et al. 2008) and the Framingham risk score (Taylor et al. 2005).

2.5.2 Early lifecourse cardiovascular disease risk factors and subclinical atherosclerosis

Two studies have examined the association of childhood or early adulthood risk factors and CAC. Increased BMI in childhood (aged 8–18 years) was associated with adult CAC measured at the mean age of 33 years in the Muscatine Study (Mahoney et al. 1996). In the CARDIA study, early adulthood risk factors quantified at 18–30 years of age, including dyslipidemia, high blood pressure, cigarette smoking and elevated plasma glucose, were found to be associated with increased CAC 15 years later independently of contemporary risk factors (Loria et al. 2007).

Levels of multiple risk factors measured in childhood or adolescence have been linked to subclinical atherosclerosis as quantified by increased IMT, decreased FMD or arterial stiffness. Previous studies in the Cardiovascular Risk in Young Finns Study have shown that levels of adolescent (aged 12-18 years) LDL-C, systolic blood pressure (SBP), BMI, and smoking were associated with carotid IMT 21 years later (Raitakari et al. 2003) and that the number of childhood risk factors defined as high LDL-C, low HDL-C, high BP, obesity, diabetes, smoking, low physical activity, and infrequent fruit consumption was associated with accelerated carotid IMT progression in adulthood (Juonala et al. 2010). Higher adult IMT was associated with elevated levels of childhood LDL-C and BMI and higher adult arterial stiffness with elevated childhood SBP in the Bogalusa Heart Study (Li et al. 2003; Li et al. 2004). Davis et al. also studied associations of childhood risk factors and adult carotid IMT among over 700 participants in the Muscatine Study and found that total cholesterol levels in both sexes and BMI among females associated with adult carotid IMT (Davis 2001).

In an analysis utilizing combined data from 4 cohorts (the Cardiovascular Risk in Young Finns, Bogalusa Heart, CDAH, and Muscatine studies, collectively the

International Childhood Cardiovascular Cohort (i3C) consortium), Juonala et al. found that the number of risk factors in childhood quantified after 9 years of age (high total cholesterol, triglycerides, BP, and BMI) was associated with elevated carotid IMT in adulthood (Juonala et al. 2010). Using data from the same i3C consortium, Juonala et al. further showed that childhood obesity was predictive of elevated carotid IMT and prevalence of type 2 diabetes, hypertension, and dyslipidemia in adulthood (Juonala et al. 2011). Collectively, these studies have further confirmed the understanding that the pathogenesis of atherosclerosis is life-long and that it is influenced by adverse risk factor levels throughout the life-course.

At the time of this study, the following gap in the literature existed: whether exposure to other risk factors than BMI in adolescence is predictive of CAC in adulthood and whether adolescence risk factors have an independent effect on the development of CAC after taking into account the change in risk factor levels during follow-up.

2.5.3 Risk factor trajectories and coronary heart disease

Risk factor trajectories over an extended period can be more informative than single measurements in predicting risk for developing CHD. One previous study has investigated the role of risk factor trajectories in the development of CAC. In the CARDIA study, five distinct blood pressure trajectories were identified from latent class analysis in participants aged 18-30 years at baseline: low-stable (22 % of participants), stable moderate (42%), moderate-increasing (12%), elevated-stable (19%), and elevated-increasing (5%). Elevated BP trajectories were associated with more advanced CAC at year 25 compared to the low-stable group (Allen et al. 2014). CARDIA participants exposed to prehypertension, defined as SBP of 120-139 mmHg or diastolic blood pressure (DBP) of 80-89 mmHg, between age 20-35 years were at increased risk of developing CAC 20 years later and the prevalence of CAC was directly associated with the extent of the exposure measured in mmHg-years, akin to pack years of cigarette exposure (Pletcher et al. 2008).

A life-course approach has also been used in studies incorporating manifest CHD or ultrasound measurements as end-points. Six long-term BMI trajectories (stable normal, resolving, progressively overweight, progressively obese, rapidly overweight/obese, and persistent increasing overweight/obese) were identified among 2631 participants aged 6-49 years in the Young Finns study. Compared with the stable normal trajectory, worsening or persistent BMI trajectories were associated with increased carotid IMT in adulthood and other CVD outcomes. Of note, the resolved BMI trajectory group was still at a significantly increased risk for increased IMT in adulthood compared to the stable normal trajectory group suggesting that changes in the arterial structure caused by elevated BMI early in the

life-course may be irreversible despite a decrease in BMI. (Buscot et al. 2018) Allen et al. identified five cardiovascular health score trajectories among 9388 participants from five studies in the United States and Finland using data on BMI, total cholesterol level, BP and glucose level from 1973 to 2015. They found that an early decline in the cardiovascular score in the life-course was associated with increased adult carotid IMT (carotid IMT 0.64 mm [95% CI 0.63-0.65 mm] in the high late-decline group vs. 0.72 mm [95% CI 0.69-0.75 mm] in the intermediate-early decline group). (Allen et al. 2020)

In the Amsterdam Growth and Health Longitudinal Study, higher longitudinal levels of central fatness and BP levels were observed during a follow-up period of 24 years among those with stiffer carotid arteries. Compared to those with less stiff arteries, increases in these levels were observed already in adolescence and the increases were steeper among those with stiffer arteries and they were independent of other risk factors (Ferreira et al. 2012). Tirosh et al. conducted a study incorporating repeated risk factor measurements from the age of 17 years over a mean follow-up period of 17.4 years among over 37,000 participants in the Staff Periodic Examination Center of the Israeli Army Medical Corps. They found that elevated adolescent BMI, albeit within the normal range, was independently associated with the risk of angiography-proven CHD in young adulthood (Tirosh et al. 2011).

The association of lifelong exposure to risk factors and cardiovascular mortality has been studied by Reinikainen et al. in a 50-year follow-up study of over 1,700 participants from the Finnish cohort of the Seven Countries Study. They found that a model using cumulative risk factor levels, including SBP and total cholesterol, was superior in predicting cardiovascular disease mortality compared with a model using only the most recent measurements (Reinikainen et al. 2015).

The following gap in the literature was identified at the time of the study: given the low likelihood of long-term pharmaceutical trials spanning youth and adulthood on later development of CAC, which risk factor trajectories from adolescence to adulthood are associated with the development of CAC in adulthood.

3 Aims of the study

The aim of this study was to investigate the associations of cardiovascular disease risk factor levels measured from adolescence to adulthood with CAC and epicardial fat volume (EFV) and the associations of EFV and CAC (Figure 1).
The specific aims of this thesis were to:

1. Investigate the prevalence of CAC in the study population and associations of CVD risk factor levels and lifestyle variables measured both in adolescence and adulthood with CAC in adulthood (I);

2. Investigate the associations of life-course risk factor and lifestyle variable trajectories and cumulative mean levels with CAC in adulthood (II); and

3. Study if epicardial fat is associated with markers of subclinical atherosclerosis (CAC, carotid artery IMT, brachial FMD, carotid distensibility) and to study the associations of risk factor levels and lifestyle variables measured in adolescence and adulthood with EFV (III).

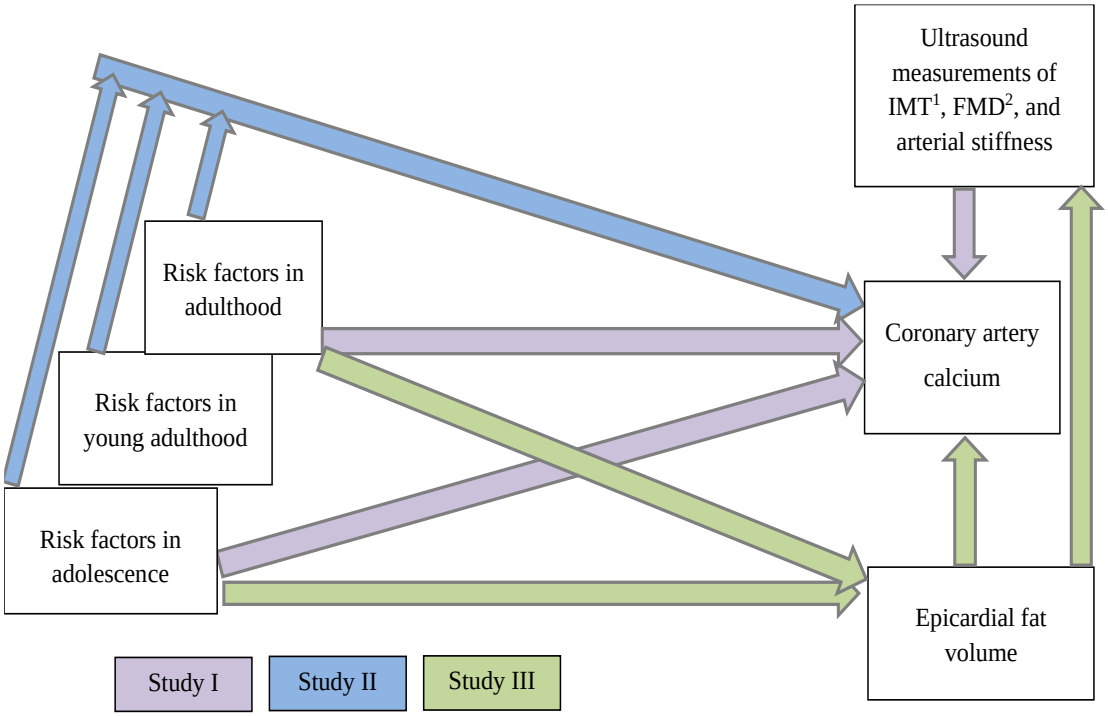


Figure 1. Schematic overview of the aims of the study. [1]IMT = Intima-media thickness, [2]FMD = Flow-mediated dilatation

4 Participants and Methods

4.1 Description of the Cardiovascular Risk in Young Finns Study

The Cardiovascular Risk in Young Finns Study is a multicenter (Helsinki, Kuopio, Oulu, Tampere, and Turku) follow-up study to identify risk factors associated with CVD. The first pilot studies were conducted in 1978 and 1979 and the first cross-sectional study in 1980, when 3,596 participants from a total of 4,320 children aged 3, 6, 9, 12, 15, and 18 years attended. The study design was approved by local ethics committees and signed informed consent from participants or their parents was obtained. Participants were randomly chosen from the population register of the aforementioned areas both from rural and urban surroundings to create a representative sample of Finnish children. Regular follow-ups have been performed since the first cross-sectional study. In 2001 and 2007, non-invasive ultrasound studies were performed to assess carotid artery IMT and distensibility and brachial artery FMD. A cardiac CT study to measure CAC and EFV was offered in 2008 for a subsample of the participants from the three oldest age-groups residing in the three study centers with CT imaging capability (N=802 invited, N=589 participated, response proportion of 73 %) (Figure 2.) Data from the 1980, 1983, 1986, 2001, and 2007 follow-ups were used in this thesis.

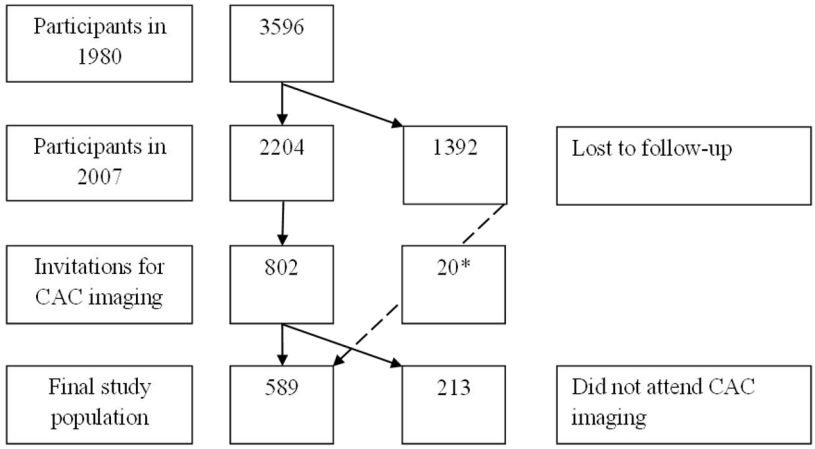


Figure 2. Flow-chart of participation in this study. *Individuals who participated in the CT study but not the clinical follow-up

4.2 Study design and participants

Study I examined the associations of risk factor levels measured in adolescence (1980) and adulthood (2007) on the presence of CAC measured in adulthood (2008). Adolescence risk factor variables were available for all 589 participants aged 12–18 years at baseline (587 for lipid variables). Adulthood risk factor variables from 2007 (or from 2001 if data from 2007 was not available) were available for 569 participants (565 for lipid variables) (i.e., there were 20 individuals with CT scan data who did not participate in the latest adulthood follow-up field clinics).

Study II examined risk factor trajectories and longitudinal risk factor levels and their associations with CAC in adulthood. In this study, repeated measurements of risk factor levels from 1980, 1983, 1986, 2001, and 2007 were used. In total, 589 participants were included in the study. On average, participants had 4.3 measurements (range 1-5, standard deviation (SD) =0.94) in total.

In Study III, associations between epicardial fat volumes, adolescence and adult risk factor levels, and markers of subclinical atherosclerosis, including CAC and vascular ultrasound measurements, were studied. A total of 557 participants with interpretable and non-missing EFV data were included in the study.

4.3 Blood samples

All venous blood samples were drawn from the right antecubital vein after a 12-hour fasting period. Total cholesterol, HDL-C and triglyceride concentrations were determined using standard enzymatic methods (Roche Diagnostics, GmbH,

Mannheim, Germany for HDL; Olympus System Reagent, Hamburg, Germany for total cholesterol and triglyceride) with a clinical chemistry analyser (Olympus, AU400, Hamburg, Germany). The Friedewald formula was used to calculate LDL-C concentration in samples where triglyceride level was below 4 mmol/l (Friedewald et al. 1972). Total cholesterol to HDL-C ratio and triglyceride to HDL-C ratio were calculated by dividing total cholesterol or triglyceride concentration by HDL-C and non-HDL-C was calculated as total cholesterol minus HDL-C. Computed values for apolipoprotein A-1 (Apo-A1) and B were created for 1980, 1983, and 1986 (Raitakari et al. 2013).

Due to changes in determination methods and reagents, lipid levels from 1980, 1983, 1986 and triglycerides from 2007 were corrected to correspond to the samples analyzed in 2001 (Juonala et al. 2004). For total cholesterol, LDL-C and HDL-C levels measured in 2007, correction equations were unnecessary. In 1980, 1983, and 1986, serum insulin was measured using a modification of the immunoassay method of Herbert et al. (Herbert et al. 1965) and in 2001 and 2007, by microparticle enzyme immunoassay kit (coefficient of variation 2.1 %) (Abbott Laboratories, Diagnostic Division, Dainabot). In 1980, serum insulin was measured using a modification of the immunoassay method. Serum high-sensitivity C-reactive protein (CRP) was analyzed by an automated analyzer (Olympus AU400) with a latex turbidimetric immunoassay kit (CRP-UL assay, Wako Chemicals, Neuss, Germany). The detection limit reported by the manufacturer for the assay was 0.06 mg/L. Glucose concentrations were analyzed enzymatically in 2001 and 2007 and with the β-D-glucose:nicotinamide adenine dinucleotide oxidoreductase method in 1986. Glucose levels were corrected due to changes in reagents from 2001 to 2007 using a correction equation. The homeostatic model assessment index (HOMA-index) was calculated as (insulin*fasting glucose)/22.5 (Matthews et al. 1985).

Participants were considered to have type 2 diabetes if they: a) had a fasting glucose ≥7.0 mmol/l (≥125 mg/dl); b) reported receiving oral hypoglycemic medication and/or insulin and did not have type 1 diabetes; or c) reported a history of physician-diagnosed type 2 diabetes (Alberti & Zimmet 1998). The modification of the original National Cholesterol Education Program definition by a joint expert group of the National Heart, Lung, and Blood Institute and the American Heart Association was used to determine those participants with MetS (Grundy et al. 2004).

4.4 Physical examination and questionnaires

Physical examination of study participants included measurement of height, weight, and waist circumference as well as SBP and DBP. Height was measured to the nearest 0.5 cm and weight to the nearest 0.1 kg. Body mass index was calculated as

weight(kg)/(height(cm))^2. Waist circumference was measured midway between the iliac crest and the lowest rib. Blood pressure was measured using a standard mercury sphygmomanometer in 1980 and 1983 and a random zero sphygmomanometer in 1986, 2001 and 2007 (Hawksley & Sons Ltd, Lansing, United Kingdom). Korotkoff´s first sound was used to record SBP and fifth sound to record DBP. Readings to the nearest even number of mmHg were recorded. Three measurements were performed on the right arm with participants in the seated position following five minutes of rest. Cuff size was determined by measured arm circumference. The average of the three measurements was used for BP in the analyses. No corrections or transformations to these values were performed.

Information on physical activity, smoking habits, alcohol intake, and use of regular medication were obtained with questionnaires. A physical activity index was calculated by assessing the duration, intensity, and frequency of physical activity and participation in organized physical activities (Telama et al. 2005). Intensity was estimated using a 3-class scale: 1) usually not becoming out of breath or sweating; 2) slightly becoming out of breath and sweating; and 3) considerably becoming out of breath and sweating. Frequency was evaluated as 1) up to once a month, 2) at least once a week but not daily, 3) daily, and duration as 1) under 20 minutes, 2) 20–60 minutes, 3) >60 minutes. Participation in organized physical activities was evaluated as 1) never or occasionally, 2) frequently, approximately once a week; and 3) several hours and times per week. The physical activity index was calculated as a sum of the individual components of the index with a higher index representing higher physical activity. Pack years of smoking were calculated as the number of cigarette packs smoked daily multiplied by the duration of daily smoking in years. Food frequency questionnaires were used to estimate the number of weekly portions or daily consumption in grams of selected food groups, e.g., fruit, vegetable, and fish. Type of spread usually used on bread (butter vs. margarine) was also inquired.

4.5 Cardiac computed tomography studies

CT scans were carried out at three study locations: Turku, Tampere, and Kuopio. The scans were performed with a GE Discovery VCT 64-slice CT/positron emission tomography device (Turku), a Philips Brilliance 64-slice CT device (Tampere), and a Siemens Somaton Sensation 16-slice CT device (Kuopio). The field of view was determined after lateral and/or frontal scout images and included the coronary vessels. The axial field of view was set at 22-25 cm and the imaging parameters were as follows: voltage 120 kV, current 200-330 mA, and slice thickness 2.5-3 mm. The acquisition time was 6-8 seconds and the scan was performed during breath hold using prospective ECG triggering. The average radiation dose received by participants was 1.43 mSv.

4.5.1 Coronary artery calcification measurements

The images were analyzed by one reader blinded to participant details (the doctoral candidate, O.H. trained by an experienced radiologist (S.K.) using the CareStream software (Rochester, NY, USA). CAC scores were calculated semi-automatically by the software using the Agatston method. First, the reader manually traced a region of interest around calcified plaques located within the path of each coronary artery and then the software calculated the CAC scores automatically using the algorithm described in 2.2. Coronary calcification, page 13 (Agatston et al. 1990). A CAC volume score was also calculated. Absence of CAC was defined as an Agatston score of 0 and presence of CAC as an Agatston score of 1 or greater (Figure 3).

A subsample of 60 scans was re-analyzed to investigate intra-observer variability. The coefficient of variation for intra-observer measurements was 4.0 %. A phantom with deposits of known calcium concentration was scanned twice using three projections at all of the study centers because of the use of different devices and the calcium scores from these scans were compared. The coefficient of variation between all of the phantom scans was 3.9 %.

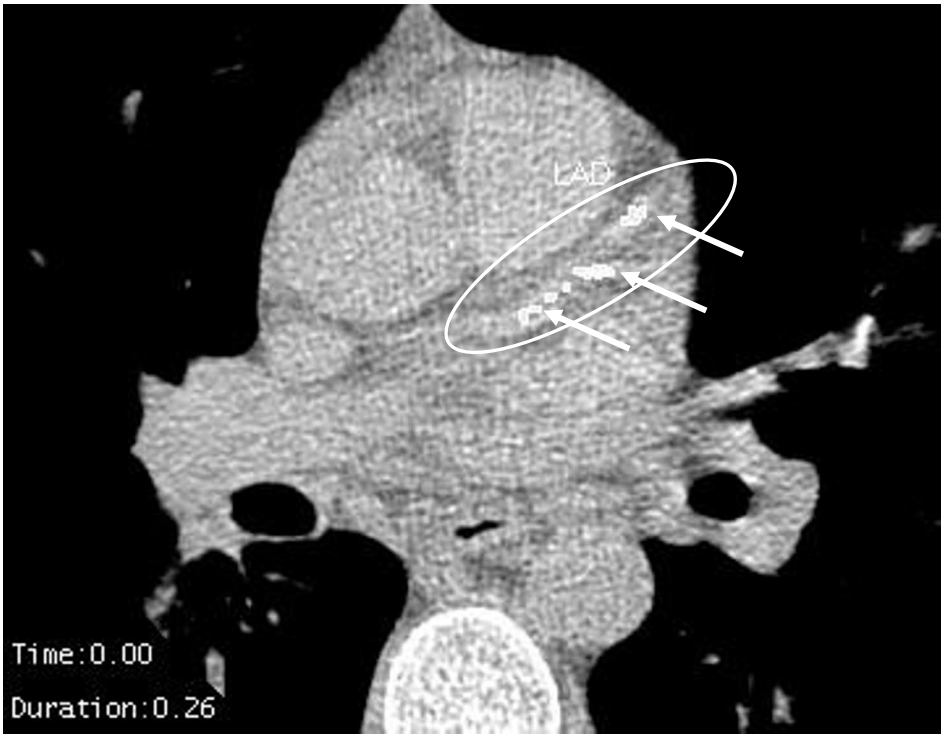
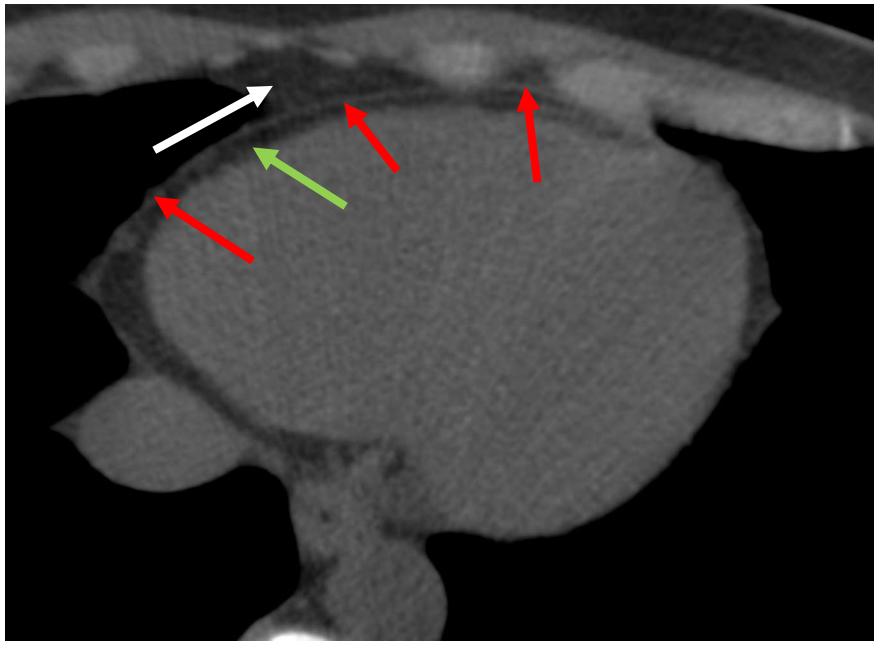


Figure 3. A CT study image frame showing calcifications (arrows) in the left anterior descending artery (LAD; ellipse). For this participant, the CAC score in the left anterior descending artery was 40 and the total CAC score 43.

4.5.2 Epicardial fat measurements

Total thoracic fat (TTFV), extra-pericardial fat volume (EPFV) and epicardial fat volume (EFV) in cm^3 were calculated from CT images using a GE Advantage Workstation (version 4.4) as described by Mahabadi et al. (Mahabadi et al. 2009). Fat volumes were assessed semi-automatically by first manually tracing regions of interest on non-consecutive slices and making necessary adjustments on the computed interpolations. The pericardial sac was then traced on the images. Within this region of interest, fat tissue was defined as voxels within a window of -195 to -45 Hounsfield units. Total thoracic fat was defined as the sum of all fat tissue deposits of any size located within the thorax from the level of the right pulmonary artery to the diaphragm and from the chest wall to the descending aorta as well as any fat tissue inside the pericardial sac. EFV was defined as fat tissue within the parietal layer of the pericardial sac (Figure 4). EPFV, fat tissue located in the thorax outside the pericardium, was calculated as the difference between TTFV and EFV. One reader (the doctoral candidate, O.H.) blinded to participant details performed all measurements. In total, scans from 32 participants were non-interpretable due to missing data. Of the three fat volumes, EFV was chosen as the primary outcome because of its reported associations with CHD development. Measurement of EFV has been shown to have high inter-reader reproducibility (Stojanovska et al. 2017).

Figure 4. A CT study image showing deposits of extra-pericardial fat (white arrow) and epicardial fat (green arrow) separated by the pericardium (red arrows).



4.6 Ultrasound measurements

4.6.1 Carotid artery intima-media thickness

Ultrasound studies were performed by trained physicians and sonographers following standardized protocols in 2007. An ultrasound imaging device with a high-resolution system (Sequoia 512; Acuson, CA, USA) and 13.0 MHz linear-array transducer was used. Mean IMT was derived using a minimum of four manual measurements using digital calipers at end-diastole from the posterior (far) wall of the left common carotid artery approximately 10 mm proximal to the carotid bifurcation. Measurements were made by one experienced reader blinded to participant details. To assess intra-observer reproducibility, a subset of 57 participants were re-examined after 3 months. The coefficient of variation was 6.4% for IMT measurements between visits.

4.6.2 Carotid artery elasticity

To assess carotid distensibility, a marker of the elasticity of an artery, the best-quality cardiac cycle was selected from a continuous 5-second moving cine clip. The common carotid diameter 10 mm proximal to the carotid bifurcation was measured at least twice during end diastole and peak-systole determined by ECG. Ultrasound and concomitant brachial blood pressure measurements were used to calculate carotid artery distensibility with the following formula: Cdist=[(Ds−Dd)/Dd]/(Ps−Pd), where Cdist is carotid distensibility, Dd is diastolic diameter, Ds is systolic diameter, Ps is systolic blood pressure, and Pd is diastolic blood pressure. The 3-month between-visit coefficient of variation was 2.7% for carotid artery diastolic diameter and 16.3% for carotid artery distensibility.

4.6.3 Brachial flow-mediated dilatation

To assess brachial FMD, the left brachial artery diameter was measured at rest and during reactive hyperemia. Increased flow was induced by inflation of a pneumatic tourniquet placed around the forearm to a pressure of 250 mmHg for 4.5 minutes followed by release. The average of three measurements at rest and 40, 60, and 80 seconds after cuff release was used to calculate maximum FMD. The maximal vessel diameter in scans after reactive hyperemia was expressed as the percentage relative to the resting scan. The 3-month between-visit coefficient of variation was 3.2% for brachial artery diameter measurement and 26.0% for FMD.

4.7 Statistical analyses

The results are expressed as mean ± SD, unless otherwise stated. Group comparisons were performed using t-tests or chi-square tests, as appropriate. The variable distributions were assessed using visual inspection of histograms to determine normality. Values for triglycerides, insulin, CRP were log-transformed before all analyses because of skewed distributions; no transformations for other variables were performed. Risk factor trajectories and their associations with chosen end-points were studied using multi-level mixed modelling with maximum-likelihood estimation as a function of age for two groups: those with CAC and those without in 2008 (Study II) or those with EFV in the lowest quarter versus those with EFV in the highest quarter in 2008 (Study III). This method allows for missing data (assuming they are random) and takes into account correlations between repeated measures on the same participant. A compound symmetry covariance structure, which assumes a constant correlation between separate measurements, was used in all models in Study II and an unstructured covariance matrix was used in Study III. A significance level of 0.05 was used for all analyses. The statistical analyses were performed with Statistical Analysis System, SAS, version 9.2 and 9.3 or STATA version 13.1.

Study I: Logistic regression analysis adjusted for sex and age was used to examine the association of risk factors on the presence or absence of CAC. A subgroup analysis was performed for those with BMI>30 to study the association of CAC and BMI (N=124) in those with a higher proportion of body fat mass. A series of multiple logistic regression models were fitted positing the dichotomous CAC variable (0=absence of CAC, 1=presence of CAC) as the outcome. First, a stepwise multivariable model was fitted adjusting for age, sex and the following adolescence risk factors: LDL-C, SBP, BMI, HDL-C, triglycerides, insulin, CRP, and smoking . Second, a multivariable model including sex, age, all significant adolescence variables from the stepwise model (SBP and LDL-C) and change in risk factor levels between adolescence and adulthood (adult level minus adolescent level) for SBP and LDL-C was performed. ORs were standardized for a 1-SD increase in risk factor levels and a 1-year increase in age.

The combined effect of LDL-C and SBP in adulthood versus adolescence on CAC prevalence was studied by dividing the participants into nine groups according to presence or absence of high levels of LDL-C, SBP or both in adolescence and adulthood. High levels of LDL-C and SBP were defined as values at or above the age- and sex-specific 75th percentile. The association of multiple high risk factor levels on prevalence of CAC was studied using a multiple logistic regression model adjusted for sex and age.

Study II: To compare the trajectories between CAC groups, interaction terms were fitted between CAC group (0 vs 1) and time. This enabled the age at which

differences in risk factor levels were apparent to be determined. First, the models were fitted with sex as a dichotomous variable and time as a multichotomous variable (model 1). The models were then fitted with additional risk factors; physical activity index as a multichotomous variable, pack years of smoking and vegetable, fruit, and fish consumption (model 2), BMI (model 3), SBP (model 4A) and LDL-C (model 4B) as continuous variables. Least square means at specific age-points as well as longitudinal mean values throughout the study period were calculated for the risk factor levels by CAC group. To study the association of risk factor levels on CAC severity, analyses were repeated using three CAC groups: no CAC, CAC score 1-99, and CAC score ≥100. This was a modification of the CAC severity classification previously used e.g. in the Multi-Ethnic Study of Atherosclerosis (Budoff et al. 2009). The two groups with the highest CAC scores (100-399 and >400) were combined in this study as the number of subjects with a CAC score of >400 was very low (N=4).

Study III: A log-transformation was used for EFV, EPFV and TTFV as they were not normally distributed. Participants were divided into sex-specific quarterts based on their epicardial fat volumes. Associations between EFV and CVD risk factors as well as ultrasound measures of subclinical atherosclerosis were assessed using age- and sex-adjusted multivariable analysis of variance models. A multiple regression model including sex, age, BMI, Apo-B and ever smoking was then created to establish the magnitude of the individual risk factors' association on EFV. Finally, in order to compare the trajectories of BMI across the EFV groups, interaction terms between EFV group (lowest versus upper quarter) and time were fitted. This allowed the age at which differences in BMI were apparent between the EFV groups.

5 Results

5.1 Clinical characteristics

5.1.1 Attrition

In 1980, 3,596 individuals aged 3-18 years participated in the first cross-sectional study. Since then, several follow-up studies have been conducted. A flow-chart of study participants as it relates to this work is shown in Figure 2 (Methods, Section 4.1.). Age and sex-adjusted clinical characteristics from 2007 were compared for participants and non-participants that were invited for the CT sub-study performed in 2008. No significant differences in risk factor levels in 2007 were observed between participants and non-participants (Table 4).

5.1.2 Coronary artery calcification

CAC was prevalent in 19.2 % of participants (N=113). 16.7 % of participants had a CAC score of 1-100, 1.9 % a score of 101-400 and 0.7 % a score of >400. (Table 5) The average CAC score was 9.6 (SD 50.5) overall and 50.1 (SD 106.5) among those with detectable calcification. The 75th percentile CAC score for men in this study was 1, the 90th 28, and the 95th 102. In women, the corresponding CAC percentile scores were 0, 2, and 5, respectively. On average, regional calcium scores were highest in the left anterior (mean CAC score 31.1, SD 72.8) and the right coronary arteries (11.5, SD 56.8) for those with detectable CAC (CAC score 1 or greater, N=113) (Table 6).

Table 4. A comparison of risk factor variables measured in 2007 for participants (N=569) and non-participants (N=142) in the CT sub-study

Risk factor	Participants (n=569)	Non-participants (n=142)	p-value[1]
Male gender, %	44.4	46.9	0.52
Age, y	41.8 (2.4)	41.9 (2.5)	0.66
Total cholesterol, mmol/l	5.20 (0.95)	5.11 (0.84)	0.50
LDL-cholesterol, mmol/l	3.21 (0.83)	3.20 (0.74)	0.67
HDL-cholesterol, mmol/l	1.33 (0.31)	1.37 (0.32)	0.34
Triglycerides, mmol/l	1.51 (1.0)	1.30 (0.73)	0.06
Apo-A1, g/l	1.60 (0.28)	1.62 (0.22)	0.55
Apo-B, g/l	1.07 (0.28)	1.01 (0.26)	0.08
SBP, mmHg	123.7 (15.9)	121.5 (15.5)	0.28
DBP, mmHg	77.6 (12.2)	76.1 (11.0)	0.35
Insulin, mU/l	9.6 (10.3)	10.3 (8.3)	0.11
Glucose, mmol/l	5.4 (0.70)	5.5 (0.9)	0.47
BMI, kg/m^2	27.0 (5.0)	26.6 (5.9)	0.51
Daily smoking, %	16.7	20.5	0.25
Physical activity index	8.6 (1.8)	8.4 (1.8)	0.16
Vegetable consumption (g/day)	277.7 (181.4)	281.0 (216.2)	0.99
Fruit consumption (g/day)	220.8 (197.7)	203.5 (213.0)	0.32
Fish consumption (g/day)	39.1 (27.1)	38.0 (29.7)	0.79
Use of butter (%)	9.6	11.6	0.44

LDL-C= low-density lipoprotein cholesterol; HDL-C= high-density lipoprotein cholesterol; Apo-A1= Apolipoprotein A1; Apo-B= Apolipoprotein B; SBP= systolic blood pressure; DBP= diastolic blood pressure; CRP= C-reactive protein; BMI= body mass index

Table 5. Prevalence of coronary artery calcification (CAC)

	n	%	p-value
Any CAC			
Overall	113	19.2	
Sex			<0.0001
Men	73	27.9	
Women	40	12.2	
Age, years			0.0169
40	35	16.3	
43	34	17.3	
46	44	24.9	
CAC score			
1-100	98	16.7	
101-400	11	1.9	
>400	4	0.7	

Table 6. Regional coronary artery calcification (CAC) scores among those with any detecable CAC (CAC score 1 or greater). N=113

	Mean	SD	75th percentile	95th percentile	Range
Total score	50.1	106.5	29	308	1-536
LMA	4.1	15.9	0	28	0-99
LAD	31.1	72.8	22	219	0-439
LCX	3.4	11.9	0	14	0-75
RCA	11.5	56.8	1	12	0-403

LAD = left anterior descending artery; LMA = left main artery; LCX = left circumflex artery; RCA= right coronary artery)

5.2 Cross-sectional risk factor levels and coronary artery calcification

Risk factor levels measured in adolescence at age 12-18 years and in adulthood at age 39-45 years among those with and without CAC are shown in Table 7. CAC was more prevalent among men than women (27.9 % vs. 12.2 %) and those with CAC were older than those without (43.2 years vs. 42.7). Adolescent and adult levels of total cholesterol, non-HDL-C and SBP were higher among individuals with CAC. In addition, their adolescent levels of LDL-C and adulthood DBP values were higher

than those without CAC. Prevalence of daily smoking was similar in both groups but the number of pack-years of smoking was higher among those with CAC. In a subgroup analysis including only participants with BMI>30 kg/mg^2, no significant differences were seen in BMI for those with CAC vs. those without (mean BMI 34.2 kg/m^2 for those without CAC vs. 33.65 kg/m^2 for those with CAC, p=0.77).

In a stepwise multivariable analysis adjusted for sex, age, LDL-C, SBP, BMI, HDL-C, triglycerides, insulin, CRP, and smoking, age, male gender, SBP and LDL-C measured in adolescence remained independent predictors of adult CAC. The association of change in risk factor levels over the follow-up period was studied in a multivariable analysis including sex, age, LDL-C and SBP measured in adolescence and change in these risk factor levels between adolescence and adulthood. Both LDL-C and SBP were independently associated with CAC in adulthood, while age or changes in these risk factor levels were not (Table 8). After further adjustment for adult and adolescent BMI, insulin, CRP, and smoking, the association of LDL-C and SBP remained statistically significant (p=0.04 for SBP and p=0.01 for LDL-C).

5.3 Coronary artery calcification and ultrasound markers of subclinical atherosclerosis

The differences in ultrasound measurements (IMT, carotid distensibility and flow-mediated dilatation) between individuals with and those without CAC were statistically non-significant. However, carotid distensibility was somewhat lower among those with CAC compared to those without (1.5 vs 1.7 %/10 mmHg, p=0.06).

Table 7. Adolescence and adult characteristics by CAC status in 2007

	Adolescence (aged 12-18 years)			Adult (aged 39-45 years)		
	CAC absent	CAC present	p-value[1]	CAC absent	CAC present	p-value[1]
N	476	113		460	109	
Male sex, %	39.7	64.6	<0.0001	39.5	65.4	<0.0001
Age, y	14.7 (2.4)	15.2 (2.5)	0.02	41.7 (2.4)	42.2 (2.5)	0.03
Weight, kg	51.9 (12.0)	56.3 (13.8)	0.14	78.7 (17.3)	83.8 (17.7)	0.43
Height, cm	161.9 (10.4)	165.6 (11.7)	0.52	170.8 (8.6)	174.2 (8.4)	0.89
BMI, kg/m^2	19.6 (2.9)	20.3 (3.2)	0.12	26.9 (5.1)	27.5 (4.7)	0.41
SBP, mmHg	116 (10)	120 (12)	0.02	122 (16)	129 (15)	0.02
DBP, mmHg	70.4 (9.8)	70.7 (9.4)	0.84	76.6 (12.0)	81.7 (12.3)	0.01
Total cholesterol, mmol/l	5.14 (0.92)	5.26 (0.90)	0.01	5.14 (0.92)	5.48 (1.07)	0.04
LDL-C, mmol/l	3.23 (0.77)	3.41 (0.80)	0.003	3.18 (0.81)	3.43 (0.92)	0.09
HDL-C, mmol/l	1.55 (0.31)	1.51 (0.30)	0.65	1.32 (0.31)	1.32 (0.34)	0.34
Triglycerides, mmol/l	0.73 (0.35)	0.74 (0.32)	0.55	1.48 (1.0)	1.63 (0.91)	0.93
Non-HDL-C, mmol/l	3.6 (0.8)	3.8 (0.9)	0.004	3.8 (0.9)	4.2 (1.0)	0.02
Total cholesterol/HDL-C	3.4 (0.7)	3.6 (0.9)	0.01	4.0 (1.2)	4.4 (1.3)	0.33
Triglycerides/HDL-C	0.5 (0.3)	0.5 (0.3)	0.66	1.2 (0.9)	1.4 (0.9)	0.80
CRP, mg/l	1.0 (3.2)	1.1 (3.7)	0.87	1.9 (2.9)	2.9 (10.1)	0.15
Insulin, mU/l	13.1 (5.8)	12.6 (5.1)	0.84	9.1 (8.4)	12.0 (15.9)	0.07
Daily smoking, %	16.2	18.9	0.97	16.4	18.3	0.99
Pack years of smoking				3.3	4.9	0.007
Antihypertensive medication, %				11.3	11.7	0.93
Lipid lowering medication, %				3.2	4.9	0.40
IMT (mm)				0.66 (0.1)	0.67 (0.1)	0.83
Carotid distensibility (%/10 mmHg)				1.7 (0.6)	1.5 (0.6)	0.06
FMD %				9.0 (4.4)	7.9 (4.1)	0.34

Values expressed as means (SD) or %. [1]p-values adjusted for sex and age, where applicable. Information on pack years of smoking, use of medication and ultrasound measurements not available in adolescence. BMI=body mass index; SBP=systolic blood pressure; DBP=diastolic blood pressure; LDL-C=low-density lipoprotein cholesterol; HDL-C=high-density lipoprotein cholesterol; CRP=C-reactive protein, IMT=Intima-media thickness; FMD=Flow-mediated dilatation

Table 8. Adolescence risk factor odds ratios (OR) and 95% confidence intervals (95%CI) for having CAC in adulthood

Model	A		B		C	
Risk factors	OR	95% CI	OR	95% CI	OR	95% CI
Age	1.12	1.02-1.21	1.10	1.00-1.21	1.09	0.99-1.20
Male sex	2.88	1.87-4.43	2.90	1.87-4.50	2.52	1.56-4.05
SBP	1.31	1.04-1.63	1.28	1.02-1.60	1.38	1.08-1.77
LDL-C	1.37	1.11-1.69	1.35	1.10-1.67	1.34	1.05-1.70
Total cholesterol	1.33	1.07-1.64				
HDL-C	0.95	0.77-1.18				
Triglycerides	1.07	0.87-1.31				
BMI	1.19	0.96-1.48				
Smoking (no/yes)	1.00	0.80-1.24				
Insulin	0.98	0.78-1.22				
CRP	1.02	0.81-1.29				
ΔLDL-C					1.07	0.84-1.37
ΔSBP					1.25	0.98-1.60

Adjusted for age and sex (A) (N=589), a stepwise multiple logistic regression analysis according to adolescence risk factor levels (B) (N=589) and a multiple logistic regression analysis adjusted for all risk factors in the model and 27-year change in LDL-C and SBP levels (C) (N=563). ORs expressed for 1-year increase in age and 1-SD increase in risk factor levels.
SBP=systolic blood pressure; LDL-C=low-density lipoprotein cholesterol; HDL-C=high-density lipoprotein cholesterol; BMI=body mass index; CRP=C-reactive protein; ΔLDL-C=27-year change in low-density lipoprotein cholesterol; ΔSBP=27-year change in systolic blood pressure

The combined effects of high LDL-C and SBP levels as defined by levels at or above the age- and sex-specific 75th percentile on the presence of CAC in adulthood was examined using a multiple logistic regression model adjusted for age and sex. The number of risk factors in adolescence was significantly associated with CAC (OR 3.5 [95% CI 1.7 – 7.2, p=0.002] between groups with zero versus both risk factors). The number of risk factors in adulthood was not associated with CAC (OR 1.1 [95% CI 0.5 to 2.5, p=0.27] between groups with zero versus both risk factors) (Figure 5).

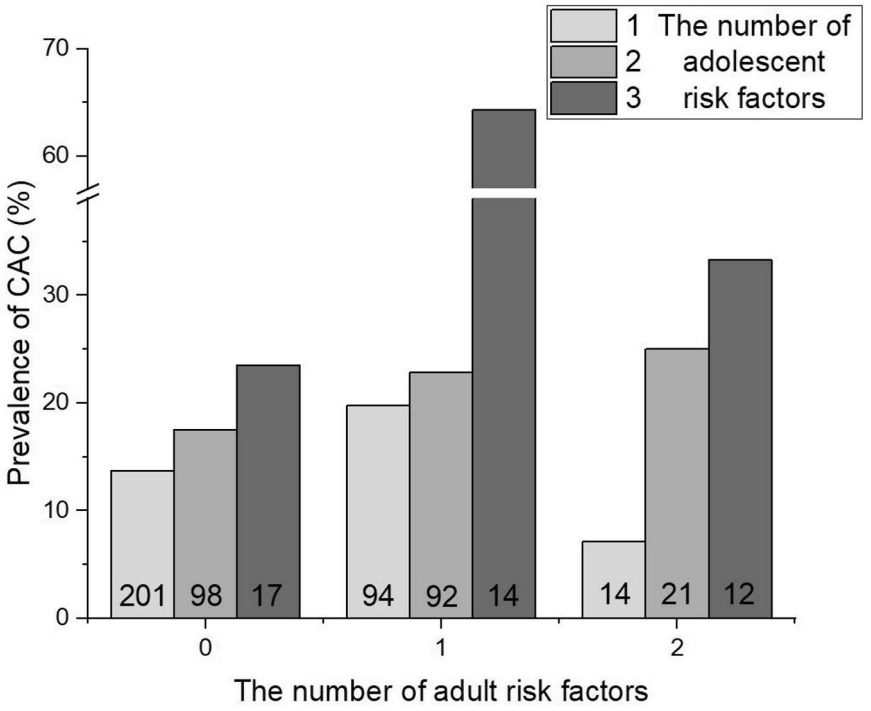


Figure 5. Prevalence of coronary artery calcification (%) in groups stratified by the number (0, 1 or 2) of risk factors (high low-density lipoprotein cholesterol and systolic blood pressure) in adolescence (colour of bar) and adulthood (groups along the x-axis). High levels were defined as values ≥ age- and sex-specific 75th percentile. The number of participants is shown in columns. p-value for trend = 0.002 (high levels in adolescence), p-value for trend = 0.27 (high levels in adulthood).

5.4 Trajectories and coronary artery calcification

Average cumulative values (using measurements from 1980, 1983, 1986, 2001, and 2007) of total cholesterol (difference between CAC groups 0.29 mmol/l; 95% CI 0.13-0.46), LDL-C (0.27 mmol/l; 95% CI 0.11-0.42), Apo-B (0.062 g/l, 95% CI 0.022-0.10), and SBP (2.25 mmHg, 95% CI 0.39-4.11) were higher across the life-course among those with CAC compared with those without CAC. These results remained consistent after adjusting for other risk factors and lifestyle variables (Table 9**)**.

Table 9. Cumulative risk factor levels from 1980, 1983, 1986, 2001, and 2007 by CAC status in 2008.

Risk factor	No CAC (N=476)	CAC present (N=113)	Model 1 p-value[4]	Model 2 p-value[5]	Model 3 p-value[6]	Model 4 p-value[7]
Male sex, %	39.7	64.6	<0.0001			
LDL-C, mmol/l	3.20±0.03	3.46±0.07	0.0006	0.0022	0.0048	0.0035
HDL-C, mmol/l	1.43±0.01	1.43±0.02	0.90			
Total cholesterol, mmol/l	5.15±0.04	5.45±0.08	0.0006	0.0023	0.0054	0.0044
Triglycerides, mmol/l	1.19±0.02	1.23±0.05	0.42			
Apo-A1[1], g/l	1.58±0.009	1.60±0.02	0.42			
Apo-B[1], g/l	1.01±0.009	1.07±0.02	0.002	0.0044	0.0183	0.0178
Glucose[2], mmol/l	5.1±0.03	5.1±0.05	0.30			
Insulin, mU/l	9.9±0.2	10.9±0.4	0.28			
BMI, kg/m^2	23.6±0.15	24.1±0.3	0.17			
Physical activity index	8.8±0.07	8.8±0.1	0.84			
SBP, mmHg	120.3±0.4	122.5±0.8	0.02	0.0149	0.0496	0.0405
DBP, mmHg	72.3±0.3	73.6±0.7	0.12			
Daily smoking, %	15.9	17.7	0.61			
Vegetable servings/week[3]	5.7±0.1	5.5±0.2	0.44			
Fruit servings/week[3]	6.0±0.1	6.0±0.2	0.87			
Use of butter (%)	42.1	46.9	0.11			
Fish servings/week[3]	1.1±0.04	1.0±0.07	0.19			

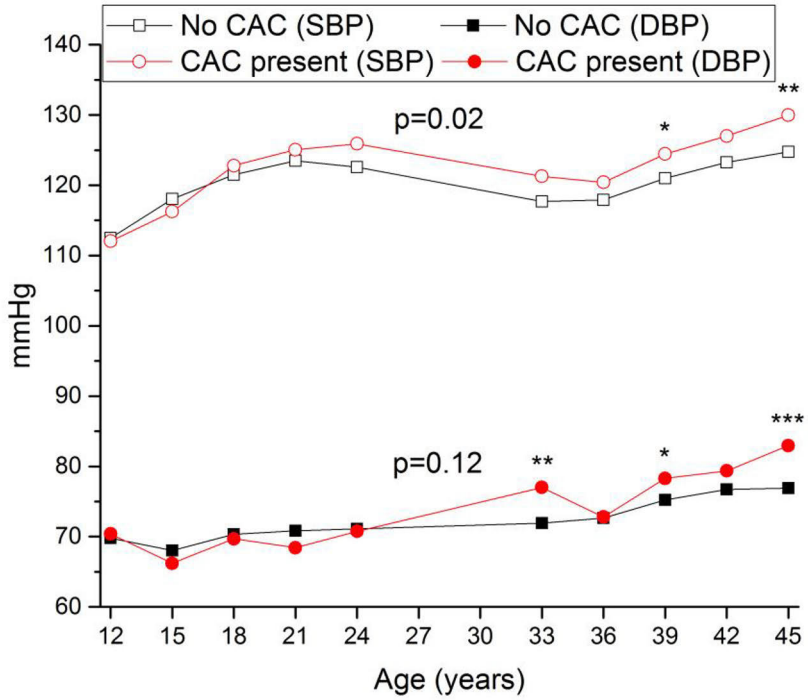
Data are mean ±SE, [1]Estimated values for Apo-A1 and Apo-B used for years 1980, 1983, and 1986. [2]Measurements available for 1986, 2001 and 2007. [3]Information not available for 2007. [4]Adjusted for age and sex, where applicable [5]Further adjusted for pack years of smoking, physical activity index, consumption of fruit, vegetables, and fish. [6]Further adjusted for BMI. [7]Further adjusted for SBP or LDL-C, where applicable.

LDL-C= low-density lipoprotein cholesterol; HDL-C= high-density lipoprotein cholesterol; Apo-A1= Apolipoprotein A1; Apo-B= Apolipoprotein B; BMI= body mass index; SBP= systolic blood pressure; DBP= diastolic blood pressure

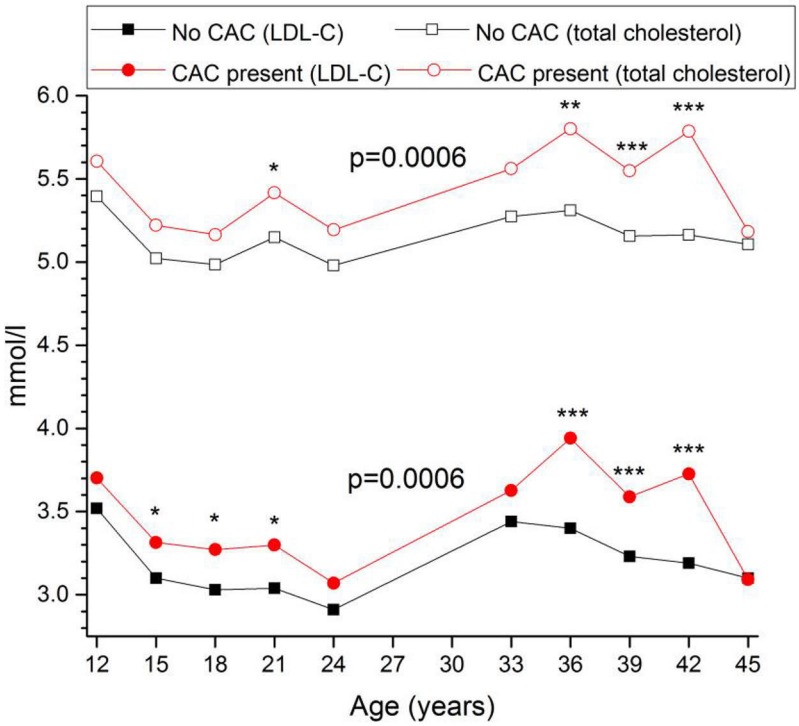
Statistically significant differences in risk factor levels between CAC groups (No CAC vs. CAC ≥1) were observed already in adolescence for LDL-C (age 15 years) and in early adulthood for total cholesterol and Apo-B (age 21 years for both). For BP measures, significant differences at specific age points were seen starting at age 33 (DBP) and 39 (SBP) years. Gaps between risk factor levels were amplified during follow-up from 0.21 mmol/l at age 15 years vs. 0.54 mmol/l at age 42 years for LDL-C, 0.27 mmol/l at age 21 years vs. 0.63 mmol/l for total cholesterol at age 42 years and 0.07 mmol/l at age 21 years vs. 0.15 mmol/l at age 42 years for Apo-B (Figure

6). Similar results were observed for risk factor trajectories based on CAC severity (No CAC, CAC score 1-99, and CAC score ≥100) for LDL-C (Figure 6D), Apo-B, and total cholesterol, while the association between CAC severity and mean longitudinal SBP was borderline significant (p=0.07). A general increase in total cholesterol, LDL-C, and Apo-B and a decrease in HDL-C and SBP levels was observed between ages 24 and 33 years. In age-stratified analyses from 1980 adjusted for sex to further study the possible association of SBP in adolescence and CAC in adulthood, only SBP among 18-year olds was associated with CAC (OR 1.8, 95% CI 1.2 – 2.7, p=0.007), while in the younger two cohorts (12- and 15-years of age) no statistically significant association was observed (OR 1.1, 95% CI 0.8-1.6, p=0.6 and OR 1.1, 95% CI 0.7-1.6, p=0.7, respectively).

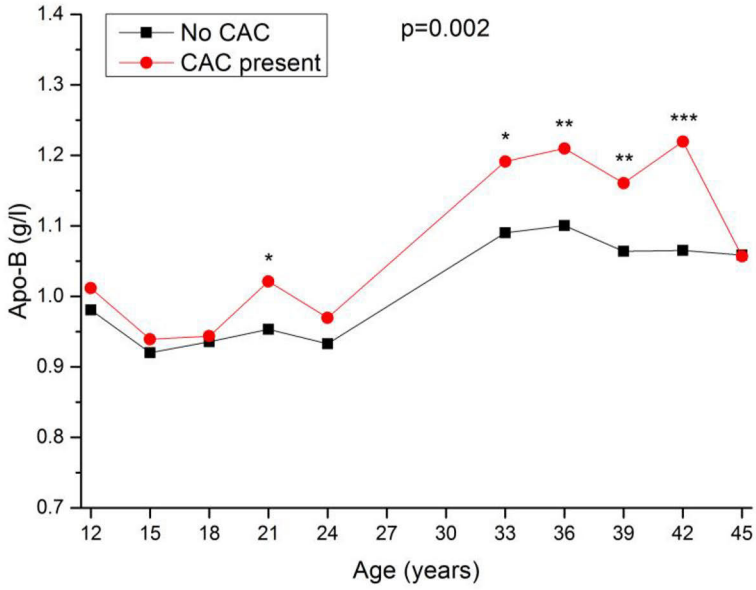
A)



B)


No CAC (LDL-C)
No CAC (total cholesterol)
CAC present (LDL-C)
CAC present (total cholesterol)
p=0.0006
p=0.0006
mmol/l
Age (years)

C)


No CAC
CAC present
p=0.002
Apo-B (g/l)
Age (years)

D)

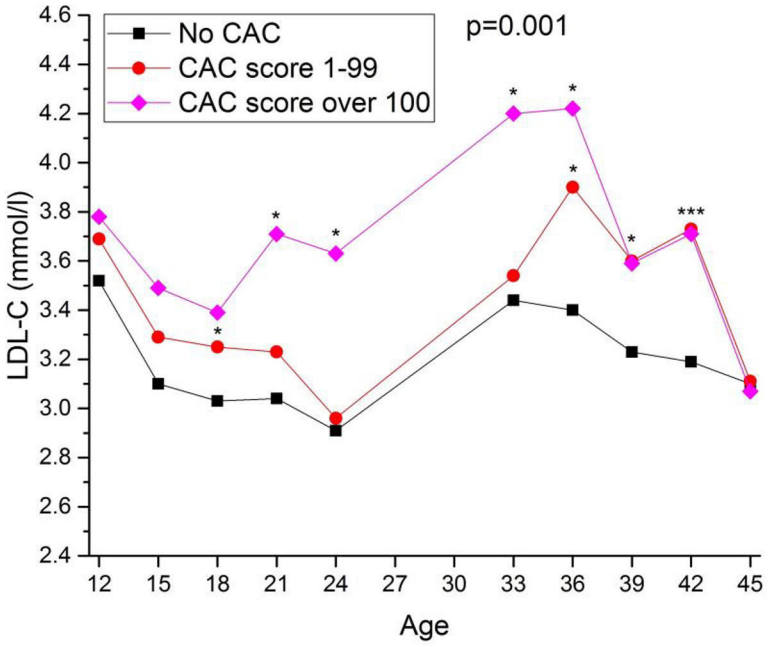


Figure 6. Risk factor trajectories (least square means) for SBP and DBP (A), LDL-C and total cholesterol (B), and Apo-B (C) based on CAC status (no vs. present) and CAC severity for LDL-C (D). *p<0.05, **p<0.01, ***p<0.001, p-value reported in the figure for average difference between groups across all time points. For D), statistically significant differences were seen between those with no CAC and those with a CAC score of 1-99 at ages 18, 36, 39, and 42. For those with a CAC score of >100 vs. 0, significant differences were seen at ages 21, 24, 33, and 36.

5.5 Risk factor levels and epicardial fat volumes

Across EFV quarters, increasing waist circumference, BMI, levels of Apo-B, total cholesterol, LDL-C, triglycerides, CRP, SBP, DBP, insulin, fasting glucose, and HOMA-index were observed (Table 10 A) In addition, a history of ever smoking, alcohol consumption, and MetS increased, while HDL-C levels and physical activity index decreased across the EFV quarters (Table 10 B). Skinfold thickness, BMI, and insulin levels measured in adolescence were higher with increasing EFV. However, after additional adjustment for BMI, only adulthood levels of Apo-B, a history of smoking, MetS, and alcohol consumption remained statistically significantly associated with EFV. In a multiple regression model including sex, age, BMI, Apo-B and a history of smoking, all risk factors except age showed a positive association with EFV.

Table 10. Adolescent (1980) and adult (2007) risk factor levels by sex-specific EFV quarters

A)

	Year	All participants (N=557)	1st quarter (N=138)	2nd quarter (N=140)	3rd quarter (N=140)	4th quarter (N=139)	p-value[1]	p-value[2]
Skinfold, cm	1980	29.8 (13.4)	26.8 (12.1)	27.9 (10.8)	30.4 (13.7)	34.1 (15.5)	<.0001	
Waist, cm	2007	92.1 (13.3)	82.3 (9.3)	88.6 (10.0)	93.4 (9.3)	103.4 (13.8)	<.0001	
BMI, kg/m^2	1980	19.7 (3.0)	18.9 (2.6)	19.5 (2.5)	19.5 (2.6)	20.7 (3.7)	<.0001	
	2007	26.9 (5.0)	23.6 (2.8)	25.5 (3.4)	27.1 (3.5)	31.3 (5.9)	<.0001	
Apo-B, g/l	1980	0.9 (0.2)	0.9 (0.2)	0.9 (0.2)	0.9 (0.2)	0.9 (0.2)	0.45	
	2007	1.1 (0.3)	0.9 (0.2)	1.1 (0.3)	1.1 (0.3)	1.2 (0.3)	<.0001	0.009
Apo-A1, g/l	1980	1.5 (0.2)	1.5 (0.2)	1.5 (0.3)	1.5 (0.2)	1.5 (0.2)	0.44	
	2007	1.6 (0.2)	1.6 (0.3)	1.6 (0.3)	1.6 (0.2)	1.6 (0.2)	0.11	
Total cholesterol, mmol/l	1980	5.1 (0.9)	5.1 (0.8)	5.1 (0.8)	5.1 (1.0)	5.1 (0.9)	0.69	
	2007	5.2 (1.0)	4.9 (0.9)	5.2 (0.9)	5.3 (0.9)	5.3 (1.0)	<.0001	0.054
LDL-C, mmol/l	1980	3.3 (0.8)	3.2 (0.7)	3.3 (0.7)	3.2 (0.9)	3.3 (0.8)	0.91	
	2007	3.2 (0.8)	3.0 (0.7)	3.3 (0.8)	3.3 (0.8)	3.3 (0.9)	0.005	0.07
HDL-C, mmol/l	1980	1.5 (0.3)	1.6 (0.3)	1.5 (0.3)	1.5 (0.3)	1.5 (0.3)	0.053	
	2007	1.3 (0.3)	1.4 (0.3)	1.4 (0.3)	1.3 (0.3)	1.3 (0.3)	<.0001	0.29
Triglycerides, mmol/l	1980	0.7 (0.3)	0.7 (0.3)	0.7 (0.3)	0.7 (0.3)	0.7 (0.4)	0.37	
	2007	1.5 (1.0)	1.1 (0.6)	1.4 (0.9)	1.6 (1.0)	1.8 (1.1)	<.0001	0.08
C-reactive protein, mg/l	1980	1.1 (3.4)	0.9 (2.6)	1.0 (3.8)	1.0 (3.0)	1.3 (3.9)	0.34	
	2007	2.1 (5.2)	1.8 (8.7)	1.5 (2.1)	2.0 (3.1)	3.2 (4.3)	0.003	0.50
SBP, mmHg	1980	116.5 (10.7)	116.1 (11.4)	117.0 (9.9)	114.8 (9.7)	118.1 (11.5)	0.20	
	2007	123.5 (16.0)	121.1 (17.3)	122.1 (15.0)	122.8 (15.3)	127.8 (15.4)	<.0001	0.51
DBP, mmHg	1980	70.5 (9.7)	70.5 (9.4)	71.1 (9.9)	69.8 (10.1)	70.6 (9.5)	0.79	
	2007	77.4 (12.3)	74.9 (12.7)	75.9 (12.2)	76.9 (11.6)	81.7 (11.7)	<.0001	0.36
Insulin, mU/l	1980	13.1 (5.8)	12.2 (5.3)	12.8 (5.4)	13.0 (5.4)	14.4 (6.8)	0.0019	0.37
	2007	9.6 (10.4)	6.6 (7.3)	7.8 (10.3)	9.6 (7.2)	14.1 (13.7)	<.0001	0.88
Fasting glucose, mmol/l	2007	5.4 (0.7)	5.3 (0.6)	5.3 (0.5)	5.5 (0.7)	5.6 (0.9)	<.0001	0.84

Data are mean (SD) or %. [1]p-values for multivariable models including age and sex where applicable, [2]p-values for multivariable models further adjusted for BMI, where applicable.
BMI= body mass index; Apo-A1= Apolipoprotein A1; Apo-B= Apolipoprotein B; LDL-C= low-density lipoprotein cholesterol; HDL-C= high-density lipoprotein cholesterol; SBP= systolic blood pressure; DBP= diastolic blood pressure

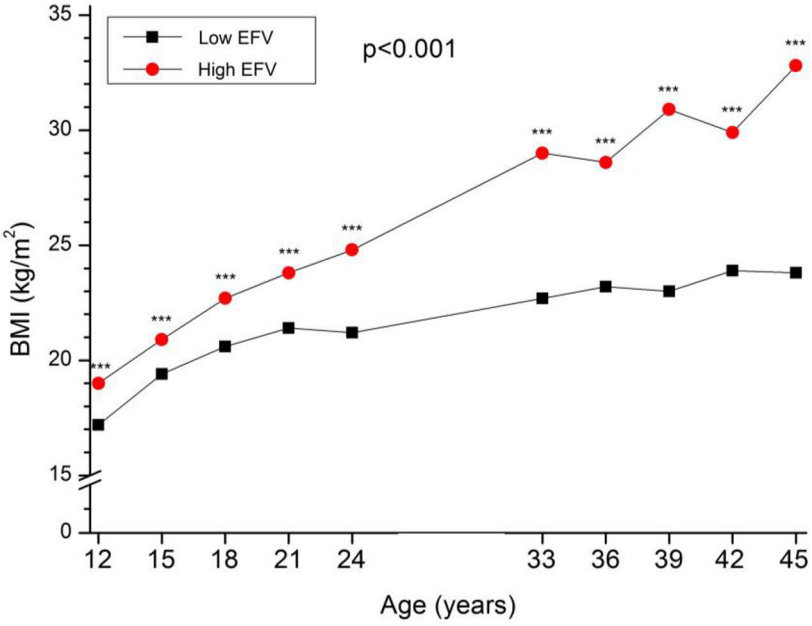
B)

	Year	All participants (N=557)	1st quarter (N=138)	2nd quarter (N=140)	3rd quarter (N=140)	4th quarter (N=139)	p-value[1]	p-value[2]
Alcohol units/day	2007	1.0 (1.2)	0.8 (1.0)	0.9 (1.1)	1.1 (1.3)	1.2 (1.4)	0.003	0.002
Physical activity index	1980	8.9 (1.8)	8.9 (1.9)	9.1 (1.8)	8.8 (1.8)	8.8 (1.8)	0.43	
	2007	8.6 (1.8)	8.9 (1.7)	8.9 (1.6)	8.5 (2.0)	8.2 (1.7)	0.0002	0.14
Daily smoking, %	1980	16.8	13.1	18.7	22.8	12.5	0.42	
	2007	17.6	14.7	18.7	19.9	17.4	0.60	
Ever smoking, %	1980	43.4	40.3	49.6	44.4	39.3	0.99	
	2007	50.6	41.7	54.0	55.4	51.1	0.044	0.0008
HOMA-index	2007	2.5 (4.2)	1.7 (2.2)	2.0 (3.3)	2.4 (2.2)	3.9 (6.8)	<.0001	0.86
Fruit intake, units (1980) or g (2007) /day	1980	6.4 (2.9)	6.7 (2.8)	6.5 (2.8)	6.4 (3.0)	6.1 (2.8)	0.26	
	2007	221 (200)	250 (205)	235 (248)	186 (147)	215 (184)	0.37	
Vegetable intake, units (1980) or g (2007) /day	1980	5.6 (2.9)	5.4 (3.0)	5.8 (3.0)	5.6 (2.9)	5.6 (2.9)	0.43	
	2007	278 (182)	276 (149)	275 (174)	266 (175)	294 (218)	0.11	
Type 2 diabetes, %	2007	2.3	1.4	0.7	2.1	5.0	0.06	
MetS prevalence, %	2007	22.4	4.0	13.9	23.8	46.6	<.0001	0.01

Data are mean (SD) or %. [1]p-values for multivariable models including age and sex where applicable, [2]p-values for multivariable models further adjusted for BMI, where applicable.
HOMA-index = Homeostatic model assessment

Those in the lowest quarter of EFV had consistently lower BMI throughout the follow-up period when compared to those in the highest quarter. The difference in average longitudinal mean levels of BMI among those from the lowest vs. highest quarter of BMI was 2.2 kg/m^2 (95%CI, 1.5–2.9, P=0.001) and the gap increased with age, especially in adulthood (from 1.8 kg/m^2 at age 12 years to 9.0 kg/m^2 at age 45 years) (Figure 7).

Figure 7. Body mass index (BMI) trajectories from childhood to adulthood among participants in the lowest vs. highest quarter of EFV in adulthood. ***p<0.001, p-value reported in the figure for average difference between groups across all time points



5.6 Epicardial fat volumes and markers of subclinical atherosclerosis

No statistically significant differences in CAC prevalence (16.7 % in the 1st quarter vs. 17.3 % in the 4th quarter, p=0.61), average CAC scores (14.4±65.2 vs. 5.5±21.6 respectively, p=0.08), or FMD (8.29±4.40 % vs. 8.61±4.55 % respectively, p=0.60) were observed across the EFV quarters. Increased carotid IMT (0.64±0.10 mm in the 1st quarter vs. 0.68±0.10 mm, in the 4th quarter p<0.001) and decreased carotid distensibility (1.83±0.58 %/10 mmHg vs. 1.63±0.62 %/10 mmHg respectively, p=0.002) were observed across the quarters. After adjustment for BMI, statistical significance was lost for both carotid IMT and carotid distensibility (Table 11).

Table 11. Subclinical markers of atherosclerosis by sex-specific epicardial fat volume quarters

	All participants (N=557)	1st quarter (N=138)	2nd quarter (N=140)	3rd quarter (N=140)	4th quarter (N=139)	p-value[1]	p-value[2]
CAC prevalence, %	18.7	16.7	20.7	20.0	17.3	0.61	
CAC score	9.9 (51.9)	14.4 (65.2)	16.7 (76.4)	3.2 (11.8)	5.5 (21.6)	0.08	
Intima-media thickness (mm)	0.66 (0.10)	0.64 (0.10)	0.67 (0.10)	0.66 (0.09)	0.68 (0.10)	<.0001	0.43
Carotid distensibility (%/10 mmHg)	1.68 (0.62)	1.83 (0.58)	1.61 (0.61)	1.66 (0.66)	1.63 (0.62)	0.002	0.89
Flow-mediated dilation (%)	8.80 (4.39)	8.29 (4.40)	8.86 (4.31)	9.47 (4.26)	8.61 (4.55)	0.60	

Data are mean (SD) for continuous variables or proportions for categorical variables. [1]p-values for multivariable models including age and sex. [2]p-values for multivariable models further adjusted for BMI.

6 Discussion

This study found that cardiovascular risk factor levels measured early in the life-course associate with subclinical atherosclerosis as shown by prevalent CAC in adulthood. Compared with those who did not have CAC in adulthood, those with CAC had higher LDL-C and SBP levels already in adolescence and higher levels of LDL-C, SBP, total cholesterol, and Apo-B, on average, throughout the study. CAC was not associated with ultrasound measures of subclinical atherosclerosis. EFV was strongly associated with body adiposity measures both in adolescence and adulthood, but associations with other risk factor levels, prevalence of CAC or ultrasound measures of subclinical atherosclerosis were largely diluted after adjustment for BMI. The main findings of the study are presented in Figure 8.

6.1 Participants

The Cardiovascular Risk in Young Finns Study is an on-going epidemiological study that began in 1980, when 3,596 participants out of a total of 4,320 randomly chosen individuals (83.2 % participation proportion) took part. The participants were chosen to represent the whole country with as many participants from rural and urban areas as well as equal numbers of males and females invited. The participants were concluded to be representative of the random sample (Åkerblom et al. 1985).

In this study, 589 of the original 3,596 participants took part in the CT sub-study when CAC and epicardial fat volumes measurements for performed. Those invited to the CT sub-study resided in Turku, Tampere, and Kuopio and represented the oldest three age-groups (birth cohorts) in the Cardiovascular Risk in Young Finns Study, aged 39, 42, and 45 years in 2007. A total of 802 were invited and the attendance proportion was 73 %. Risk factor levels at follow-up did not differ between those who participated and those who did not participate, as shown in Table 4.

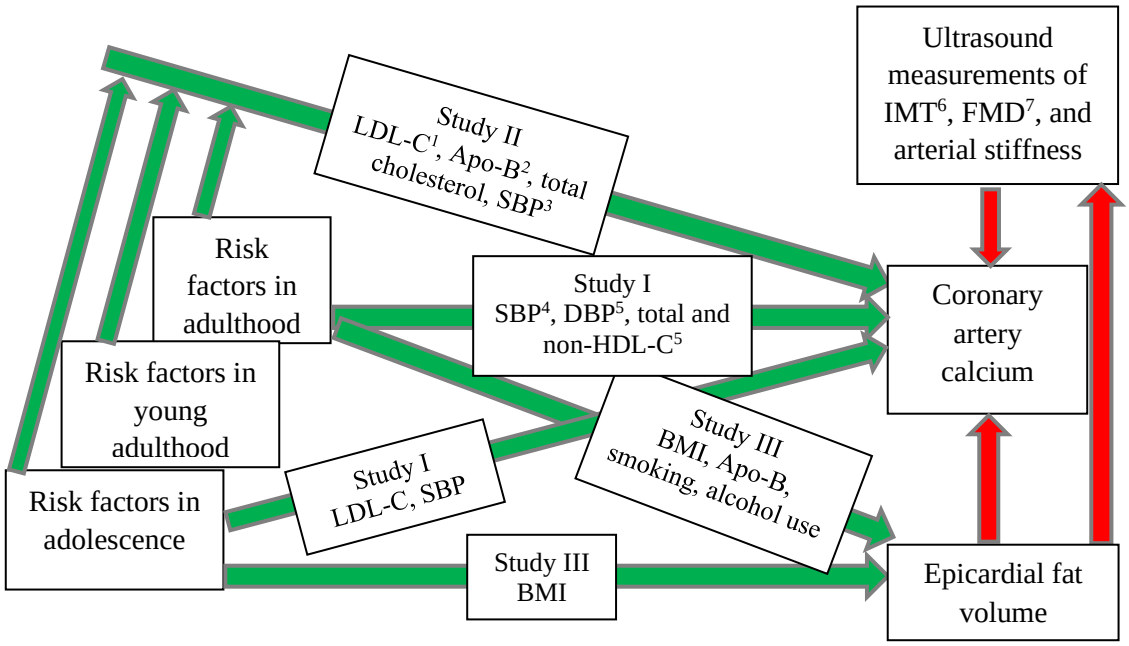


Figure 8. Main findings of the study, green arrow = independent association, red arrow = lack of independent association. [1]LDL-C = low-density lipoprotein cholesterol, [2]Apo-B = apolipoprotein B, [3]SBP = systolic blood pressure, [4]BP = blood pressure, [5]Non-HDL-C = non-high-density lipoprotein cholesterol, [6]IMT = intima-media thickness, [7]FMD = flow-mediated dilatation

.

6.2 Results

6.2.1 Prevalence of coronary artery calcification

In this study, 19.2 % of participants had visible CAC and it was more prevalent among males and those of older age. This is in line with other studies with participants of similar age. In the CARDIA study the overall prevalence of CAC was 9.6 % in participants aged 33–45 years and 13.3 % in participants aged 40–45 years (Loria et al. 2007). In the Muscatine Study, CAC was prevalent in 20.6 % of their 384 participants who were aged 29–37 years (Mahoney et al. 1996). In both studies, CAC was approximately three times more prevalent in males than in females (31 % vs. 10 % in the Muscatine study and 15.0 % vs 5.1 % in the CARDIA study), while

CAC prevalence was about two and a half times higher in males vs. females in this study. In addition, compared to two large studies of over 35,000 and 18,000 participants free of known CAD or CHD events or coronary interventions, participants in this study had comparable, albeit slightly lower, CAC scores stratified by age and sex (Hoff et al. 2001; Mitchell et al. 2001). This is probably because participants in the aforementioned studies were not randomly chosen but rather self-referred or referred by their physicians.

6.2.2 Cardiovascular risk factors associated with coronary artery calcification

In this study, levels of LDL-C and SBP measured already in adolescence were associated with CAC after a 27-year follow-up, even after adjustment for change in these risk factors between adolescence and adulthood. In addition to these risk factors, cumulative mean values of total cholesterol and Apo-B were higher among those with CAC at follow-up compared with those free of CAC. Collectively, these data imply that cumulative elevated risk factor levels play a role in the development of CAC starting in adolescence and their effect is at least partly independent of adult levels.

In general, risk factors that have been associated with CAC are similar to those for CHD (Hoff et al. 2003). Only one previous study has addressed if childhood risk factors associate with CAC in adulthood. An association between higher BMI in those aged 8-18 years and CAC measured at the mean age of 33 years was observed in the Muscatine Study (Mahoney et al. 1996). Statistically significant associations between childhood BP or lipid levels and CAC were not observed in the Muscatine Study, although levels of total cholesterol were higher among those with CAC in adulthood compared to those without. Higher BMI and BP levels as well as decreased HDL-C levels in young adulthood (aged 20-34 years) were also associated with subsequent CAC in the study. Information on childhood levels of HDL-C and LDL-C was not available in the Muscatine Study and the number of participants was lower and they were younger at follow-up compared to participants in the present study. In the CARDIA study, participants were followed for 15 years from early adulthood (mean age 25.1 years) to middle-age (mean age 40.3 years), when a CT scan was performed (Loria et al. 2007). In the study, early adulthood levels of LDL-C, SBP, and glucose as well as smoking were associated with CAC measured in mid-adulthood. Similar to the findings of the present study, baseline levels of LDL-C were associated with CAC independently of change in risk factor levels from early to mid-adulthood. Change in SBP levels was independently associated with CAC; whereas this association was borderline significant in the present study. BMI was not associated with CAC in the overall study population nor in a subgroup only

including those with BMI>30 in this study, which is surprising given the well-established adverse effects of adiposity on cardiovascular health.

The roles of serum total cholesterol, LDL-C, Apo-B, and SBP in the pathogenesis of CHD are well documented. Increased concentration of serum cholesterol is associated with higher prevalence of CHD (Keys et al. 1984). As the majority of circulating cholesterol is contained in LDL-particles, the unfavourable effect of LDL-C on CHD pathogenesis has also been well established. An increased concentration of LDL-C in the plasma accelerates the rate of LDL-C particle accumulation into the arterial wall (Tabas et al. 2007), which is considered the initial phase of atherogenesis (Lusis 2000). Lowering LDL-C levels using statin therapy has been shown to substantially reduce the risk of CHD events and all-cause mortality in both men and women (Fulcher et al. 2015). Furthermore, a meta-analysis using Mendelian randomization showed that long-term exposure to low LDL-C levels was associated with a significantly greater risk reduction in CHD risk when compared to statin treatment to achieve lower LDL-C levels initiated later in life (Ference et al. 2012). Apo-B is the primary structural component of LDL and other atherogenic lipoproteins and has been shown to be a better predictor of elevated CHD risk than LDL-C levels (Sniderman et al. 2019; Holme et al. 2008). Childhood levels of Apo-B have been associated with subclinical atherosclerosis in the Bogalusa and Young Finns studies (Frontini et al. 2008; Juonala et al. 2008). Increased levels of plasma lipoprotein a have also been associated with CAC (Garg et al. 2021; Greif et al. 2013).

Elevated blood pressure has several effects on the development of CHD. Hypertension causes increased endothelial permeability and is associated with macrophage accumulation, stimulation of smooth muscle cell proliferation, and elevated expression of inflammatory mediators in the arterial wall (Safar et al. 1998; Chobanian & Alexander 1996). Antihypertensive therapy by antihypertensive drugs has been shown to significantly reduce the risk of ischemic stroke and cardiovascular events (Thomopoulos et al. 2015). The American Academy of Pediatrics recommends that blood pressure should be measured annually in children and adolescents >3 years of age because of growing evidence suggesting prevention and intervention efforts should start a young age. In children and adolescents who are diagnosed with hypertension, a target blood pressure of <90th percentile or <130/80 mmHg, whichever is lowest, should be set. Recommended treatment options are lifestyle interventions, specifically increased physical activity and changes in diet. Pharmacologic treatment should be considered if treatment targets are not met after lifestyle interventions. (Flynn et al. 2017)

Guidelines concerning lipid measurements, however, are somewhat controversial. The National Heart, Lung, and Blood Institute recommends that in otherwise healthy individuals, a universal lipid screening should be performed at age

9-11 years and repeated at age 17-21, while the US Preventive Services Task Force found no direct evidence in their systematic review for benefits or harms of childhood screening or treatment on adult outcomes. (Expert Panel on Integrated Guidelines for Cardiovascular Health and Risk Reduction in Children and Adolescents, & National Heart, Lung, and Blood Institute 2011; Lozano et al. 2016) Considering the possible benefits of measuring risk factor levels in informing an individual on their risk of developing CHD, screening could nevertheless be recommended at an earlier age than is presently customary.

6.2.3 Risk factor trajectories and coronary artery calcification

Statistically significant differences at specific age-points between groups with CAC compared to those without were seen for LDL-C, total cholesterol and Apo-B levels already starting in adolescence or early adulthood. Using repeated measurements, a difference in BP levels between CAC groups was observed starting at age 33 years for DBP and 39 years for SBP. The results were similar for total cholesterol, LDL-C, and Apo-B when a three-scale measure of CAC severity was used instead of presence (yes/no) of CAC. For SBP, increases in mean longitudinal levels across groups based on CAC severity were borderline significant.

The age at which significantly different SBP levels were seen across CAC groups was in contrast to the findings of Study I where a significant difference in SBP was seen between CAC groups in 1980 (age 12-18). Further analyses revealed that this difference was driven by the oldest age cohort (age 18), whereas no statistically significant difference in SBP across CAC groups was seen in the two younger cohorts (age 12 and 15). This could be due to many factors: a cohort effect, random fluctuation or the association could be confounded by other risk factors. Most likely, however, adolescence SBP level is reflective of adult SBP and therefore the association of adolescence SBP and CAC is best seen in the oldest 18-year-old age cohort whose SBP levels correlate more strongly with adult SBP levels (Chen et al. 2008). Similarly in the Muscatine Study, no significant difference was seen between CAC groups in SBP measured at 8-18 years of age (Mahoney et al. 1996). Furthermore, using data from the i3C consortium, Juhola et al. found that the effect of elevated childhood BP on carotid atherosclerosis was significantly reduced in subjects whose BP levels were normalized in adulthood compared to those with constantly high BP levels and those whose BP levels first became high in adulthood. This suggests that even though BP levels early in life have an effect on subsequent development of atherosclerosis, they are at least partially reversible (Juhola et al. 2013).

The association of risk factor trajectories and CAC has previously been studied in the CARDIA study, where those with elevated BP trajectories were at increased odds of developing CAC compared with those in the low-stable BP group (Allen et al. 2014). Previously, Pletcher et al. have shown that the extent of exposure to prehypertension in young adulthood was associated with increasing prevalence of CAC 20 years later in the same study (Pletcher et al. 2008). A recent study using data from the Young Finns study show that exposure to non-HDL cholesterol was associated with CAC at all life stages but the strongest association was shown for exposure in adolescence (Armstrong et al. 2021). Similarly, life-long exposure to elevated BMI, central fatness, or BP has been linked to arterial stiffness, prevalence of CHD and cardiovascular mortality (Ferreira et al. 2012; Tirosh et al. 2011; Reinikainen et al. 2015).

The changes in risk factor levels in young adulthood are partly explained by age-related effects, especially increases in LDL-C, total cholesterol, and Apo-B and the decrease in HDL-C levels. Secular trends observed in the Young Finns Study, including decreasing BP, are most likely partially responsible for the observed decrease in SBP levels in young adulthood (Raiko et al. 2010; Juonala et al. 2004; Porkka et al. 1997). In addition, blood pressure was measured using a standard mercury sphygmomanometer in 1980 and 1983 and a random zero sphygmomanometer thereafter. This may have contributed to a slight decrease in blood pressure levels across the study population as blood pressure levels obtained using a random zero sphygmomanometer tend to be somewhat lower compared to those obtained with a standard mercury sphygmomanometer (Parker et al. 1988).

6.2.4 Association of coronary artery calcification and ultrasound measurements

In this study, a statistically significant association between CAC and ultrasound measurements of IMT, FMD and arterial stiffness was not observed despite the documented associations with CHD-related events and the extent of atherosclerotic burden (Burke et al. 1995; Bots et al. 1997; Lorenz et al. 2007; Matsuzawa et al. 2015; van Sloten et al. 2014). The lack of an association between IMT and CAC was surprising given the noted difference in SBP levels across the CAC groups as IMT and childhood as well as adulthood BP measures have been previously shown to be associated in the i3C consortium (Juhola et al. 2013). However, the observed differences in the ultrasound measurements between those with and without CAC were in the anticipated direction and a borderline significant difference was seen across CAC groups for carotid distensibility. Associations between carotid artery calcification and CAC were not studied but could provide insight into possible shared mechanisms in the development of calcified plaques in the vasculature.

6.2.5 Fat volumes

The mean fat volumes in this study were comparable to those reported in other studies. According to a review of EFV studies, mean fat volumes ranged from 65 to 240 cm^3 (mean age of participants was 50.2–65 years, 50.1% females) (Spearman et al. 2015). Given the somewhat younger population and the greater proportion of females (mean age 41.8 years, 56.3 % females) in the Young Finns Study, the relatively low EFV values compared with those from other studies could be expected.

6.2.6 Association of fat volumes and risk factors

Several risk factors measured in adulthood were less favorably associated with increasing EFV but after adjustment for BMI, the associations became non-significant for most risk factors. BMI and waist circumference were most strongly associated with EFV, while associations were also observed for adult levels of Apo-B, prevalence of MetS alcohol consumption, and history of smoking. Skinfold thickness and BMI were the only adolescent risk factors associated with EFV. These data suggest that the associations observed between risk factors and EFV are confounded via the effect of overall adiposity on EFV. Several studies have previously addressed the association of EFV and CVD risk factor levels, including reported associations with elevated triglyceride and lower HDL-C concentrations, hypertension, impaired fasting glucose, and adiposity measures (Mahabadi et al. 2013; Rosito et al. 2008). Prevalence of MetS increased markedly between the highest and lowest EFV quarters, which could be expected considering the strong association of BMI and waist circumference measured with EFV in the study. This is also in agreement with other studies (Pierdomenico et al. 2013, Yorgun et al. 2013). Most likely EFV serves as an indicator of overall visceral fat accumulation which at least partly explains its association with MetS.

6.2.7 Fat volumes and subclinical coronary heart disease

EFV was not associated with CAC or brachial FMD and associations with carotid distensibility and IMT became non-significant after adjustment for BMI in this study. Therefore, EFV was not independently associated with subclinical atherosclerosis in this study despite other data linking measured EFV with cardiac events or CAC (Ding et al. 2009; Mahabadi et al. 2013; Nagy et al. 2017; Rosito et al. 2008). Considering the current hypothesis that epicardial fat affects atherogenesis predominantly through local signalling (Mazurek et al. 2003; Sacks & Fain 2007), the lack of an independent association with carotid ultrasound measurements in this study is somewhat expected. Additionally, the associations observed between EFV

and carotid distensibility and IMT measurements appeared to be confounded by central adiposity.

Studies that have reported an association between EFV and CAC or CHD events have generally included individuals who have been either referred by their physicians or self-referred, rather than being population-based. There has been considerable variation in the study designs and methods, which has complicated comparison of results and meta-analysis of the data (Spearman et al. 2015). In addition, participants in this study were younger and CAC less prevalent than in most other studies, which might partly explain the differing results. Considerable overlap in EFV has been noted between those with and without events during follow-up, which indicates that EFV measurement alone is not sufficient in estimating risk in individuals but combined with risk factor and CAC data it can improve risk assessment (Spearman et al. 2015). In contrast to most studies on EFV and CHD, the association of EFV and CAC has been attenuated in a number of studies after adjustment for other risk factors (Bucci et al. 2011; Tanami et al. 2015; Pracon et al. 2011) and a trend for decreasing EFV was seen for increasing CAC after adjustment for CHD risk factors in the Heinz Nixdorf Recall Study (Mahabadi et al. 2013). In addition, a study by Alexopoulos using contrast-enhanced CT angiography found that EFV was larger in those with non-calcified or mixed plaques and obstructive CHD compared with those with calcified or no plaques (Alexopoulos et al. 2010). Therefore, it is possible that epicardial fat has a greater influence on the development of mixed and non-calcified plaques, which may in turn, be more prone to rupture and CHD events.

6.3 Implications of the study

The findings of this study add to the growing understanding of the development of CHD and its origins in childhood. Significant differences in risk factor levels between those who developed CAC in adulthood and those who did not were seen nearly three decades prior to the measurement of CAC. Therefore, identification of individuals at an elevated risk of developing CHD could be commenced relatively early in the life-course. Moreover, the results of the study indicate that maintaining lower risk factor levels throughout the life-course might be important in the prevention of CHD. In 2010, the American Heart Association introduced the concept of ideal cardiovascular health which is based on seven metrics: smoking, BMI, diet, physical activity, cholesterol, fasting glucose and blood pressure levels, all categorized into ideal, intermediate or poor levels. The focus of the concept of ideal cardiovascular health is promoting primordial prevention in the population, i.e. preventing the development of risk factors and therefore preventing the adverse effects of the risk factors during the life-course. (Lloyd-Jones et al. 2010)

Intervention has been shown to be a safe and effective method to influence risk factor profiles by delaying the onset of smoking and reducing cholesterol levels in the North Karelia Youth Project (Vartiainen et al. 1991) and the STRIP study (Kaitosaari et al. 2003). A favorable effect on BP was also seen in the STRIP study from infancy to young adulthood following a 20-year dietary intervention initiated at infancy (Laitinen et al. 2020). A 2-year dietary and physical activity intervention in the Physical Activity and Nutrition in Children Study resulted in a decrease in LDL-C levels among children aged 6-9 years at baseline (Eloranta et al. 2021). This presents a question as to when screening of risk factor levels should commence, and when intervention or treatment should be initiated based on those measurements. Findings from this thesis suggest that CHD has its roots in childhood and therefore intervention and prevention would likely be most effective if initiated early, either primordial, or when suboptimal risk factor levels are first identified. On the other hand, the possible outcomes of the intervention are remote, often several decades away.

EFV was not associated with measures of subclinical atherosclerosis and was strongly associated with BMI in this study. EFV measurement is substantially more time-consuming than measuring CAC when performed user-dependently, as in this study. Therefore, the usefulness of measuring EFV compared to measuring CAC alone in this otherwise relatively healthy population was unclear.

6.4 Strengths and limitations

Strengths of this study were the longitudinal, population-based design of the Young Finns Study as well as the long follow-up from adolescence to adulthood with comparably low drop-out rates; and the extensive data on physical, laboratory, lifestyle, ultrasound, and CT measures that were acquired using well-established methods.

A limitation of this study is that cardiovascular events were not used as an end-point because of the relatively young age, and therefore lack of events, of the participants. Nevertheless, presence of CAC has been shown to be a robust marker of coronary atherosclerosis and a predictor of cardiovascular events. In this study, the CAC score was calculated by computer software (CareStream) used after manually defining regions of interest using the Agatston method (Agatston et al. 1990), which is the most widely used measure of arterial calcification. However, the Agatston method has limitations. For example, it does not take into account the distribution of CAC or the number of coronary arteries with CAC. It also has fixed imaging parameters including 3 mm slice thickness and 120 kV voltage as well as a higher score assigned to higher density plaques. Using data on the number of vessels with CAC in addition to the CAC score alone improved the ability to predict CHD

and CVD events in the Multi-Ethnic Study of Atherosclerosis (Blaha et al. 2016). Modifying the imaging parameters could lead to increased sensitivity and reduced radiation doses (Greenland et al. 2018). Furthermore, CAC density was inversely associated with CHD and CVD risk in the Multi-Ethnic Study of Atherosclerosis, whereas the Agatston score assigns a higher score for increased plaque density (Criqui et al. 2014). The software also calculated a volume score, but not a mass score which has been reported to be a considerably accurate estimation of the physical mass of coronary calcium deposits (Oudkerk et al. 2008). Nevertheless, use of the Agatston score in this study allowed direct comparison with other studies and databases. As different CT devices were used in this study, a phantom with known calcium deposits was scanned by all CT devices. The coefficient of variation, 3.9 %, between all of the phantom scans is considered acceptable.

In the present study, EFV was measured from CT scans using the method introduced in participants of the Framingham Heart Study (Mahabadi et al. 2009). However, the term epicardial fat was used instead of pericardial fat for consistency with the corresponding original publication even though fat located within the parietal and not only the visceral layer of the pericardium was included in the measurement of EFV. Quantification of epicardial fat using CT is considered more accurate than echocardiography and provides a measure of volume, in contrast to fat thickness (Iacobellis et al. 2009). It is more time-consuming and expensive than echocardiography, which, in contrast, is more readily available and does not subject participants to radiation. Automated software to measure EFV has, however, proven time-saving and accurate and could provide a more efficient way of quantifying fat volumes compared to the manual approach (Spearman et al. 2014). TTFV and EPFV measurements included patchy fat deposits within the thoracic cavity without a minimum size for deposits and are likely more prone to measurement inaccuracies than EFV measurement. However, EFV was used as the primary outcome in the analyses.

Another potential limitation is bias due to differential loss to follow-up, though no differences in risk factor levels at follow-up were observed between those who did, and those who did not, participate in the CT sub-study. In addition, previous analyses of baseline data have shown that those lost to follow-up have not differed with regards to the major risk factor levels (Raitakari et al. 2008) and that loss to follow-up in this cohort is lower than those of other similar cohorts with similar follow-up time periods (Dwyer et al. 2013). Therefore, bias due to differential loss to follow-up is expected to be minimal. The number of participants in this study was relatively large (N=589) but given their young age, the number of subjects with detectable CAC was rather low (19.2%, N=113) and therefore, the study may have been underpowered to detect some differences in risk factor levels. Furthermore, some statistical models including many variables may have been overfitted. Also,

Apo-B and Apo-A1 measurements were not available for all participants from 1980–1986 and computationally estimated values were used instead. This may have influenced the results concerning these risk factors, though, according to previous data in the Young Finns Study, correlations between estimated and measured levels were strong and were replicated in a large sample of over 15,000 participants (Raitakari et al. 2013).

Reproducibility data for CAC and EFV measurements was limited as inter- or intra-observer variability for epicardial fat measurements and inter-observer variability for CAC measurements was not measured. BP was measured only during the visits and no ambulatory monitoring was used; therefore no data on circadian BP variability was available. BMI and waist circumference in adulthood were used as a proxy for adiposity instead of direct measurements of total body fat mass, body composition or visceral fat distribution, which could better differentiate participants with normal or slightly overweight BMI. Finally, despite the use of a vast number of risk factor variables, some possibly relevant risk factors, such as exposure to air pollution or passive smoking, psychosocial and socioeconomic factors or genetics, were not included in this thesis.

6.5 Future research perspectives

The results of this study indicate that adolescent levels of LDL-c are associated with the development of CAC in adulthood. At the time of this study, no data directly linking childhood or adolescence risk factors to CHD events exist. As the participants in the longitudinal follow-up studies, such as the Cardiovascular Risk in Young Finns Study, age, associations between cardiovascular risk factors and CHD events can be studied instead of using surrogate markers, such as CAC used in this study. This will potentially confirm the results obtained from longitudinal studies linking childhood and youth risk factors with the development of CHD. Furthermore, data from on-going intervention studies, such as STRIP and the Physical Activity and Nutrition in Children Study, will show the possible effects of early lifestyle intervention on CHD outcomes. This data is needed to clarify screening and intervention guidelines in children, adolescents and young adults. Before that data becomes available, measuring CAC in other on-going cohort studies with data from childhood and adolescence could further our understanding on the development of CHD as only two such studies exist to date. Data on genetics could bring insight into gene-lifestyle interactions in the development of CAC in studies with long-term data.

Cumulative risk factor levels were associated with CAC in adulthood in this thesis. More studies are needed to address whether the effect of unfavorable risk factor levels in childhood on the development of CHD is reversible if better risk factor levels are achieved or if there is a vulnerable period in the life-course where

adverse effects on the development of CHD are more likely in the presence of adverse risk factors as suggested by recent data (Armstrong et al. 2021; Buscot et al. 2018). Moreover, new lifecourse modelling approaches could help determine the relative importance of exposure to risk factors across the lifecourse and shed light on the possible importance of SBP measured in adolescence on CHD development.

In this study, an association between EFV and subclinical atherosclerosis quantified by CAC or ultrasound measurements after adjustment for BMI, was not observed. More studies are needed to clarify the role of epicardial fat tissue in the development of CHD in apparently healthy young individuals and whether the measurement of EFV gives additional information on an individual's risk for developing CHD than measurement of CAC and risk factor levels.

7 Summary and Conclusions

1. CAC was prevalent in 19.2 % of participants aged 39-45 years. Elevated LDL-C levels measured in adolescence were associated with CAC measured in adulthood independent of the change in levels during follow-up. These findings add to the evidence base that adolescence risk factor levels play an important role in the pathogenesis of CHD.

2. Higher risk factor trajectories for LDL-C, total cholesterol, and Apo-B were identified starting already in adolescence and in adulthood for SBP in those with CAC in adulthood versus those without CAC and the differences in risk factor levels increased during the life-course. This further emphasizes the role of life-long exposure to adverse risk factor levels on the development of CHD.

3. No statistically significant associations were found between EFV and CAC or carotid ultrasound measurements after adjustment for BMI.
BMI and other markers of adiposity measured both in adolescence and adulthood were strongly associated with EFV. Most other associations with risk factors were attenuated in multivariable analyses that adjusted for adiposity measures.

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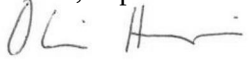

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References

Agatston, A.S., Janowitz, W.R., Hildner, F.J., Zusmer, N.R., Viamonte, M. & Detrano, R. 1990, "Quantification of coronary artery calcium using ultrafast computed tomography.", *Journal of the American College of Cardiology*, vol. 15, no. 4, pp. 827-832.

Alberti, K.G. & Zimmet, P.Z. 1998, "Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation.", *Diabetic Medicine*, vol. 15, no. 7, pp. 539-53.

Alexopoulos, N., McLean, D. S., Janik, M., Arepalli, C. D., Stillman, A. E., & Raggi, P. 2010, "Epicardial adipose tissue and coronary artery plaque characteristics." *Atherosclerosis*, vol. 210, no. 1, pp. 150–154.

Allen, N., Siddique, J., Wilkins, J., Shay, C., Lewis, C., Goff, D., Jacobs, D., Liu, K. & Lloyd Jones, D. 2014, "Blood pressure trajectories in early adulthood and subclinical atherosclerosis in middle age.", *JAMA: the Journal of the American Medical Association*, vol. 311, no. 5, pp. 490-497.

Allen, N., Krefman, A., Labarthe, D., Greenland, P., Juonala, M., Kähönen, M., Lehtimäki, T., Day, R., Bazzano, L., Van Horn, L., Liu, L., Alonso, C., Webber, L., Pahkala, K., Laitinen, T., Raitakari, O., & Lloyd-Jones, D. 2020, "Cardiovascular Health Trajectories From Childhood Through Middle Age and Their Association With Subclinical Atherosclerosis", *JAMA cardiology*, vol. 5, no 5, pp. 557–566.

Anderson, T.J., Uehata, A., Gerhard, M.D., Meredith, I.T., Knab, S., Delagrange, D., Lieberman, E.H., Ganz, P., Creager, M.A. & Yeung, A.C. 1995, "Close relation of endothelial function in the human coronary and peripheral circulations.", *Journal of the American College of Cardiology*, vol. 26, no. 5, pp. 1235-1241.

Arad, Y., Spadaro, L.A., Goodman, K., Newstein, D. & Guerci, A.D. 2000, "Prediction of coronary events with electron beam computed tomography.", *Journal of the American College of Cardiology*, vol. 36, no. 4, pp. 1253-1260.

Armstrong, M. K., Fraser, B. J., Hartiala, O., Buscot, M. J., Juonala, M., Wu, F., Koskinen, J., Hutri-Kähönen, N., Kähönen, M., Laitinen, T. P., Lehtimäki, T., Viikari, J., Raitakari, O. T., & Magnussen, C. G. 2021, "Association of Non-High-Density Lipoprotein Cholesterol Measured in Adolescence, Young Adulthood, and Mid-Adulthood With Coronary Artery Calcification Measured in Mid-Adulthood." *JAMA cardiology*, e207238. Advance online publication.

Avolio, A., Jones, D. & Tafazzoli Shadpour, M. 1998, "Quantification of alterations in structure and function of elastin in the arterial media.", *Hypertension*, vol. 32, no. 1, pp. 170-175.

Baker, A., da Silva, N., Quinn, D., Harte, A., Pagano, D., Bonser, R., Kumar, S. & McTernan, P. 2006, "Human epicardial adipose tissue expresses a pathogenic profile of adipocytokines in patients with cardiovascular disease.", *Cardiovascular Diabetology*, vol. 5, pp. 1-1.

Beckman, J.A., Ganz, J., Creager, M.A., Ganz, P. & Kinlay, S. 2001, "Relationship of clinical presentation and calcification of culprit coronary artery stenoses.", *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 21, no. 10, pp. 1618-1622.

Berenson, G.S., Srinivasan, S.R., Bao, W., Newman, W.P., Tracy, R.E. & Wattigney, W.A. 1998, "Association between multiple cardiovascular risk factors and atherosclerosis in children and

young adults. The Bogalusa Heart Study.", *The New England journal of medicine*, vol. 338, no. 23, pp. 1650-1656.

Blaha, M. J., Budoff, M. J., Tota-Maharaj, R., Dardari, Z. A., Wong, N. D., Kronmal, R. A., Eng, J., Post, W. S., Blumenthal, R. S., & Nasir, K. 2016, "Improving the CAC Score by Addition of Regional Measures of Calcium Distribution: Multi-Ethnic Study of Atherosclerosis." *JACC. Cardiovascular imaging*, vol. 9, no. 12, pp. 1407–1416

Block, G. A., Spiegel, D. M., Ehrlich, J., Mehta, R., Lindbergh, J., Dreisbach, A., & Raggi, P. 2005, "Effects of sevelamer and calcium on coronary artery calcification in patients new to hemodialysis." *Kidney international*, vol. 68, no. 4, pp. 1815–1824.

Bots, M.L., Hoes, A.W., Koudstaal, P.J., Hofman, A. & Grobbee, D.E. 1997, "Common carotid intima-media thickness and risk of stroke and myocardial infarction: the Rotterdam Study.", *Circulation*, vol. 96, no. 5, pp. 1432-1437.

Brinkley, T.E., Hsu, F., Carr, J.J., Hundley, W.G., Bluemke, D.A., Polak, J.F. & Ding, J. 2011, "Pericardial fat is associated with carotid stiffness in the Multi-Ethnic Study of Atherosclerosis.", *Nutrition Metabolism and Cardiovascular Diseases*, vol. 21, no. 5, pp. 332-338.

Bucci, M., Joutsiniemi, E., Saraste, A., Kajander, S., Ukkonen, H., Saraste, M., Pietilä, M., Sipilä, H., Teräs, M., Mäki, M., Airaksinen, K.E.J., Hartiala, J., Knuuti, J. & Iozzo, P. 2011, "Intrapericardial, but not extrapericardial, fat is an independent predictor of impaired hyperemic coronary perfusion in coronary artery disease.", *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 31, no. 1, pp. 211-218.

Budoff, M. J., Nasir, K., McClelland, R. L., Detrano, R., Wong, N., Blumenthal, R. S., Kondos, G., & Kronmal, R. A. 2009, "Coronary calcium predicts events better with absolute calcium scores than age-sex-race/ethnicity percentiles: MESA (Multi-Ethnic Study of Atherosclerosis)." *Journal of the American College of Cardiology*, vol. 53, no.4, pp. 345–352.

Budoff, M.J., Shaw, L., Liu, S., Weinstein, S., Mosler, T., Tseng, P., Flores, F., Callister, T., Raggi, P. & Berman, D. 2007, "Long-term prognosis associated with coronary calcification: observations from a registry of 25,253 patients.", *Journal of the American College of Cardiology*, vol. 49, no. 18, pp. 1860-1870.

Budoff, M. J., Yu, D., Nasir, K., Mehrotra, R., Chen, L., Takasu, J., Agrawal, N., Liu, S. T., & Blumenthal, R. S. 2005, "Diabetes and progression of coronary calcium under the influence of statin therapy.", *American heart journal*, vol.149, pp. 695–700.

Burke, G.L., Evans, G.W., Riley, W.A., Sharrett, A.R., Howard, G., Barnes, R.W., Rosamond, W., Crow, R.S., Rautaharju, P.M. & Heiss, G. 1995, "Arterial wall thickness is associated with prevalent cardiovascular disease in middle-aged adults. The Atherosclerosis Risk in Communities (ARIC) Study.", *Stroke*, vol. 26, no. 3, pp. 386-391.

Buscot, M. J., Thomson, R. J., Juonala, M., Sabin, M. A., Burgner, D. P., Lehtimäki, T., Hutri-Kähönen, N., Viikari, J., Raitakari, O. T., & Magnussen, C. G. 2018, "Distinct child-to-adult body mass index trajectories are associated with different levels of adult cardiometabolic risk.", *European heart journal*, vol. 39, no. 24, pp. 2263–2270.

Callister, T. Q., Cooil, B., Raya, S. P., Lippolis, N. J., Russo, D. J., & Raggi, P. 1998, "Coronary artery disease: improved reproducibility of calcium scoring with an electron-beam CT volumetric method." *Radiology*, vol. 208, no. 3, pp. 807–814.

Carr, J. J., Jacobs, D. R., Jr, Terry, J. G., Shay, C. M., Sidney, S., Liu, K., Schreiner, P. J., Lewis, C. E., Shikany, J. M., Reis, J. P., & Goff, D. C., Jr. 2017, "Association of Coronary Artery Calcium in Adults Aged 32 to 46 Years With Incident Coronary Heart Disease and Death." *JAMA cardiology*, vol. 2, no. 4, pp. 391–399.

Celermajer, D.S., Sorensen, K.E., Gooch, V.M., Spiegelhalter, D.J., Miller, O.I., Sullivan, I.D., Lloyd, J.K. & Deanfield, J.E. 1992, "Non-invasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis.", *The Lancet*, vol. 340, no. 8828, pp. 1111-1115.

Chen X, Wang Y. 2008 "Tracking of blood pressure from childhood to adulthood: a systematic review and meta-regression analysis." *Circulation*, vol. 117, no. 25, pp. 3171-3180.

Chobanian, A.V. & Alexander, R.W. 1996, "Exacerbation of atherosclerosis by hypertension. Potential mechanisms and clinical implications.", *Archives of Internal Medicine*, vol. 156, no. 17, pp. 1952-1956.

Criqui, M. H., Denenberg, J. O., Ix, J. H., McClelland, R. L., Wassel, C. L., Rifkin, D. E., Carr, J. J., Budoff, M. J., & Allison, M. A. 2014, "Calcium density of coronary artery plaque and risk of incident cardiovascular events." *JAMA: the Journal of the American Medical Association* , vol. 311, no. 3, pp. 271–278.

Cutter, G.R., Burke, G.L., Dyer, A.R., Friedman, G.D., Hilner, J.E., Hughes, G.H., Hulley, S.B., Jacobs, D.R., Liu, K. & Manolio, T.A. 1991, "Cardiovascular risk factors in young adults. The CARDIA baseline monograph.", *Controlled clinical trials*, vol. 12, no. 1 Suppl, pp. 1S-77S.

Davis, P.H., Dawson, J.D., Riley, W.A. & Lauer, R.M. 2001, "Carotid intimal-medial thickness is related to cardiovascular risk factors measured from childhood through middle age: The Muscatine Study.", *Circulation*, vol. 104, no. 23, pp. 2815-2819.

Demer, L. & Tintut, Y. 2008, "Vascular calcification: pathobiology of a multifaceted disease.", *Circulation*, vol. 117, no. 22, pp. 2938-2948.

Detrano, R., Guerci, A., Carr, J.J., Bild, D., Burke, G., Folsom, A., Liu, K., Shea, S., Szklo, M., Bluemke, D., O'Leary, D., Tracy, R., Watson, K., Wong, N. & Kronmal, R. 2008, "Coronary calcium as a predictor of coronary events in four racial or ethnic groups.", *The New England journal of medicine*, vol. 358, no. 13, pp. 1336-1345.

Ding, J., Hsu, F. C., Harris, T. B., Liu, Y., Kritchevsky, S. B., Szklo, M., Ouyang, P., Espeland, M. A., Lohman, K. K., Criqui, M. H., Allison, M., Bluemke, D. A., & Carr, J. J. 2009, "The association of pericardial fat with incident coronary heart disease: the Multi-Ethnic Study of Atherosclerosis (MESA).", *The American Journal of Clinical Nutrition*, vol. 90, no. 3, pp. 499-504.

Dwyer, T., Sun, C., Magnussen, C., Raitakari, O., Schork, N., Venn, A., Burns, T., Juonala, M., Steinberger, J., Sinaiko, A., Prineas, R., Davis, P., Woo, J., Morrison, J., Daniels, S., Chen, W., Srinivasan, S., Viikari, J. & Berenson, G. 2013, "Cohort Profile: the international childhood cardiovascular cohort (i3C) consortium.", *International journal of epidemiology*, vol. 42, no. 1, pp. 86-96.

Ehara, S., Kobayashi, Y., Yoshiyama, M., Shimada, K., Shimada, Y., Fukuda, D., Nakamura, Y., Yamashita, H., Yamagishi, H., Takeuchi, K., Naruko, T., Haze, K., Becker, A., Yoshikawa, J. & Ueda, M. 2004, "Spotty calcification typifies the culprit plaque in patients with acute myocardial infarction: an intravascular ultrasound study.", *Circulation*, vol. 110, no. 22, pp. 3424-3429.

Eloranta, A. M., Sallinen, T., Viitasalo, A., Lintu, N., Väistö, J., Jalkanen, H., Tompuri, T. T., Soininen, S., Haapala, E. A., Kiiskinen, S., Schnurr, T. M., Kilpeläinen, T. O., Mikkonen, S., Savonen, K., Atalay, M., Brage, S., Laaksonen, D. E., Lindi, V., Ågren, J., Schwab, U., Jääskeläinen J. & Lakka, T. A. 2021, "The effects of a 2-year physical activity and dietary intervention on plasma lipid concentrations in children: the PANIC Study." *European journal of nutrition*, vol 60, no. 1, pp. 425–434.

Enos, W.F., Holmes, R.H. & Beyer, J. 1953, "Coronary disease among United States soldiers killed in action in Korea; preliminary report.", *Journal of the American Medical Association*, vol. 152, no. 12, pp. 1090-1093.

Eroglu, S., Sade, L., E Yildirir, A., Bal, U., Ozbicer, S., Ozgul, A.S., Bozbas, H., Aydinalp, A. & Muderrisoglu, H. 2009, "Epicardial adipose tissue thickness by echocardiography is a marker for the presence and severity of coronary artery disease.", *NMCD.Nutrition Metabolism and Cardiovascular Diseases*, vol. 19, no. 3, pp. 211-217.

Expert Panel on Integrated Guidelines for Cardiovascular Health and Risk Reduction in Children and Adolescents, & National Heart, Lung, and Blood Institute 2011, "Expert panel on integrated guidelines for cardiovascular health and risk reduction in children and adolescents: summary report." *Pediatrics*, vol. *128 Suppl 5*, S213–S256.

Ference, B., Yoo, W., Alesh, I., Mahajan, N., Mirowska, K., Mewada, A., Kahn, J., Afonso, L., Williams, K. & Flack, J. 2012, "Effect of long-term exposure to lower low-density lipoprotein

cholesterol beginning early in life on the risk of coronary heart disease: a Mendelian randomization analysis.", *Journal of the American College of Cardiology*, vol. 60, no. 25, pp. 2631-2639.

Ferreira, I., van de Laar, R.J., Prins, M., Twisk, J. & Stehouwer, C. 2012, "Carotid stiffness in young adults: a life-course analysis of its early determinants: the Amsterdam Growth and Health Longitudinal Study.", *Hypertension*, vol. 59, no. 1, pp. 54-61.

Flynn, J., Kaelber, D., Baker-Smith, C., Blowey, D., Carroll, A., Daniels, S., de Ferranti, S., Dionne, J., Falkner, B., Flinn, S., Gidding, S., Goodwin, C., Leu, M., Powers, M., Rea, C., Samuels, J., Simasek, M., Thaker, V., Urbina, E., & subcommittee on screening and management of high blood pressure in children. 2017, "Clinical Practice Guideline for Screening and Management of High Blood Pressure in Children and Adolescents." *Pediatrics*, vol. 140, no. 3: e20171904

Friedewald, W.T., Levy, R.I. & Fredrickson, D.S. 1972, "Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge.", Clinical chemistry, vol. 18, no. 6, pp. 499-502.

Frontini, M., Srinivasan, S., Xu, J., Tang, R., Bond, M.G. & Berenson, G. 2008, "Usefulness of childhood non-high density lipoprotein cholesterol levels versus other lipoprotein measures in predicting adult subclinical atherosclerosis: the Bogalusa Heart Study.", *Pediatrics*, vol. 121, no. 5, pp. 924-929.

Fulcher, J., O'Connell, R., Voysey, M., Emberson, J., Blackwell, L., Mihaylova, B., Simes, J., Collins, R., Kirby, A., Colhoun, H., Braunwald, E., La Rosa, J., Pedersen, T.R., Tonkin, A., Davis, B., Sleight, P., Franzosi, M., Baigent, C. & Keech, A. 2015, "Efficacy and safety of LDL-lowering therapy among men and women: meta-analysis of individual data from 174,000 participants in 27 randomised trials.", *The Lancet*, vol. 385, no. 9976, pp. 1397-1405.

Gall S.L., Jose K., Smith K., Dwyer T. &Venn A. 2009, "The childhood determinants of adult health study: a profile of a cohort study to examine the childhood influences on adult cardiovascular health." Australas Epidemiol; vol. 16 pp. 35-39.

Garg, P.K., Guan, W., Karger, A.B., Steffen, B.T., Budoff, M., & Tsai, M.Y. 2021, "Lipoprotein (a) and risk for calcification of the coronary arteries, mitral valve, and thoracic aorta: The Multi-Ethnic Study of Atherosclerosis." Journal of cardiovascular computed tomography, vol. 15, no. 2, pp. 154–160.

Gorter, P., de Vos, A., van der Graaf, Y., Stella, P., Doevendans, P., Meijs, M.F.L., Prokop, M. & Visseren, F.L.J. 2008, "Relation of epicardial and pericoronary fat to coronary atherosclerosis and coronary artery calcium in patients undergoing coronary angiography.", *The American Journal of Cardiology*, vol. 102, no. 4, pp. 380-385.

Greenland, P., Blaha, M.J., Budoff, M.J., Erbel, R., & Watson, K.E. 2018, "Coronary Calcium Score and Cardiovascular Risk." *Journal of the American College of Cardiology*, vol. 72, no. 4, pp. 434–447.

Greenland, P., Bonow, R., Brundage, B., Budoff, M., Eisenberg, M., Grundy, S., Lauer, M., Post, W., Raggi, P., Redberg, R., Rodgers, G., Shaw, L., Taylor, A. & Weintraub, W. 2007, "ACCF/AHA 2007 clinical expert consensus document on coronary artery calcium scoring by computed tomography in global cardiovascular risk assessment and in evaluation of patients with chest pain: a report of the American College of Cardiology Foundation Clinical Expert Consensus Task Force (ACCF/AHA Writing Committee to Update the 2000 Expert Consensus Document on Electron Beam Computed Tomography) developed in collaboration with the Society of Atherosclerosis Imaging and Prevention and the Society of Cardiovascular Computed Tomography.", *Journal of the American College of Cardiology*, vol. 49, no. 3, pp. 378-402.

Greif, M., Arnoldt, T., von Ziegler, F., Ruemmler, J., Becker, C., Wakili, R., D'Anastasi, M., Schenzle, J., Leber, A. W., & Becker, A. 2013, "Lipoprotein (a) is independently correlated with coronary artery calcification." *European journal of internal medicine*, vol. 24, no. 1, pp. 75–79.

Grundy, S., Brewer, H.B., Cleeman, J., Smith, S. & Lenfant, C. 2004, "Definition of metabolic syndrome: Report of the National Heart, Lung, and Blood Institute/American Heart Association conference on scientific issues related to definition.", *Circulation*, vol. 109, no. 3, pp. 433-438.

Halon, D.A., Sapoznikov, D., Lewis, B.S. & Gotsman, M.S. 1983, "Localization of lesions in the coronary circulation.", *The American Journal of Cardiology*, vol. 52, no. 8, pp. 921-926.

Herbert, V., Lau, K.S., Gottlieb, C.W. & Bleicher, S.J. 1965, "Coated charcoal immunoassay of insulin.", *The Journal of clinical endocrinology and metabolism*, vol. 25, no. 10, pp. 1375-1384.

Hirata, Y., Kurobe, H., Akaike, M., Chikugo, F., Hori, T., Bando, Y., Nishio, C., Higashida, M., Nakaya, Y., Kitagawa, T. & Sata, M. 2011, "Enhanced inflammation in epicardial fat in patients with coronary artery disease.", *International Heart Journal*, vol. 52, no. 3, pp. 139-142.

Hoff, J.A., Chomka, E.V., Krainik, A.J., Daviglus, M., Rich, S. & Kondos, G.T. 2001, "Age and gender distributions of coronary artery calcium detected by electron beam tomography in 35,246 adults.", *The American Journal of Cardiology*, vol. 87, no. 12, pp. 1335-1339.

Hoff, J., Daviglus, M., Chomka, E., Krainik, A., Sevrukov, A. & Kondos, G. 2003, "Conventional coronary artery disease risk factors and coronary artery calcium detected by electron beam tomography in 30,908 healthy individuals.", *Annals of Epidemiology*, vol. 13, no. 3, pp. 163-169.

Holme, I., Aastveit, A. H., Jungner, I., & Walldius, G. 2008, "Relationships between lipoprotein components and risk of myocardial infarction: age, gender and short versus longer follow-up periods in the Apolipoprotein MOrtality RISk study (AMORIS)." *Journal of internal medicine*, vol 264, no. 1, pp. 30–38.

Iacobellis, G., Pistilli, D., Gucciardo, M., Leonetti, F., Miraldi, F., Brancaccio, G., Gallo, P. & di Gioia, C.R.T. 2005, "Adiponectin expression in human epicardial adipose tissue in vivo is lower in patients with coronary artery disease.", *Cytokine*, vol. 29, no. 6, pp. 251-255.

Iacobellis, G. & Willens, H. 2009, "Echocardiographic epicardial fat: a review of research and clinical applications.", *Journal of the American Society of Echocardiography*, vol. 22, no. 12, pp. 1311-1319.

Jang, S., Kim, S., Sung, J., Cho, S. & Choe, Y. 2016, "Coronary artery calcium scores and cardiovascular risk factors in 31,545 asymptomatic Korean adults.", *The international journal of cardiovascular imaging*, vol. 32 Suppl 1, pp. 139-145.

Juhola, J., Magnussen, C., Berenson, G., Venn, A., Burns, T., Sabin, M., Srinivasan, S., Daniels, S., Davis, P., Chen, W., Kähönen, M., Taittonen, L., Urbina, E., Viikari, J., Dwyer, T., Raitakari, O., & Juonala, M. 2013, "Combined effects of child and adult elevated blood pressure on subclinical atherosclerosis: the International Childhood Cardiovascular Cohort Consortium." *Circulation*, vol 128, no 3, pp. 217–224.

Juonala, M., Magnussen, C., Berenson, G., Venn, A., Burns, T., Sabin, M., Srinivasan, S., Daniels, S., Davis, P., Chen, W., Sun, C., Cheung, M., Viikari, J.S.A., Dwyer, T. & Raitakari, O. 2011, "Childhood adiposity, adult adiposity, and cardiovascular risk factors.", *The New England journal of medicine*, vol. 365, no. 20, pp. 1876-1885.

Juonala, M., Viikari, J.S.A., Kähönen, M., Taittonen, L., Laitinen, T., Hutri Kähönen, N., Lehtimäki, T., Jula, A., Pietikäinen, M., Jokinen, E., Telama, R., Räsänen, L., Mikkilä, V., Helenius, H., Kivimäki, M. & Raitakari, O. 2010, "Life-time risk factors and progression of carotid atherosclerosis in young adults: the Cardiovascular Risk in Young Finns study.", *European heart journal*, vol. 31, no. 14, pp. 1745-1751.

Juonala, M., Viikari, J.S.A., Hutri-Kähönen, N., Pietikäinen, M., Jokinen, E., Taittonen, L., Marniemi, J., Rönnemaa, T. & Raitakari, O.T. 2004, "The 21-year follow-up of the Cardiovascular Risk in Young Finns Study: risk factor levels, secular trends and east-west difference.", *Journal of internal medicine*, vol. 255, no. 4, pp. 457-468.

Juonala, M., Magnussen, C., Venn, A., Dwyer, T., Burns, T., Davis, P., Chen, W., Srinivasan, S., Daniels, S., Khnen, M., Laitinen, T., Taittonen, L., Berenson, G., Viikari, J.S.A. & Raitakari, O. 2010, "Influence of age on associations between childhood risk factors and carotid intima-media thickness in adulthood: the Cardiovascular Risk in Young Finns Study, the Childhood Determinants of Adult Health Study, the Bogalusa Heart Study, and the Muscatine Study for the International Childhood Cardiovascular Cohort (i3C) Consortium.", *Circulation*, vol. 122, no. 24, pp. 2514-2520.

Juonala, M., Viikari, J.S.A., Kähönen, M., Solakivi, T., Helenius, H., Jula, A., Marniemi, J., Taittonen, L., Laitinen, T., Nikkari, T. & Raitakari, O. 2008, "Childhood levels of serum apolipoproteins B and A-I predict carotid intima-media thickness and brachial endothelial function in adulthood: the cardiovascular risk in young Finns study.", *Journal of the American College of Cardiology*, vol. 52, no. 4, pp. 293-299.

Kaitosaari, T., Rönnemaa, T., Raitakari, O., Talvia, S., Kallio, K., Volanen, I., Leino, A., Jokinen, E., Välimäki, I., Viikari, J. & Simell, O. 2003, "Effect of 7-year infancy-onset dietary intervention on serum lipoproteins and lipoprotein subclasses in healthy children in the prospective, randomized Special Turku Coronary Risk Factor Intervention Project for Children (STRIP) study.", *Circulation*, vol. 108, no. 6, pp. 672-677.

Karwowski, W., Naumnik, B., Szczepański, M., & Myśliwiec, M., 2012, "The mechanism of vascular calcification - a systematic review." *Medical science monitor : international medical journal of experimental and clinical research*, vol. 18, no.1, pp. 1-11.

Keys, A., Menotti, A., Aravanis, C., Blackburn, H., Djordevic, B.S., Buzina, R., Dontas, A.S., Fidanza, F., Karvonen, M.J. & Kimura, N. 1984, "The seven countries study: 2,289 deaths in 15 years.", *Preventive medicine*, vol. 13, no. 2, pp. 141-154.

Kim, B. & Kang, J. 2013, "Echocardiographic epicardial fat thickness is associated with arterial stiffness.", *International journal of cardiology*, vol. 167, no. 5, pp. 2234-2238.

Kondos, G.T. 2003, "Electron-beam tomography coronary artery calcium and cardiac events: a 37-month follow-up of 5635 initially asymptomatic low- to intermediate-risk adults.", *Circulation*, [Online], vol. 107, no. 20, pp. 2571-2576.

Laitinen, T., Nuotio, J., Niinikoski, H., Juonala, M., Rovio, S., Viikari, J., Rönnemaa, T., Magnussen, C. G., Sabin, M., Burgner, D., Jokinen, E., Lagström, H., Jula, A., Simell, O., Raitakari, O., & Pahkala, K. 2020, "Attainment of Targets of the 20-Year Infancy-Onset Dietary Intervention and Blood Pressure Across Childhood and Young Adulthood: The Special Turku Coronary Risk Factor Intervention Project (STRIP)." *Hypertension*, vol. 76, no. 5, pp. 1572–1579

LaMonte, M., FitzGerald, S., Church, T., Barlow, C., Radford, N., Levine, B., Pippin, J., Gibbons, L., Blair, S. & Nichaman, M. 2005, "Coronary artery calcium score and coronary heart disease events in a large cohort of asymptomatic men and women.", *American Journal of Epidemiology*, vol. 162, no. 5, pp. 421-429.

Lapinleimu, H., Viikari, J., Jokinen, E., Salo, P., Routi, T., Leino, A., Rönnemaa, T., Seppänen, R., Välimäki, I. & Simell, O. 1995, "Prospective randomised trial in 1062 infants of diet low in saturated fat and cholesterol.", *The Lancet*, vol. 345, no. 8948, pp. 471-476.

Lauer, R.M., Connor, W.E., Leaverton, P.E., Reiter, M.A. & Clarke, W.R. 1975, "Coronary heart disease risk factors in school children: the Muscatine study.", *The Journal of pediatrics*, vol. 86, no. 5, pp. 697-706.

Li, S., Chen, W., Srinivasan, S. & Berenson, G. 2004, "Childhood blood pressure as a predictor of arterial stiffness in young adults: the Bogalusa heart study.", *Hypertension*, vol. 43, no. 3, pp. 541-546.

Li, S., Chen, W., Srinivasan, S., Bond, M.G., Tang, R., Urbina, E. & Berenson, G. 2003, "Childhood cardiovascular risk factors and carotid vascular changes in adulthood: the Bogalusa Heart Study.", *JAMA: the Journal of the American Medical Association* , vol. 290, no. 17, pp. 2271-2276.

Liang, Y., Shiel, L., Teede, H., Kotsopoulos, D., McNeil, J., Cameron, J. & McGrath, B. 2001, "Effects of Blood Pressure, Smoking, and Their Interaction on Carotid Artery Structure and Function.", *Hypertension*, vol. 37, no. 1, pp. 6-11.

Lloyd-Jones, D.M., Hong, Y., Labarthe, D., Mozaffarian, D., Appel, L.J., Van Horn, L., Greenlund, K., Daniels, S., Nichol, G., Tomaselli, G.F., Arnett, D.K., Fonarow, G.C., Ho, P.M., Lauer, M.S., Masoudi, F.A., Robertson, R.M., Roger, V., Schwamm, L.H., Sorlie, P., Yancy, C.W., Rosamond W. D. American Heart Association Strategic Planning Task Force and Statistics Committee 2010, "Defining and setting national goals for cardiovascular health promotion and disease reduction:

the American Heart Association's strategic Impact Goal through 2020 and beyond." *Circulation*, vol 121, no. 4, pp. 586–613.

Lorenz, M., Markus, H., Bots, M., Rosvall, M. & Sitzer, M. 2007, "Prediction of clinical cardiovascular events with carotid intima-media thickness: a systematic review and meta-analysis.", *Circulation*, vol. 115, no. 4, pp. 459-467.

Loria, C., Liu, K., Lewis, C., Hulley, S., Sidney, S., Schreiner, P., Williams, O.D., Bild, D. & Detrano, R. 2007, "Early adult risk factor levels and subsequent coronary artery calcification: the CARDIA Study.", *Journal of the American College of Cardiology*, vol. 49, no. 20, pp. 2013-2020.

Lozano, P., Henrikson, N. B., Morrison, C. C., Dunn, J., Nguyen, M., Blasi, P.R., & Whitlock, E.P. 2016, "Lipid Screening in Childhood and Adolescence for Detection of Multifactorial Dyslipidemia: Evidence Report and Systematic Review for the US Preventive Services Task Force." *JAMA*, vol. 316, no. 6, pp. 634–644.

Luo, G., Ducy, P., McKee, M.D., Pinero, G.J., Loyer, E., Behringer, R.R. & Karsenty, G. 1997, "Spontaneous calcification of arteries and cartilage in mice lacking matrix GLA protein.", *Nature*, vol. 386, no. 6620, pp. 78-81.

Lusis, A.J. 2000, "Atherosclerosis.", *Nature*, vol. 407, no. 6801, pp. 233-241.

Mahabadi, A.A., Berg, M. H., Lehmann, N., Kälsch, H., Bauer, M., Kara, K., Dragano, N., Moebus, S., Jöckel, K. H., Erbel, R., & Möhlenkamp, S. 2013, "Association of epicardial fat with cardiovascular risk factors and incident myocardial infarction in the general population: the Heinz Nixdorf Recall Study.", *Journal of the American College of Cardiology*, vol. 61, no. 13, pp. 1388-1395.

Mahabadi, A.A., Massaro, J. M., Rosito, G. A., Levy, D., Murabito, J. M., Wolf, P. A., O'Donnell, C. J., Fox, C. S., & Hoffmann, U. 2009, "Association of pericardial fat, intrathoracic fat, and visceral abdominal fat with cardiovascular disease burden: the Framingham Heart Study.", *European heart journal*, vol. 30, no. 7, pp. 850-856.

Mahoney, L.T., Burns, T.L., Stanford, W., Thompson, B.H., Witt, J.D., Rost, C.A. & Lauer, R.M. 1996, "Coronary risk factors measured in childhood and young adult life are associated with coronary artery calcification in young adults: the Muscatine Study.", *Journal of the American College of Cardiology*, vol. 27, no. 2, pp. 277-284.

Matsuzawa, Y., Kwon, T. G., Lennon, R. J., Lerman, L. O., & Lerman, A. 2015, Prognostic Value of Flow-Mediated Vasodilation in Brachial Artery and Fingertip Artery for Cardiovascular Events: A Systematic Review and Meta-Analysis. *Journal of the American Heart Association*, vol. 4, no. 11

Matthews, D.R., Hosker, J.P., Rudenski, A.S., Naylor, B.A., Treacher, D.F. & Turner, R.C. 1985, "Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man.", *Diabetologia*, vol. 28, no. 7, pp. 412-419.

Mazurek, T., Zhang, L., Zalewski, A., Mannion, J., Diehl, J., Arafat, H., Sarov Blat, L., O'Brien, S., Keiper, E., Johnson, A., Martin, J., Goldstein, B. & Shi, Y. 2003, "Human epicardial adipose tissue is a source of inflammatory mediators.", *Circulation*, vol. 108, no. 20, pp. 2460-2466.

McGill, H.C., McMahan, C.A., Zieske, A.W., Sloop, G.D., Walcott, J.V., Troxclair, D.A., Malcom, G.T., Tracy, R.E., Oalmann, M.C. & Strong, J.P. 2000, "Associations of coronary heart disease risk factors with the intermediate lesion of atherosclerosis in youth. The Pathobiological Determinants of Atherosclerosis in Youth (PDAY) Research Group.", *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 20, no. 8, pp. 1998-2004.

McNamara, J.J., Molot, M.A., Stremple, J.F. & Cutting, R.T. 1971, "Coronary artery disease in combat casualties in Vietnam.", *JAMA: the Journal of the American Medical Association*, vol. 216, no. 7, pp. 1185-1187.

Mitchell, T.L., Pippin, J.J., Devers, S.M., Kimball, T.E., Cannaday, J.J., Gibbons, L.W. & Cooper, K.H. 2001, "Age- and sex-based nomograms from coronary artery calcium scores as determined by electron beam computed tomography.", *The American Journal of Cardiology*, vol. 87, no. 4, pp. 453-456.

Mullen, M.J., Kharbanda, R.K., Cross, J., Donald, A.E., Taylor, M., Vallance, P., Deanfield, J.E. & MacAllister, R.J. 2001, "Heterogenous nature of flow-mediated dilatation in human conduit arteries in vivo: relevance to endothelial dysfunction in hypercholesterolemia.", *Circulation research*, vol. 88, no. 2, pp. 145-151.

Nagy, E., Jermendy, A.L., Merkely, B., & Maurovich-Horvat, P. 2017, "Clinical importance of epicardial adipose tissue.", *Archives of medical science : AMS*, vol. 13, no 4, pp. 864–874.

Oliver, J. & Webb, D. 2003, "Noninvasive assessment of arterial stiffness and risk of atherosclerotic events.", *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 23, no. 4, pp. 554-566.

Oudkerk, M., Stillman, A., Halliburton, S., Kalender, W., Möhlenkamp, S., McCollough, C., Vliegenthart, R., Shaw, L., Stanford, W., Taylor, A., van Ooijen, Peter M A, Wexler, L. & Raggi, P. 2008, "Coronary artery calcium screening: current status and recommendations from the European Society of Cardiac Radiology and North American Society for Cardiovascular Imaging.", *European radiology*, vol. 18, no. 12, pp. 2785-2807.

Parker, D., Liu, K., Dyer, A. R., Giumetti, D., Liao, Y. L., & Stamler, J. 1988, "A comparison of the random-zero and standard mercury sphygmomanometers." *Hypertension*, vol. 11, no. 3, pp. 269–272.

Pierdomenico, S. D., Pierdomenico, A. M., Neri, M., & Cuccurullo, F. 2011, "Epicardial adipose tissue and metabolic syndrome in hypertensive patients with normal body weight and waist circumference.", *American journal of hypertension*, vol. 24, no. 11, pp. 1245-1249.

Pierdomenico S.D., Pierdomenico A.M., Cuccurullo F., Iacobellis G. 2013, "Meta-analysis of the relation of echocardiographic epicardial adipose tissue thickness and the metabolic syndrome." *The American Journal of Cardiology*, vol. 111,1, pp. 73-78.

Pignoli, P., Tremoli, E., Poli, A., Oreste, P. & Paoletti, R. 1986, "Intimal plus medial thickness of the arterial wall: a direct measurement with ultrasound imaging.", *Circulation*, vol. 74, no. 6, pp. 1399-1406.

Pletcher, M., Tice, J., Pignone, M., McCulloch, C., Callister, T. & Browner, W. 2004, "What does my patient's coronary artery calcium score mean? Combining information from the coronary artery calcium score with information from conventional risk factors to estimate coronary heart disease risk.", *BMC medicine*, vol. 2, pp. 31-31.

Pletcher, M. J., Bibbins-Domingo, K., Lewis, C. E., Wei, G. S., Sidney, S., Carr, J. J., Vittinghoff, E., McCulloch, C. E., & Hulley, S. B. 2008, "Prehypertension during young adulthood and coronary calcium later in life." *Annals of internal medicine*, vol. 149, no. 2, pp. 91–99.

Porkka, K.V., Raitakari, O.T., Leino, A., Laitinen, S., Räsänen, L., Rönnemaa, T., Marniemi, J., Lehtimäki, T., Taimela, S., Dahl, M., Uhari, M., Åkerblom, H.K. & Viikari, J.S. 1997, "Trends in serum lipid levels during 1980-1992 in children and young adults. The Cardiovascular Risk in Young Finns Study.", *American Journal of Epidemiology*, vol. 146, no. 1, pp. 64-77.

Pracon, R., Kruk, M., Kepka, C., Pregowski, J., Opolski, M., Dzielinska, Z., Michalowska, I., Chmielak, Z., Demkow, M. & Ruzyllo, W. 2011, "Epicardial adipose tissue radiodensity is independently related to coronary atherosclerosis. A multidetector computed tomography study.", *Circulation Journal*, vol. 75, no. 2, pp. 391-397.

Pugliese, G., Iacobini, C., Blasetti Fantauzzi, C. & Menini, S. 2015, "The dark and bright side of atherosclerotic calcification.", *Atherosclerosis*, vol. 238, no. 2, pp. 220-230.

Raggi, P., Callister, T.Q., Cooil, B., He, Z.X., Lippolis, N.J., Russo, D.J., Zelinger, A. & Mahmarian, J.J. 2000, "Identification of patients at increased risk of first unheralded acute myocardial infarction by electron-beam computed tomography.", *Circulation*, vol. 101, no. 8, pp. 850-855.

Raiko, J.R.H., Viikari, J.S.A., Ilmanen, A., Hutri-Kähönen, N., Taittonen, L., Jokinen, E., Pietikäinen, M., Jula, A., Loo, B., Marniemi, J., Lehtimäki, T., Kähönen, M., Rönnemaa, T., Raitakari, O.T. & Juonala, M. 2010, "Follow-ups of the Cardiovascular Risk in Young Finns Study in 2001 and 2007: levels and 6-year changes in risk factors.", *Journal of internal medicine*, vol. 267, no. 4, pp. 370-384.

Raitakari, O. & Celermajer, D.S. 2000, "Testing for endothelial dysfunction.", *Annals of medicine*, vol. 32, no. 5, pp. 293-304.

Raitakari, O., Mäkinen, V., McQueen, M., Niemi, J., Juonala, M., Jauhiainen, M., Salomaa, V., Hannuksela, M., Savolainen, M., Kesäniemi, Y.A., Kovanen, P., Sundvall, J., Solakivi, T., Loo, B., Marniemi, J., Hernesniemi, J., Lehtimäki, T., Kähönen, M., Peltonen, M., Leiviskä, J., Jula, A., Anand, S., Miller, R., Yusuf, S., Viikari, J.S.A. & Ala Korpela, M. 2013, "Computationally estimated apolipoproteins B and A1 in predicting cardiovascular risk.", *Atherosclerosis*, vol. 226, no. 1, pp. 245-51.

Raitakari, O., Juonala, M., Kähönen, M., Taittonen, L., Laitinen, T., Mäki-Torkko, N., Järvisalo, M.J., Uhari, M., Jokinen, E., Rönnemaa, T., Åkerblom, H.K. & Viikari, J.S. 2003, "Cardiovascular risk factors in childhood and carotid artery intima-media thickness in adulthood: the Cardiovascular Risk in Young Finns Study", *JAMA : the journal of the American Medical Association*, vol. 290, no. 17, pp. 2277-2283.

Raitakari, O., Juonala, M., Rönnemaa, T., Keltikangas-Järvinen, L., Räsänen, L., Pietikäinen, M., Hutri-Kähönen, N., Taittonen, L., Jokinen, E., Marniemi, J., Jula, A., Telama, R., Kähönen, M., Lehtimäki, T., Åkerblom, H. & Viikari, J.S.A. 2008, "Cohort profile: the cardiovascular risk in Young Finns Study.", *International journal of epidemiology*, vol. 37, no. 6, pp. 1220-1226.

Rego, J.O.C., Iacobellis, G., Sarmientos, J.C.G., Mustelier, J., Aquiles, E.W.O., Rodríguez, V. & Rojas, N. 2011, "Epicardial fat thickness correlates with ApoB/ApoA1 ratio, coronary calcium and carotid intima media thickness in asymptomatic subjects.", *International journal of cardiology*, vol. 151, no. 2, pp. 234-236.

Reinikainen, J., Laatikainen, T., Karvanen, J. & Tolonen, H. 2015, "Lifetime cumulative risk factors predict cardiovascular disease mortality in a 50-year follow-up study in Finland.", *International journal of epidemiology*, vol. 44, no. 1, pp. 108-116.

Riley, W.A., Barnes, R.W., Evans, G.W. & Burke, G.L. 1992, "Ultrasonic measurement of the elastic modulus of the common carotid artery. The Atherosclerosis Risk in Communities (ARIC) Study.", *Stroke*, vol. 23, no. 7, pp. 952-956.

Rosito, G.A., Massaro, J.M., Hoffmann, U., Ruberg, F.L., Mahabadi, A.A., Vasan, R.S., O'Donnell, C. J., & Fox, C.S. 2008, "Pericardial fat, visceral abdominal fat, cardiovascular disease risk factors, and vascular calcification in a community-based sample: the Framingham Heart Study.", *Circulation*, vol. 117, no. 5, pp. 605-613.

Rumberger, J.A., Schwartz, R.S., Simons, D.B., Sheedy, P.F.,3rd, Edwards, W.D. & Fitzpatrick, L.A. 1994, "Relation of coronary calcium determined by electron beam computed tomography and lumen narrowing determined by autopsy", *The American Journal of Cardiology*, vol. 73, no. 16, pp. 1169-1173.

Rutsch, F., Ruf, N., Vaingankar, S., Toliat, M., Suk, A., Höhne, W., Schauer, G., Lehmann, M., Roscioli, T., Schnabel, D., Epplen, J., Knisely, A., Superti Furga, A., McGill, J., Filippone, M., Sinaiko, A., Vallance, H., Hinrichs, B., Smith, W., Ferre, M., Terkeltaub, R. & Nürnberg, P. 2003, "Mutations in ENPP1 are associated with 'idiopathic' infantile arterial calcification.", *Nature genetics*, vol. 34, no. 4, pp. 379-381.

Sacks, H. & Fain, J. 2007, "Human epicardial adipose tissue: a review.", *The American Heart Journal*, vol. 153, no. 6, pp. 907-917.

Safar, M.E., London, G.M., Asmar, R. & Frohlich, E.D. 1998, "Recent advances on large arteries in hypertension.", *Hypertension*, vol. 32, no. 1, pp. 156-161.

Sandfort, V., & Bluemke, D. A. 2017, "CT calcium scoring. History, current status and outlook." *Diagnostic and interventional imaging*, vol. 98, no. 1, pp. 3–10.

Sangiorgi G, Rumberger J.A., Severson A., Edwards W.D., Gregoire J., Fitzpatrick L.A., Schwartz R.S., 1998, "Arterial calcification and not lumen stenosis is highly correlated with atherosclerotic plaque burden in humans: a histologic study of 723 coronary artery segments using nondecalcifying methodology.", *Journal of the American College of Cardiology*, vol. 31, no. 1, pp. 126-133.

Schmermund, A., Baumgart, D., Möhlenkamp, S., Kriener, P., Pump, H., Grönemeyer, D., Seibel, R. & Erbel, R. 2001, "Natural history and topographic pattern of progression of coronary calcification in symptomatic patients: An electron-beam CT study.", *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 21, no. 3, pp. 421-426.

Sengul, C., Cevik, C., Ozveren, O., Oduncu, V., Sunbul, A., Akgun, T., Can, M., Semiz, E. & Dindar, I. 2011, "Echocardiographic epicardial fat thickness is associated with carotid intima-media thickness in patients with metabolic syndrome.", *Echocardiography*, vol. 28, no. 8, pp. 853-858.

Shaw, L., Raggi, P., Schisterman, E., Berman, D. & Callister, T. 2003, "Prognostic value of cardiac risk factors and coronary artery calcium screening for all-cause mortality.", *Radiology*, vol. 228, no. 3, pp. 826-833.

Simons, D.B., Schwartz, R.S., Edwards, W.D., Sheedy, P.F., Breen, J.F., & Rumberger, J.A. 1992, "Noninvasive definition of anatomic coronary artery disease by ultrafast computed tomographic scanning: A quantitative pathologic comparison study." Journal of the American College of Cardiology vol. 20, no. 5, pp. 1118-1126.

Sniderman, A.D., Thanassoulis, G., Glavinovic, T., Navar, A. M., Pencina, M., Catapano, A., & Ference, B. A. 2019, "Apolipoprotein B Particles and Cardiovascular Disease: A Narrative Review." JAMA cardiology, vol 4, no. 12, pp. 1287–1295.

Spearman, J., Meinel, F., Schoepf, U.J., Apfaltrer, P., Silverman, J., Krazinski, A., Canstein, C., De Cecco, C., Costello, P. & Geyer, L. 2014, "Automated quantification of epicardial adipose tissue using CT angiography: evaluation of a prototype software.", *European radiology*, vol. 24, no. 2, pp. 519-526.

Spearman, J., Renker, M., Schoepf, U.J., Krazinski, A., Herbert, T., De Cecco, C., Nietert, P. & Meinel, F. 2015, "Prognostic value of epicardial fat volume measurements by computed tomography: a systematic review of the literature.", *European radiology*, vol. 25, no. 11, pp. 3372-3381.

Srinivasan, S.R., Frerichs, R.R., Webber, L.S. & Berenson, G.S. 1976, "Serum lipoprotein profile in children from a biracial community: the Bogalusa Heart Study.", *Circulation*, vol. 54, no. 2, pp. 309-318.

Stary, H.C., Chandler, A.B., Dinsmore, R.E., Fuster, V., Glagov, S., Insull, W., Rosenfeld, M.E., Schwartz, C.J., Wagner, W.D. & Wissler, R.W. 1995, "A definition of advanced types of atherosclerotic lesions and a histological classification of atherosclerosis. A report from the Committee on Vascular Lesions of the Council on Arteriosclerosis, American Heart Association.", *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 15, no. 9, pp. 1512-1531.

Stojanovska, J., Ibrahim, E. H., Chughtai, A. R., Jackson, E. A., Gross, B. H., Jacobson, J. A., Tsodikov, A., Daneshvar, B., Long, B. D., Chenevert, T. L., & Kazerooni, E. A. 2017, Intrathoracic Fat Measurements Using Multidetector Computed Tomography (MDCT): Feasibility and Reproducibility. *Tomography*, vol 3, no. 1, pp. 33–40.

Tabas, I., Williams, K. & Born, J. 2007, "Subendothelial lipoprotein retention as the initiating process in atherosclerosis: update and therapeutic implications.", *Circulation*, vol. 116, no. 16, pp. 1832-1844.

Tanami, Y., Jinzaki, M., Kishi, S., Matheson, M., Vavere, A., Rochitte, C., Dewey, M., Chen, M., Clouse, M., Cox, C., Kuribayashi, S., Lima, J.A.C. & Arbab Zadeh, A. 2015, "Lack of association between epicardial fat volume and extent of coronary artery calcification, severity of coronary artery disease, or presence of myocardial perfusion abnormalities in a diverse, symptomatic patient population: results from the CORE320 multicenter study.", *Circulation: Cardiovascular Imaging*, vol. 8, no. 3, pp. 2676-2685.

Taylor, A., Bindeman, J., Feuerstein, I., Cao, F., Brazaitis, M. & O'Malley, P. 2005, "Coronary calcium independently predicts incident premature coronary heart disease over measured cardiovascular risk factors: mean three-year outcomes in the Prospective Army Coronary Calcium (PACC) project.", *Journal of the American College of Cardiology*, vol. 46, no. 5, pp. 807-814.

Telama, R., Yang, X., Viikari, J., Välimäki, I., Wanne, O. & Raitakari, O. 2005, "Physical activity from childhood to adulthood: a 21-year tracking study.", *American Journal of Preventive Medicine*, vol. 28, no. 3, pp. 267-273.

Thomopoulos, C., Parati, G. & Zanchetti, A. 2015, "Effects of blood pressure lowering on outcome incidence in hypertension: 4. Effects of various classes of antihypertensive drugs--overview and meta-analyses.", *Journal of hypertension*, vol. 33, no. 2, pp. 195-211.

Tirosh, A., Shai, I., Afek, A., Dubnov Raz, G., Ayalon, N., Gordon, B., Derazne, E., Tzur, D., Shamis, A., Vinker, S. & Rudich, A. 2011, "Adolescent BMI trajectory and risk of diabetes versus coronary disease.", *The New England journal of medicine*, vol. 364, no. 14, pp. 1315-1325.

van Sloten, T.T., Schram, M.T., van den Hurk, K., Dekker, J.M., Nijpels, G., Henry, R.M., & Stehouwer, C.D. 2014, "Local stiffness of the carotid and femoral artery is associated with incident cardiovascular events and all-cause mortality: the Hoorn study." *Journal of the American College of Cardiology*, vol. 63, no. 17, pp. 1739–1747.

Vartiainen, E., Tossavainen, K., Viri, L., Niskanen, E. & Puska, P. 1991, "The North Karelia Youth Programs.", *Annals of the New York Academy of Sciences*, vol. 623, pp. 332-349.

Wang, C.P., Hsu, H.L., Hung, W.C., Yu, T. H., Chen, Y.H., Chiu, C.A., Lu, L.F., Chung, F.M., Shin, S.J., & Lee, Y.J. 2009, "Increased epicardial adipose tissue (EAT) volume in type 2 diabetes mellitus and association with metabolic syndrome and severity of coronary atherosclerosis.", *Clinical endocrinology*, vol. 70, no. 6, pp. 876-882.

Wolf, D. & Ley, K. 2019, "Immunity and Inflammation in Atherosclerosis." *Circulation Research*, vol 124, no. 2, pp. 315-327.

World Health Organization, World Health Federation, World Stroke Organization. 2011. Global atlas on cadiovascular disease prevention and control.

Yorgun H, Canpolat U, Hazırolan T, Ateş AH, Sunman H, Dural M, et al. Increased epicardial fat tissue is a marker of metabolic syndrome in adult patients. *Int J Cardiol*. 2013;165(2):308-313.

Åkerblom, H.K., Viikari, J., Uhari, M., Räsänen, L., Byckling, T., Louhivuori, K., Pesonen, E., Suoninen, P., Pietikäinen, M. & Lähde, P.L. 1985, "Atherosclerosis precursors in Finnish children and adolescents. I. General description of the cross-sectional study of 1980, and an account of the children's and families' state of health.", *Acta pædiatrica Scandinavica.Supplement*, vol. 318, pp. 49-63.

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